Discovery of *Omecamtiv Mecarbil* the First, Selective, Small Molecule Activator of Cardiac Myosin for the Treatment of Systolic Heart Failure.

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Contents

Summary of Biological Assays:

Biochemical Assay: The reconstituted sarcomere consisted of actin filaments decorated with tropomyosin and troponin complex and an active cardiac myosin subfragment-1 all purified from readily available bovine sources. Biochemical activity was quantitated as an AC_{40}, the compound concentration resulting in a 40% increase in the cardiac sarcomere ATPase activity at the calcium concentration midway up the calcium

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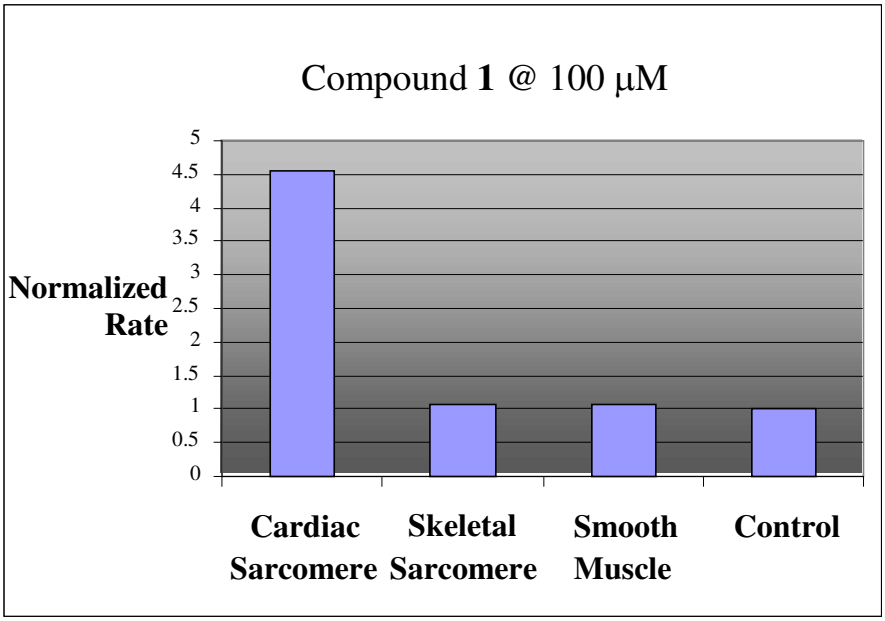
activation curve (½ maximal calcium). ATPase activity was measured using a pyruvate kinase-lactate dehydrogenase detection system that couples the production of ADP to oxidation of NADH. Change in absorbance was monitored at 340 nM. Reactions were carried out at 25 ^{o}C in a buffer consisting of 12 mM Pipes, 2 mM $MgCl_2$, 1 mM DTT at pH 6.8 (PM12 buffer) and contained saturating amounts of ATP (1 mM). Compound specificity with respect to muscle type was evaluated by comparing the effect of the compound on actin-stimulated ATPase activity of a panel of myosin isoforms including cardiac [bovine], skeletal [rabbit], and smooth muscle [bovine uterus or chicken gizzard]) as well as thin filament isoforms (cardiac [bovine] and skeletal [rabbit]) at a single dose of 50 μM or 100 μM of compound. Typical myosin swapping results are shown in Figure S1, where compound **1** increases ATPase activity only in the presence of cardiac myosin but not in the presence of fast skeletal or smooth muscle myosin isoforms.

Figure S1. Cardiac Myosin Selectivity of compound **1**.



Normalized rate = rate of ATP hydrolysis normalized to DMSO control

Rat Cardiomyocyte Experiments:

Preparations of adult cardiac ventricular rat myocytes: Adult male Sprague-Dawley rats were anesthetized with a mixture of isoflurane gas and oxygen. Hearts were quickly excised, rinsed and the ascending aorta cannulated. Continuous retrograde perfusion was initiated on the hearts at 5 ml/min. Hearts were first perfused with a nominally Ca^{2+} free modified Krebs solution of the following composition: 110 mM NaCl, 2.6 mM KCL, 1.2 mM KH_2PO_4 7 H_20, 1.2 mM $MgSO_4$, 2.1 mM $NaHCO_3$, 11 mM glucose and 4 mM Hepes (all purchased from Sigma). This medium was not recirculated and was continually gassed with O_2. After approximately 3-5 minutes the heart was perfused with modified Krebs buffer supplemented with 3.3% collagenase (169 u/mg activity, Class II, Worthington Biochemical Corp., Freehold, NJ) and 25 μM final calcium concentration until the heart became sufficiently blanched and soft. The heart was removed from the cannula, the atria and vessels discarded, and the ventricles cut into small pieces. The myocytes were dispersed by gentle agitation of the ventricular tissue in fresh Krebs solution containing collagenase, prior to being gently forced through a 200 μm nylon mesh in a 50 mL tube. The resulting myocytes were resuspended in modified Krebs' solution containing 25 μM calcium. Myocytes were made calcium tolerant by addition of a calcium solution (100 mM stock) at 10 minute intervals until 100 μM calcium was achieved. After 30 minutes the supernatant was discarded, and 30 – 50 mL of Tyrode's buffer (137 mM NaCl, 3.7 mM KCl, 0.5 mM $MgCl_2$, 11 mM glucose, 4 mM Hepes, and 1.2 mM $CaCl_2$, pH 7.4) added to cells. Cells were kept for 60 min at 37 ^{o}C prior to initiating experiments and used within 5 hours of isolation.

Adult ventricular myocyte contractility experiments: Aliquots of Tyrode's buffer containing myocytes were placed in perfusion chambers (Series 20 RC-27NE; Warner Instruments) complete with heating platforms. Myocytes were allowed to attach, the chambers heated to 37 ºC, and the cells then perfused with 37 ºC Tyrode's buffer. Myocytes were field stimulated at 1 Hz with platinum electrodes (20% above threshold). Only cells that had clear striations and that were quiescent prior to pacing were used for contractility experiments. To determine basal contractility, myocytes were imaged through a 40x objective and using a variable frame rate (60-240 Hz) charge-coupled device camera. The images were digitized and displayed on the computer screen at a sampling speed of 240 Hz. Frame grabber, myopacer, acquisition, and analysis software for cell contractility were obtained from IonOptix (Milton, MA). After an initial basal contractility period, compounds were perfused on the myocytes as defined below. Using this edge detection strategy, contractility of the myocytes and contraction and relaxation velocities were recorded continuously.

Contractility analysis: For each cell, twenty or more contractility transients at basal (defined as 1 minute prior to compound infusion) and after compound addition were averaged and compared. These average transients were analyzed to determine changes in diastolic length, and using the Ionwizard analysis program (IonOptix), fractional shortening (% decrease in the diastolic length), and maximum contraction and relaxation velocities (um/sec) were determined. Baseline levels of fractional shortening are defined as 100%. Preparations of cell were used only if cells first passed QC criteria by responding to a CK standard (>150% of basal) and isoproterenol (ISO; > 250% of basal). Compounds that increased fractional shortening of the cells resulted in values >100% and

compounds that decreased fractional shortening of the cells resulted in values <100% at the applied compound concentration (FS%@μM). Additionally, only cells whose basal contractility was between 3 and 8% were used in the following experiments.

Calcium transient analysis:

Fura loading: Cell permeable Fura-2 (Molecular Probes) was dissolved in equal amounts of pluronic (Mol Probes) and FBS for 10 min at RT. A 1 μM Fura stock solution was made in Tyrode buffer containing 500 mM probenecid (Sigma). To load cells, this solution was added to myocytes at RT. After 10 min the buffer was removed, the cells were washed with Tyrode containing probenecid, and they were incubated at RT for 10 min. This wash and incubation cycle was repeated. Simultaneous contractility and calcium measurements were determined within 40 min of loading.

Imaging: A test compound was perfused on cells and simultaneous contractility and calcium transient ratios determined at baseline and after compound addition. Cells were digitally imaged and contractility determined as described above except that a red filter was in the light path so that it would not interfere with fluorescent calcium measurements. Acquisition, analysis software and hardware for calcium transient analysis were obtained from IonOptix. The instrumentation for fluorescence measurement included a xenon arc lamp and a Hyperswitch dual excitation light source that alternates between 340 and 380 nm wavelengths at 100 Hz by a galvo-driven mirror. A liquid filled light guide delivered the dual excitation light to the microscope and the emission fluorescence was determined using a photomultiplier tube (PMT). The fluorescence system interface routes the PMT signal and the ratios were recorded using the IonWizard acquisition program.

Analysis: For each cell, ten or more contractility and calcium ratio transients at basal and after compound addition were averaged and compared. Contractility average transients were analyzed using the Ionwizard analysis program to determine changes in diastolic length and fractional shortening (% decrease in the diastolic length). The averaged calcium ratio transients were also analyzed using the Ionwizard analysis program to determine changes in diastolic and systolic ratios and the 75% time to baseline (T_{75}).

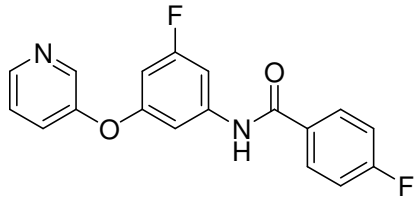
Durability: To determine the durability of response, myocytes were challenged with the compound for 25 minutes followed by a 2 minute washout period. Contractility response was compared at 5 and 25 minutes of compound infusion.

Threshold potential: In other experiments, myocytes were field stimulated at a voltage approximately 20% above threshold. In these experiments the threshold voltage (minimum voltage to pace cell) was empirically determined, the cell was paced at that threshold and then the compound was infused. After the compound activity was at steady state, the voltage was decreased for 20 seconds and then restarted. If the compound altered ion channel function, that effect would result in increasing or lowering the threshold action potential.

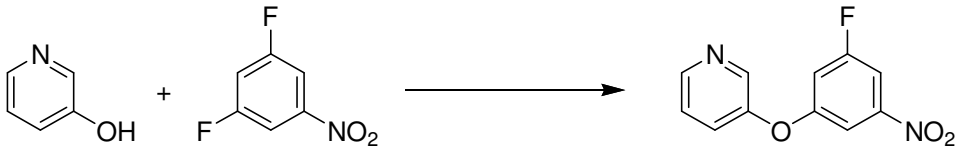
Hz frequency: Contractility of myocytes was determined at 3 Hz as follows: a 1 minute basal time point followed by perfusion of the compound for 5 minutes followed by a 2 minute washout. After the cell contractility has returned completely to baseline the Hz frequency is decreased to 1. After an initial acclimation period the cell is challenged by the same compound. As this preparation exhibits a negative force frequency at 1 Hz, at 3 Hz the FS of the cell should be lower, but should respond well to the compound.

Synthesis:

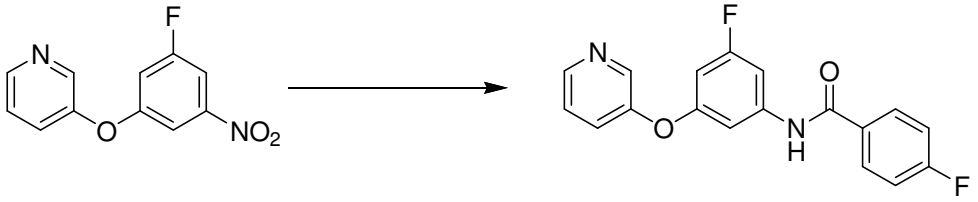
General. Unless otherwise noted, all reagents were commercially obtained and, where appropriate, purified prior to use. Anhydrous THF, Et_2O, Toluene, and CH_2Cl_2 were purchased from standard commercial vendors. All air or moisture sensitive reactions were carried out under an atmosphere of nitrogen in glassware that had been oven- or flame-dried. ^{1}H NMR and ^{13}C NMR spectra were recorded at ambient temperature at 400 MHz and 100 MHz, respectively, using a Bruker or DRX 400 spectrometer. The data are reported as follows: chemical shift in ppm from internal tetramethylsilane on the δ scale, multiplicity (br = broad, s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet), coupling constants (Hz), and integration. Infrared (IR) spectra were obtained using a Perkin Elmer Spectrum One FT-IR spectrometer with a Universal ATR Sampling Accessory. High resolution mass spectrometry data were obtained with an Agilent 1200 HPLC/MS system equipped with a Zorbax SB-C18 column and an Agilent 6210 electrospray time-of-flight mass spectrometer. Melting points were obtained using an SRS Optimelt MPA-100 Automated Melting Point System. Normal phase liquid chromatography was performed using forced flow (flash chromatography) of the indicated solvent system on EM Reagents silica gel (SiO_2) 60 (230–400 mesh) or using a Biotage Horizon MPLC with Biotage KP-Sil silica gel columns. Reverse phase HPLC purification was performed with a Gilson HPLC with a YMC-ODS-A C18 Column (5 uM, 75 x 30 mm) with UV detection.



4-Fluoro-N-(3-fluoro-5-(pyridin-3-yloxy)phenyl)benzamide (2)



3-(3-Fluoro-5-nitrophenoxy)pyridine. A mixture of 3-hydroxypyridine (11.9 g, 126 mmol, 1.0 equiv), 3,5-difluoronitrobenzene (20.0 g, 126 mmol, 1.0 equiv) and potassium carbonate (34.8 g, 252 mmol, 2.0 equiv) in dry *N,N*-dimethylformamide (200 mL) was heated to 100 ^{o}C overnight. The reaction was allowed to cool to room temperature, and the solvent was removed *in vacuo*. The mixture was diluted with EtOAc and water, and the layers were separated. The organic layer was washed twice with water. The combined aqueous layers were extracted once with EtOAc and the combined organic layers were washed once with brine, dried over anhydrous sodium sulfate, filtered, and concentrated *in vacuo*. Purification by silica gel chromatography (50% EtOAc/hexanes) provided the title compound as a yellow oil (14.1 g, 48%).

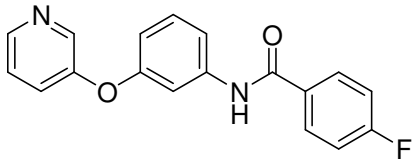


4-Fluoro-N-(3-fluoro-5-(pyridin-3-yloxy)phenyl)benzamide (2). To a 0 ^{o}C suspension of 3-(3-fluoro-5-nitrophenoxy)pyridine (6.0 g, 25.6 mmol, 1.0 equiv) in 1:1: MeOH: conc. HCl (200 mL) was added Fe powder (5.69 g, 100 mmol, 4.0 equiv) as a solid

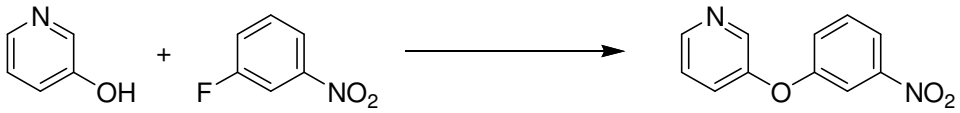
portion-wise. After stirring at 0 ^{o}C for 30 min, the reaction was allowed to warm to room temperature and stirred for 1.5 h. The MeOH was then removed *in vacuo*, and the pH of the resulting mixture was adjusted to 10 by the addition 50% aq. NaOH. The aqueous suspension was then extracted 3 times with EtOAc. The combined organic layers were washed with brine, dried over anhydrous sodium sulfate, filtered and concentrated *in vacuo* to yield the aniline as an orange solid which was used without further purification (3.15 g, 61%).

To a solution of 3-(3-fluoro-5-aminophenoxy)pyridine (141 mg, 0.60 mmol, 1.0 equiv) and N,N-diisopropylethylamine (160 μL, 0.90 mmol, 1.5 equiv) in THF (3 mL) was added 4-fluorobenzoyl chloride (79 μL, 0.66 mmol, 1.1 equiv) by syringe. The reaction mixture was allowed to stir at room temperature overnight. The reaction was diluted with EtOAc and sat'd. aq. $NaHCO_3$, and the layers were separated. The organic layer was washed twice with sat'd. aq. $NaHCO_3$, and the combined aqueous layers were extracted with EtOAc. The combined organic layers were washed with brine, dried over Na_2SO_4, filtered, and concentrated *in vacuo*. Purification by silica gel chromatography (25 – 50% EtOAc/ hexanes) provided compound **2** as a white solid (156 mg, 80%). mp 127 - 129 ^{o}C; IR (Solid) 3276, 3235, 3183, 3100, 3068, 1677, 1602, 1574, 1537, 1505, 1476, 1425, 1371, 1274, 1257, 1146, 1158, 1094, 1043, 1024, 979, 872, 847, 806, 872, 759, 708, 691 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-*d6*) δ 10.47 (s, 1H), 8.51 – 8.43 (m, 2H), 8.09 – 7.83 (m, 2H), 7.59 (ddd, J = 5.4, 4.1, 2.0 Hz, 2H), 7.50 (dd, J = 8.4, 4.6 Hz, 1H), 7.44 – 7.24 (m, 3H), 6.75 (dt, J = 9.9, 2.3 Hz, 1H); ^{13}C NMR (100 MHz, DMSO-*d6*) δ 165.5, 164.7, 163.5, 157.8, 152.2, 145.3, 141.7, 130.7, 126.8, 124.7, 115.2, 104.9, 102.1, 101.9, 100.8,

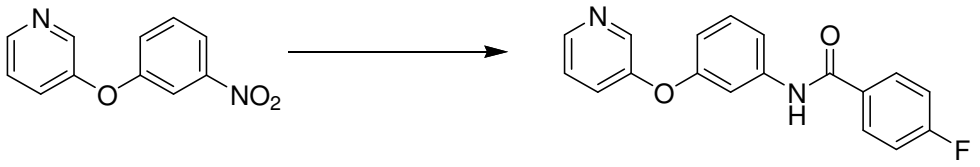
99.5. Exact mass calc'd for $C_{18}H_{13}F_2N_2O_2$ requires *m/z* 327.0945 found *m/z* 327.1213 (ESI, $M+H^+$).



