Supporting Information

Discovery of the First Potent and Selective Inhibitor of Centromere-Associated Protein E (CENP-E): 3-Chloro-*N*-[(1*S*)-2-[[2-(dimethylamino)acetyl]amino]-1-[[4-[8-[(1*S*)-1-hydroxyethyl]imidazo[1,2-a]pyridin-2-yl]phenyl]methyl]ethyl]-4-(1-methylethoxy)benzamide (GSK923295)

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Contents

Biological Assays

All data in the manuscript is an average of at least two individual measurements ($n \geq 2$). In all cases, individual measurements were within three-fold for each compound.

Expression and Purification

The motor domains of KSP (amino acids 1–360), MKLP1 (amino acids 4–433), CENP-E (amino acids 2–340), Kif1A (amino acids 3–353), MCAK (amino acids 257-617) and RabK6 (amino acids 59-506) were expressed in *Escherichia coli* BL21(DE3) as a C-terminal 6-his fusion proteins. Bacterial pellets were lysed with a microfluidizer (Microfluidics Corp.) in lysis buffer [50 mM Tris-HCl; 50 mM KCl, 10 mM imidazole, 2 mM $MgCl_2$, 8 mM ß-mercaptoethanol, 0.1 mM ATP (pH 7.4)], and the resulting proteins were further purified using Ni-NTA agarose affinity chromatography, with an elution buffer consisting of 50 mM PIPES, 10% sucrose, 300 mM imidazole, 50 mM KCl, 2 mM $MgCl_2$, 8 mM ß-mercaptoethanol, and 0.1 mM ATP (pH 6.8).

Steady-state Kinetic Analysis of Human CENP-E ATPase Activity and Inhibition by Compounds

Kinesin specificity analysis was carried out using a pyruvate kinase-lactate dehydrogenase detection system that couples the production of ADP to oxidation of NADH. Absorbance changes were monitored at 340 nm. Steady-state studies using nM concentrations of CENP-E were performed using a sensitive fluorescence-based assay utilizing a pyruvate kinase, pyruvate oxidase and horseradish peroxidase coupled detection system that couples the generation of ADP to oxidation of Amplex Red to fluorescent resorufin. Generation of resorufin was monitored by fluorescence ($\lambda_{excitation}$ = 520 nm and $\lambda_{emission}$ = 580 nm). Steady-state biochemical experiments were performed in PEM25 buffer [25 mM $PipesK^+$ (pH 6.8), 2 mM $MgCl_2$, 1 mM EGTA] supplemented with 10 μM paclitaxel for experiments involving microtubules. The IC_{50} for steady-state

inhibition was determined at 500 μM ATP, 5 μM MTs and 1 nM CENP-E in PEM25 buffer. K_{iapp} (apparent inhibitor dissociation constant) estimate of the inhibitor was extracted from the concentration-response curves, with explicit correction for enzyme concentration by using the Morrison equation:

$$\frac{v}{v_o} = 1 - \frac{([E]+[I]+K_{iapp}) - \sqrt{([E]+[I]+K_{iapp})^2 - 4[E][I]}}{2[E]} \qquad \text{(Eq. 1)}$$

Where ν is the reaction velocity at different concentrations of inhibitor I, ν_o is the control velocity in the absence of inhibitor and E is the total enzyme concentration.
Inhibitor modality (e.g. competitive, noncompetitive, uncompetitive, or mixed) under steady-state conditions was determined by measuring the effect of inhibitor concentration on initial velocity as a function of substrate concentrations. Data were fit using equations in GraFit (version 5.0.11, Erithiacus Software Ltd.) to velocity equations for the various modes of inhibition.

Cell culture and Growth Inhibition

Cell line growth inhibition assays were performed on 214 solid and 19 hematological tumor cell lines by MDS (Irvine, CA). Each cell line was seeded into 384 well plates in triplicate at two densities, where the lower density had half the number of cells as the high density. Densities ranged from 300 to 3600 cells per well. After 24 hours, a serially diluted compound was added to cells. One set of wells for each cell line (with no added compound) was analyzed for DNA content using the method below to give a time zero (t = 0) value. After a further 72 hours medium was aspirated and cells were washed and fixed. Cell nuclei were stained using DAPI and counted by real-time imaging using an Incell 1000 (GE, Piscataway, NJ). All data were normalized to t = 0. Curves were analyzed using the XLfit (IDBS Ltd.) curve-fitting tool for Microsoft Excel to determine the IC_{50} (concentration of 50% growth inhibition relative to t = 0 and Y_{max} values), the IC-Maximum (concentration giving maximum growth inhibition), and the Y_{min} (bottom of the four-parameter curve at IC-Maximum). For subsequent analyses, IC_{50} values obtained at low and high density plating were averaged.

Tumor Xenograft Studies

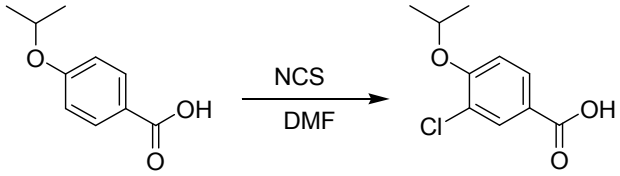
Cells were implanted in Nude mice and grown as tumor xenografts. Dosing began when tumors achieved ~100 mm^3. GSK923295A (62.5, 125, or 250 mg/kg) or vehicle (4% DMA/Cremaphore (50/50) pH5.6) was administered intraperitoneally in two cycles of three daily injections separated by one week. Results are reported as median tumor volume for 7-8 mice. *Paclitaxel* (Mead Johnson; 30mg/kg *i.v.*; q4dx3) was used as a positive control for comparison. Tumors were measured 3x/week with Vernier calipers, and tumor volume was calculated from two-dimensional measurements using an equation approximating the volume of a prolate ellipsoid (tumor volume mm^3 = (length x $width^2$) x 0.5). Antitumor activity was defined as tumor growth delay (TGD), partial regression (PR), or complete regression (CR). TGD represents the time differential between the treated and control tumors to reach a predetermined tumor volume of 1000 mm^3. PR was defined as a decrease in an individual tumor volume to one half the initial starting volume for at least 1 week (three consecutive measurements). CR was defined as a decrease in an individual tumor volume to less than 13 mm^3 for at least 1 week. All *in vivo* procedures were carried out in accordance with protocols approved by the GSK Institutional Animal Care and Use Committee.

Experimental Procedures

Unless otherwise noted, all solvents and reagents were purchased from commercially available sources and used without further purification. Air or moisture sensitive reactions were carried out under a nitrogen atmosphere. Flash chromatography was performed using silica gel (EM Science, 230-400 mesh) with standard techniques or using silica gel cartridges (RediSep or Varian normal phase disposable flash columns) on an Isco CombiFlash or Analogix Intelliflash. Reverse phase HPLC purification was conducted with a Gilson HPLC (monitoring at a wavelength of 214 or 254 nm) with a YMC ODS-A C18 column (5 μm, 75 x 30 mm), eluting with 5-90% acetonitrile in water with 0.1% TFA. ^{1}H NMR spectra were recorded on a Bruker 400 MHz spectrometer.

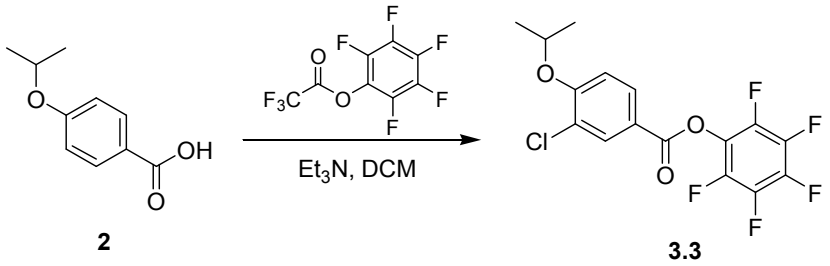
Mass spectra were recorded on an Applied Biosystems MSD Sciex or Agilent 1100 series. Analytical HPLC was conducted on an Agilent 1100 Series HPLC with a Zorbax Eclipse XDB-C18 column (5 μm, 4.6 x 150 mm), eluting over 10 minutes with 10-90% acetonitrile in water with 0.1% TFA. Elemental analyses were performed by Quantitative Technologies, Inc., Whitehouse, NJ. Optical rotations were determined on a Jasco P-2000 polarimeter. All compounds that appear in SAR tables in the manuscript were determined to be ≥95% pure by HPLC analysis.

Preparations of Compounds 3a-3i:



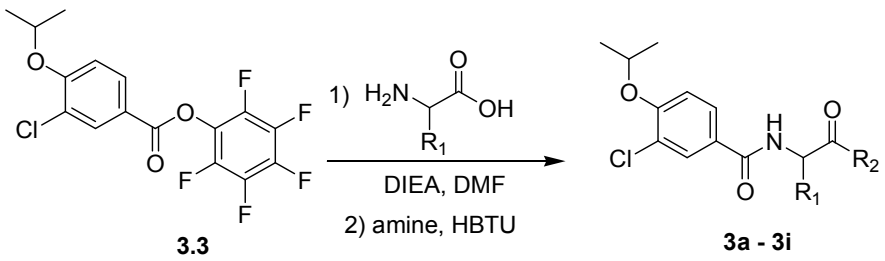
3.1 **2**

To a solution of 4-isopropoxylbenzoic acid **3.1** (25 g, 140 mmol) in DMF (150mL) was added NCS (24 g, 182 mmol). The reaction mixture was stirred overnight. H_2O (500mL) was added to the reaction mixture, and the precipitate was collected, washed with water, and dried *in vacuo* to give **2** (26.4 g, 88 %) as a white solid, which was used in the next step without further purification. LRMS ($M+H^+$) *m/z* 213.0.



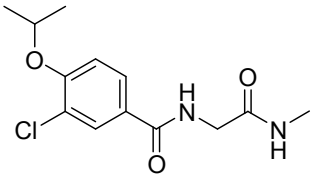
2 **3.3**

To a solution of **2** (20 g, 93 mmol) in DCM were added pentafluorophenyltrifluoroacetate (20 mL, 112 mmol) and triethylamine (17 mL, 112 mmol) at 0 ^oC. The reaction mixture was stirred for 1 h. The solution was concentrated and the mixture purified by flash column chromatography (100% DCM) to give **1.3** (35 g, quant.) as a white solid.



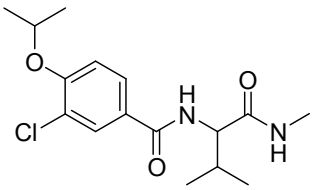
To a solution of **3** in DMF (0.2 M) were added amino acid (1.2 equiv.) and *N*, *N*-diisopropylethylamine (3 equiv.). The reaction was monitored by LC/MS. After completion, amine (1.5 equiv.) and HBTU (1.5 equiv.) were added to the reaction solution. The reaction mixture was stirred for 4 h. The product was purified by either HPLC or flash column chromatography to give **3a** – **3i** (60-95% yield).

3-Chloro-4-isopropoxy-*N*-(2-(methylamino)-2-oxoethyl)benzamide (3a)



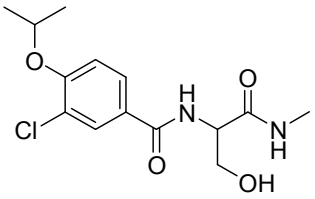
^{1}H NMR (400 MHz, MeOD) δ 7.94 (d, *J* = 2.3, 1H), 7.80 (dd, *J* = 2.3, 8.7, 1H), 7.15 (d, *J* = 8.8, 1H), 4.76 (dt, *J* = 6.1, 12.1, 1H), 3.97 (s, 2H), 2.75 (s, 3H), 1.38 (d, *J* = 6.0, 6H); LRMS (M+H$^+$) *m/z* 285.1.

3-Chloro-4-isopropoxy-*N*-(3-methyl-1-(methylamino)-1-oxobutan-2-yl)benzamide (3b)



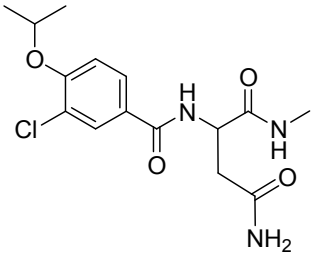
^{1}H NMR (400 MHz, MeOD) δ 7.71 (d, *J* = 2.2, 1H), 7.57 (dd, *J* = 2.2, 8.7, 1H), 6.93 (d, *J* = 8.7, 1H), 4.55 (dt, *J* = 6.1, 12.1, 1H), 4.05 (d, *J* = 8.3, 1H), 2.54 (s, 3H), 2.01 – 1.88 (m, 1H), 1.17 (d, *J* = 6.0, 6H), 0.78 (dd, *J* = 6.8, 11.2, 6H); LRMS (M+H$^+$) *m/z* 327.1.