4-Fluoro-N-(3-(pyridin-3-yloxy)phenyl)benzamide (3)



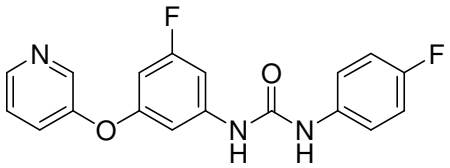
3-(3-nitrophenoxy)pyridine. To a 0 °C solution of 3-hydroxypyridine (1.99 g, 21 mmol, 1.1 equiv) in dry DMF (12 mL) was added NaH (1.8 g of a 60% dispersion in mineral oil, 45.6 mmol, 2.4 equiv) as a solid portion-wise over 5 minutes. The resulting mixture was allowed to warm to room temperature and stir for an additional 10 minutes. 3-Fluoronitrobenzene (2.68 g, 19 mmol, 1.0 equiv) was then added as a solution in dry DMF (5 mL) by syringe. The resulting mixture was heated to 100 °C overnight. After the reaction mixture was cooled to room temperature, and the excess NaH was quenched by the careful addition of water to the reaction mixture. The solvent was removed *in vacuo*, and the residue was re-dissolved in EtOAc and water. The layers were separated, and the water layer was extracted once with EtOAc. The combined organic layers were then washed three times with sat'd. aq. $NaHCO_3$ and once with brine. The organic layer was then dried over Na_2SO_4, filtered, and concentrated *in vacuo*. Silica gel chromatography (25-50% EtOAc/hexanes) provided the title compound as an orange oil (2.58 g, 61%). LRMS $(M+H)^+$ *m/z* 217.2.



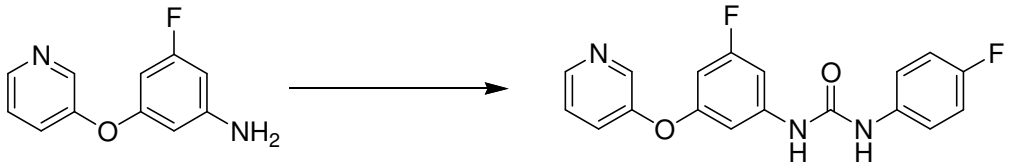
4-Fluoro-N-(3-(pyridin-3-yloxy)phenyl)benzamide (3). To a solution of 3-(3-nitrophenoxy)pyridine (2.5 g, 11.6 mmol, 1.0 equiv) in EtOH (25 mL) was added 10% Pd/C (2.5 g), and the mixture was placed in a Parr bomb and pressurized to ~ 45 psi of H_2 at RT. The reaction was stirred for 2h under hydrogen, after which the Parr bomb was depressurized, and the reaction mixture was filtered through a pad of celite. The resulting solution was concentrated *in vacuo* to provide the aniline as a light yellow solid, which was used in the next step without further purification (2.17 g, 100%). LRMS $(M+H)^+$ *m/z* 187.3.

To a solution of 3-(3-aminophenoxy)pyridine (125 mg, 0.67 mmol, 1.0 equiv) and *N*,*N*-diisopropylethylamine (195 μL, 1.33 mmol, 2.0 equiv) in dry THF (3 mL) was added 4-fluorobenzoyl chloride (88 μL, 0.74 mmol, 1.1 equiv) by syringe. The reaction was allowed to stir for 2 h at room temperature. The reaction mixture was then diluted with EtOAc and sat'd. aq. $NaHCO_3$. The layers were separated, and the aqueous layer was extracted with EtOAc. The combined organic layers were washed once with brine, dried over anhydrous sodium sulfate, filtered, and concentrated *in vacuo*. Purification by silica gel chromatography (50% EtOAc/hexanes) provided compound **3** as a white solid (181 mg, 82%). mp 109 - 112 ^oC; IR (Solid) 3302, 3260, 3127, 3088, 1680, 1614, 1601, 1575, 1549, 1504, 1472, 1456, 1425, 1304, 1265, 1254, 1221, 1165, 1151, 1115, 1099, 1000, 886, 857, 849, 833, 800, 759, 701, 677 cm^{-1}; ^1H NMR (400 MHz, DMSO-*d6*) δ 10.36 (s, 1H), 8.46 – 8.37 (m, 2H), 8.14 – 7.93 (m, 2H), 7.61 (m, 1 H), 7.56 (m, 1H), 7.53 - 7.45

(m, 2H), 7.43 – 7.34 (m, 3H), 6.84 (m, 1H); ^{13}C NMR (100 MHz, DMSO-*d6*) δ 165.3, 164.6, 162.9, 156.4, 153.0, 144.7, 141.2, 140.8, 131.1, 130.4, 130.2, 126.0, 124.6, 115.5, 113.6, 110.0. Exact mass calc'd for $C_{18}H_{14}FN_2O_2$ requires *m/z* 309.1039 found *m/z* 309.1831 (ESI, $M+H^+$).

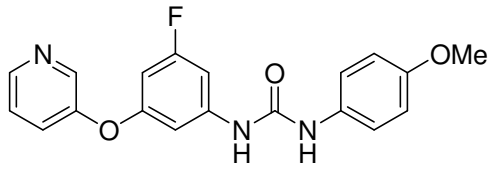


1-(3-Fluoro-5-(pyridin-3-yloxy)phenyl)-3-(4-fluorophenyl)urea (4)

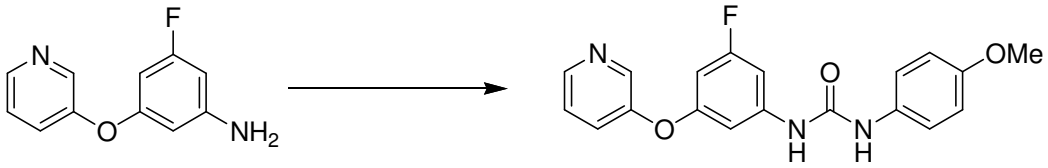


1-(3-Fluoro-5-(pyridin-3-yloxy)phenyl)-3-(4-fluorophenyl)urea (4). To a solution of 3-(3-fluoro-5-aminophenoxy)pyridine (86 mg, 0.42 mmol, 1.0 equiv) and DIPEA (100 μL, 0.6 mmol, 1.5 equiv) in dry THF (2.0 mL) was added 4-fluorophenyl isocyanate (48 μL, 0.42 mmol, 1.0 equiv) by syringe. After stirring for 2 h, the reaction was diluted with sat'd. aq. $NaHCO_3$ and EtOAc. The layers were separated and the organic layer was washed twice with sat'd. aq. $NaHCO_3$ and once with brine. The combined aqueous layers were washed extracted with EtOAc once and the combined organic layers were dried over anhydrous sodium sulfate, filtered and concentrated *in vacuo*. Purification by silica gel chromatography (30-50% EtOAc/hexanes) provided the title compound as a white solid (116 mg, 65%). mp 122 - 123 ^{o}C (dec); IR (Solid) 3295, 3199, 3100, 3001, 1676, 1625, 1566, 1517, 1476, 1437, 1403, 1315, 1211, 1153, 1123, 1010, 986, 833, 803, 706 cm^{-1}; 1H NMR (400 MHz, DMSO) δ 9.04 (s, 1H), 8.79 (s, 1H), 8.49 – 8.41 (m, 2H), 7.57 (ddd, J = 8.4, 2.8, 1.4 Hz, 1H), 7.52 – 7.36 (m, 3H), 7.23 – 7.06 (m, 3H), 6.92 (s,

1H), 6.56 (dt, J = 9.9, 2.3 Hz, 1H); ^{13}C NMR (100 MHz, DMSO-*d6*) δ 165.6, 162.5, 157.4, 153.0, 145.0, 141.6, 136.8, 130.9, 125.5, 124.9, 115.6, 105.3, 102.0, 101.7, 100.8, 99.9. Exact mass calc'd for $C_{18}H_{14}F_2N_3O_2$ requires *m/z* 342.1054 found *m/z* 342.1047 (ESI, $M+H^+$).

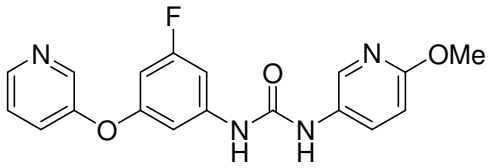


1-(3-Fluoro-5-(pyridin-3-yloxy)phenyl)-3-(4-methoxyphenyl)urea (5).

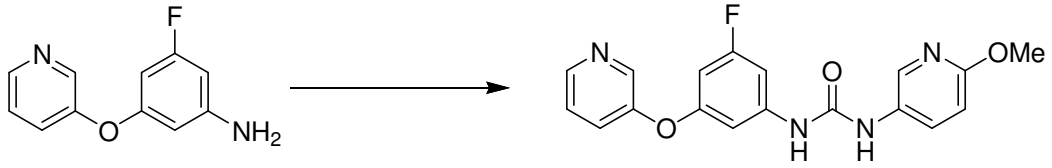


1-(3-Fluoro-5-(pyridin-3-yloxy)phenyl)-3-(4-methoxyphenyl)urea (5). To a solution of 3-(3-fluoro-5-aminophenoxy)pyridine (86 mg, 0.42 mmol, 1.0 equiv) and DIPEA (100 μL, 0.6 mmol, 1.5 equiv) in dry THF (2.0 mL) was added 4-methoxyphenyl isocyanate (55 μL, 0.42 mmol, 1.0 equiv) by syringe. After stirring for 2 h, the reaction was diluted with sat'd. aq. $NaHCO_3$ and EtOAc. The layers were separated, and the organic layer was washed twice with sat'd. aq. $NaHCO_3$ and once with brine. The combined aqueous layers were extracted with EtOAc once, and the combined organic layers were dried over anhydrous sodium sulfate, filtered, and concentrated *in vacuo*. Purification by silica gel (30-50% EtOAc/hexanes) provided compound **5** as a white solid (96 mg, 81%). mp 163 - 165 ^{o}C (dec); IR (Solid) 3294, 3194, 3111, 3090, 3023, 3008, 2945, 2827, 1668, 1626, 1612, 1560, 1532, 1512, 1475, 1439, 1422, 1318, 1245, 1223, 1183, 1175, 1152, 1122, 1029, 1008, 986, 816, 806, 776, 708, 681 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-*d6*) δ 8.97 (s, 1H), 8.56 (s, 1H), 8.50 – 8.41 (m, 2H), 7.58 (ddd, J = 8.4, 2.8, 1.4 Hz, 1H), 7.49 (ddd,

J = 8.4, 4.6, 0.5 Hz, 1H), 7.36 – 7.28 (m, 2H), 7.18 (dt, J = 11.4, 2.1 Hz, 1H), 6.93 – 6.83 (m, 3H), 6.54 (dt, J = 9.9, 2.3 Hz, 1H); ^{13}C NMR (100 MHz, DMSO-*d6*) δ 13C NMR (100 MHz, DMSO-d6) δ 165.9, 162.0, 158.5, 155.9, 143.0, 140.4, 136.8, 130.9, 128.5, 126.0, 115.9, 108.7, 103.9, 101.7, 101.1, 99.7, 53.5. Exact mass calc'd for $C_{19}H_{17}FN_3O_3$ requires *m/z* 354.1254 found *m/z* 354.1242 (ESI, $M+H^+$).

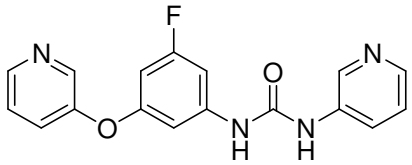


1-(3-Fluoro-5-(pyridin-3-yloxy)phenyl)-3-(6-methoxypyridin-3-yl)urea (6).

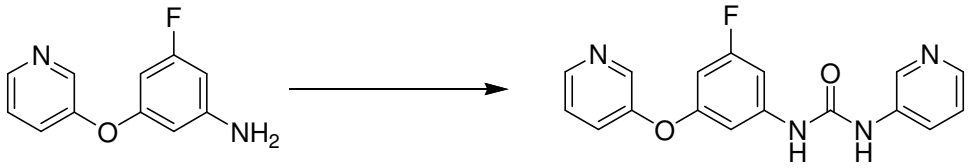


1-(3-Fluoro-5-(pyridin-3-yloxy)phenyl)-3-(6-methoxypyridin-3-yl)urea (6). To a solution of triphosgene (145 mg, 0.49 mmol, 0.40 equiv) in dry DCM (2.0 mL) was added via cannula a solution of 2-methoxy-5-aminopyridine (150 mg, 1.22 mmol, 1.0 equiv) and DIPEA (469 μL, 2.69 mmol, 2.2 equiv) in dry DCM (2.0 mL) over 10 min. The solution was allowed to stir for an additional 10 minutes. A solution of 3-(3-fluoro-5-aminophenoxy)pyridine (250 mg, 1.22 mmol, 1.0 equiv) in DCM (2.0 mL) was then added to the resulting mixture via cannula over 10 min. The reaction mixture was stirred for an additional 2 h, and the resulting solution was diluted with EtOAc and sat'd. aq. $NaHCO_3$. The layers were separated, and the organic layer was washed twice with sat'd. aq. $NaHCO_3$. The combined aqueous layers were extracted once with EtOAc, and the combined organic layers were washed once with brine, dried over anhydrous sodium sulfate, filtered, and concentrated *in vacuo*. Purification by silica gel chromatography

(50% EtOAc/hexanes) furnished compound **6** as a white solid (266 mg, 62%). mp 134 - 141 ^{o}C; IR (Solid) 3367, 3185, 3066, 3008, 1716, 1604, 1557, 1472, 1438, 1420, 1382, 1313, 1234, 1200, 1154, 1123, 1051, 1019, 1004, 982, 880, 855, 794, 825, 706, 687, 666 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-*d6*) δ 9.12 (s, 1H), 8.69 (s, 1H), 8.48 – 8.41 (m, 2H), 8.16 (d, J = 2.4 Hz, 1H), 7.78 (dd, J = 8.9, 2.8 Hz, 1H), 7.57 (ddd, J = 8.4, 2.8, 1.4 Hz, 1H), 7.52 – 7.44 (m, 1H), 7.18 (dt, J = 11.3, 2.1 Hz, 1H), 6.92 (s, 1H), 6.78 (d, J = 8.8 Hz, 1H), 6.56 (dt, J = 9.9, 2.3 Hz, 1H), 3.81 (s, 3H); ^{13}C NMR (100 MHz, DMSO-*d6*) δ 161.8, 159.3, 152.6, 152.3, 146.9, 145.3, 141.6, 137.5, 131.7, 129.9, 126.7, 124.8, 118.1, 117.8, 110.0, 103.2, 99.1, 53.1. Exact mass calc'd for $C_{18}H_{14}FN_4O_3$ requires *m/z* 355.1206 found *m/z* 355.1200 (ESI, M+H^{+}).

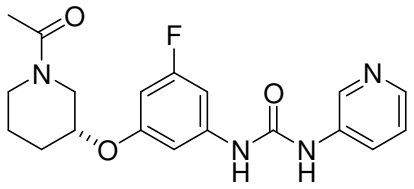


1-(3-Fluoro-5-(pyridin-3-yloxy)phenyl)-3-(pyridin-3-yl)urea (7).

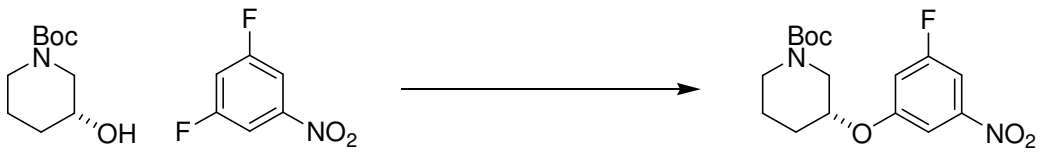


1-(3-Fluoro-5-(pyridin-3-yloxy)phenyl)-3-(pyridin-3-yl)urea (7). To a solution of triphosgene (145 mg, 0.49 mmol, 0.40 equiv) in dry DCM (2.0 mL) was added via cannula a solution of 3-aminopyridine (93 mg, 0.98 mmol, 1.0 equiv) and DIPEA (375 μL, 2.15 mmol, 2.2 equiv) in dry DCM (2.0 mL) over 10 min. The solution was allowed to stir for an additional 10 minutes. A solution of 3-(3-fluoro-5-aminophenoxy)pyridine (200 mg, 0.98 mmol, 1.0 equiv) in dry DCM (2.0 mL) was then added to the resulting mixture via cannula over 10 min. The reaction mixture was stirred for an additional 30

min, and the resulting solution was diluted with EtOAc and sat'd. aq. $NaHCO_3$. The layers were separated, and the organic layer was washed twice with sat'd. aq. $NaHCO_3$. The combined aqueous layers were extracted once with EtOAc, and the combined organic layers were washed once with brine, dried over anhydrous sodium sulfate, filtered, and concentrated *in vacuo*. Purification by silica gel chromatography (50% EtOAc/hexanes) furnished compound **7** as a white solid (229 mg, 72%). mp 185 - 186 ^{o}C; IR (Solid) 3312, 3250, 3189, 3127, 3169, 1667, 1607, 1548, 1472, 1410, 1254, 1223, 1156, 1130, 1156, 1130, 1009, 987, 863, 832, 809, 718 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-*d6*) δ 9.17 (s, 1H), 8.93 (s, 1H), 8.57 (d, J = 2.5 Hz, 1H), 8.49 – 8.41 (m, 2H), 8.21 (dd, J = 4.7, 1.4 Hz, 1H), 7.91 (ddd, J = 8.3, 2.6, 1.5 Hz, 1H), 7.58 (ddd, J = 8.4, 2.8, 1.4 Hz, 1H), 7.49 (dd, J = 8.4, 4.6 Hz, 1H), 7.32 (dd, J = 8.3, 4.7 Hz, 1H), 7.20 (dt, J = 11.3, 2.1 Hz, 1H), 6.93 (s, 1H), 6.59 (dt, J = 9.9, 2.3 Hz, 1H); ^{13}C NMR (100 MHz, DMSO-*d6*) δ 161.9, 158.0, 157.8, 152.3, 152.2, 145.4, 143.2, 142.3, 142.2, 140.4, 135.9, 126.8, 125.5, 124.8, 113.6, 110.2, 103.3. Exact mass calc'd for $C_{17}H_{14}FN_4O_2$ requires *m/z* 325.1101 found *m/z* 325.1010 (ESI, $M+H^+$).

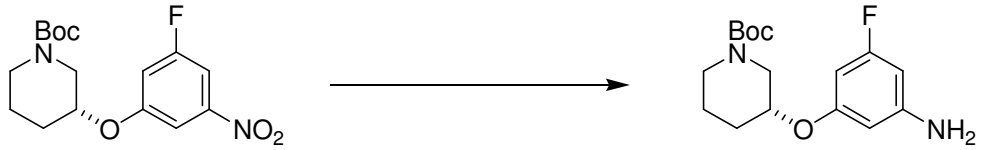


(*R*)-1-(3-(1-Acetylpiperidin-3-yloxy)-5-fluorophenyl)-3-(pyridin-3-yl)urea (8)

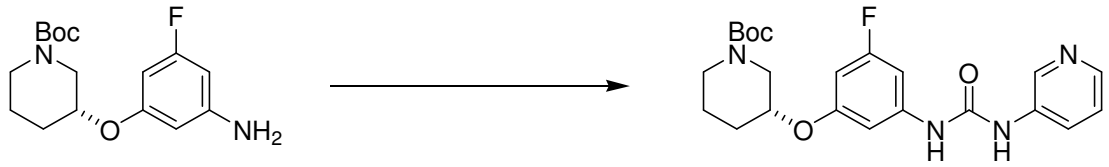


(*R*)-*tert*-Butyl 3-(3-fluoro-5-nitrophenoxy)piperidine-1-carboxylate. To a 0 ^{o}C solution of (*R*)-*tert*-butyl-3-hydroxypiperidine (34.2 g, 170 mmol, 1.0 equiv) in dry DMF

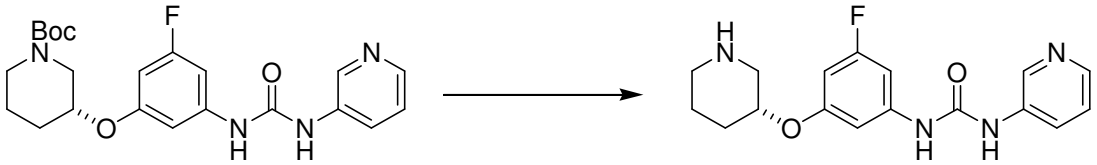
(350 mL) was added solid sodium hydride (7.14 g of a 60% dispersion in mineral oil, 175 mmol, 1.0 equiv) in portions. After stirring an additional 30 minutes, 3,5-difluoronitrobenzene (19.3 mL, 170 mmol, 1.0 equiv) was added by syringe, and the reaction was heated to 80 ^{o}C for 1 h. The reaction was allowed to cool to room temperature, and a small amount of water was slowly added. The solvent was then removed *in vacuo*. The mixture was diluted with EtOAc and water, and the layers were separated. The organic layer was washed twice with water. The combined aqueous layers were extracted once with EtOAc, and the combined organic layers were washed once with brine, dried over anhydrous sodium sulfate, filtered, and concentrated *in vacuo*. Purification by silica gel chromatography (20-100% DCM/hexanes) provided the title compound as a brown oil (39.5 g, 70 %).



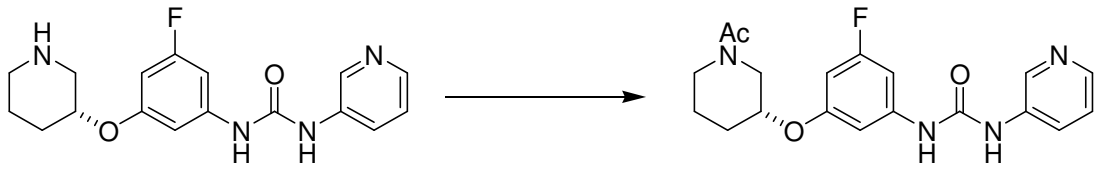
(*R*)-*tert*-Butyl 3-(3-amino-5-fluorophenoxy)piperidine-1-carboxylate. To a solution of (*R*)-*tert*-butyl 3-(3-fluoro-5-nitrophenoxy)piperidine-1-carboxylate (39.5 g, 116 mmol) in MeOH (200 mL) was added wet Pd/C (20 g, 10% wt/wt) as a solid in one portion. The reaction mixture was pressurized to ~60 psi H_2 and stirred for 1.5 h. The reaction was depressurized, and the mixture was filtered through a pad of celite. The yellow solution was concentrated *in vacuo*, and orange oil was dissolved in dichloromethane and passed through a pad of silica gel. The resulting yellow solution was concentrated *in vacuo* to provide the title compound as an orange oil which was used without further purification (32.2 g, 92%).



(*R*)-*tert*-Butyl 3-(3-fluoro-5-(3-pyridin-3-ylureido)phenoxy)piperidine-1-carboxylate. To a solution of triphosgene (529 mg, 1.8 mmol, 0.48 equiv) in dry THF (6 mL) was added via cannula a solution of 3-aminopyridine (425 mg, 4.5 mmol, 1.2 equiv) and triethyl amine (1.67 mL, 12.6 mmol, 3.34 equiv) in dry THF (6 mL) over 5 minutes. The solution was stirred for an additional 15 min, and a solution of (*R*)-*tert*-butyl 3-(3-amino-5-fluorophenoxy)piperidine-1-carboxylate (1.17 g, 3.77 mmol, 1.0 equiv) in dry THF (5 mL) was added by syringe. The reaction was stirred for 1 h, and the solvent was removed *in vacuo*. Purification by silica gel chromatography (5% MeOH, EtOAc) furnished the title compound as a thick colorless foam (1.27 g, 78%).

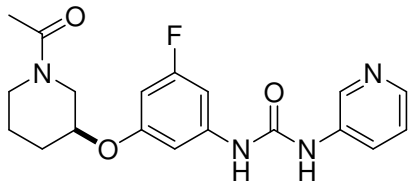


(*R*)-1-(3-(1-Piperidin-3-yloxy)-5-fluorophenyl)-3-(pyridin-3-yl)urea. To a solution of (*R*)-*tert*-butyl 3-(3-fluoro-5-(3-pyridin-3-ylureido)phenoxy)piperidine-1-carboxylate (1.27 g, 2.94 mmol) in DCM (15 mL) was added trifluoroacetic acid (15 mL). The mixture was stirred for 1 h and concentrated *in vacuo*. The residue was dissolved in EtOAc and aq. NaOH (1 N). The layers were separated and the organic layer was washed once with sat'd. aq. $NaHCO_3$ and once with brine. The organic layer was then dried over anhydrous sodium sulfate, filtered, and concentrated *in vacuo* to provide (*R*)-1-(3-(1-piperidin-3-yloxy)-5-fluorophenyl)-3-(pyridin-3-yl)urea as a foam which was used in subsequent diversifications without further purification (970 mg, 99%).



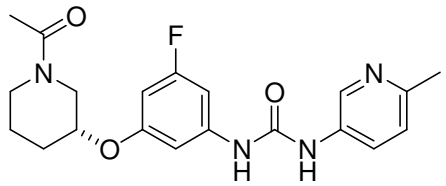
(*R*)-1-(3-(1-Acetylpiperidin-3-yloxy)-5-fluorophenyl)-3-(pyridin-3-yl)urea (8). To a solution of (*R*)-1-(3-(1-piperidin-3-yloxy)-5-fluorophenyl)-3-(pyridin-3-yl)urea (105 mg, 0.32 mmol, 1.0 equiv) and DIPEA (224 μL, 1.29 mmol, 4.0 equiv) in dry THF (2 mL) was added acetic anhydride (91 μL, 0.97 mmol, 3.0 equiv) by syringe. After stirring for 3 hours, sat'd. aq. $NaHCO_3$ was poured into the reaction mixture, and the mixture was then diluted with EtOAc. The layers were separated, and the organic layer was washed twice with sat'd. aq. $NaHCO_3$ and once with brine. The organic layer was dried over anhydrous sodium sulfate, filtered, and concentrated *in vacuo*. Purification by silica gel chromatography (2-5%MeOH/DCM) provided compound **8** as a white solid (86 mg, 72%). mp 138 - 143 ^{o}C; IR (Solid) 3304, 3295, 3127, 2863, 1712, 1623, 1596, 15596, 1547, 1474, 1422, 1368, 1198, 1165, 1123, 797,706, 680 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-*d6*) δ 9.01 (d, J = 21.7 Hz, 1H), 8.95 (d, J = 7.3 Hz, 1H), 8.60 (d, J = 2.5 Hz, 1H), 8.21 (dd, J = 4.7, 1.4 Hz, 1H), 7.93 (d, J = 8.4 Hz, 1H), 7.33 (dd, J = 8.3, 4.6 Hz, 1H), 7.03 – 6.88 (m, 2H), 6.84 (s, 1H), 6.53 (ddt, J = 20.5, 11.0, 2.2 Hz, 1H), 4.63 – 4.38 (m, 1H), 4.50 (m, 0.5H), 4.28 (m, 0.5H), 3.94 (dd, J = 12.8, 2.9 Hz, 1H), 3.70 – 3.46 (m, 2H), 3.40 – 3.20 (m, 2H), 2.65 (d, J = 25.7 Hz, 0H), 2.61 – 2.53 (m, 0H), 2.51 (dt, J = 3.6, 1.8 Hz, 7H), 3.70 – 3.45 (m, 2H), 3.30 (m, 2H), 2.03 (s, 1.4 H, Ac methyl rotamer), 1.91 (s, 1.6 H, Ac methyl rotamer) 1.78 (m, 1H), 1.67 (m, 1H), 1.55 (m, 1H), 1.44 (m, 1H); ^{13}C NMR (100 MHz, DMSO-*d6*) δ 168.6, 152.4, 151.2, 148.1, 141.1, 140.5, 139.8, 126.3, 122.6, 101.6, 101.4, 100.0, 71.1, 49.1, 45.8, 29.1, 28.4, 21.2, 21.0; $[\alpha]^{D}_{23}$ = + 40.8

^{o} (c 0.26, DCM). Exact mass calc'd for $C_{19}H_{22}FN_4O_3$ requires *m/z* 373.1676 found *m/z* 373.1670 (ESI, $M+H^+$).



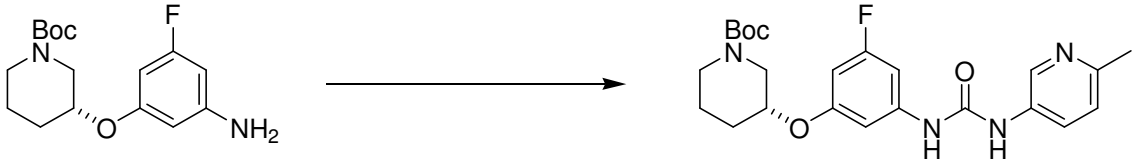
(*S*)-1-(3-(1-Acetylpiperidin-3-yloxy)-5-fluorophenyl)-3-(pyridin-3-yl)urea (9)

(*S*)-1-(3-(1-Acetylpiperidin-3-yloxy)-5-fluorophenyl)-3-(pyridin-3-yl)urea (9). The title compound was synthesized in the same manner described for its enantiomer, (*R*)-1-(3-(1-acetylpiperidin-3-yloxy)-5-fluorophenyl)-3-(pyridin-3-yl)urea, compound (**8**). mp 137 - 141 ^{o}C; IR (Solid) 3329, 3302, 3296, 2868, 1712, 1622, 1599, 1546, 1474, 1199, 1165, 1126, 998, 706 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-*d6*) δ 8.98 (dd, J = 21.3, 7.3 Hz, 2H), 8.60 (d, J = 2.4 Hz, 1H), 8.21 (dd, J = 4.7, 1.4 Hz, 1H), 7.94 (d, J = 8.4 Hz, 1H), 7.33 (dd, J = 8.3, 4.7 Hz, 1H), 7.03 – 6.88 (m, 2H), 6.84 (s, 0H), 6.53 (ddt, J = 20.4, 11.0, 2.2 Hz, 1H), 4.50 (dd, J = 5.6, 2.9 Hz, 1H), 4.28 (dd, J = 7.4, 3.7 Hz, 1H), 3.94 (dd, J = 12.9, 2.9 Hz, 1H), 3.67 – 3.43 (m, 3H), 3.41 – 3.17 (m, 4H), 2.51 (dt, J = 3.6, 1.8 Hz, 5H), 2.08 – 1.84 (m, 2H), 1.85 – 1.26 (m, 1H); ^{13}C NMR (100 MHz, DMSO-*d6*) δ 168.6, 152.3, 151.2, 148.1, 141.1, 140.3, 139.9, 126.3, 122.6, 101.7, 101.3, 100.1, 71.1, 49.1, 45.8, 29.1, 28.4, 21.2, 21.0; $[\alpha]^{D}_{23}$ = - 39.7 ^{o} (c 0.28, DCM). Exact mass calc'd for $C_{19}H_{22}FN_4O_3$ requires *m/z* 373.1676 found *m/z* 373.1777 (ESI, $M+H^+$).

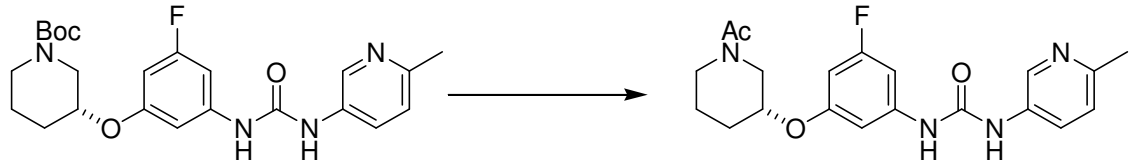


(*R*)-1-(3-(1-Acetylpiperidin-3-yloxy)-5-fluorophenyl)-3-(6-methylpyridin-3-yl)

urea (10)

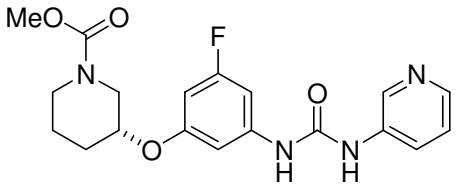


(*R*)-*tert*-Butyl 3-(3-fluoro-5-(3-(6-methylpyridin-3-yl)ureido)phenoxy)piperidine-1-carboxylate. To a solution of triphosgene (38.2 mg, 0.129 mmol, 0.4 equiv) in dry THF (2 mL) was added via cannula a solution of 2-methyl-5-aminopyridine (38.3 mg, 0.354 mmol, 1.1 equiv) and DIPEA (135 mL, 0.77 mmol, 2.4 equiv) in dry THF (2 mL) over 5 minutes. The solution was stirred for an additional 20 min, and a solution of (*R*)-*tert*-butyl 3-(3-amino-5-fluorophenoxy)piperidine-1-carboxylate (100 g, 0.322 mmol, 1.0 equiv) in dry THF (3 mL) was added by syringe. The reaction was stirred for 1 h, and the solvent was removed *in vacuo*. Purification by silica gel chromatography (5% MeOH, EtOAc) furnished the title compound as a white foam (111 g, 77%).

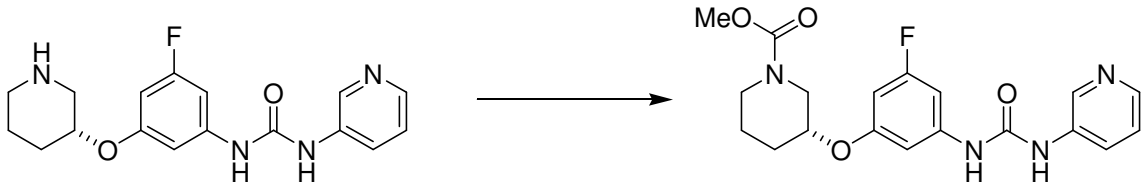


(*R*)-1-(3-(1-Acetylpiperidin-3-yloxy)-5-fluorophenyl)-3-(6-methylpyridin-3-yl)urea (10). To a solution of (*R*)-*tert*-butyl 3-(3-fluoro-5-(3-(6-methylpyridin-3-yl)ureido)phenoxy)piperidine-1-carboxylate (111 mg, 0.25 mmol, 1.0 equiv) in DCM (1 mL) was added TFA (1 mL). The reaction mixture was allowed to stir for 1 h, and then the solvents were removed *in vacuo*. The residue was dissolved in EtOAc and sat'd. aq. $NaHCO_3$. The layers were separated, and the aq. layer was extracted once with EtOAc. The combined organic layers were washed once with sat'd. aq. $NaHCO_3$ and once with brine. The solution was dried over anhydrous sodium sulfate, filtered, and concentrated *in vacuo*. The free amine was dissolved in THF (2.0 mL), and DIPEA (104 μL, 0.6

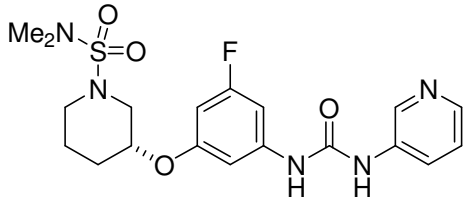
mmol, 2.4 equiv) was added by syringe. To the resulting solution was added acetic anhydride (33 μL, 0.35 mmol, 1.4 equiv). Purification by reverse phase HPLC provided compound **10** as a white solid (38 mg, 39%). mp 156 - 160 ^{o}C; IR (Solid) 3292, 3100, 2949, 2919, 2852, 1712, 1602, 1540, 1475, 1427, 1373, 1194, 1165, 1125, 1021, 997, 829, 724, 678 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-*d6*) δ 8.99 (d, J = 13.3 Hz, 1H), 8.86 (s, 1H), 8.46 (d, J = 2.6 Hz, 1H), 7.80 (dd, J = 8.4, 2.6 Hz, 1H), 7.18 (d, J = 8.4 Hz, 1H), 7.02 – 6.83 (m, 2H), 6.51 (ddt, J = 20.0, 11.0, 2.2 Hz, 1H), 5.92 (d, J = 11.1 Hz, 1H), 4.50 (m, 0.6H), 4.28 (m, 0.6H), 3.93 (dd, J = 12.9, 2.8 Hz, 0.6H), 3.70 – 3.46 (m, 2.4H), 3.40 – 3.22 (m, 2H), 3.05 (dd, J = 47.3, 40.2 Hz, 0.6H), 2.41 (s, 3H), 2.03 (s, 1H, Ac Rotamer), 1.91 (s, 2H, Ac Rotamer), 1.77 (m, 1H), 1.66 (m, 1H), 1.54 (m, 1H), 1.43 (m, 1H); ^{13}C NMR (100 MHz, DMSO-*d6*) δ 168.6, 152.4, 151.2, 148.4, 141.8, 139.5, 133.5, 126.3, 122.7, 101.6, 101.4, 100.0, 71.1, 49.1, 45.8, 41.0, 29.1, 28.4, 23.2, 22.5; $[\alpha]^{D}_{23}$ = + 31.2 ^{o} (c 0.30, DCM). Exact mass calc'd for $C_{20}H_{24}FN_4O_3$ requires *m/z* 387.1832 found *m/z* 387.1856 (ESI, M+H^{+}).