3-Chloro-*N*-(3-hydroxy-1-(methylamino)-1-oxopropan-2-yl)-4-isopropoxybenzamide (3c)



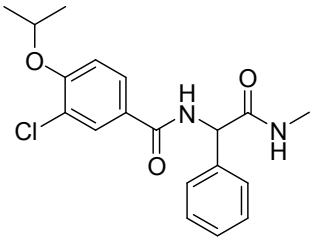
^{1}H NMR (400 MHz, MeOD) δ 7.87 (d, *J* = 2.3, 1H), 7.72 (dd, *J* = 2.3, 8.7, 1H), 7.05 (d, *J* = 8.8, 1H), 4.66 (dt, *J* = 6.0, 12.1, 1H), 4.47 (t, *J* = 5.4, 1H), 3.77 (d, *J* = 5.3, 2H), 2.66 (s, 3H), 1.28 (d, *J* = 6.0, 6H); LRMS (M+H$^+$) *m/z* 315.0.

2-(3-Chloro-4-isopropoxybenzamido)-*N*^{1}-methylsuccinamide (3d)



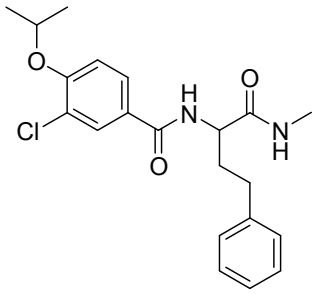
^{1}H NMR (400 MHz, MeOD) δ 7.92 (d, *J* = 2.3, 1H), 7.78 (dd, *J* = 2.3, 8.7, 1H), 7.15 (d, *J* = 8.8, 1H), 4.86 (d, *J* = 6.0, 1H), 4.76 (dt, *J* = 6.0, 12.1, 1H), 2.82 – 2.75 (m, 2H), 2.75 (s, 3H), 1.38 (d, *J* = 6.0, 6H); LRMS (M+H$^+$) *m/z* 342.1.

3-Chloro-4-isopropoxy-*N*-(2-(methylamino)-2-oxo-1-phenylethyl)benzamide (3e)



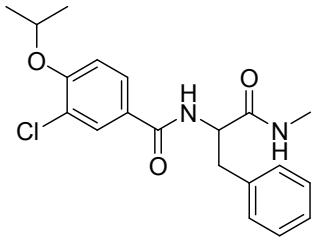
^{1}H NMR (400 MHz, CDCl3) δ 7.86 (d, *J* = 2.2, 1H), 7.69 (dd, *J* = 2.2, 8.6, 1H), 7.55 (d, *J* = 6.2, 1H), 7.48 – 7.41 (m, 2H), 7.39 – 7.28 (m, 3H), 7.26 (s, 1H), 6.93 (d, *J* = 8.7, 1H), 5.90 (s, 1H), 5.58 (d, *J* = 6.3, 1H), 5.30 (s, 0H), 4.63 (m, 1H), 2.82 (d, *J* = 4.8, 3H), 1.39 (d, *J* = 6.1, 6H); LRMS (M+H$^+$) *m/z* 361.0.

3-Chloro-4-isopropoxy-*N*-(1-(methylamino)-1-oxo-4-phenylbutan-2-yl)benzamide (3f)



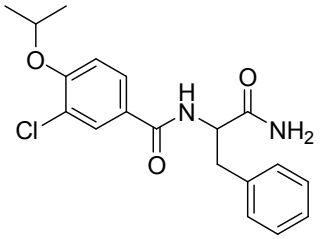
^{1}H NMR (400 MHz, MeOD) δ 7.94 (d, *J* = 2.2, 1H), 7.81 (dd, *J* = 2.2, 8.7, 1H), 7.22 (m, 6H), 4.78 (dt, *J* = 6.1, 12.1, 1H), 4.50 (dd, *J* = 5.2, 9.2, 1H), 2.82 – 2.63 (m, 5H), 2.26 – 2.05 (m, 2H), 1.40 (d, *J* = 6.0, 6H); LRMS (M+H$^+$) *m/z* 389.0.

3-Chloro-4-isopropoxy-*N*-(1-(methylamino)-1-oxo-3-phenylpropan-2-yl)benzamide (3g)



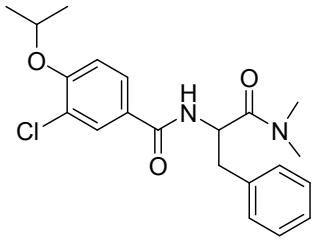
^{1}H NMR (400 MHz, MeOD) δ 7.80 (d, *J* = 2.2, 1H), 7.66 (dd, *J* = 2.2, 8.7, 1H), 7.29 – 7.24 (m, 4H), 7.22 – 7.17 (m, 1H), 7.10 (d, *J* = 8.7, 1H), 4.72 (m, 2H), 3.21 (dd, *J* = 6.2, 13.7, 1H), 3.01 (dd, *J* = 9.1, 13.7, 1H), 2.69 (s, 3H), 1.36 (d, *J* = 6.0, 6H); LRMS (M+H$^+$) *m/z* 375.0.

***N*-(1-Amino-1-oxo-3-phenylpropan-2-yl)-3-chloro-4-isopropoxybenzamide (3h)**



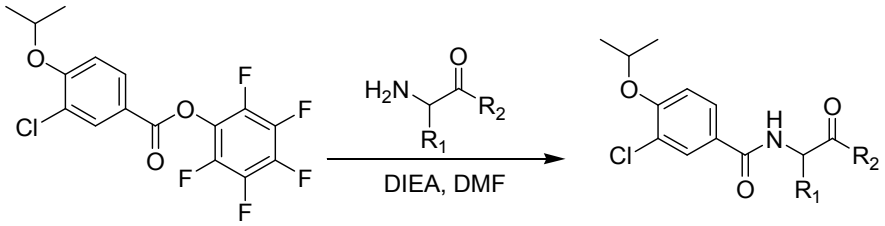
^{1}H NMR (400 MHz, MeOD) δ 7.78 (d, *J* = 2.2, 1H), 7.65 (dd, *J* = 2.2, 8.7, 1H), 7.31 – 7.23 (m, 4H), 7.22 – 7.15 (m, 1H), 7.09 (d, *J* = 8.8, 1H), 4.80 (dd, *J* = 5.5, 9.4, 1H), 4.73 (dt, *J* = 6.1, 12.1, 1H), 3.26 (dd, *J* = 5.5, 13.8, 1H), 3.03 (dd, *J* = 9.5, 13.8, 1H), 1.36 (d, *J* = 6.0, 6H); LRMS (M+H$^+$) *m/z* 361.0.

3-Chloro-*N*-(1-(dimethylamino)-1-oxo-3-phenylpropan-2-yl)-4-isopropoxybenzamide (3i)



^{1}H NMR (400 MHz, MeOD) δ 7.87 (d, *J* = 2.2, 1H), 7.73 (dd, *J* = 2.2, 8.7, 1H), 7.34 – 7.21 (m, 5H), 7.13 (d, *J* = 8.7, 1H), 5.19 (t, *J* = 7.6, 1H), 4.75 (dt, *J* = 6.0, 12.0, 1H), 3.09 (ddd, *J* = 7.7, 13.2, 20.3, 2H), 2.87 (d, *J* = 2.0, 6H), 1.37 (d, *J* = 6.0, 6H); LRMS ($M+H^+$) *m/z* 389.0.

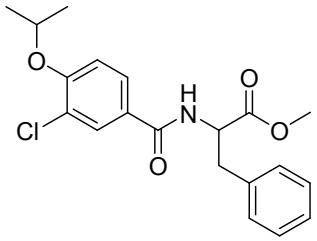
Preparations of Compounds 3j and 3k:



3.3 **3j, 3K**

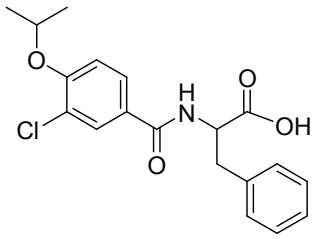
To a solution of **3** in DMF (0.2 M) were added amino acid or amino ester (1.2 equiv.) and *N*, *N*-diisopropylethylamine (3 equiv.). The reaction was monitored by LC/MS. The product was purified by either HPLC or flash column chromatography to give **3j** or **3k**.

Methyl 2-(3-chloro-4-isopropoxybenzamido)-3-phenylpropanoate (3j)



^{1}H NMR (400 MHz, MeOD) δ 7.79 (d, *J* = 2.3, 1H), 7.66 (dd, *J* = 2.3, 8.7, 1H), 7.30 – 7.17 (m, 5H), 7.10 (d, *J* = 8.8, 1H), 4.80 (dd, *J* = 5.6, 9.6, 1H), 4.73 (dt, *J* = 6.0, 12.1, 1H), 3.71 (s, 3H), 3.29 – 3.22 (m, 1H), 3.09 (dd, *J* = 9.6, 13.8, 1H), 1.36 (dd, *J* = 2.4, 6.0, 6H); LRMS (M+H$^+$) *m/z* 376.1.

2-(3-Chloro-4-isopropoxybenzamido)-3-phenylpropanoic acid (3k)

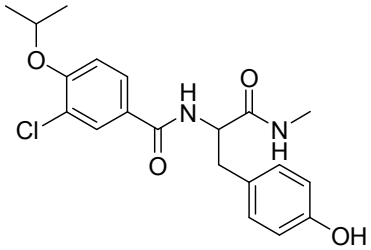


^{1}H NMR (400 MHz, MeOD) δ 7.78 (d, *J* = 2.2, 1H), 7.64 (dd, *J* = 2.3, 8.7, 1H), 7.31 – 7.20 (m, 5H), 7.07 (d, *J* = 8.8, 1H), 4.82 (dd, *J* = 4.9, 9.8, 1H), 4.70 (dq, *J* = 6.1, 12.2, 1H), 3.33 (dd, *J* = 3.1, 12.1, 1H), 3.09 (dd, *J* = 9.8, 13.9, 1H), 1.36 d, *J* = 7.2, 6H); LRMS (M+H$^+$) *m/z* 362.0.

Preparation of Compounds 4a – 4i

Compounds **4a** – **4i** were prepared as described for 3a – 3h (70-95% yield).

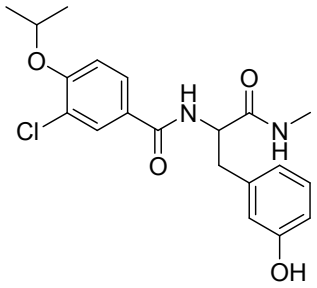
3-Chloro-*N*-(3-(4-hydroxyphenyl)-1-(methylamino)-1-oxopropan-2-yl)-4-isopropoxybenzamide (4a)



^{1}H NMR (400 MHz, MeOD) δ 7.82 (d, *J* = 2.2, 1H), 7.68 (dd, *J* = 2.3, 8.7, 1H), 7.09 (dd, *J* = 8.6, 14.3, 3H), 6.69 (m, 2H), 4.79 – 4.69 (m, 1H), 4.68 – 4.58 (m, 1H), 3.10 (dd, *J* = 6.4, 13.7, 1H), 2.92 (dd, *J* = 8.7, 13.8, 1H), 2.69 (s, 3H), 1.37 (d, *J* = 6.0, 6H); LRMS

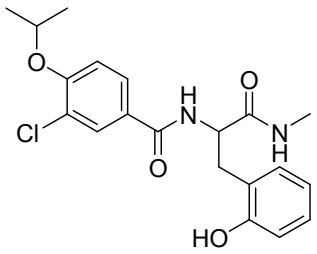
(M+H$^+$) *m/z* 391.0.

3-Chloro-*N*-(3-(3-hydroxyphenyl)-1-(methylamino)-1-oxopropan-2-yl)-4-isopropoxybenzamide (4b)



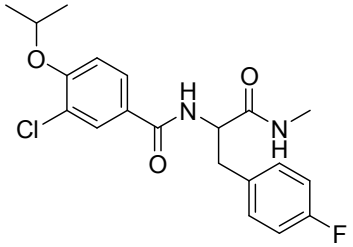
^{1}H NMR (400 MHz, CDCl3) δ 7.77 (s, 1H), 7.65 – 7.52 (m, 1H), 7.17 (m, 1H), 6.87 (m, 5H), 6.10 – 5.83 (m, 2H), 4.86 – 4.54 (m, 2H), 3.26 – 3.08 (m, 1H), 3.06 – 2.90 (m, 1H), 2.72 (d, *J* = 4.8, 3H), 1.39 (d, *J* = 6.1, 6H); LRMS (M+H$^+$) *m/z* 391.0.

3-Chloro-*N*-(3-(2-hydroxyphenyl)-1-(methylamino)-1-oxopropan-2-yl)-4-isopropoxybenzamide (4c)



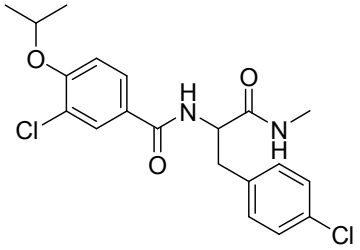
^{1}H NMR (400 MHz, CDCl3) δ 9.20 (s, 1H), 7.84 (d, *J* = 2.3, 1H), 7.64 (dd, *J* = 2.3, 8.6, 1H), 7.25 – 7.17 (m, 2H), 7.05 (dd, *J* = 1.6, 7.5, 1H), 6.99 (d, *J* = 7.1, 1H), 6.93 (d, *J* = 8.8, 1H), 6.88 – 6.82 (m, 1H), 6.09 (s, 1H), 4.71 – 4.55 (m, 2H), 3.39 (dd, *J* = 2.9, 14.3, 1H), 2.88 (dd, *J* = 9.0, 14.3, 1H), 2.80 (d, *J* = 4.9, 3H), 1.59 (s, 3H), 1.39 (d, *J* = 6.1, 6H); LRMS (M+H$^+$) *m/z* 391.0.

3-Chloro-*N*-(3-(4-fluorophenyl)-1-(methylamino)-1-oxopropan-2-yl)-4-isopropoxybenzamide (4d)



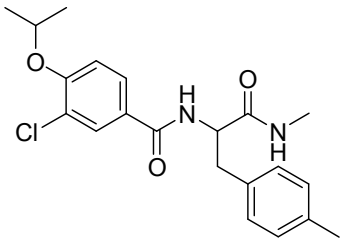
^{1}H NMR (400 MHz, MeOD) δ 7.83 (d, *J* = 2.2, 1H), 7.68 (dd, *J* = 2.3, 8.7, 1H), 7.27 (m, 2H), 7.11 (d, *J* = 8.8, 1H), 6.99 (m, 2H), 4.79 – 4.66 (m, 2H), 3.20 (dd, *J* = 6.3, 13.8, 1H), 3.00 (dd, *J* = 9.0, 13.7, 1H), 2.70 (s, 3H), 1.37 (d, *J* = 6.0, 6H); LRMS (M+H$^+$) *m/z* 393.0.

3-Chloro-*N*-(3-(4-chlorophenyl)-1-(methylamino)-1-oxopropan-2-yl)-4-isopropoxybenzamide (4e)



^{1}H NMR (400 MHz, CDCl3) δ 7.86 (d, *J* = 2.2, 1H), 7.66 (dd, *J* = 2.3, 8.6, 1H), 7.33 – 7.27 (m, 3H), 7.21 (d, *J* = 8.39, 1H), 7.09 (d, *J* = 7.89, 1H), 6.98 (d, *J* = 8.71, 1H), 6.24 (d, *J* = 4.6, 1H), 4.90 – 4.63 (m, 2H), 3.26 – 3.06 (m, 2H), 2.82 (d, *J* = 4.8, 3H), 1.71 (s, 1H), 1.48 (t, *J* = 9.0, 6H), 1.31 (s, 1H); LRMS (M+H$^+$) *m/z* 409.1.

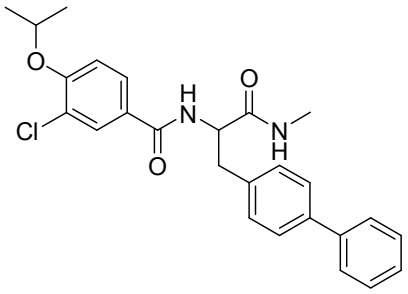
3-Chloro-4-isopropoxy-*N*-(1-(methylamino)-1-oxo-3-p-tolylpropan-2-yl)benzamide (4f)



^{1}H NMR (400 MHz, CDCl3) δ 7.76 (d, *J* = 2.2, 1H), 7.59 (dd, *J* = 2.2, 8.6, 1H), 7.11 (d, *J*

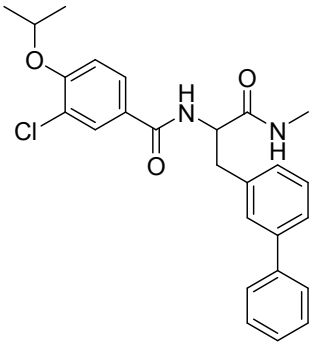
= 2.0, 4H), 6.92 (d, *J* = 8.7, 1H), 6.77 (m, *J* = 7.7, 1H), 5.69 (m, 1H), 4.65 (m, 2H), 3.18 (dd, *J* = 5.8, 13.6, 1H), 3.01 (dd, *J* = 8.3, 13.6, 1H), 2.72 (d, *J* = 4.9, 3H), 2.31 (s, 3H), 1.39 (d, *J* = 6.1, 6H); LRMS ($M+H^+$) *m/z* 389.1.

_N_-(3-(Biphenyl-4-yl)-1-(methylamino)-1-oxopropan-2-yl)-3-chloro-4-isopropoxybenzamide (4g)



^{1}H NMR (400 MHz, CDCl3) δ 7.80 (d, *J* = 2.2, 1H), 7.63 – 7.51 (m, 5H), 7.43 (m, 2H), 7.34 (m, 3H), 6.92 (d, *J* = 8.7, 1H), 6.84 (m, 1H), 5.81 (m, 1H), 4.78 (dd, *J* = 7.9, 13.8, 1H), 4.63 (m, 1H), 3.27 (dd, *J* = 5.7, 13.5, 1H), 3.13 (dd, *J* = 8.3, 13.6, 1H), 2.76 (d, *J* = 4.9, 3H), 1.40 (d, *J* = 6.1, 6H); LRMS ($M+H^+$) *m/z* 451.1.

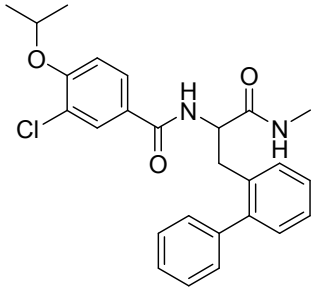
_N_-(3-(Bihenyl-3-yl)-1-(methylamino)-1-oxopropan-2-yl)-3-chloro-4-isopropoxybenzamide (4h)



^{1}H NMR (400 MHz, MeOD) δ 7.84 (d, *J* = 2.2, 1H), 7.67 (dd, *J* = 2.3, 8.7, 1H), 7.53 (m, 3H), 7.46 (m, 1H), 7.42 – 7.23 (m, 5H), 7.07 (d, *J* = 8.7, 1H), 5.49 (s, 1H), 4.83 – 4.68 (m, 2H), 3.13 – 3.02 (m, 1H), 2.73 (s, 3H), 1.36 (d, *J* = 6.0, 6H); LRMS ($M+H^+$) *m/z* 451.1.

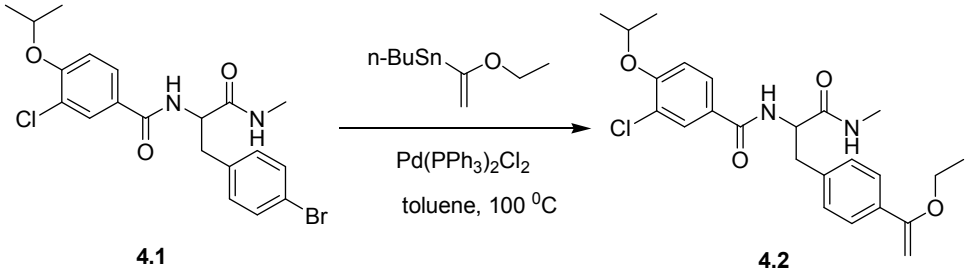
_N_-(3-(Bihenyl-2-yl)-1-(methylamino)-1-oxopropan-2-yl)-3-chloro-4-

isopropoxybenzamide (4i)



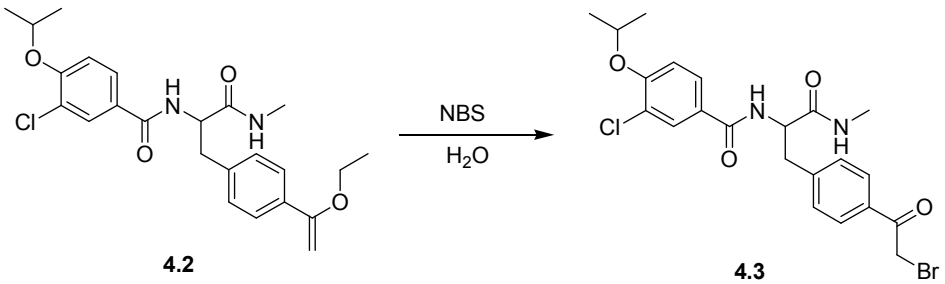
^{1}H NMR (400 MHz, MeOD) δ 7.78 (d, *J* = 2.2, 1H), 7.65 (m, 1H), 7.50 – 7.33 (m, 7H), 7.31 (m, 2H), 7.21 (m, 1H), 7.13 (d, *J* = 8.7, 1H), 4.78 (m, 2H), 4.60 (m, 1H), 3.05 (m, 1H), 2.67 (s, 3H), 1.41 (d, *J* = 6.0, 7H); LRMS (M+H$^+$) *m/z* 451.1.

Preparation of Compounds 4j and 4k



4.1 **4.2**

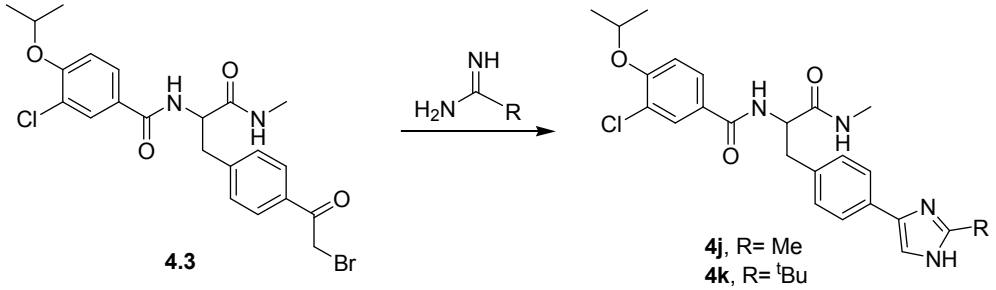
A solution of bromide **4.1** (1.0 g, 2.20 mol), dichlorobis(triphenylphosphine)palladium (II) (154 mg, 0.220 mol), tributyl(1-ethoxyvinyl)tin (1.49 ml, 4.41 mmol), and toluene (15 mL) under N_2 was stirred at 100 ^{o}C for 6 hours. Upon completion, as monitored by LCMS, the reaction mixture was cooled, filtered through Celite, and concentrated *in vacuo*. The resulting residue was purified by flash column chromatography (silica gel, 2:1:0.1 EtOAc: hexanes: triethylamine) to give **4.2** (540 mg, 55%). LRMS (M+H$^+$) *m/z* 445.2.



4.2 **4.3**

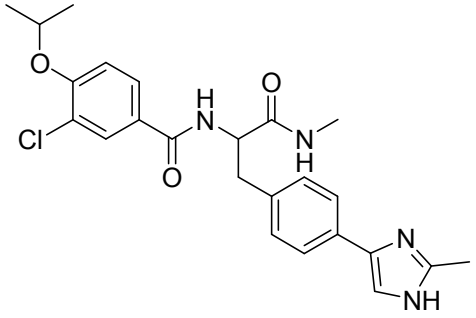
A solution of compound **4.2** (540 mg, 1.21.mmol), THF:H_2O (3:1, 12 mL), and N-bromo-

succinimide (216 mg, 1.21 mmol) was stirred at 23 °C for 15 min. The reaction mixture was then concentrated *in vacuo* and the crude residue diluted with EtOAc (30 mL), washed with brine (10 mL), and concentrated *in vacuo*. The resulting residue was purified by flash column chromatography (silica gel, 1:1 EtOAc:hexanes) to give **4.3** (210 mg, 35%). LRMS ($M+H^+$) *m/z* 495.1.



A solution of **4.3** (210 mg, 0.42 mmol), K_2CO_3 (174 mg 1.26 mmol), amidine hydrochloride (2 equiv.), and DMF (4 mL) was stirred at 23 °C under N_2 for 18 hours. The reaction mixture was concentrated *in vacuo*, and the resulting residue was purified by column chromatography (silica gel, 4:1 EtOAc:hexanes) to give compound 4j or 4k.