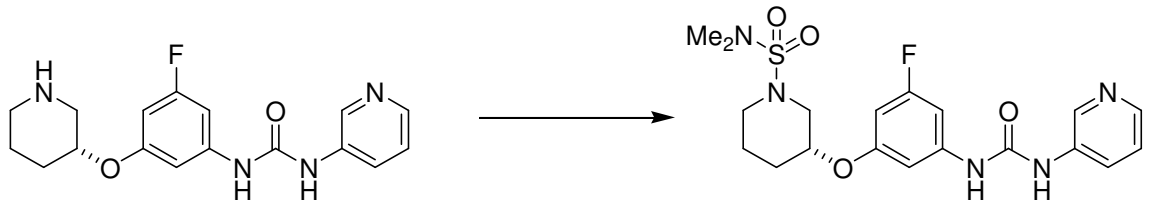
(*R*)-Methyl 3-(3-fluoro-5-(3-pyridin-3-ylureido)phenoxy)piperidine-1-carboxylate (11)



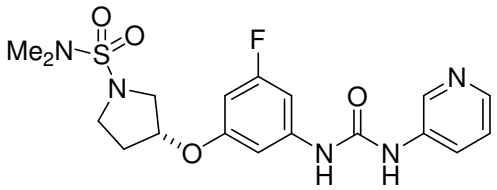
(*R*)-Methyl 3-(3-fluoro-5-(3-pyridin-3-ylureido)phenoxy)piperidine-1-carbox-ylate (11). To a 0 °C solution of (*R*)-1-(3-(1-piperidin-3-yloxy)-5-fluorophenyl)-3-(pyridin-3-yl)urea (195 mg, 0.59 mmol, 1.0 equiv) and DIPEA (125 μL, 0.70 mmol, 1.2 equiv) in DCM (3 mL) was added methyl chloroformate (50 μL, 0.66 mmol, 1.1 equiv). After 10 min, the reaction was quenched by the addition of sat'd aq. $NaHCO_3$. The mixture was diluted with EtOAc, and the layers were separated. The organic layer was washed with brine, dried over anhydrous sodium sulfate, filtered, and concentrated *in vacuo*. Purification by silica gel chromatography (10% MeOH/DCM) provided compound **11** as a white solid (119 mg, 52%). mp 95 - 100 °C; IR (Solid) 3333, 3304, 2946, 2858, 1698, 1601, 1556, 1473, 1457, 1444, 1420, 1366, 1234, 1207, 1185, 1147, 1134, 1086, 1043, 1059, 1008, 984, 958, 844, 823, 806, 703, 675 cm^{-1}; ^1H NMR (400 MHz, DMSO-*d6*) δ 9.02 (s, 1H), 8.76 (s, 1H), 8.47 (d, J = 2.5 Hz, 1H), 7.81 (dd, J = 8.4, 2.7 Hz, 1H), 7.37 (dt, J = 11.5, 2.1 Hz, 1H), 7.18 (d, J = 8.4 Hz, 1H), 7.11 (s, 1H), 6.74 (d, J = 9.5 Hz, 1H), 3.59 (s, 3H), 3.46 (s, 2H), 3.43 – 3.32 (m, 4H), 2.41 (s, 3H), 2.35 (m, 4H); ^{13}C NMR (100 MHz, DMSO-*d6*) δ 161.9, 158.5, 158.3, 155.3, 152.4, 143.1, 140.2, 136.1, 125.4, 123.6, 101.8, 99.7, 96.3, 70.6, 47.3, 46.7, 45.5, 28.5, 18.5; $[\alpha]^D_{23}$ = + 16.5 ° (c 0.23, DCM). Exact mass calc'd for $C_{19}H_{22}FN_4O_4$ requires *m/z* 389.1625 found *m/z* 389.1621 (ESI, $M+H^+$).



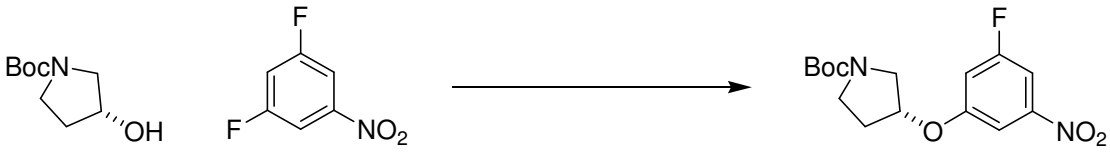
(*R*)-3-(3-Fluoro-5-(3-pyridin-3-ylureido)phenoxy)-N,N-dimethylpiperidine-1-sulfonamide (12)



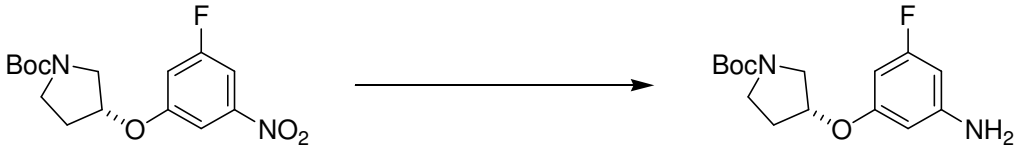
(*R*)-3-(3-Fluoro-5-(3-pyridin-3-ylureido)phenoxy)-N,N-dimethylpiperidine-1-sulfonamide (12). To a solution of (*R*)-1-(3-(1-piperidin-3-yloxy)-5-fluorophenyl)-3-(pyridin-3-yl)urea (1.15 g, 3.5 mmol, 1.0 equiv), DMAP (43 mg, 0.35 mmol, 0.10 equiv) and DIPEA (1.34 mL, 7.7 mmol, 2.2 equiv) in dry DCM (20 mL) was added *N*,*N*-dimethylsulfamoyl chloride (0.560 mL, 5.25 mmol, 1.5 equiv) by syringe. The reaction was allowed to stir overnight. The reaction mixture was diluted with 200 mL DCM and extracted three times with 50 mL water. The organic layer was dried over anhydrous sodium sulfate, filtered, and concentrated *in vacuo*. Purification by silica gel chromatography (3%MeOH/EtOAc) furnished compound **12** as a white solid (844 mg, 55%). mp 158 - 160 ^{o}C; IR (Solid) 3343, 3263, 3307, 3105, 2951, 2853, 2703, 1724, 1588, 1548, 1473, 1422, 1342, 1199, 1143, 1131, 1020, 971, 928, 859, 839, 813, 677 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-*d6*) δ 9.02 (s, 1H), 8.95 (s, 1H), 8.60 (d, J = 2.4 Hz, 1H), 8.21 (dd, J = 4.7, 1.4 Hz, 1H), 7.93 (ddd, J = 8.4, 2.6, 1.5 Hz, 1H), 7.33 (dd, J = 8.3, 4.6 Hz, 1H), 6.98 (d, J = 11.2 Hz, 1H), 6.89 (s, 1H), 6.53 (d, J = 11.0 Hz, 1H), 4.51 – 4.43 (m, 1H), 3.44 (m, 1H), 3.30 – 3.12 (m, 3H), 2.74 (s, 6H), 1.98 – 1.52 (m, 4H); ^{13}C NMR (100 MHz, DMSO-*d6*) δ 158.2, 158.1, 152.4, 143.1 141.8, 141.7, 140.3, 136.1, 125.4, 123.6, 101.6, 96.6, 70.6, 48.9, 45.9, 37.7, 28.0, 21.5; $[\alpha]^{D}_{23}$ = + 21.6 ^{o} (c 0.21, Acetone). Exact mass calc'd for $C_{19}H_{25}FN_5O_4S$ requires *m/z* 438.1611 found *m/z* 438.1596 (ESI, M+H^{+}).



(*R*)-3-(3-Fluoro-5-(3-pyridin-3-ylureido)phenoxy)-N,N-dimethylpyrrolidine-1-sulfonamide (13)

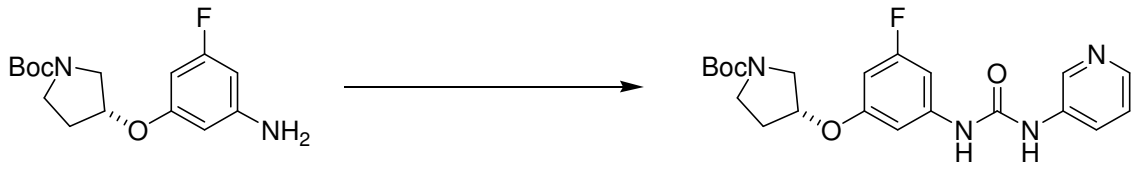


(*R*)-*tert*-Butyl 3-(3-fluoro-5-nitrophenoxy)pyrrolidine-1-carboxylate. To an ice-bath cooled solution of (*R*)-*tert*-butyl-3-hydroxypyrrolidine (10 g, 53.4 mmol, 1.0 equiv) in dry DMF (130 mL) was added solid NaH (2.56 g of 60% dispersion in mineral oil, 64 mmol, 1.2 equiv) portion-wise. After stirring for 10 minutes, 3,5-difluoronitrobenzene was added. The reaction was stirred at room temperature for 5 hours. After the mixture was carefully quenched with water, it was diluted with EtOAc and additional water. The layers were separated, and the organic layer was washed repeatedly with water and once with brine, dried over anhydrous sodium sulfate, filtered, and concentrated *in vacuo*. Purification by silica gel chromatography (14-50%EtOAc/hexanes) furnished the title compound as an orange syrup (15.3 g, 88%).

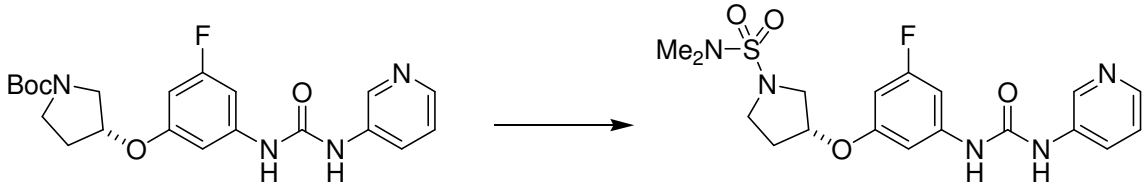


(*R*)-*tert*-Butyl 3-(3-fluoro-5-aminophenoxy)pyrrolidine-1-carboxylate. To a solution of (*R*)-*tert*-butyl 3-(3-fluoro-5-nitrophenoxy)pyrrolidine-1-carboxylate (1.77 g, 5.43 mmol) in MeOH (35 mL) was added wet Pd/ (~1 g of 10% wt/wt) as a solid in one

portion. The reaction mixture was pressurized to ~60 psi in a Parr bomb and stirred for 1 h. The mixture was filtered through a pad of celite, and the solvent was removed *in vacuo*. The residue was diluted with 10 mL MeOH and passed through a 0.45 micron Teflon® syringe filter. The solution was concentrated *in vacuo* to provide the title compound as a light orange syrup which was used without further purification (1.61 g, 89%).



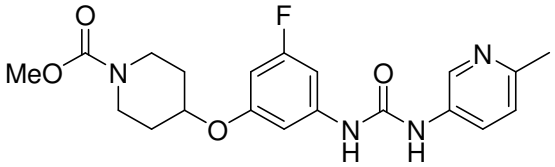
(*R*)-*tert*-Butyl 3-(3-fluoro-5-(3-pyridin-3-ylureido) phenoxy)pyrrolidine-1-carboxylate. To a solution of triphosgene (1.30 g, 4.4 mmol, 0.40 equiv) in dry THF (11 mL) was added a solution of 3-aminopyridine (1.04 g, 11.1 mmol, 1.1 equiv) and DIPEA (3.83 mL, 22 mmol, 2.2 equiv) in dry THF (11 mL) via cannula over 15 minutes. After stirring for an additional 20 minutes, a solution (*R*)-*tert*-butyl 3-(3-fluoro-5-aminophenoxy)pyrrolidine-1-carboxylate (3.25 g, 10.0 mmol, 1.0 equiv) in dry THF (10 mL) was added by syringe. After 2 h, the reaction mixture was diluted with EtOAc and sat'd. aq. $NaHCO_3$. The layers were separated and the organic layer was washed twice with sat'd. aq. $NaHCO_3$ and once with brine. The organic layer was dried over anhydrous sodium sulfate, filtered, and concentrated *in vacuo*. Purification by silica gel chromatography (2.5-10% MeOH/EtOAc) provided the title compound as a foam (3.0 g, 72%).



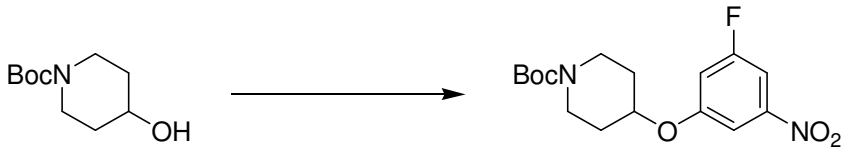
(*R*)-3-(3-Fluoro-5-(3-pyridin-3-ylureido)phenoxy)-N,N-dimethylpyrrolidine-1-sulfonamide (13). To a solution of (*R*)-*tert*-butyl 3-(3-fluoro-5-(3-pyridin-3-ylureido) phenoxy)pyrrolidine-1-carboxylate (2.00 mg, 4.63 mmol, 1.0 equiv) in DCM (10 mL) was added TFA (10 mL) in one portion. After stirring for 1 h, the solvents were removed *in vacuo* and the residue was dissolved in EtOAc and sat'd. aq. $NaHCO_3$. The layers were separated, and the organic layer was washed three times with sat'd. aq. $NaHCO_3$ and once with brine, dried over anhydrous sodium sulfate, filtered, and concentrated *in vacuo*. The free amine was used in subsequent diversification steps without further purification (1.53 g, 99%).

To a solution of the free amine (900 mg, 2.85 mmol, 1.0 equiv) and triethyl amine (1.13 mL, 8.56 mmol, 3.0 equiv) in dry DCM (5 mL) was added *N*,*N*-dimethylsulfamoyl chloride (0.367 mL, 2.38 mmol , 1.2 equiv) by syringe, and the reaction was stirred overnight. The mixture was then diluted with EtOAc and sat'd. aq. $NaHCO_3$. The layers were separated, and the organic layer was washed with brine, dried over anhydrous sodium sulfate, filtered, and concentrated *in vacuo*. Purification by reverse phase preparative HPLC furnished compound **13** as a white solid (632 mg, 52%). mp 150 - 151 ^{o}C; IR (Solid) 3254, 31700, 3121, 3069, 2935, 2863, 1671, 1624, 1606, 1571, 1548, 1481, 1442, 1427, 1408, 1322, 1300, 1258, 1223, 1172, 1141, 1123, 1018, 970, 856, 843, 813, 791, 718, 735, 691 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-*d6*) δ 9.05 (s, 1H), 8.97 (s, 1H), 8.60 (d, J = 2.5 Hz, 1H), 8.21 (dd, J = 4.7, 1.4 Hz, 1H), 7.97 – 7.89 (m, 1H), 7.33 (dd, J = 8.3, 4.7 Hz, 1H), 6.99 (d, J = 11.2 Hz, 1H), 6.87 (s, 1H), 6.51 (d, J = 10.9 Hz, 1H), 5.03 (s, 1H), 3.58 (dd, J = 11.3, 4.4 Hz, 1H), 3.54 – 3.32 (m, 3H), 2.75 (s, 6H), 2.22 (td, J = 9.2, 4.8 Hz, 1H), 2.10 (d, J = 13.4 Hz, 1H); ^{13}C NMR (100 MHz, DMSO-*d6*) δ

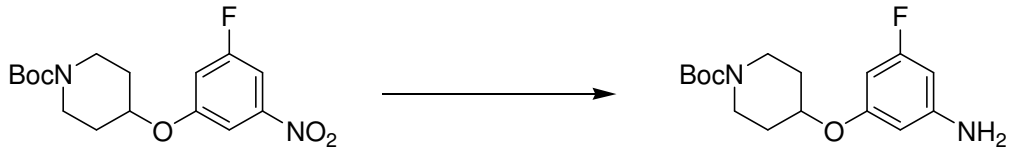
158.4, 159.1, 153.1, 144.0, 141.9, 141.6, 140.0, 137.2, 126.9, 124.1, 101.4, 96.6, 74.4, 49.9, 45.9, 35.7, 35.1; $[\alpha]^{D}_{23}$ = - 12.4 ^{o} (c 0.28, Acetone). Exact mass calc'd for $C_{18}H_{23}FN_5O_4S$ requires *m/z* 424.1455 found *m/z* 424.1582 (ESI, $M+H^+$).



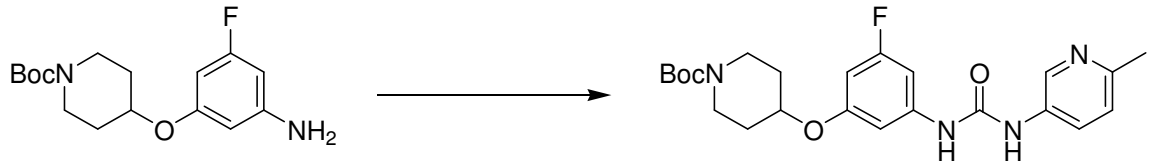
Methyl 4-(3-fluoro-5-(3-(6-methylpyridin-3-yl)ureido)phenoxy)piperidine-1-carboxylate (14)



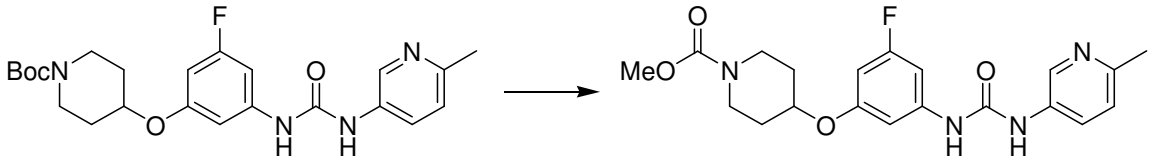
***tert*-Butyl 4-(3-fluoro-5-nitrophenoxy)piperidine-1-carboxylate.** To a 0 ^{o}C solution of *N*-Boc-4-hydroxypiperidine (3.45 g, 17.1 mmol, 1.0 equiv) in dry DMF (100 mL) was added NaH (823 mg of a 60% dispersion in mineral oil, 20.6 mmol, 1.2 equiv) portion-wise. After addition, the mixture was allowed to stir for an additional 10 min, after which 3,5-difluoronitrobenzene (3.0 g, 18.9 mmol, 1.1 equiv) was added and the reaction mixture was allowed to stir at room temperature for 1.5 h. The excess NaH was quenched by the careful addition of water, and the mixture was diluted with EtOAc and additional water. The organic layer was washed 4 times with water. The combined aqueous layers were washed once with EtOAc, and the combined organic layers were then washed with brine, dried over anhydrous sodium sulfate, filtered, and concentrated *in vacuo*. Purification by silica gel chromatography (10 – 40% EtOAc/hexanes) provided the title compound as an orange oil (3.22 g, 55%).



***tert*-Butyl 4-(3-amino-5-fluorophenoxy)piperidine-1-carboxylate.** To a solution of *tert*-butyl 4-(3-fluoro-5-nitrophenoxy)piperidine-1-carboxylate (3.22 g, 9.5 mmol, 1.0 equiv) in MeOH (30 mL) was added wet Pd/C (~200 mg of 10% wt/wt) as a solid in one portion. The solution was pressurized with 45 psi H_2 and stirred for 1 h. The mixture was filtered through a plug of celite and concentrated *in vacuo* to furnish the title compound as a yellow oil (2.94 g, 97%).

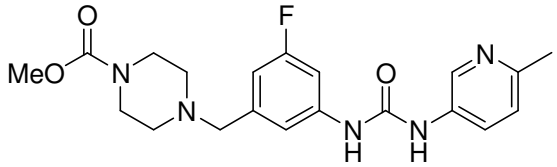


***tert*-Butyl 4-(3-fluoro-5-(3-(6-methylpyridin-3-yl)ureido)phenoxy)piperidine-1-carboxylate.** To a solution of triphosgene (547 mg, 1.84 mmol, 0.4 equiv) in dry THF (23 mL) was added a solution of 6-methylpyridin-3-amine (499 mg, 4.61 mmol, 1.0 equiv) and DIPEA (1.608 mL, 9.23 mmol, 2.0 equiv) in dry THF (8 mL) via cannula over 20 minutes. The reaction mixture was stirred for an additional 20 min, and *tert*-butyl 4-(3-amino-5-fluorophenoxy)piperidine-1-carboxylate (1.43 g, 4.61 mmol, 1.0 equiv) was then added as a solution in dry THF (5 mL) by syringe. The mixture was stirred for 2 h and then diluted with EtOAc and sat'd. aq. $NaHCO_3$. The layers were separated, and the organic layer was washed with brine, dried over anhydrous sodium sulfate, filtered, and concentrated *in vacuo*. Purification by silica gel chromatography (2-10% MeOH/DCM) provided the title compound as a foam (1.59 g, 78%).

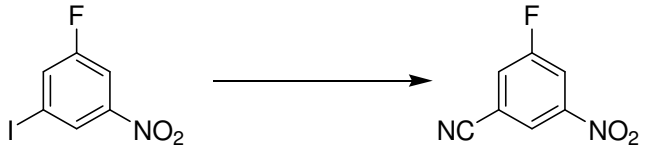


Methyl 4-(3-fluoro-5-(3-(6-methylpyridin-3-yl)ureido)phenoxy)piperidine-1-carboxylate (14). To a solution of *tert*-butyl 4-(3-fluoro-5-(3-(6-methylpyridin-3-yl)ureido)phenoxy)piperidine-1-carboxylate (1.49 g, 3.35 mmol, 1.0 equiv) in DCM (5 mL) was added TFA (5 mL) in one portion. The mixture was stirred for 30 min and the solvents were removed *in vacuo*. The residue was dissolved in EtOAc and sat'd. aq. $NaHCO_3$. The organic layer was washed with brine, dried over anhydrous sodium sulfate, filtered, and concentrated *in vacuo* to provide the free amine. The free amine was dissolved in dry THF (5 mL) and DIPEA (1.4 mL) was added. The solution was cooled to 0 ^oC, and methyl chloroformate (260 μL, 3.35 mmol, 1.0 equiv) was then added as a DCM solution (2.0 mL) drop-wise by syringe. The reaction was quenched after 30 min by the addition of sat'd. aq. $NaHCO_3$. The mixture was diluted with EtOAc, and the layers were separated. The organic layer was washed with brine, dried over anhydrous sodium sulfate, filtered and concentrated *in vacuo*. Purification by silica gel chromatography (15% MeOH/DCM) provided compound **14** as a white solid (929 mg, 69%). mp 156 - 160 ^oC; IR (Solid) 3302, 2863, 1706, 1640, 1558, 1475, 1341, 1171, 1134, 1051, 950, 837, 814 cm^{-1}; ^1H NMR (400 MHz, DMSO-*d6*) δ 8.94 (s, 1H), 8.82 (s, 1H), 8.46 (d, J = 2.5 Hz, 1H), 7.80 (dd, J = 8.4, 2.7 Hz, 1H), 7.18 (d, J = 8.4 Hz, 1H), 6.96 (dt, J = 11.3, 2.0 Hz, 1H), 6.86 (s, 1H), 6.53 (dt, J = 11.0, 2.2 Hz, 1H), 4.55 (dd, J = 7.9, 4.0 Hz, 1H), 3.74 – 3.62 (m, 2H), 3.60 (s, 3H), 3.36 – 3.11 (m, 2H), 2.40 (s, 3H), 2.04 – 1.82 (m, 2H), 1.55 (ddd, J = 13.1, 8.7, 4.6 Hz, 2H); ^{13}C NMR (100 MHz, DMSO-*d6*) δ 160.0, 159.1, 155.8, 143.0, 141.9, 141.8, 139.9, 136.6, 133.1, 127.9, 126.1, 102.4,

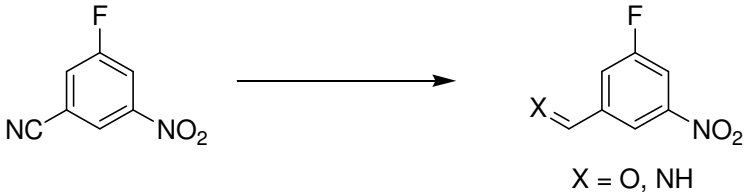
95.6, 74.0, 52.1, 47.5, 33.7, 23.8. Exact mass calc'd for $C_{20}H_{24}FN_4O_4$ requires *m/z* 403.1782 found *m/z* 403.2198 (ESI, $M+H^+$).



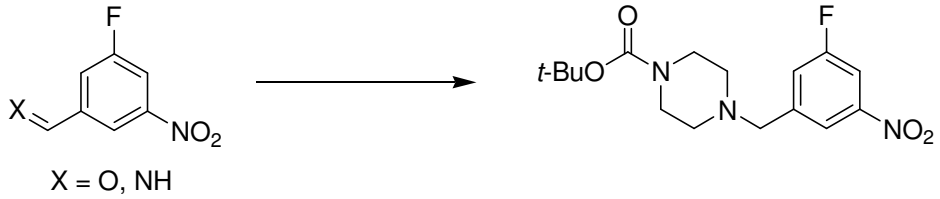
Methyl 4-(3-fluoro-5-(3-(6-methylpyridin-3-yl)ureido)benzyl)piperazine-1-carboxylate (CK-1316189)



3-Fluoro-5-nitrobenzonitrile. To a solution of 3-iodo-5-fluoronitrobenzene (20 g, 75 mmol, 1.0 equiv) in dry DMF (200 mL) was added $Zn(CN)_2$ (8.1 g, 69 mmol, 0.92 equiv) and $Pd(PPh_3)_4$ (5.0 g, 4.4 mmol, 0.058 equiv). The reaction mixture was purged with nitrogen and heated to 80 °C overnight. An additional 0.023 equiv of $Pd(PPh_3)_4$ was then added and the reaction was heated at 80 °C for additional 6 hrs. The reaction mixture was then cooled to RT, diluted with 15 volumes of EtOAc (based on starting material) and the organic layer was washed 3 times with water and once with brine. The organic layer was dried over anhydrous sodium sulfate, filtered and concentrated *in vacuo*. Purification by chromatography over silica gel (10% Et_2O/hexane) provided the title compound as an off-white solid (11.2 g, 67%).

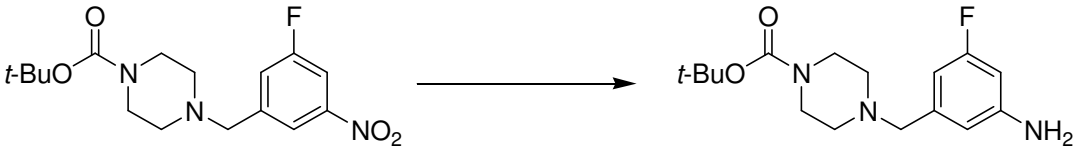


3-Fluoro-5-nitrobenzaldehyde/aldimine. To solution of 3-fluoro-5-nitrobenzonitrile (5.0 g, 30 mmol, 1.0 equiv) in dry Et_2O (500 mL) at 0 °C was added drop-wise a solution of diisobutylaluminum hydride (33 mL, 33 mmol, 1.1 equiv, 1.0 M in hexanes) by syringe. The resulting solution was kept at 0 °C overnight. The reaction mixture was then added to a mixture of ice and glacial acetic acid. The reaction mixture was then diluted with ethyl acetate, and the aqueous layer was extracted with ethyl acetate two additional times. The combined organic layers were washed twice with saturated sodium bicarbonate, and once with brine. The organic layers were then dried over anhydrous sodium sulfate, filtered and concentrated *in vacuo*. Purification over silica gel (10-30% EtOAc/hexanes) afforded a yellow solid (5.04 g, 100%) as a mixture of the aldehyde and imine. Approximately 20% of the material was the nitrile starting material as judged by HPLC analysis, however the material was used without further purification.

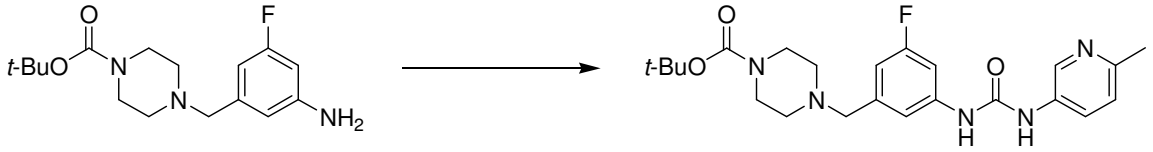


***tert*-Butyl 4-(3-fluoro-5-nitrobenzyl)piperazine-1-carboxylate.** To a cooled (0 °C) slurry of the aldehyde/imine mixture (5.0 g, 30 mmol, 1.0 equiv) and boc-piperazine (10.8 g, 58 mmol, 2 equiv) in a mixture of HOAc (5 mL) and DCM (7 mL) was added sodium triacetoxyborohydride (14.0 g, 66 mmol, 2.2 equiv) as a solid over approximately 15 minutes. The reaction was allowed to warm to RT and stirred for two additional hours. The reaction mixture was quenched with sat'd. aq. sodium bicarbonate and then diluted with ethyl acetate. The layers were separated, and the aqueous layer was washed three times with ethyl acetate. The organic layers were combined and washed with brine,

dried over anhydrous sodium sulfate, and concentrated *in vacuo*. Purification by chromatography over silica gel (50% ethyl acetate/hexanes) provided the title compound (6.8 g, 68%) as a yellow oil.

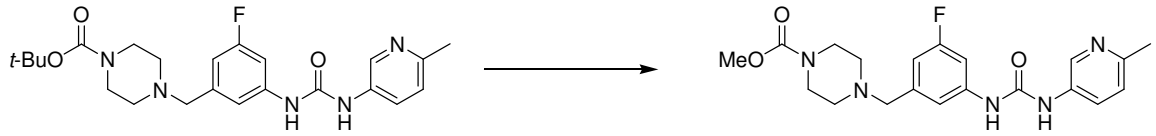


***tert*-Butyl 4-(3-amino-5-fluorobenzyl)piperazine-1-carboxylate.** A mixture of *tert*-butyl 4-(3-fluoro-5-nitrobenzyl)piperazine-1-carboxylate (5.0 g, 14.7 mmol, 1.0 equiv), and a catalytic amount of 10% Pd/C (500 mg, 10 wt/wt %) in MeOH (0.6 M in MeOH) was stirred over an atmosphere of 50 psi H_2 for 45 min. After replacement of the H_2 atmosphere with N_2, the reaction mixture was filtered through diatomaceous earth and the diatomaceous earth was washed with MeOH. Concentration of the MeOH *in vacuo* resulted in the isolation of the title compound as a beige foam (4.9 g, 99%).



tert-Butyl 4-(3-fluoro-5-(3-(6-methylpyridin-3-yl)ureido)benzyl)piperazine-1-carboxylate. To a solution of *tert*-butyl 4-(3-amino-5-fluorobenzyl)piperazine-1-carboxylate (4.7 g, 15.2 mmol, 1.0 equiv) in dry DCM (~150 mL) at RT under a N_2 atmosphere was added the 2-methyl-5-isocyanatopyridine (2.9 g, 18.0 mmol, 1.2 equiv) by syringe. The mixture was stirred for 1 hour. The reaction mixture was then diluted with sat'd. aq. sodium bicarbonate and ethyl acetate. The layers were separated and the organic layer was washed twice with sat'd. aq. $NaHCO_3$ and once with brine. The organic layer was dried over anhydrous sodium sulfate, filtered, and concentrated *in*

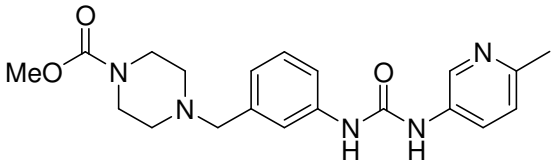
vacuo. Purification by chromatography over silica gel (5% methanol/DCM) provided the title compound as a foam (4.58 g, 68%).



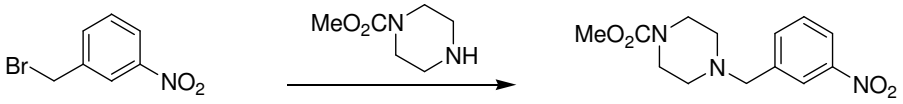
Methyl 4-(3-fluoro-5-(3-(6-methylpyridin-3-yl)ureido)benzyl)piperazine-1-carboxylate (CK-1316189). To a solution of *tert*-butyl 4-(3-fluoro-5-(3-(6-methylpyridin-3-yl)ureido)benzyl)piperazine-1-carboxylate (1.89 mg, 4.26 mmol, 1.0 equiv) in CH_2Cl_2 (5 mL) was added trifluoroacetic acid (5 mL). The reaction mixture was stirred for 30 min and concentrated *in vacuo*. The resulting residue was dissolved in EtOAc and washed sequentially with 3N NaOH (2 times) and brine. The organic layer was dried over anhydrous sodium sulfate, filtered, and concentrated *in vacuo* to provided the desired free amine that was used without further purification.

To a 0 ^{o}C solution of the above amine (1.3 g, 3.80 mmol, 1.0 equiv) and DIPEA (1.2 equiv) in dry THF (20 mL) was added methyl chloroformate (1.1 equiv) by syringe and the resultant mixture stirred for 1h. The reaction mixture was then diluted with EtOAc and sat'd. aq. sodium bicarbonate. The layers were separated and the organic layer was washed twice with sat'd. aq. sodium bicarbonate and once with brine. The combined aqueous layers were extracted once with ethyl acetate. The combined organic layers were dried over anhydrous sodium sulfate, filtered, and concentrated *in vacuo*. Purification by chromatography over silica gel (5% MeOH/DCM) provided the title compound as a white solid (1.1 g, 72%). mp 149 - 150 ^{o}C; IR (Solid) 3288, 3006, 2858, 2791, 2703, 2687, 1721, 1637, 1583, 1561, 1456, 1406, 1348, 1145, 1124, 1094, 1013, 858,833, 814,

681cm^{-1}; ^{1}H NMR (400 MHz, DMSO-*d6*) δ 9.02 (s, 1H), 8.76 (s, 1H), 8.47 (d, J = 2.5 Hz, 1H), 7.81 (dd, J = 8.4, 2.7 Hz, 1H), 7.37 (dt, J = 11.5, 2.1 Hz, 1H), 7.18 (d, J = 8.4 Hz, 1H), 7.11 (s, 1H), 6.74 (d, J = 9.5 Hz, 1H), 3.59 (s, 3H), 3.46 (s, 2H), 3.37 (m, 4H), 2.41 (s, 3H), 2.35 (d, J = 4.5 Hz, 4H).; ^{13}C NMR (100 MHz, DMSO-*d6*) δ 155.0, 152.3, 151.1, 149.5, 139.4, 134.1, 127.6, 125.2, 125.1, 123.1, 123.0, 121.8, 118.0, 62.0, 52.2, 52.1, 43.4, 23.2. Exact mass calc'd for $C_{20}H_{25}FN_5O_3$ requires *m/z* 402.1941 found *m/z* 402.1930 (ESI, M+H^{+}).

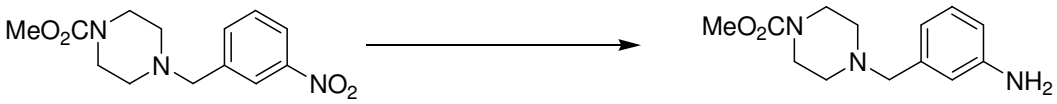


Methyl 4-(3-(3-(6-methylpyridin-3-yl)ureido)benzyl)piperazine-1-carboxylate (CK-1317138)



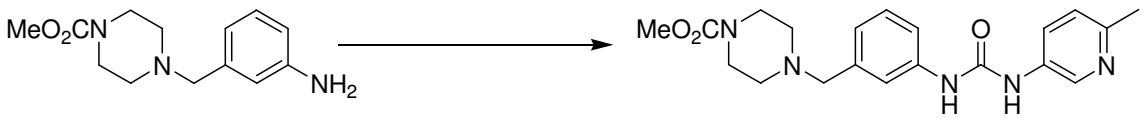
Methyl 4-(3-nitrobenzyl)piperazine-1-carboxylate. A 22 L 3-neck RBF equipped with an addition funnel, mechanical stirrer, and nitrogen bubbler was charged with 3-nitrobenzyl bromide (1.00 kg, 4.63 mol, 1 equiv) and anhydrous CH_2Cl_2 (4 L). Diisoproplyethylamine (DIEA, 886.6 mL, 5.09 mol, 1.1 equiv) was added. To the resultant solution was added, drop wise methyl piperazine-1-carboxylate (734.1 g, 5.09 mol, 1.1 equiv) in CH_2Cl_2 (1 L). When the internal temperature of the reaction mixture reached 30 ^{o}C, the reaction mixture was placed over an ice-water bath. Addition of the methyl piperazine-1-carboxylate in CH_2Cl_2 was continued while maintaining the internal temperature below 17 ^{o}C. The reaction mixture was warmed to room temperature for approximately 19 h, heated to reflux for 8 h, and then cooled to room temperature. An

additional 33.4 g (0.05 equiv) of methyl piperazine-1-carboxylate in CH_2Cl_2 (0.05 L).was added and the resultant mixture stirred at room temperature for approximately 15 h. The reaction mixture was washed with water (5 L) and the water layer was extracted with CH_2Cl_2 (5 L). The combined organic layers were washed sequentially with 10% acetic acid in water (5 L), saturated $NaHCO_3$ (5 L), and brine (5 L) then concentrated to colorless oil. This oil was dissolved in methyl *t*-butyl ether (MTBE, 1 L). To this solution hexane (5 L) was added drop wise resulting in a white suspension. This suspension was stirred for 16 h. The solids were isolated by vacuum filtration and dried at room temperature under vacuum (~30 mmHg) resulting in methyl 4-(3-nitrobenzyl)piperazine-1-carboxylate (1.11 Kg, 85.9 % yield) as a white solid.