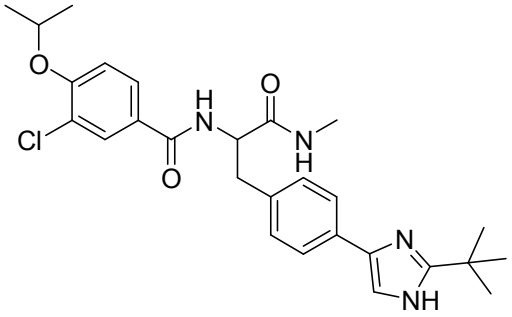
3-Chloro-4-isopropoxy-*N*-(3-(4-(2-methyl-1H-imidazol-4-yl)phenyl)-1-(methylamino)-1-oxopropan-2-yl)benzamide (4j)



^{1}H NMR (400 MHz, CDCl3) δ 7.83 (d, *J* = 2.2, 1H), 7.67 – 7.58 (m, 3H), 7.28 – 7.22 (m, 2H), 7.18 (s, 1H), 6.94 (d, *J* = 8.7, 2H), 5.88 (s, 1H), 4.83 – 4.55 (m, 2H), 3.25 (dd, *J* = 5.8, 13.6, 1H), 3.06 (dd, *J* = 8.5, 13.5, 1H), 2.74 (d, *J* = 4.9, 3H), 2.49 (s, 3H), 1.42 (t, *J* = 6.4, 6H); LRMS ($M+H^+$) *m/z* 455.2.

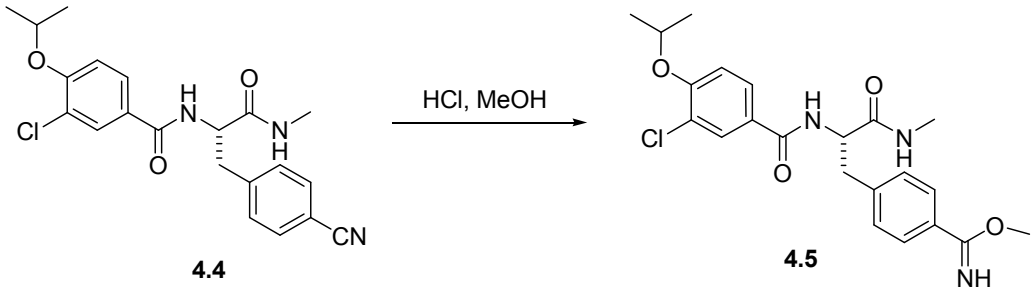
***N*-(3-(4-(2-tert-Butyl-1H-imidazol-4-yl)phenyl)-1-(methylamino)-1-oxopropan-2-yl)-**

3-chloro-4-isopropoxybenzamide (4k)

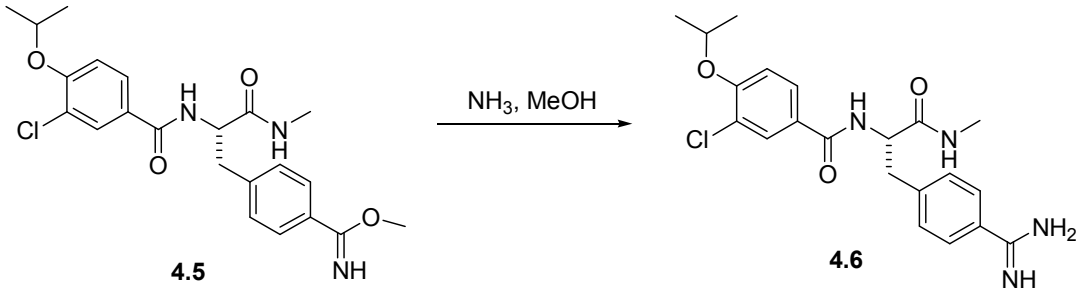


1H NMR (400 MHz, CDCl3) δ 7.91 (d, J = 8.1, 1H), 7.82 (m, 1H), 7.72 – 7.44 (m, 3H), 7.34 (m, 1H), 7.24 – 7.11 (m, 2H), 6.93 (m, 1H), 6.61 (bs, 1H), 6.06 – 5.98 (m, 0H), 4.82 (m, 1H), 4.63 (m, 1H), 3.14 (m, 2H), 2.75 (m, 3H), 1.50 – 1.33 (m, 15H); LRMS ($M+H^+$) *m/z* 497.2.

Preparation of Compound 4l

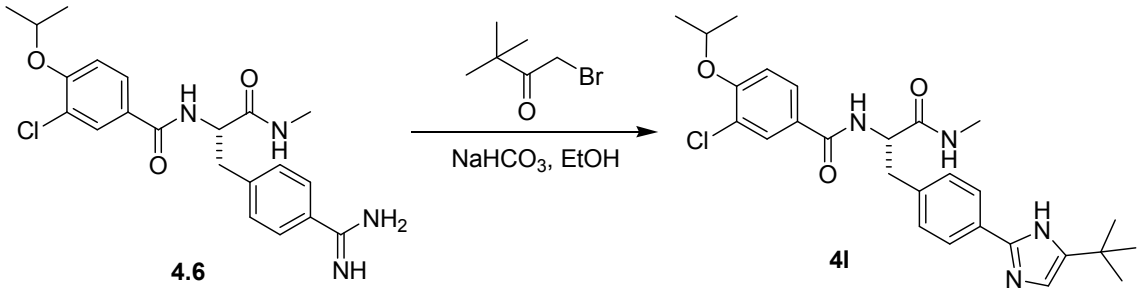


To a solution of **4.4** (1.6 g, 4.0 mmol) in methanol (80 mL) was saturated with HCl (gas) for 15 minutes at 0 ^{o}C. The resulting yellow solution was stirred at room temperature for 3 hours, and the solvents were removed. The residual viscous oil **26** was dried and used without further purification.



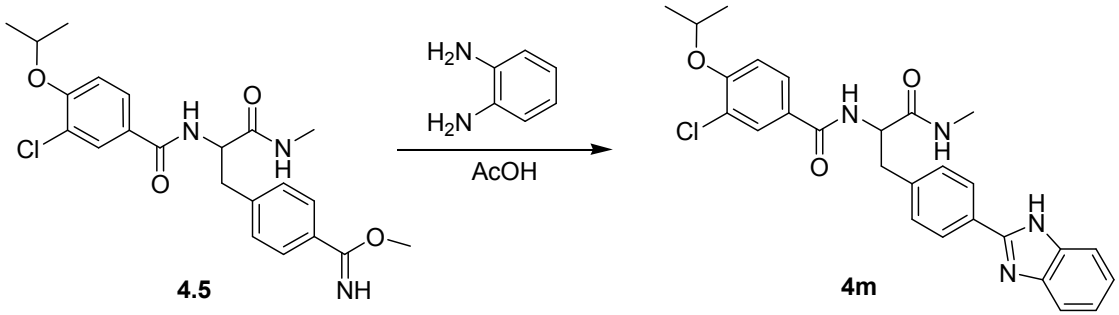
A mixture of **4.5** (4.0 mmol) and ammonia in methanol (10 mL, 2M) was stirred at room temperature for overnight, and the solvents were removed. The residue was purified by

flash chromatography (silica gel, DCM/MeOH) to provide **4.6** (1.1 g, 66% over 2 steps). LRMS ($M+H^+$) *m/z* 417.2



A solution of **4.6** (40 mg, 0.096 mmol) in ethanol (50 mL) was stirred with DBU (58 uL, 0.096 mmol) at room temperature for 5 minutes, followed by the addition of 1-bromo-2-butanone (0.029 mL, 0.192 mmol). The reaction mixture was stirred at 115 C for 2 hours, and the solvents were removed. The residue was purified by reverse-phase HPLC using a mobile phase gradient consisting of acetonitrile and water to provide **4l** (25 mg, 52.5%). LRMS ($M+H^+$) *m/z* 497.3.

Preparation of Compound 4m



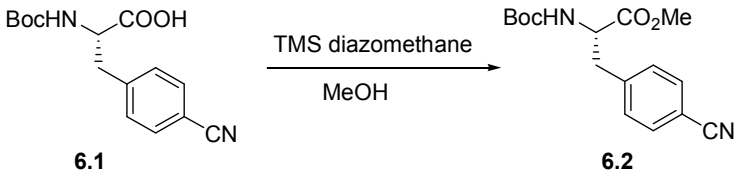
A solution of crude imidate **4.2** (50 mg, 0.12 mmol), phenylene diamine (36 mg, 0.32 mmol) and acetic acid (1 mL) was stirred at 80 ^oC for 30 min. The reaction mixture was then concentrated *in vacuo,* and the resulting residue was dissolved in EtOAc (10 mL) and washed with NaOH (1 N, 5 mL) and brine (5 mL). The organic layer was dried ($MgSO_4$), filtered, and concentrated *in vacuo*. The resulting residue was purified by flash column chromatography (silica gel, 1:3 hexanes:EtOAc) to yield **4m** (20 mg, 34%). LRMS ($M+H^+$) *m/z* 491.2. ^1H NMR (400 MHz, CDCl3) δ 7.96 (d, *J* = 8.0, 2H), 7.79 (m, 1H), 7.62 – 7.48 (m, 4H), 7.25 – 7.12 (m, 4H), 6.85 (d, *J* = 8.8, 1H), 6.56 (bs, 1H), 4.84

(d, *J* = 7.3, 1H), 4.66 – 4.47 (m, 1H), 3.25 – 3.06 (m, 2H), 2.70 (d, *J* = 4.7, 3H), 1.36 (d, *J* = 6.0, 6H); LRMS ($M+H^+$) *m/z* 491.0.

Preparation of Compounds 5a and 5b

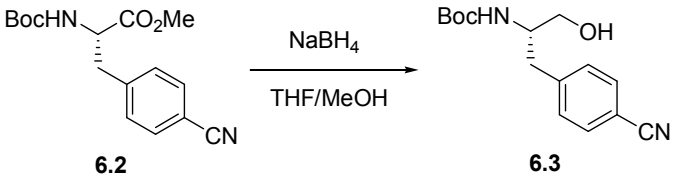
Resolution of the racemic mixture of **4m** was accomplished by chiral HPLC using a Chiralpac AD column (20x250 mm; lot#63-20-20911) with an isocratic mobile phase (25:75 MeOH:CH3CN with 0.1% ispropylamine; 20 mL/min) to provide both enantiomers **5a** (RT = 12.4 min) and **5b** (RT = 5.5 min). LRMS ($M+H^+$) *m/z* 491.2. ^1H NMR (400 MHz, CDCl3) δ 7.96 (d, *J* = 8.0, 2H), 7.79 (m, 1H), 7.62 – 7.48 (m, 4H), 7.25 – 7.12 (m, 4H), 6.85 (d, *J* = 8.8, 1H), 6.56 (bs, 1H), 4.84 (d, *J* = 7.3, 1H), 4.66 – 4.47 (m, 1H), 3.25 – 3.06 (m, 2H), 2.70 (d, *J* = 4.7, 3H), 1.36 (d, *J* = 6.0, 6H). LRMS ($M+H^+$) *m/z* 491.2.

Preparation of Compounds 6a and 6b



6.1 **6.2**

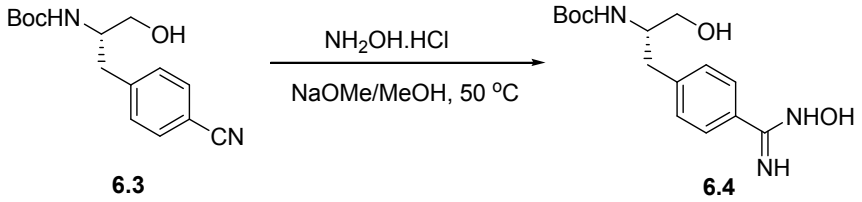
To a room temperature solution of **6.1** (4.96 g, 17 mmol) in methanol (15 mL) was added dropwise a solution of TMS diazomethane in hexanes (17.0 mL, 34 mmol, 2 M). The resulting yellow solution was stirred at ambient temperature for 30 minutes. The solvents were removed and dried under high vacuum to give **6.2** (5.19 g, 17 mmol). LRMS ($M+H^+$) *m/z* 305.3.



6.2 **6.3**

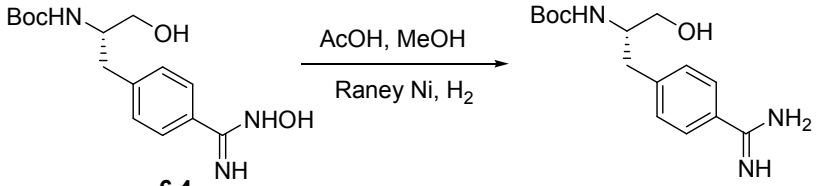
A solution of **6.2** (5.19 g, 17 mmol) and sodium borohydride (3.23 g, 85 mmol) in methanol (50 mL) and tetrahydrofuran (50 mL) was stirred at room temperature for 2 h. The solvents were removed, and the resulting residue was partitioned between ethyl

acetate (50 mL) and water (50 mL). The layers were separated and the aqueous phase was extracted with ethyl acetate (3 x 50 mL), and the combined organic layers were dried over sodium sulfate and concentrated *in vacuo* to yield **6.3** (4.71 g, 17 mmol) as a white solid. LRMS ($M+H^+$) *m/z* 277.3.



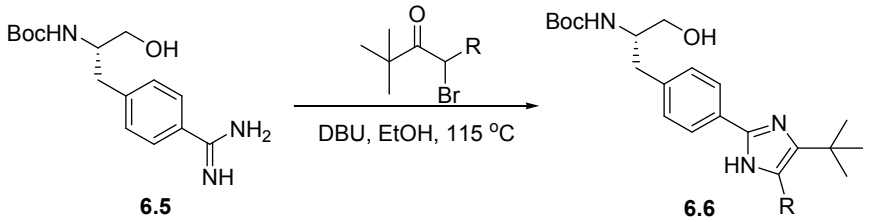
6.3 **6.4**

A solution of **6.3** (1.92 g, 6.9 mmol) was stirred with sodium methoxide in methanol (27.7 mL, 13.9 mmol, 0.5 M) and hydroxylamine hydrochloride (964 mg, 13.9 mmol) under an atmosphere of nitrogen at 50 °C for 2 h. It was then cooled to room temperature and the solvents were removed. The resulting residue was partitioned between saturated aqueous ammonium chloride solution (30 mL) and ethyl acetate (30 mL). The layers were separated and the aqueous phase was extracted with ethyl acetate (2 x 30 mL). The combined organic extracts were dried over sodium sulfate and concentrated *in vacuo*, and the residue was purified by flash chromatography (silica gel, hexanes/ethyl acetate) to yield **6.4** (1.08 g, 51%). LRMS ($M+H^+$) *m/z* 310.2.



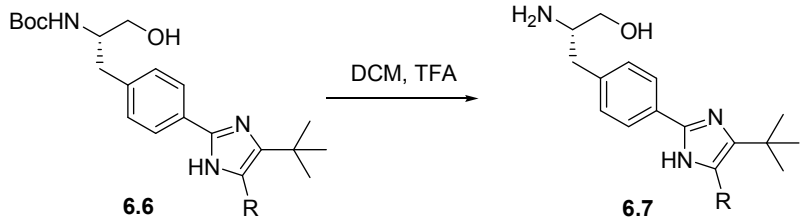
6.4 **6.5**

To a solution of **6.4** (1.08 g, 3.5 mmol) in methanol (30 mL) was added Raney nickel (200 mg) and acetic acid (1 mL). The resulting mixture was stirred under an atmosphere of hydrogen at room temperature for 2 h. It was filtered through Celite and concentrated *in vacuo* to provide **6.5** (1.02 g, quant.) as a white solid. LRMS ($M+H^+$) *m/z* 294.3.

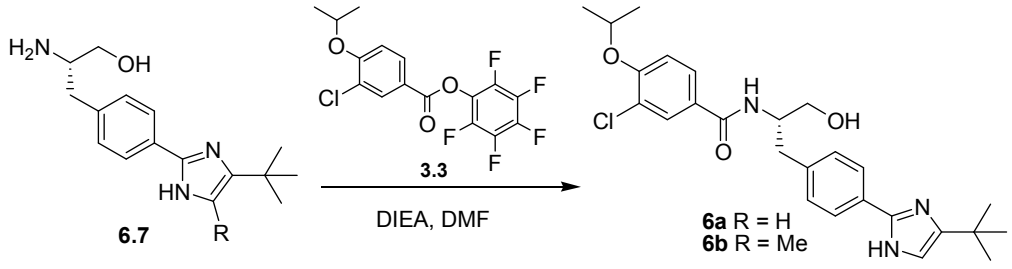


6.5 **6.6**

To a room temperature solution of amidine **6.5** (304 mg, 1.0 mmol) in anhydrous ethanol (15 mL) was added 1,8-diazabicyclo[5.4.0]undec-7-ene (622 μL, 4.2 mmol) and 3-bromoketone (2.1 equiv.). The resulting mixture was stirred under an atmosphere of nitrogen at 115 °C for 30 minutes. It was then cooled to room temperature and the solvents removed *in vacuo*. The residue was purified by reverse-phase HPLC to provide **6.6**.

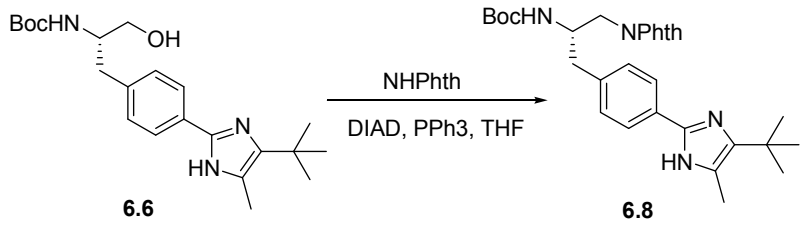


To a solution of **6.6** (0.2 mmol) in DCM (2 mL) was added trifluoroacetic acid (2 mL). The resulting mixture was stirred at room temperature for 45 minutes. The solvents were removed and dried under high vacuum to provide **6.7**.



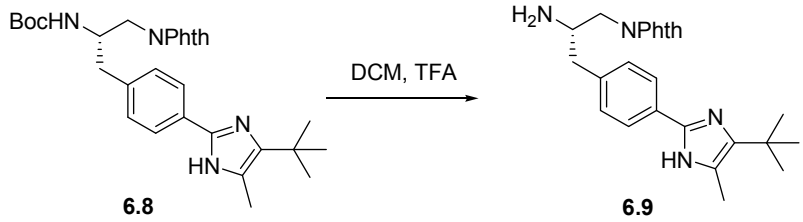
Amine was coupled with **3.3** as described for **3j.** The crude product was purified by reverse-phase HPLC to provide (*S*)-*N*-(1-(4-(4-tert-butyl-1*H*-imidazol-2-yl)phenyl)-3-hydroxypropan-2-yl)-3-chloro-4-isopropoxybenzamide (**6a**)(20% over three steps) and (*S*)-*N*-(1-(4-(4-tert-butyl-5-methyl-1*H*-imidazol-2-yl)phenyl)-3-hydroxypropan-2-yl)-3-chloro-4-isopropoxybenzamide (**6b**) (15% over three steps). LRMS ($M+H^+$) *m/z* 470.1 (**6a**); 484.4 (**6b**).

Preparation of Compound 6c



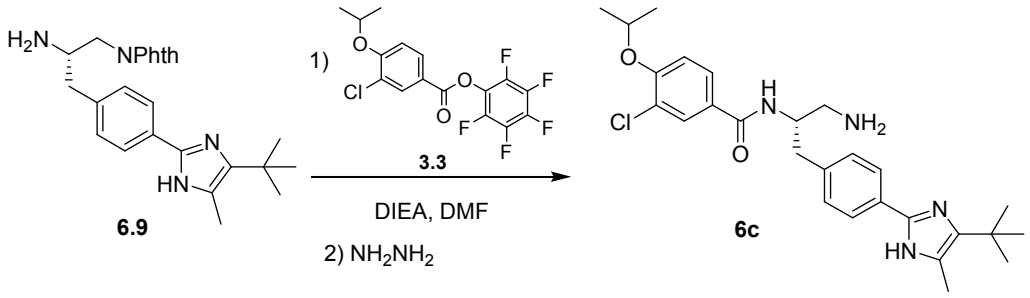
6.6 **6.8**

A solution of **6.7** (3.00 g, 7.74 mmol), phthalimide (1.37 g, 9.29 mmol) and triphenyl phosphine (2.23 g, 8.51 mmol) in THF (100 mL) was stirred at room temperature for 5 minutes. To the reaction solution was then added DIAD (1.72 mL, 8.51 mmol) dropwise. The reaction mixture was stirred for an additional 30 minutes. The solvents were removed, and the resulting residue was purified by flash chromatography (silica gel, hexanes/ethyl acetate) to provide **12** (2.85 g, 71.3%) as a white solid.



6.8 **6.9**

A solution of **6.8** (1.0g, 1.94 mmol) in TFA (25 mL) and DCM (25 mL) was stirred at room temperature for 30 minutes, and the solvents were removed and dried under high vacuum to provide **6.9** (quant.) as a TFA salt.

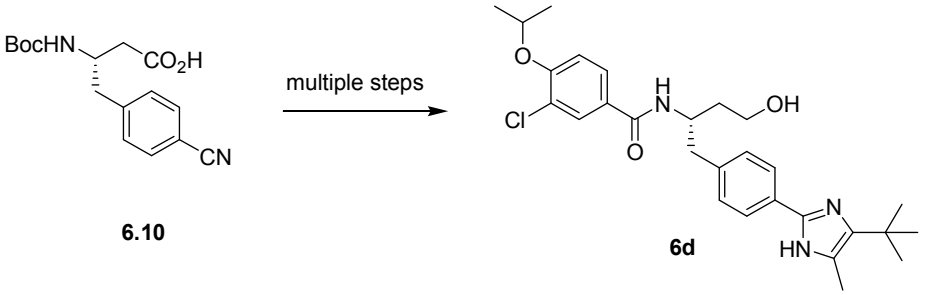


6.9 **6c**

A solution of amine **6.9** (1.94 mmol) in DMF (5 mL) was stirred with DIEA (0.507 mL, 2.91 mmol) at room temperature, **3.3** (886 mg, 2.33 mmol) was then added. The reaction mixture was stirred for 30 minutes. The reaction mixture was diluted with methanol (10 mL), and hydrazine (1.21 mL, 3.88 mmol) was then added. The resulting mixture was stirred for 4 h. The solvents were removed, and the resulting residue was purified by reverse-phase HPLC using a mixture of acetonitrile and water to provide (*S*)-*N*-(1-amino-3-(4-(4-tert-butyl-5-methyl-1*H*-imidazol-2-yl)phenyl)propan-2-yl)-3-chloro-4-isopropoxybenzamide **6c** (500 mg, 53% over 3 steps) as a white solid. ^{1}H NMR (400

MHz, MeOD) δ 7.72 (d, *J* = 2.2, 1H), 7.63 (d, *J* = 8.2, 2H), 7.57 (dd, *J* = 2.2, 8.6, 1H), 7.22 (d, *J* = 8.2, 2H), 7.00 (d, *J* = 8.8, 1H), 4.67 – 4.58 (m, 1H), 4.25 (m, 1H), 2.92 – 2.67 (m, 4H), 2.22 (s, 3H), 1.30 – 1.23 (m, 15H); LRMS ($M+H^+$) *m/z* 483.4.

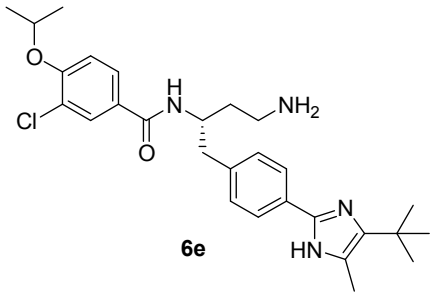
Preparation of Compound 6d



6.10 **6d**

(*S*)-*N*-(1-(4-(4-tert-Butyl-5-methyl-1*H*-imidazol-2-yl)phenyl)-4-hydroxybutan-2-yl)-3-chloro-4-isopropoxybenzamide (**6d**) was prepared as described for **6a** starting from **6.10**. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.20 (d, *J* = 8.59 Hz, 1H), 7.88 (d, *J* = 2.02 Hz, 1H), 7.74 (dd, *J* = 2.02, 8.59 Hz, 2H), 7.23 (dd, *J* = 3.92, 8.72 Hz, 3H), 4.76 (dt, *J* = 6.06, 12.13 Hz, 1H), 4.42 (t, *J* = 4.80 Hz, 1H), 4.16 - 4.31 (m, *J* = 7.58 Hz, 1H), 3.40 - 3.51 (m, 2H), 2.82 (t, *J* = 6.19 Hz, 2H), 2.27 (s, 3H), 1.62 - 1.76 (m, 2H), 1.27 - 1.33 (m, 15H). LRMS ($M+H^+$) *m/z* 498.1.

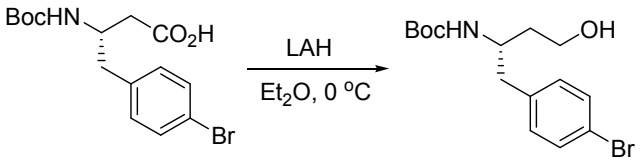
Preparation of Compound 6e



6e

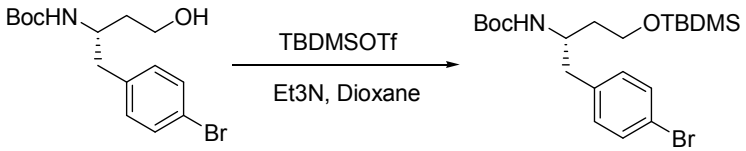
(*S*)-*N*-(4-Amino-1-(4-(4-tert-butyl-5-methyl-1*H*-imidazol-2-yl)phenyl)butan-2-yl)-3-chloro-4-isopropoxybenzamide (**6e**) was prepared as described for **6c**. ^{1}H NMR (400 MHz, MeOD) δ 7.75-7.85 (m, 3H), 7.67 (dd, *J* = 2.2, 8.7, 1H), 7.57 (d, *J* = 8.3, 2H), 7.12 (d, *J* = 8.8, 1H), 4.75 (m, 1H), 4.47 (m, 1H), 3.21 – 2.90 (m, 4H), 2.47 (s, 3H), 2.13 (m, 1H), 2.00 (m, 1H), 1.57 – 1.25 (m, 15H); LRMS ($M+H^+$) *m/z* 497.4.