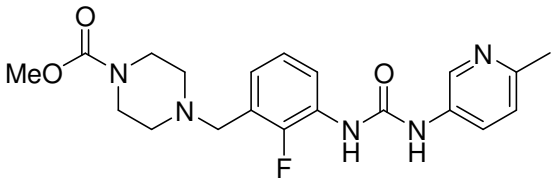
Methyl 4-(3-aminobenzyl)piperazine-1-carboxylate. Two 8 L Parr hydrogenators were each charged with 1% platinum on carbon (1% Pt/C, 138.83g, 25 wt %) and K_2CO_3 (413.4 g, 2.99 mol, 1.5 equiv). The reaction vessels were placed under vacuum followed by purging with nitrogen (3 times). A solution of methyl 4-(3-nitrobenzyl)piperazine-1-carboxylate (555.15g, 1.99 mol, 1 equiv) in THF (5.5 L) was added to the reaction vessel under vacuum. The reaction vessels were placed under vacuum followed by purging with Hydrogen (3 times). The reaction mixture was stirred vigorously under 50 psi H_2 at room temperature for approximately 14 h. The reaction vessels were placed under vacuum followed by purging with nitrogen (3 times), combined, and filtered through a pad of celite while under an atmosphere of nitrogen. The filtrate was concentrated *in vacuo* and the resultant solid was dried *in vacuo* (~ 30 mm Hg) at room temperature for

approximately 20 h resulting in methyl 4-(3-aminobenzyl)piperazine-1-carboxylate (801.9 g, 80.8 % yield) as a white solid.

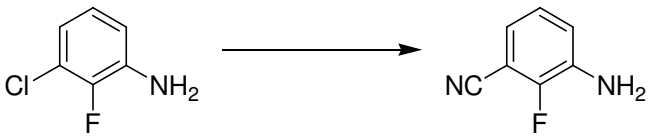


Methyl 4-(3-(3-(6-methylpyridin-3-yl)ureido)benzyl)piperazine-1-carboxylate (CK-1317138): A 12L 3-neck RBF equipped with a heating mantle, mechanical stirrer, thermal well, condenser, and nitrogen bubbler was charged with methyl 4-(3-aminobenzyl)piperazine-1-carboxylate (249.31 g, 2.41 mol, 1 equiv) and acetone (32 L). The solution was warmed to 35 ± 5 °C to form a solution and 5-isocyanato-2-methyl pyridine (323.3 g, 2.41 mol, 1.0 equiv) was added over ~ 30 min. An additional 0.6 L of acetone was to the resultant viscous slurry and the suspension was stirred for approximately 14h. The suspension was heated to reflux than allowed to cool to room temperature (3 times). The reaction mixture was filtered and the resulting solid was rinsed with acetone (0.6 L), EtOAc (3 x 1 L), and hexanes (3 x 1 L). The solids were dried under vacuum (~30 in Hg) at ≤ 30 °C to afford methyl 4-(3-(3-(6-methylpyridin-3-yl)ureido)benzyl)piperazine-1-carboxylate (**CK-1317138**) as a white powder (779.01 g, 84 % yield). IR (KBR) 3291, 2949, 2795, 1720, 1635, 1560, 1600, 1488, 1244, 1123, 800, 770, 691, 668 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6) δ 8.76 (s, 1 H, 2-pyridyl **H**), 8.68 (s, 1 H, Urea N-**H**), 8.47 (d, 1 H, J = 2.6 Hz, Urea N-**H**), 7.82 (dd, 1 H, J = 2.6 Hz, 8.4 Hz, phenyl **H**), 7.43 (s, 1 H, phenyl **H**), 7.43 (s, 1 H, phenyl **H**), 7.34 (d, 1 H, J = 8.2 Hz, pyridyl **H**), 7.24-7.15 (m, 3H, phenyl **H,** pyridyl **H**), 6.91 (d, J = 7.5 Hz, phenyl **H**),

3.59 (s, 3 H, OC**H**$_3$), 3.36 (m, 4 H, piperazine **H**s), 2.40 (s, 3 H, pyridine-C**H3**), 2.33 (br m, 4 H, piperazine **H**s); ^{13}C NMR (100 MHz, DMSO-d_6) δ 155.0,152.6, 150.4, 139.5, 139.3, 138.5, 133.8, 128.6, 126.0, 122.7, 122.5, 113.6, 117.0, 62.0, 52.2, 52.2, 43.4, 23.2; Anal. Calcd. For $C_{20}H_{25}N_5O_3$: C, 62.65; H, 6.57; N, 18.26. Found: C, 62.82; H, 6.71; N, 18.41.

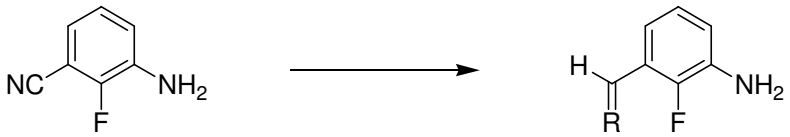


Methyl 4-(2-fluoro-3-(3-(6-methylpyridin-3-yl)ureido)benzyl)piperazine-1-carboxylate (Omecamtiv Mecarbil)

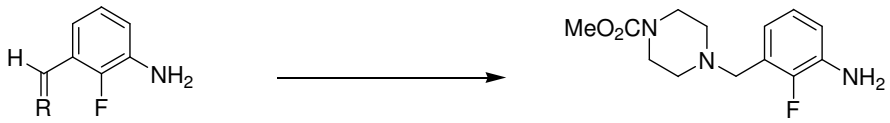


3-Amino-2-fluorobenzonitrile. A 22 L 3-neck RBF equipped with a mechanical stirrer, thermowell, condenser, nitrogen bubbler, and heating mantle was charged with 3-chloro-2-fluoroaniline (1.0 kg, 6.87 mol, 1 equiv), 1-methyl-2-pyrrolidinone (5 L), NaCN (741 g, 15.1 mol, 2.2 equiv), and $NiBr_2$ (2.026 kg, 9.27 mol, 1.35 equiv). Finally, additional NMP (5 L) was added, and the solution was gently warmed to 200 °C and stirred for 4 days. The reaction was deemed complete by TLC (100% dichloromethane (DCM), UV/ninhydrin stain). The heat was turned off and the solution was allowed to cool to room temperature. The reaction was diluted with MTBE (30 L) and filtered through celite. The celite pad was then rinsed with MTBE (10 L). The organics were washed with 25% sodium chloride (wt %) aqueous solution (40 L), water (3x 40 L) and once

again with 25% sodium chloride (wt%) aqueous solution (40 L). The combined organics were dried over sodium sulfate and concentrated to afford 3-amino-2-fluorobenzonitrile as a brown solid, which was dried under vacuum (~30 in Hg) at ≤40°C to afford 0.665 kg (71% yield).

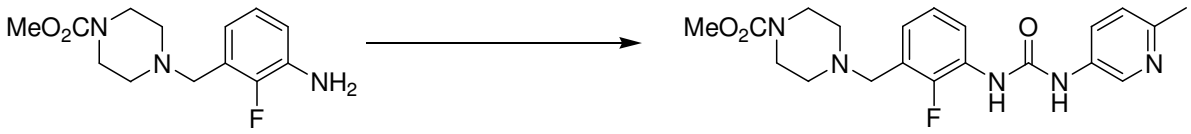


3-Amino-2-fluorobenzaldehyde. A 50 L 3-neck RBF equipped with a mechanical stirrer, thermowell, condenser, nitrogen bubbler and 2 L addition funnel was charged with 3-amino-2-fluorobenzonitrile (1.30 kg, 9.55 mol, 1 equiv) and DCM (6.5 L). The reaction was cooled to ~0 °C in an ice/brine/acetone bath, and DIBALH (19.1 L, 1 M in DCM, 19.1 mol, 2.0 equiv) was added drop-wise over ~3.5 hours, maintaining an internal reaction temperature ≤ 0 °C. The reaction was deemed complete by HPLC/TLC (100% DCM, UV/ninhydrin stain) upon completion of the DIBALH addition and was quenched *via* an inverse quench. Thus, the reaction mixture was added drop-wise with vigorous stirring to a cooled solution (~0 °C) of 15% (wt %) aq. potassium sodium tartrate (52 L) and DCM (13 L), maintaining an internal reaction temperature below 10 °C. The flask was rinsed with DCM (13 L) and the mixture was allowed to warm to room temperature and stirred for 4 hours. The layers were separated, and the aqueous layers were back extracted with DCM (13 L). The combined organics were washed with water (26 L), dried over sodium sulfate, and concentrated to afford a brown foam, which was dried under vacuum (~30 in Hg) at RT to afford 1.22 kg of the title compound (92% yield) as a mixture of the aldehyde and imine, which was used in the next step without further purification.



Methyl 4-(3-amino-2-fluorobenzyl)piperazine-1-carboxylate. A 50 L 3-neck RBF equipped with a cooling bath, addition funnel, mechanical stirrer, thermal well, condenser, and nitrogen bubbler was charged with 3-amino-2-fluorobenzaldehyde/imine mixture (3.60 kg, 25.9 mol, 1 equiv), anhydrous THF (18 L), and methylpiperazine-1-carboxylate (3.92 kg, 27.2 mol, 1.05 equiv). The solution stirred for 4.5 h at room temperature. A room-temperature water bath was then added to regulate the temperature, and sodium triacetoxyborohydride (7.21 kg, 34.0 mol, 1.3 equiv) was added portion-wise over 1 hour, maintaining an internal reaction temperature below 40 °C. The reaction stirred for 15 hours, and an additional portion of sodium triacetoxyborohydride (1.02 kg, 4.81 mol, 1.5 equiv) was added. The reaction was stirred for an additional 5 hours and was deemed complete by HPLC/TLC (4:1 DCM:acetonitrile, UV/ninhydrin stain). The reaction was then added to water (27 L) and EtOAc (27 L) dropwise over 45 min, maintaining an internal reaction temperature below 30 °C. The reaction mixture was allowed to stir for 90 min before the layers were separated. The aqueous layers were then extracted with EtOAc (18 L). The combined organics were washed with aq. 8% sodium bicarbonate (wt %) (36 L) and the pH of the aqueous layer was 6 (Micro Essential Labs pHydrion paper). The layers were separated, and the organics were washed with aq. 25% sodium chloride (wt %) (18 L). The organics were dried over sodium sulfate (5.4 kg) and activated carbon (5.4 kg). The organics were filtered and rinsed with EtOAc (41 L). The organics were concentrated in vacuo and dried overnight on a rotary evaporator (~30 in Hg at RT) to afford 4.22 kg of the title compound as an amber-brown oil. A 50 L 3-neck

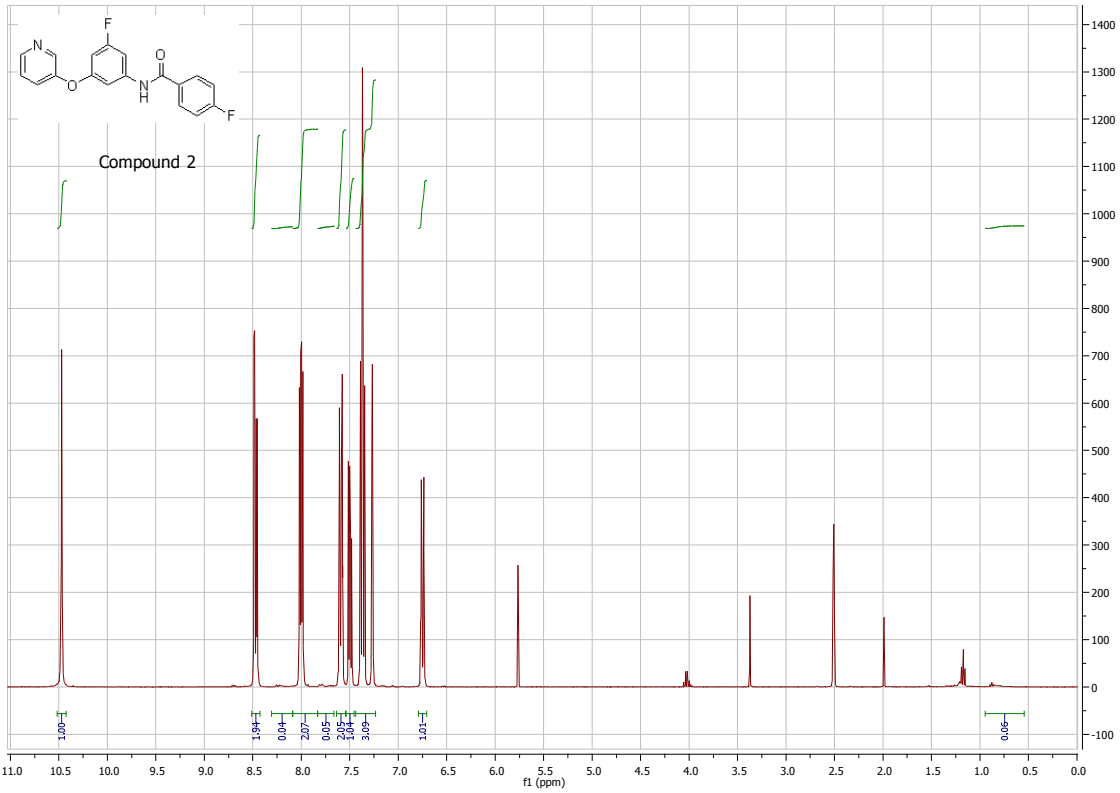
RBF equipped with a cooling bath, mechanical stirrer, thermal well, 2 L addition funnel, and nitrogen bubbler was charged with MeOH (10.8 L). The solution was cooled to 0 ± 5 °C in an ice/brine/acetone bath, and AcCl (5.5 L, 77.7 mol, 3 equiv) was added drop-wise over ~4 h, maintaining an internal reaction temperature below 10 °C. The solution was then stirred for an additional hour below 5 °C, then a solution of unpurified methyl 4-(3-amino-2-fluorobenzyl)piperazine-1-carboxylate (4.22 kg, 1.0 eq) in MeOH (7.2 L) was added dropwise over 30 min, maintaining an internal reaction temperature below 15 °C. The reaction was allowed to warm to room temperature over 45 h. The solids were filtered the and rinsed with MeOH (2 x 1.8 L), MTBE:MeOH (1:5, 18 L), and MTBE (18 L). The solids were then taken up in EtOAc (18 L) and aq. 8% sodium bicarbonate (wt %, 18 L). Sodium bicarbonate was added to bring the pH to 8 (Micro Essential Labs pHydrion paper), and the layers were separated. The aqueous layer was extracted with EtOAc (18 L). The combined organics were washed with 25% sodium chloride (wt%) aqueous solution (18 L), dried over sodium sulfate (5.4 kg), and concentrated in vacuo (~30 in Hg) to afford the title compound as a pale orange solid (2.72 kg, 39% yield).

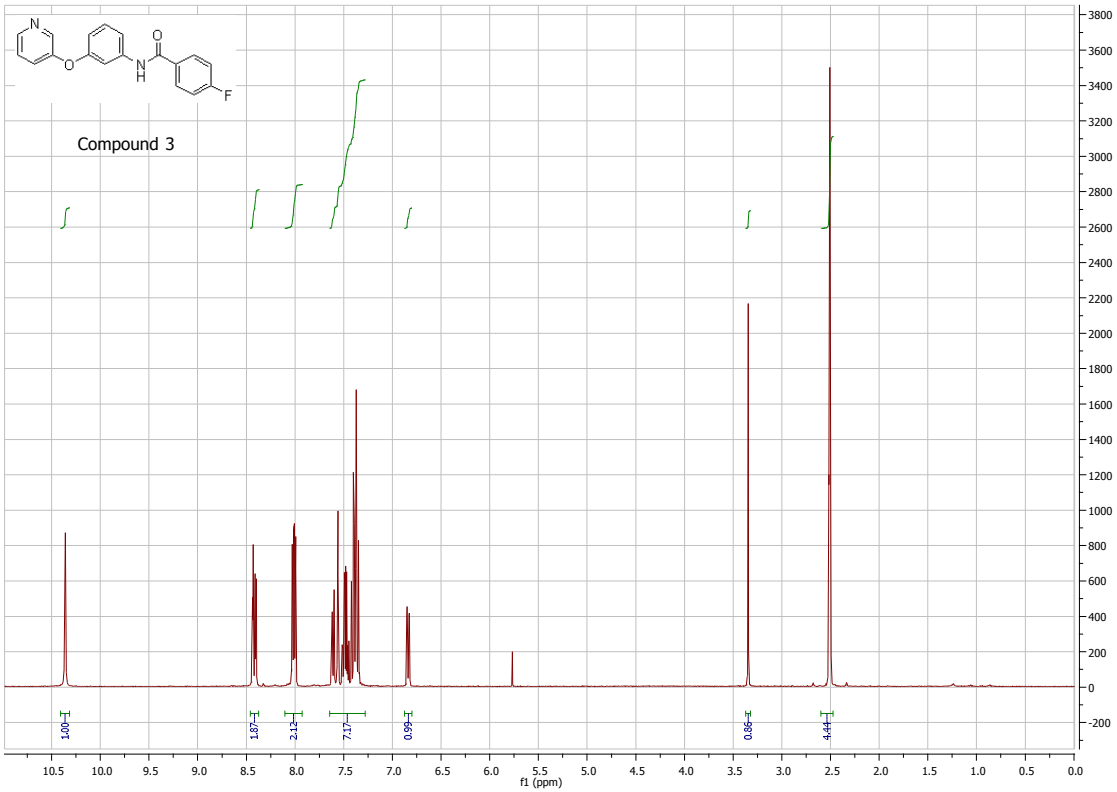


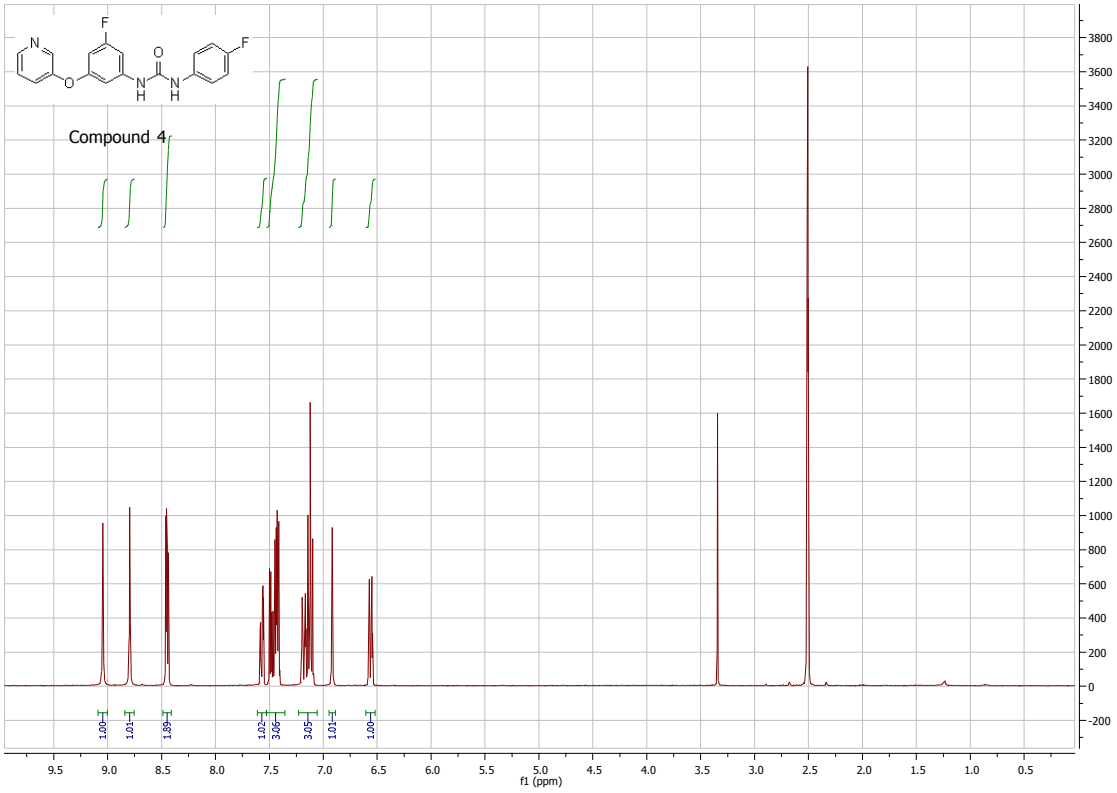
Methyl 4-(2-fluoro-3-(3-(6-methylpyridin-3-yl)ureido)benzyl)piperazine-1-carboxylate (Omecamtiv Mecarbil). A 50L 3-neck RBF equipped with a heating mantle, mechanical stirrer, thermal well, condenser, and nitrogen bubbler was charged with methyl 4-(3-amino-2-fluorobenzyl)piperazine-1-carboxylate (2.70 kg, 10.10 mol, 1 equiv) and acetone (27 L). The solution was warmed to 40±5 °C, and 5-isocyanato-2-