Preparation of Compound 7



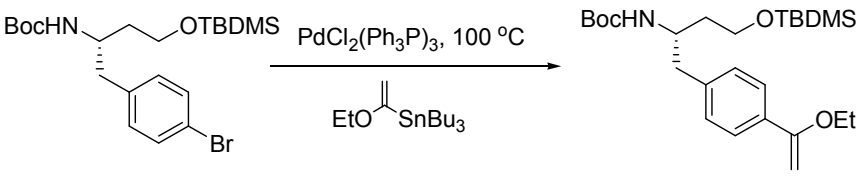
7.1 **7.2**

To a solution of carboxylic acid **7.1** (10.0 g, 28 mmol) in anhydrous diethyl ether (200 mL) at 0 °C was added dropwise a solution of lithium aluminum hydride in tetrahydrofuran (40 mL, 40 mmol, 1 M). The resulting solution was then stirred for an additional 2 h at 0 °C. It was carefully quenched with water (2.5 mL), aqueous sodium hydroxide (2.5 mL, 1 M) and water (3.0 mL). The solution was then dried over sodium sulfate, and removal of the solvents yielded intermediate **2** (9.2 g, 96%). LRMS ($M+H^+$) *m/z* 344.1.



7.2 **7.3**

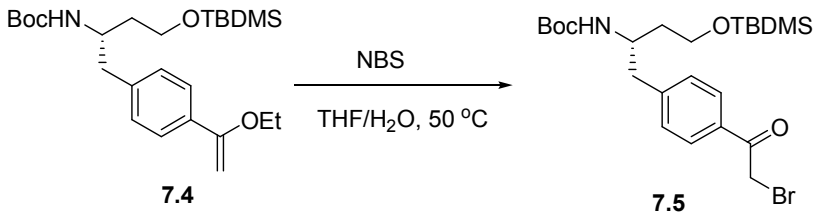
To a solution of **7.2** in anhydrous dioxane (200 mL) were added triethylamine (6 mL, 40 mmol) and *tert*-butyldimethylsilyltrifluoro methanesulfonate (8.6 g, 32 mmol). The resulting solution was then stirred overnight and quenched with saturated aqueous sodium bicarbonate solution. It was extracted with dichloromethane (3 x 100 mL), and the combined organic layers were dried over sodium sulfate and concentrate *in vacuo*. The residue was purified by flash chromatography (silica gel, hexane/ethyl acetate) to provide **7.3** (9.2 g, 72%). LRMS ($M+H^+$) *m/z* 458.2.



7.3 **7.4**

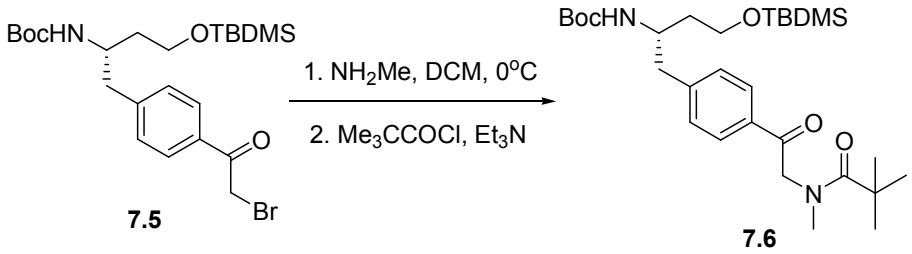
To a solution of **7.3** (6.0 g, 13 mmol) in dioxane (100 mL) were added *trans*-

dichlorobis(triphenylphosphine)palladium(II) (500 mg) and 1-ethoxyvinyltri-n-butyltin (12.3 g, 34 mmol). The resulting solution was heated to 100 ^{o}C for 4 h. The solvents were removed and the resulting residue was purified by flash chromatography (silica gel, hexanes/ethyl acetate/5% triethylamine) to provide **7.4** (5.4 g). LRMS (M+H^{+}) *m/z* 450.3.



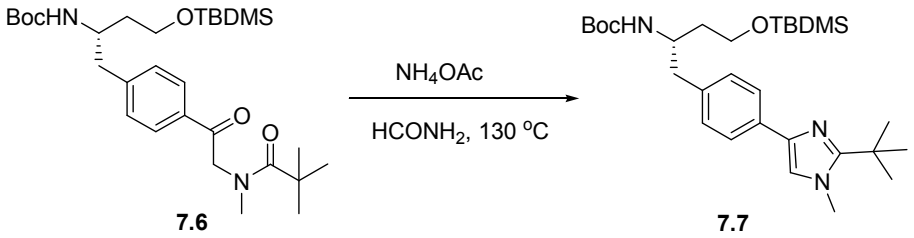
7.4 **7.5**

A solution of **7.4** and *N*-bromosuccinimide (5.9 g, 33 mmol) in methanol (100 mL) and water (50 mL) was stirred at 50 ^{o}C for 4 h. The solvents were removed, and the resulting residue was extracted with ethyl acetate (3 x 50 mL). The combined organic layers were dried over sodium sulfate and concentrate *in vacuo*. The residue was then purified by flash chromatography (silica gel, hexanes/ethyl acetate) to provide **7.5** (4.5 g, 69% over 2 steps). LRMS (M+H^{+}) *m/z* 500.5.

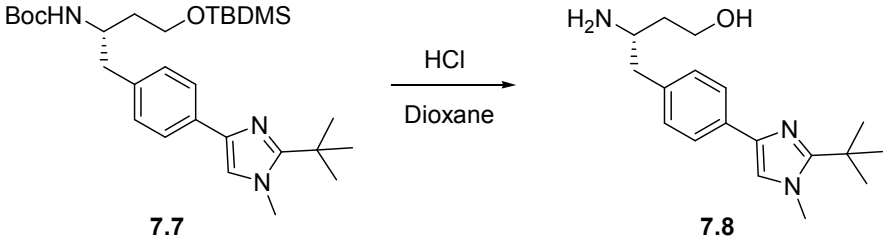


7.5 **7.6**

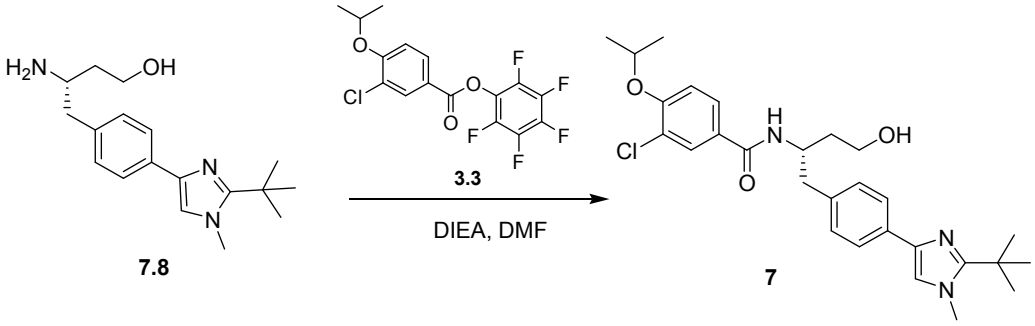
Under a nitrogen atmosphere, a pressure-equalizing dropping funnel charged with the **7.5** (2.5 g, 5.0 mmol) in dichloromethane (40 mL) was attached to a 150-mL flask which contains a solution of methylamine (15 mL, 30 mmol, 1 M in THF). The flask was cooled to 0 ^{o}C, and the bromide solution was added dropwise over 2 h. The resulting solution was stirred for one more hour, after which triethylamine (1 mL) and a solution of trimethylacetyl chloride (4.8 mL, 40 mmol) in dichloromethane (10 mL) were added. The resulting mixture was stirred for another 2 h and then quenched with saturated sodium bicarbonate solution. The mixture was extracted with ethyl acetate (3 x 50 mL), and the combined organic layers were dried over sodium sulfate and concentrated. The resulting reside was purified by flash chromatography (silica gel, ethyl acetate/hexanes) to provide **7.6** (1.3 g, 49%).LRMS (M+H^{+}) *m/z* 535.4.



A solution of **7.6** (1.3 g, 2.6 mmol) in an excess of ammonium acetate in formamide (10 mL) was heated to 130 ^{o}C under a nitrogen atmosphere for 3 h. The resulting mixture was cooled to room temperature, partitioned between water and extracted with dichloromethane (3 x 50 mL). The combined organic layers were dried over sodium sulfate and concentrated *in vacuo*. The resulting residue was purified by flash chromatography (silica gel, ethyl acetate/hexane) to provide **7.7** (0.8 g, 60%). LRMS ($M+H^+$) *m/z* 516.4.



To a solution of **7.7** (800 mg, 1.55 mmol) in THF (10 mL) was added hydrogen chloride in 1,4-dioxane (10 mL, 4.0 M). The solution was stirred with at room temperature for 1 h. The solvents were removed, and the resulting residue was dried under high vacuum overnight to provide **7.8** (600 mg). LRMS ($M+H^+$) *m/z* 302.2.

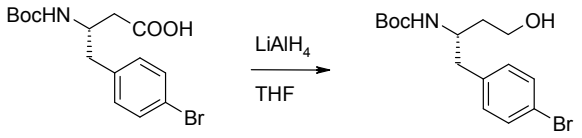


Amine **7.8** was coupled with **3.3** as described for **3j.** The crude product was purified by reverse-phase HPLC to provide (*S*)-*N*-(1-(4-(2-tert-butyl-1-methyl-1*H*-imidazol-4-

yl)phenyl)-4-hydroxybutan-2-yl)-3-chloro-4-isopropoxybenzamide (**7**) (36%). ^{1}H NMR (400 MHz, MeOD) δ 7.80 (d, *J* = 2.2, 1H), 7.70-7.50 (m, 3H), 7.30-7.15 (m, 3H), 7.12 (d, *J* = 8.8, 1H), 4.75 (m, 1H), 4.40 (m, 1H), 3.82 (s, 3H), 3.65 (m, 2H), 2.92 (m, 2H), 1.92 (m, 1H), 1.78 (m, 1H), 1.51 – 1.30 (m, 15H); LRMS (M+H$^+$) *m/z* 498.5.

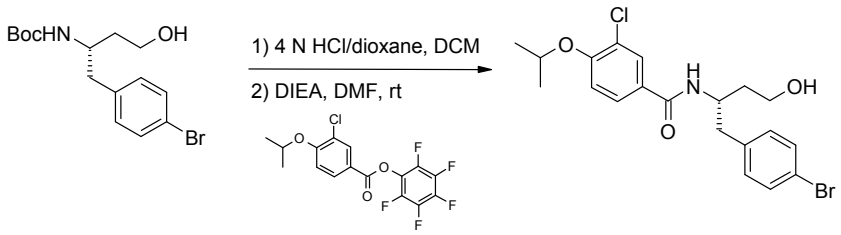
Preparation of Compound 8a

a) 1,1-dimethylethyl {(1*S*)-1-[(4-bromophenyl)methyl]-3-hydroxypropyl}carbamate



A slurry of lithium aluminum hydride (8.9 g, 0.23 mol) in dry THF (800 mL) was cooled to 0 ^{o}C, then slowly treated with a solution of (3*S*)-4-(4-bromophenyl)-3-({[(1,1-dimethylethyl)oxy]carbonyl}amino)butanoic acid (60 g, 0.17 mmol) in THF (200 mL). The reaction was allowed to slowly warm to room temperature and was stirred for 16 h. The reaction was cooled to 0 ^{o}C and treated with water (12 mL), 1 N NaOH (24 mL), and water again (12 mL). The ice bath was removed and the reaction stirred for 3 h, at which time the solids were filtered and rinsed with THF (2 x 100 mL). The filtrate was concentrated. The residue was dissolved in methylene chloride (250 mL), dried (Na_2SO_4), and concentrated to give the title compound (45 g, 78% yield) as a white solid.

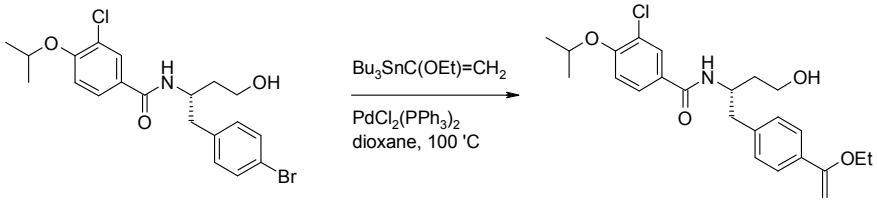
b) *N*-{(1*S*)-1-[(4-bromophenyl)methyl]-3-hydroxypropyl}-3-chloro-4-[(1-methylethyl)oxy]benzamide



To a cooled (0 ^{o}C) solution of 1,1-dimethylethyl {(1*S*)-1-[(4-bromophenyl)methyl]-3-hydroxypropyl}carbamate (55 g, 0.16 mol) in methylene chloride (250 mL) was added 4 N HCl/dioxane (40 mL, 0.16 mol) and the reaction

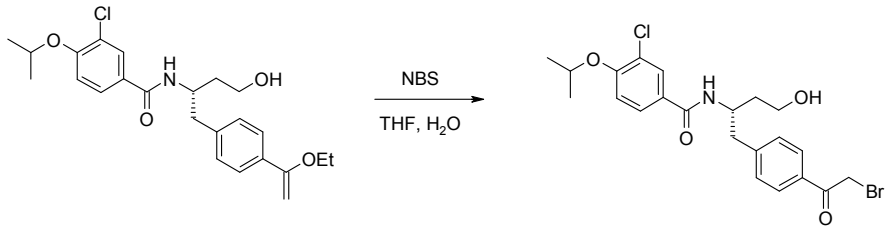
allowed to warm to room temperature. After 4 h, additional 4 N HCl/dioxane (20 mL, 0.08 mol) was added and the reaction maintained for 16 h, at which time it was concentrated. The residue was dissolved in DMF (450 mL), treated with diisopropylethylamine (33 mL, 0.19 mol), and cooled to 0 ^{o}C. To the reaction was added pentafluorophenyl 3-chloro-4-[(1-methylethyl)oxy]benzoate (66 g, 0.17 mol). The reaction was allowed to slowly warm to room temperature and was maintained for 2 days. The reaction was then concentrated and the residue diluted with ethyl acetate. The solution was washed with saturated $NaHCO_3$ and brine, dried (Na_2SO_4) and concentrated. Purification of the residue by flash chromatography (3:2 hexanes/EtOAc to 1:3 hexanes/EtOAc) gave the title compound (61 g, 87% yield) as pale yellow solid.

c) 3-chloro-*N*-[(1*S*)-1-({4-[1-(ethyloxy)ethenyl]phenyl}methyl)-3-hydroxypropyl]-4-[(1-methylethyl)oxy]benzamide



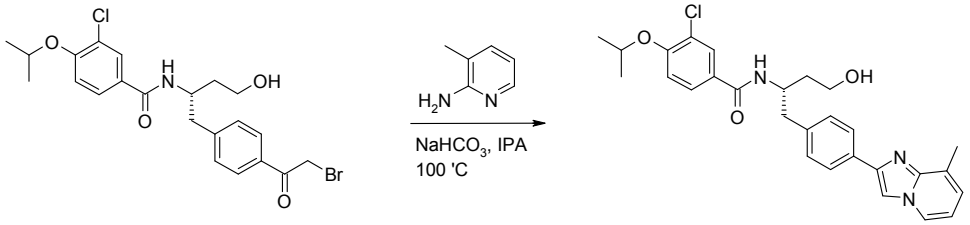
To a solution of *N*-{(1*S*)-1-[(4-bromophenyl)methyl]-3-hydroxypropyl}-3-chloro-4-[(1-methylethyl)oxy]benzamide (20 g, 46 mmol) and tributyl[1-(ethyloxy)ethenyl]stannane (31 mL, 91 mmol) in dioxane (400 mL) was added $PdCl_2(PPh_3)_2$ (1.6 g, 2.3 mmol). The reaction was heated at 100 ^{o}C for 4 h, at which time it was allowed to cool to room temperature and concentrated. Purification of the residue by flash chromatography (47.5 : 47.5 : 5 hexanes/EtOAc/Et_3N) gave the title compound (16 g, 80% yield).

d) *N*-((1*S*)-1-{[4-(bromoacetyl)phenyl]methyl}-3-hydroxypropyl)-3-chloro-4-[(1-methylethyl)oxy]benzamide



To a cooled (0 °C) solution of 3-chloro-*N*-[(1*S*)-1-({4-[1-(ethyloxy)ethenyl]phenyl}methyl)-3-hydroxypropyl]-4-[(1-methylethyl)oxy]benzamide (16 g, 37 mmol) in THF (250 mL) and water (150 mL) was added a solution of NBS (6.9 g, 39 mmol) in THF (200 mL). The reaction was allowed to warm to room temperature and was maintained for 2 h, at which time it was concentrated. The residue was diluted with water (700 mL) and extracted with EtOAc (2 x 700 mL). The combined organics were washed with brine (700 mL), dried ($MgSO_4$), and concentrated. Purification of the residue by flash chromatography (10% EtOAc/hexanes to 70% EtOAc/hexanes) gave the title compound (11.8 g, 66% yield).

e) 3-chloro-*N*-((1*S*)-3-hydroxy-1-{[4-(8-methylimidazo[1,2-*a*]pyridin-2-yl)phenyl]methyl}propyl)-4-[(1-methylethyl)oxy]benzamide

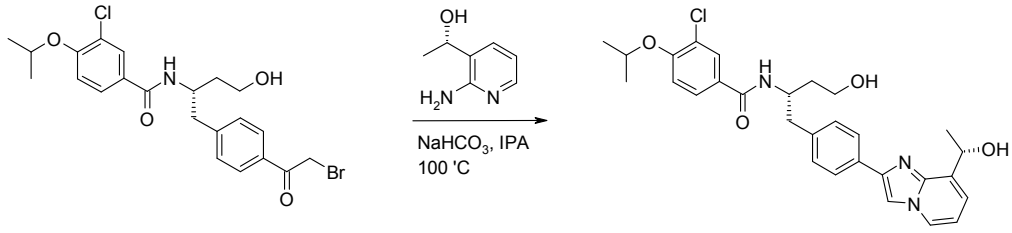


A mixture of *N*-((1*S*)-1-{[4-(bromoacetyl)phenyl]methyl}-3-hydroxypropyl)-3-chloro-4-[(1-methylethyl)oxy]benzamide (300 mg, 0.62 mmol), 3-methyl-2-pyridinamine (67 mg, 0.62 mmol), and $NaHCO_3$ (63 mg, 0.75 mmol) in isopropanol (6 mL) was heated in a sealed reaction vessel at 80 °C for 5 h. The reaction was allowed to cool to room temperature and concentrated. The residue was dissolved in EtOAc and washed with water and brine. The combined aqueous layers were extracted with 3%MeOH/EtOAc and EtOAc. The combined organics were dried (Na_2SO_4) and concentrated. Purification of the residue by flash chromatography (3% MeOH/EtOAc) gave the title compound (260 mg, 84% yield) as a white solid. >95% pure by HPLC. ^{1}H NMR (400 MHz, DMSO-d_6)

δ 8.35 (d, 1H), 8.32 (s, 1H), 8.22 (d, *J* = 8.59 Hz, 1H), 7.89 (d, *J* = 2.27 Hz, 1H), 7.86 (d, *J* = 8.08 Hz, 2H), 7.76 (dd, *J* = 2.15, 8.72 Hz, 1H), 7.29 (d, *J* = 8.08 Hz, 2H), 7.23 (d, *J* = 8.84 Hz, 1H), 7.03 (d, *J* = 6.82 Hz, 1H), 6.78 (t, *J* = 6.82 Hz, 1H), 5.77 (s, 1H), 4.77 (dt, *J* = 5.97, 12.06 Hz, 1H), 4.42 (t, *J* = 5.18 Hz, 1H), 4.20 - 4.32 (m, 1H), 3.39 - 3.52 (m, 2H), 2.78 - 2.95 (m, 2H), 1.64 - 1.80 (m, 2H), 1.31 (d, 6H). MS(ES+) m/e 522 $[M+H]^+$.

Preparation of Compound 8b

a) 3-chloro-*N*-{(1*S*)-3-hydroxy-1-[(4-{8-[(1*S*)-1-hydroxyethyl]imidazo[1,2-*a*]pyridin-2-yl}phenyl)methyl]propyl}-4-[(1-methylethyl)oxy]benzamide

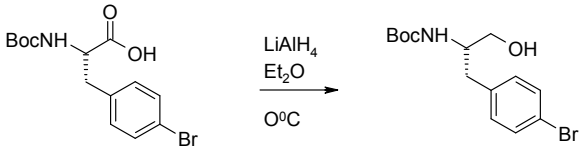


A mixture of *N*-((1*S*)-1-{[4-(bromoacetyl)phenyl]methyl}-3-hydroxypropyl)-3-chloro-4-[(1-methylethyl)oxy]benzamide (10.5 g, 22 mmol), (1*S*)-1-(2-amino-3-pyridinyl)ethanol (3 g, 22 mmol), and $NaHCO_3$ (2.2 g, 26 mmol) in isopropanol (300 mL) was heated in a sealed reaction vessel at 100 °C for 4 h. The reaction was allowed to cool to room temperature and concentrated. Purification of the residue by flash chromatography (EtOAc to 3% MeOH/EtOAc) gave the title compound (7 g, 62% yield) as a white solid. Anal. ($C_{29}H_{32}ClN_3O_4$) Theory C: 66.72, H: 6.18, N: 8.05; Found C: 66.52, H: 6.28, N: 7.95. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.38 (d, *J* = 5.81 Hz, 1H), 8.32 (s, 1H), 8.23 (d, *J* = 8.59 Hz, 1H), 7.90 (d, *J* = 2.02 Hz, 1H), 7.86 (d, *J* = 8.08 Hz, 2H), 7.76 (dd, *J* = 2.27, 8.59 Hz, 1H), 7.19 - 7.34 (m, 4H), 6.88 (t, *J* = 6.82 Hz, 1H), 5.36 (d, *J* = 4.55 Hz, 1H), 5.31 (app quin, *J* = 5.87 Hz, 1H), 4.76 (dt, *J* = 6.06, 12.13 Hz, 1H), 4.43 (t, *J* = 5.18 Hz, 1H), 4.19 - 4.33 (m, 1H), 3.38 - 3.52 (m, 2H), 2.79 - 2.94 (m, 2H), 1.64 - 1.79 (m, 2H), 1.51 (d, *J* = 6.32 Hz, 3H), 1.31 (d, *J* = 5.81 Hz, 6H)98.74% pure by HPLC A% at 260 nm. MS *m/z* 522.6 $[M+H]^+$. mp 161-162 °C. $[\alpha]_D$ = -145.8° (methanol, 18 °C, c = 1.00).

Preparation and Characterization of GSK923295 (1)

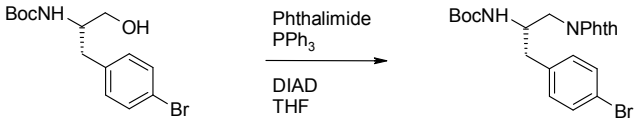
Preparation of Racemic GSK923295:

a) 1,1-Dimethylethyl [(1*S*)-2-(4-bromophenyl)-1-(hydroxymethyl)ethyl]carbamate:



To a solution of 4-bromo-*N*-{[(1,1-dimethylethyl)oxy]carbonyl}-L-phenylalanine (72.6 mmol), in anhydrous diethyl ether (550 mL) at 0 ^{o}C was added slowly lithium aluminum hydride, 95% (108.9 mmol). The resulting solution was stirred for an additional 2 h at 0 ^{o}C. The reaction was then carefully quenched with a saturated aqueous solution of sodium bicarbonate (73 mL) which stirred at RT for half an hour. Lithium aluminum salts crashed out of solution and were removed by filtration. The filtrate was concentrated and vacuum pumped for 24 h to afford the title product as a white solid (97%). ESMS $[M+H]^{+}$: 331.2.

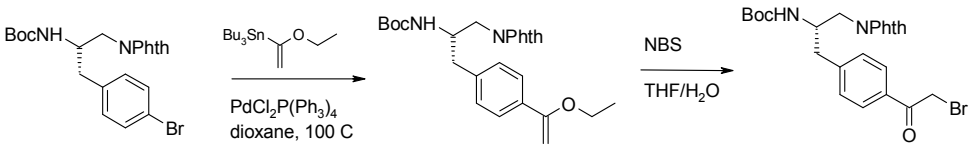
b) 1,1-Dimethylethyl {(1*S*)-2-(4-bromophenyl)-1-[(1,3-dioxo-1,3-dihydro-2*H*-isoindol-2-yl)methyl]ethyl}carbamate:



To a solution of 1,1-dimethylethyl [(1*S*)-2-(4-bromophenyl)-1-(hydroxymethyl)ethyl]carbamate (70.6 mmol), tripheylphosphine (84.7 mmol), and phthalimide (84.7 mmol) in anhydrous tetrahydrofuran (550 mL) at 0 ^{o}C was added dropwise diisopropyl azodicarboxylate (84.7 mmol) over 10 minutes. The reaction continued to stir allowing to warm to RT over 5 h. The reaction was then concentrated in vacuo and product was triturated out of solution using acetate (500 mL). The precipitate was filtered, washed with ethyl acetate (3 x 100 mL), and dried to afford the title product as a white solid (57%). ESMS $[M+H]^{+}$: 460.4.