methyl pyridine (1.355 kg, 10.10 mol, 1.0 equiv) was added over 4 min. A voluminous precipitate formed during the addition, and the heat was turned off, allowing the reaction exotherm to warm the reaction to reflux over 5 min. The reaction was allowed to reflux for a total of 1.5 hours and was deemed incomplete by HPLC/TLC (1:1 DCM:ACN, UV/ninhydrin stain). The reaction was allowed to reflux for an additional 2 h 40 min. The reaction was still deemed incomplete, and an additional (0.10 mol, 0.01 equiv) 5-isocyanato-2-methyl pyridine was added and the reaction was allowed to cool to ≤ 30 °C over 7 h 40 min. The reaction was kept at ≤ 30 °C for 5 hours. The reaction was filtered and the resulting solid was rinsed with acetone (3 L), then EtOAc (3 x 5 L). The solids were dried under vacuum (~30 in Hg) at ≤ 30 °C to afford omecamtiv mecarbil as a white powder (3.64 kg, 90% yield). IR (KBR) 3292, 2950, 2866, 2833, 1720, 1640, 1550, 1600, 1490, 1455, 1406, 1378, 1352, 1274, 1244, 1191, 1125, 815, 769, 725, 668 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.12 (s, 1 H, 2-pyridyl **H**), 8.59 (d, 1 H, J = 2.5 Hz, Urea N-**H**), 8.47 (d, 1 H, J = 2.6 Hz, Urea N-**H**), 8.04 (dt, 1 H, J = 1.5 Hz, 7.8 Hz, phenyl **H**), 7.83 (dd, 1 H, J = 2.6 Hz, 8.4 Hz, 4-pyridyl **H**), 7.18 (d, 1 H, J = 8.4 Hz, 5-pyridyl **H**), 7.10 (app t, 1 H, J = 7.8 Hz, phenyl **H**), 7.02 (app p, 1 H, J = 1.5 Hz, 6.3 Hz, 7.8 Hz, phenyl **H**), 3.58 (s, 3 H, OC**H₃**), 3.56 (m, 4 H, piperazine **H**s), 2.41 (s, 3 H, pyridine-C**H3**), 2.37 (br m, 4 H, piperazine **H**s); ^{13}C NMR (100 MHz, DMSO-d_6) δ 155.0,152.3, 151.1 150.7, 139.1, 133.6, 127.3, 127.2, 125.8, 124.1, 123.7, 122.8, 119.5, 54.5, 52.2, 52.0, 43.4, 23.2; Exact mass calcd for $C_{20}H_{24}FN_5O_3$ requires *m/z* 402.1926. Found m/z 402.1940. Anal. Calcd. For $C_{20}H_{24}FN_5O_3$: C, 59.84; H, 6.03; N, 17.45. Found: C, 59.99; H, 6.07; N, 17.41.

^{1}H NMR Spectra:



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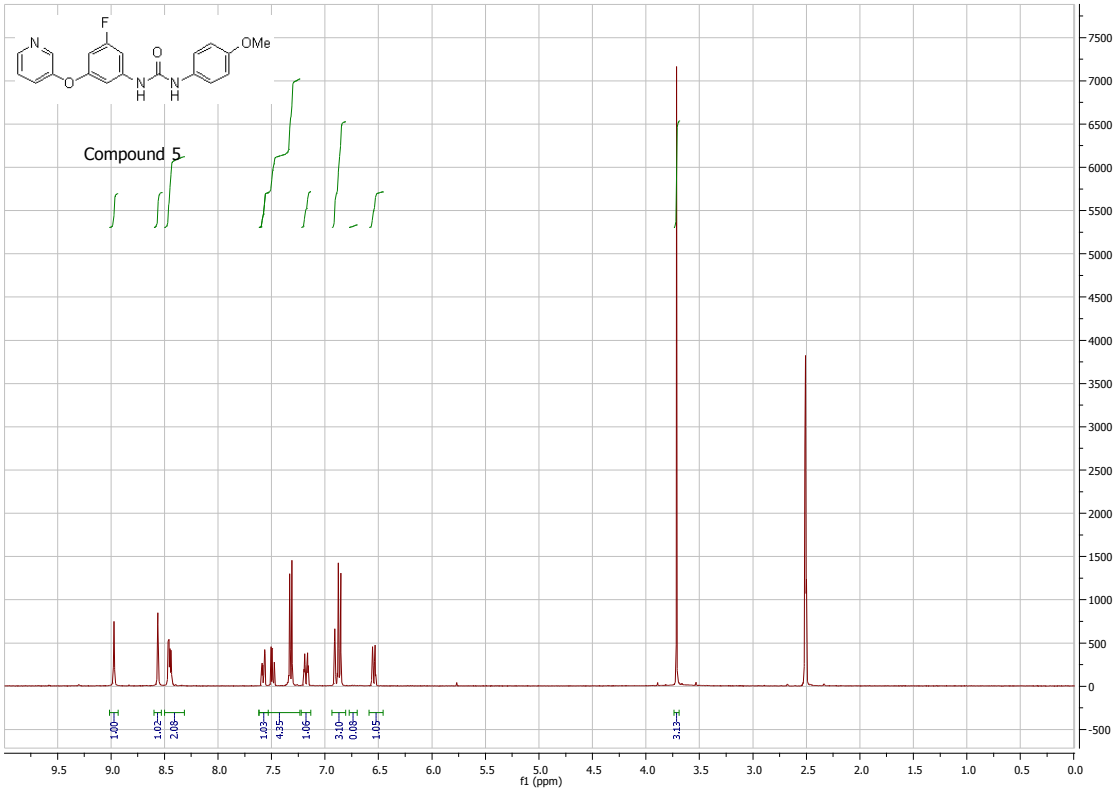
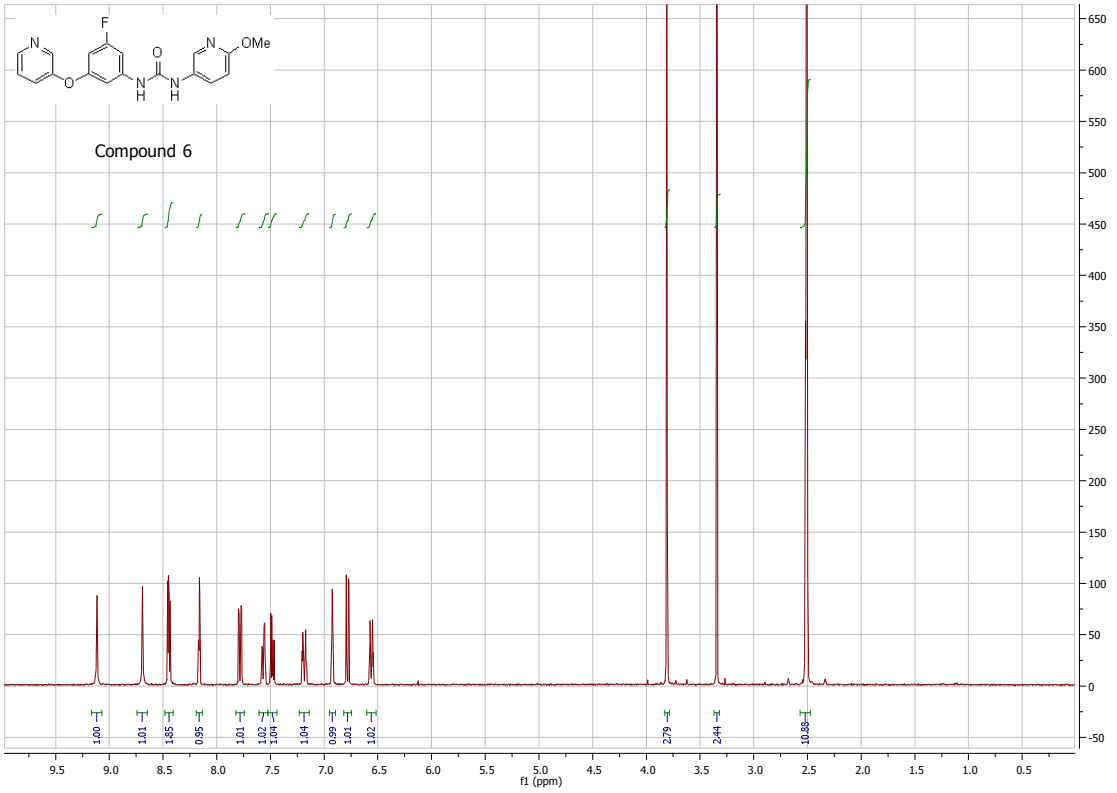
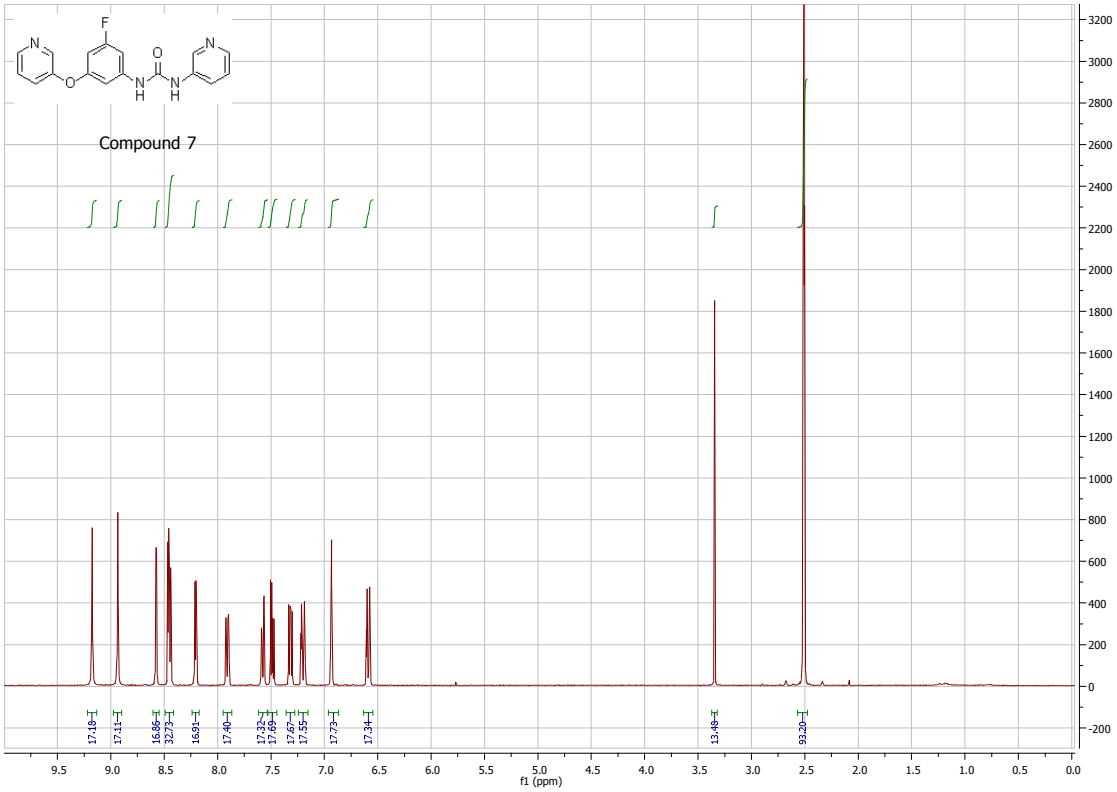

Compound 5
f1 (ppm)


F
N
O
N
OMe
O
N
H
N
H
Compound 6
1.00
1.01
1.85
0.95
1.01
1.02
1.04
1.04
0.99
1.01
1.02
2.79
2.44
10.88
f1 (ppm)


F
N
O
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H
N
H
Compound 7
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93.20
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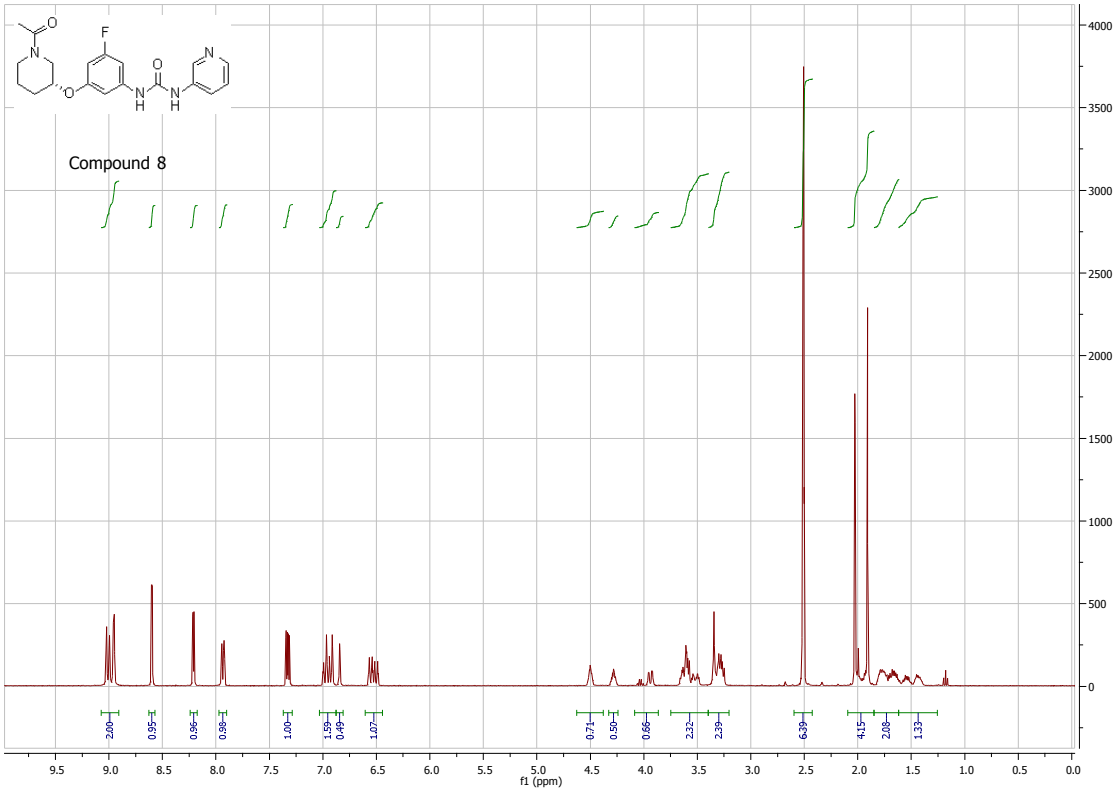
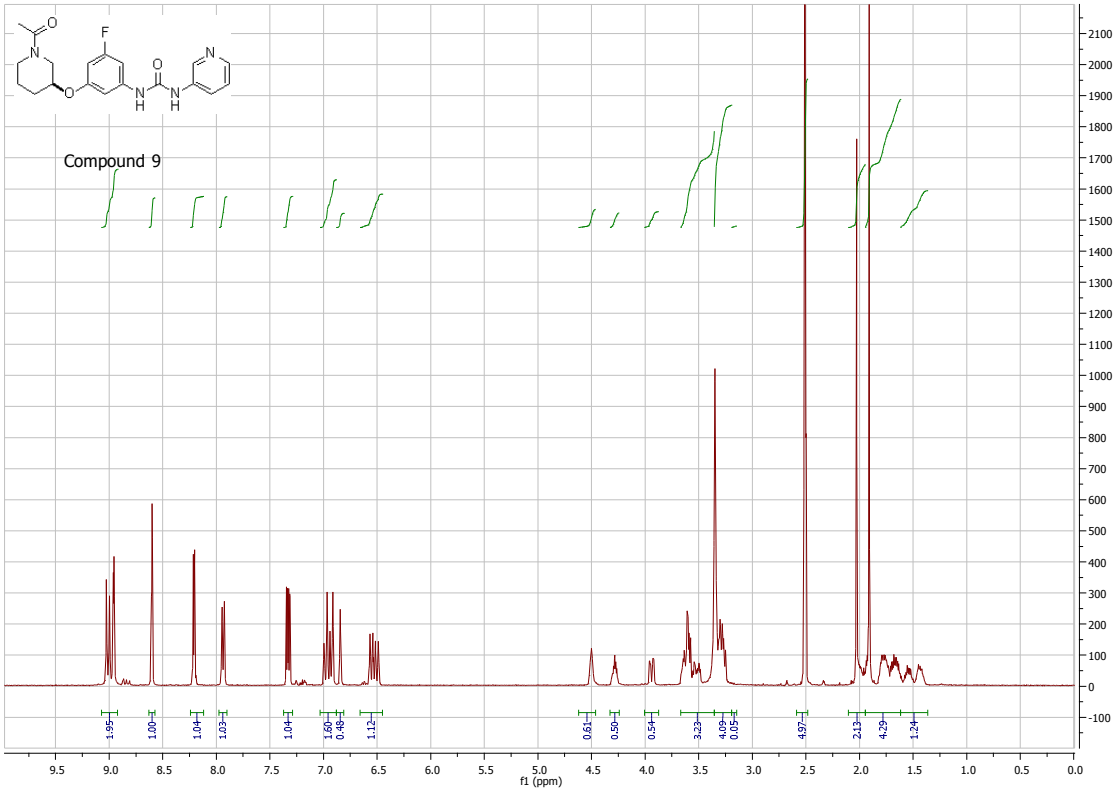
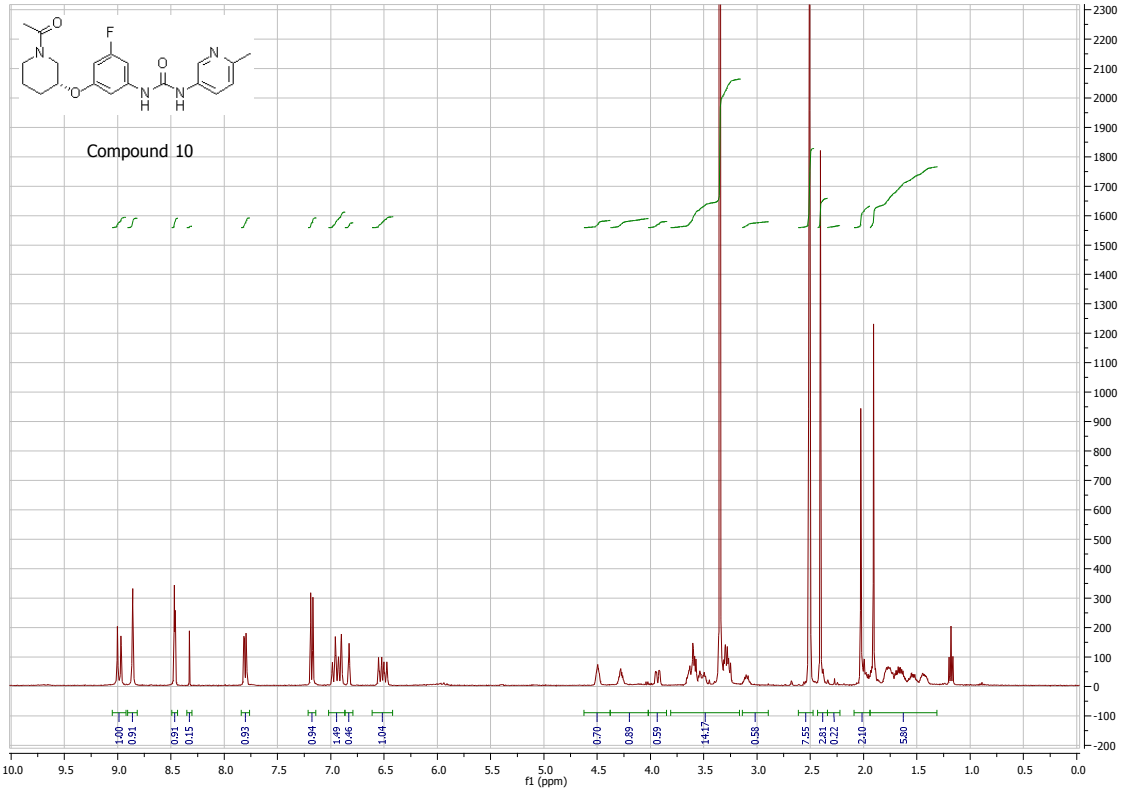
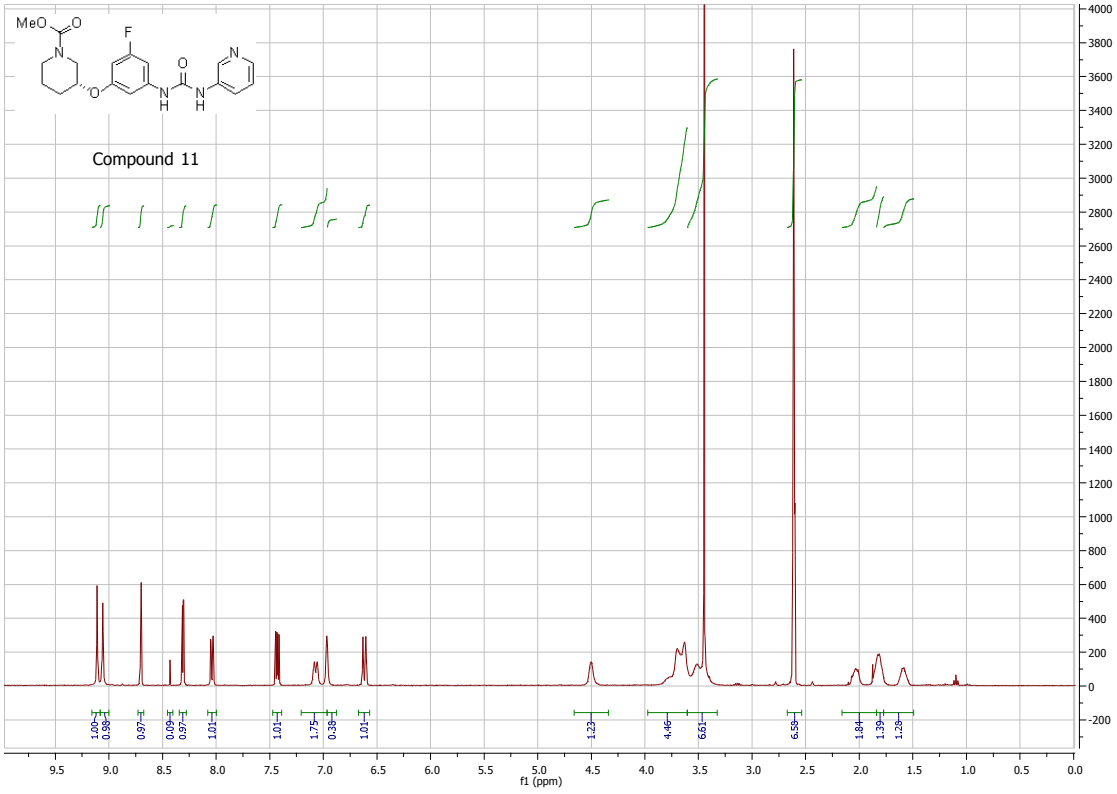
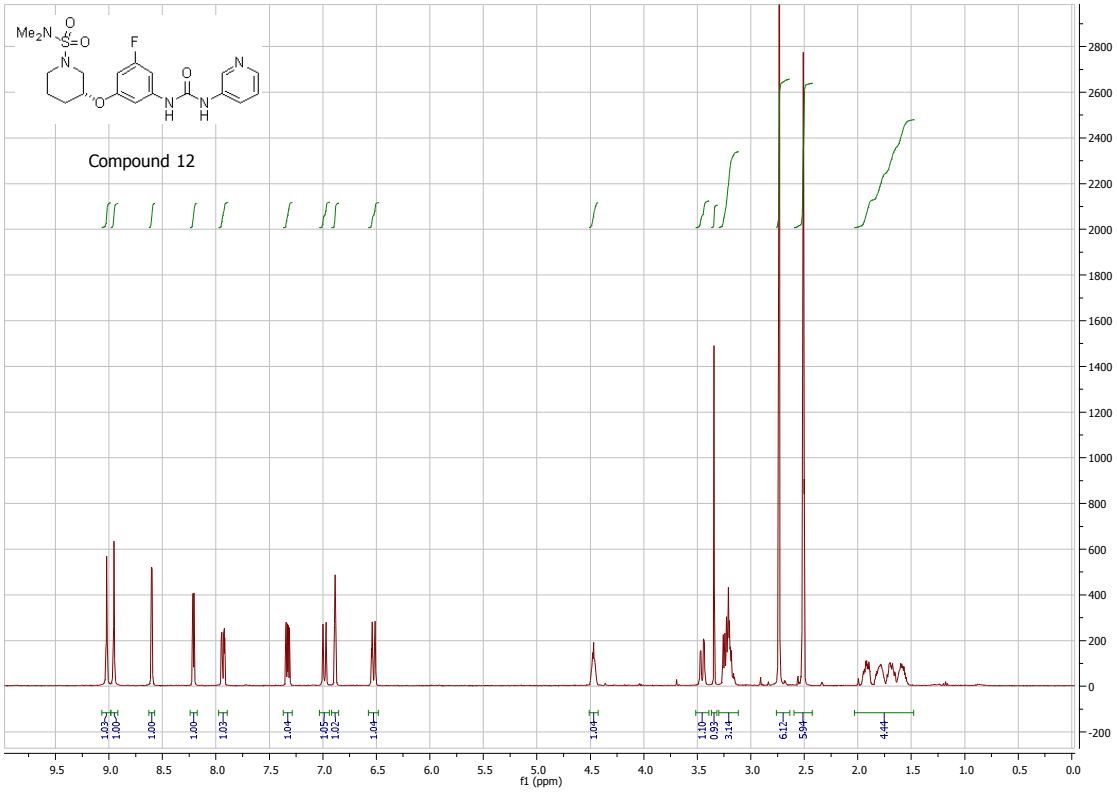
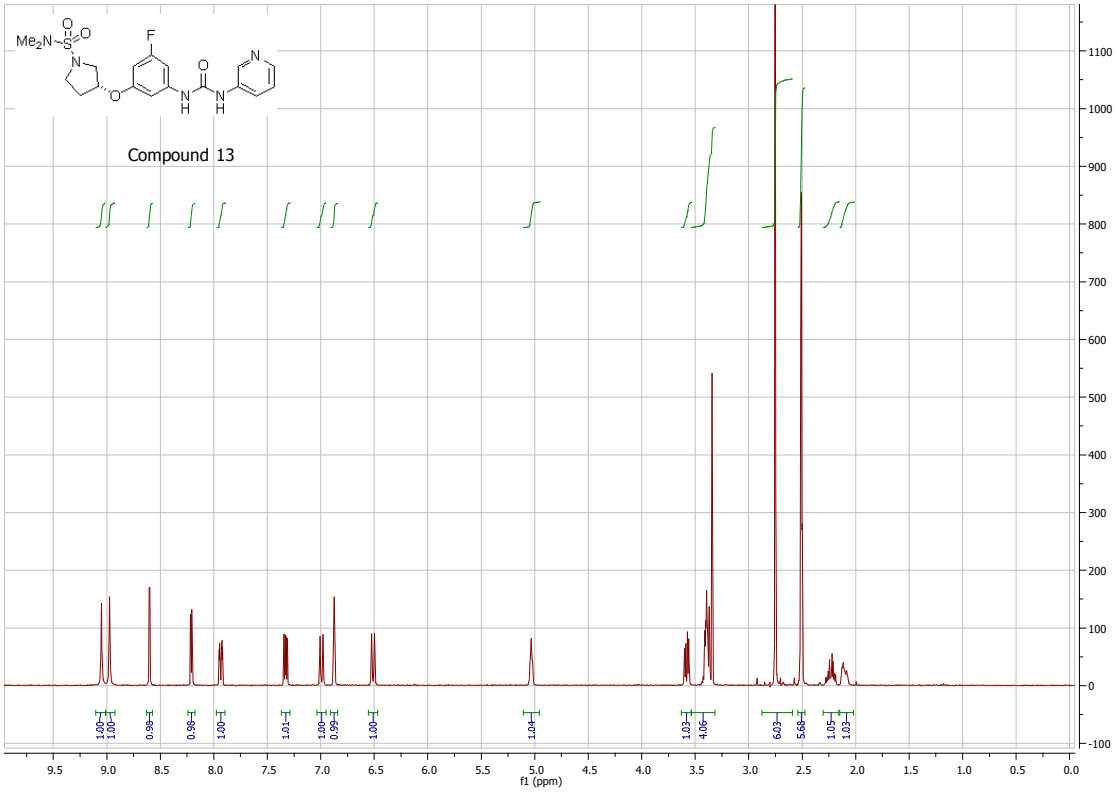

Compound 8
f1 (ppm)

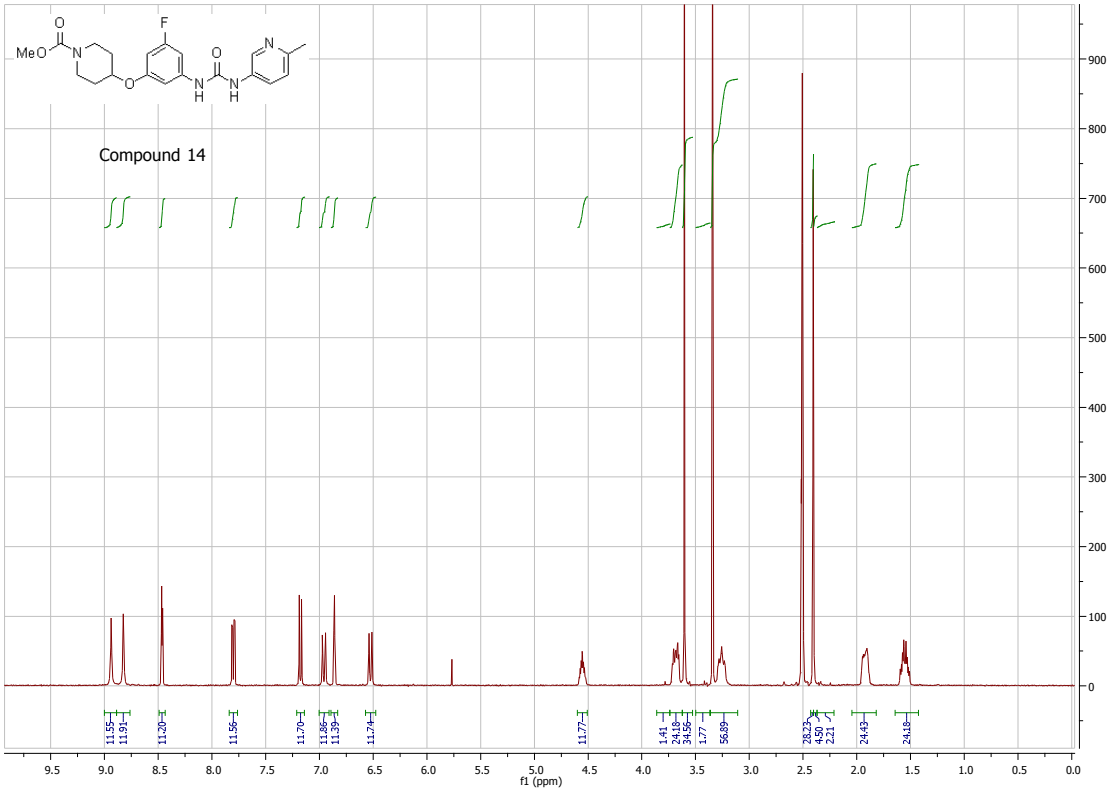

Compound 9
f1 (ppm)

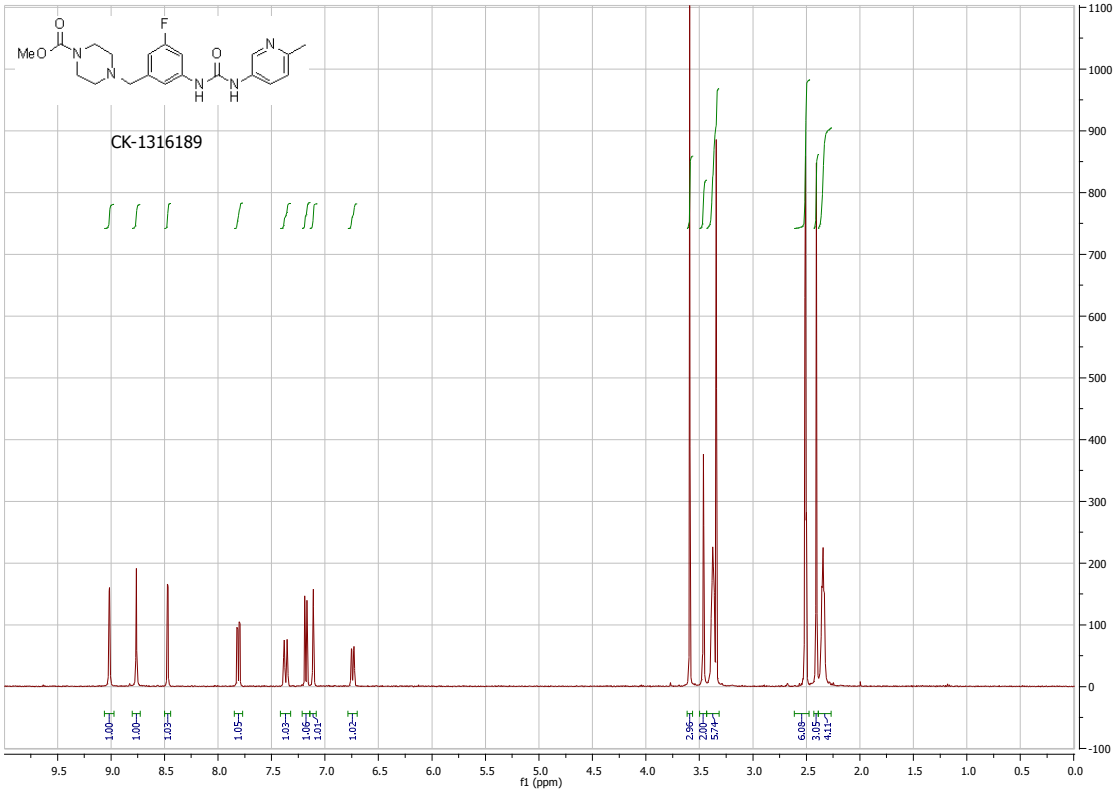

Compound 10
f1 (ppm)


MeO
Compound 11
f1 (ppm)


Compound 12
f1 (ppm)


Compound 13
f1 (ppm)


Compound 14
f1 (ppm)


CK-1316189
f1 (ppm)