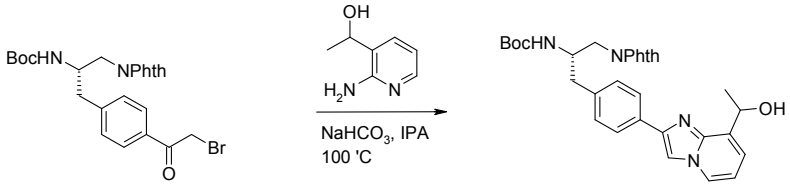
c) 1,1-Dimethylethyl {(1*S*)-2-[4-(bromoacetyl)phenyl]-1-[(1,3-dioxo-1,3-dihydro-2*H*-

isoindol-2-yl)methyl]ethyl}carbamate:



A solution of 1,1-dimethylethyl {(1*S*)-2-(4-bromophenyl)-1-[(1,3-dioxo-1,3-dihydro-2*H*-isoindol-2-yl)methyl]ethyl}carbamate (21.7 mmol), 1-ethoxyvinyltri-n-butylin (43.5 mmol), and *trans*-dichlorobis(triphenylphosphine)palladium(II) (5 mol%) were stirred in anhydrous dioxane (300 mL) at 100 ^{o}C for 3h. The reaction was then concentrated in vacuo and redissolved in a solution of tetrahydrofuran and water (3:1, 400 mL) and treated with *N*-bromosuccinimide (108.8 mmol) and stirred at RT for half an hour. The reaction solution was then concentrated to dryness and redissolved in ethyl acetate (150 mL) and precipitate formed upon addition of hexanes (350 mL). The precipitate was filtered and dried to afford the title product as yellow solid (71%). ESMS $[M+H]^{+}$: 502.4.

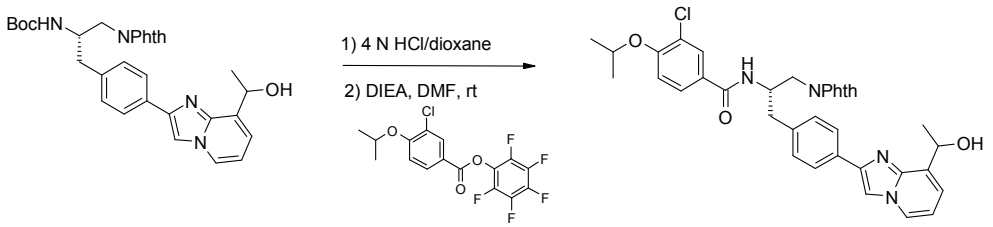
d) 1,1-Dimethylethyl [(1*S*)-2-(1,3-dioxo-1,3-dihydro-2*H*-isoindol-2-yl)-1-({4-[8-(1-hydroxyethyl)imidazo[1,2-*a*]pyridin-2-yl]phenyl}methyl)ethyl]carbamate**:**



A mixture of 1,1-dimethylethyl{(1S)-2-{4-(bromoacetyl)phenyl]-1-[(1,3-dioxo-1,3-dihydro-2*H*-isoindol-2-yl)methyl]ethyl}carbamate (1.90 g, 3.79 mmol), 1-(2-amino-3-pyridinyl)ethanol (0.523 g, 3.79 mmol), and solid sodium bicarbonate (0.398 g, 4.72 mmol) in isopropanol (24 mL) was refluxed for 3.0 h. and concentrated in vacuo. The residue was dissolved in ethyl acetate, washed with water and saturated sodium chloride, dried (Na_2SO_4), and concentrated to give the title compound (1.79 g, 87%) as a light pink solid. MS(ES+) m/e 541 $[M+H]^{+}$.

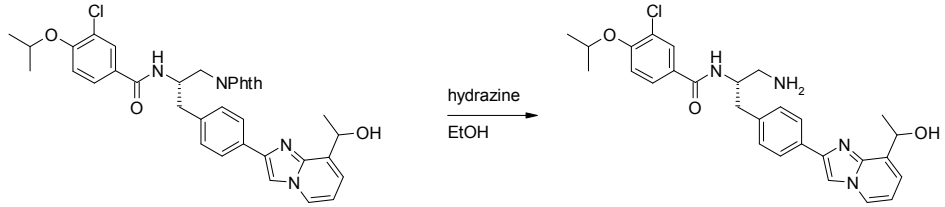
e) 3-Chloro-*N*-[(1*S*)-2-(1,3-dioxo-1,3-dihydro-2*H*-isoindol-2-yl)-1-({4-[8-(1-hydroxyethyl)imidazo[1,2-*a*]pyridin-2-yl]phenyl}methyl)ethyl]-4-[(1-

methylethyl)oxy]benzamide**:**



A mixture of 1,1-dimethylethyl [(1*S*)-2-(1,3-dioxo-1,3-dihydro-2*H*-isoindol-2-yl)-1-({4-[8-(1-hydroxyethyl)imidazo[1,2-*a*]pyridin-2-yl]phenyl}methyl)ethyl]carbamate (1.79 g, 3.31 mmol) and 4M HCl in 1,4-dioxane (20 mL, 80 mmol) was stirred at room temperature for 45 minutes. The reaction was concentrated to dryness ,redissolved in DMF (30 mL), and to this solution was added *N,N*-diisopropylethylamine (2.14 g, 16.55 mmol) and pentafluorophenyl 3-chloro-4 [(1-methylethyl)oxy]benzoate (1.36 g, 3.31 mmol). The mixture was stirred overnight at room temperature, diluted with water, and extracted into ethyl acetate. The extracts were washed with water, dried (Na_2SO_4), and concentrated in vacuo to give the title compound (2.10 g, 100%) as a tan solid. MS(ES+) m/e 637 $[M+H]^+$.

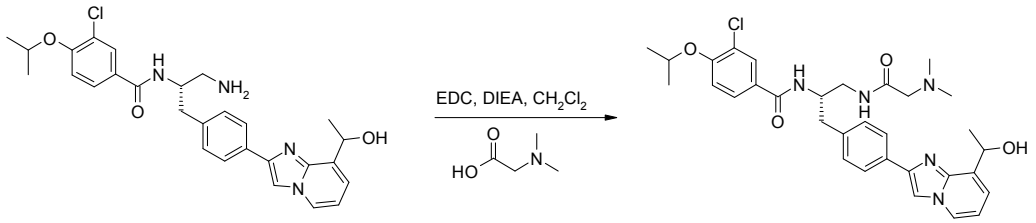
f) *N*-[(1*S*)-2-Amino-1-({4-[8-(1-hydroxyethyl)imidazo[1,2-*a*]pyridin-2-yl]phenyl}methyl)ethyl]-3-chloro-4-[(1-methylethyl)oxy]benzamide:



A mixture of 3-chloro-*N*-[(1*S*)-2-(1,3-dioxo-1,3-dihydro-2*H*-isoindol-2-yl)-1-({4-[8-(1-hydroxyethyl)imidazo[1,2-*a*]pyridin-2-yl]phenyl}methyl)ethyl]-4-[(1-methylethyl)oxy]benzamide (2.10 g, 3.30 mmol) and hydrazine monohydrate (0.83 g, 16.5 mmol) in ethanol (30 mL) was heated at 57 ^oC overnight. The reaction was cooled, diluted with ethanol, filtered, and concentrated to give the title compound (1.67 g, 100%) as a pale yellow powder. MS(ES+) m/e 507 $[M+H]^+$.

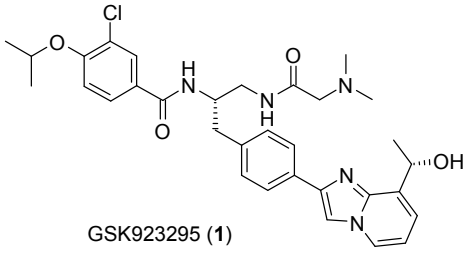
g) 3-Chloro-*N*-[(1*S*)-2-[(*N,N*-dimethylglycyl)amino]-1-({4-[8-(1-hydroxyethyl)imidazo[1,2-*a*]pyridin-2-yl]phenyl}methyl)ethyl]-4-[(1-

methylethyl)oxy]benzamide:



A mixture of *N*-[(1*S*)-2-amino-1-({4-[8-(1-hydroxyethyl)imidazo[1,2-*a*]pyridin-2-yl]phenyl}methyl)ethyl]-3-chloro-4-[(1-methylethyl)oxy]benzamide (0.912 g, 1.80 mmol), EDCI (0.69 g, 3.6 mmol), *N,N*-diisopropylethylamine (0.466 g, 3.6 mmol), and *N,N*-dimethylglycine (0.372 g, 3.6 mmol) in methylene chloride (17 mL) was stirred overnight at room temperature. The reaction was diluted with water, washed with brine, dried (Na_2SO_4), and concentrated. The residue was purified by flash chromatography on silica gel (8%-10% MeOH:CH_2Cl_2) to give the title compound (0.515 g, 48%) as a pale yellow solid. MS(ES+) m/e 592 $[M+H]^+$.

Enatiomerically pure 3-chloro-*N*-{(1*S*)-2-[(*N,N*-dimethylglycyl)amino]-1-[(4-{8-[(1*S*)-1-hydroxyethyl]imidazo[1,2-*a*]pyridin-2-yl}phenyl)methyl]ethyl}-4-[(1-methylethyl)oxy]benzamide (**GSK923295**) was prepared using (S)-1-(2-amino-3-pyridinyl)ethanol (Enantiomer **A** below) following the procedures described above.

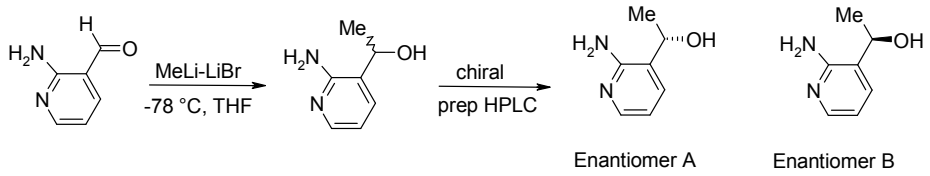


GSK923295 (**1**)

^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 1.30 (d, *J*=5.31 Hz, 6 H), 1.51 (d, *J*=6.57 Hz, 3 H), 2.14 (s, 6 H), 2.82 (s, 2 H), 2.85 (d, *J*=7.07 Hz, 2 H), 3.19 - 3.31 (m, 1 H), 3.33 - 3.45 (m, 1 H), 4.26 - 4.40 (m, 1 H), 4.76 (dt, *J*=12.13, 6.06 Hz, 1 H), 5.30 (q, *J*=6.23 Hz, 1 H), 6.88 (t, *J*=6.95 Hz, 1 H), 7.18 - 7.28 (m, 2 H), 7.30 (d, *J*=8.34 Hz, 2 H), 7.72 (dd, *J*=8.72, 2.15 Hz, 1 H), 7.81 - 7.89 (m, 3 H), 8.22 (d, *J*=8.34 Hz, 1 H), 8.31 (s, 1 H), 8.37 (d, *J*=6.82 Hz, 1 H). Anal. ($C_{32}H_{38}ClN_5O_4$) Theory C: 64.91, H: 6.47, N: 11.83; Found C: 64.73, H: 6.50, N: 11.68. 99.3% pure by HPLC A% at 260 nm. MS *m/z* 592.0 $[M+H]^+$. mp 197-198 ^oC. $[\alpha]_D$ = -116^o (methanol, 20 ^oC, c = 1.00).

Preparation of Homochiral Alcohol Intermediate:

1-(2-amino-3-pyridinyl)ethanol**:**



To a dry flask (dried with a heat gun under argon purge) was added dry THF (400 mL) and MeLi-LiBr (137 mL of a 1.5M solution in Et_2O, 204.9 mmol) *via* cannula. This solution was cooled to -78 °C when a solution of 2-aminopyridine-3-carboxaldehyde (10.0 g, 82.0 mmol) in THF (150 mL) was added dropwise via a pressure equalizing addition funnel over ~45 with vigorous stirring (exotherm observed, orange color persisted). Upon complete addition, the solution was allowed to stir for 1 hour at -78 °C, at which time TLC ($KMnO_4$ stain with heat) indicated that most of the starting material had been converted to product. The reaction was quenched very carefully with water (200 mL; dropwise initially), diluted with EtOAc (200 mL) and allowed to warm to rt. The layers were separated and the aqueous layer was extracted with 3% MeOH in EtOAc. The combined extracts were dried over sodium sulfate, filtered and concentrated under reduced pressure. The residue was purified by silica gel chromatography (Analogix; 0 to 5% MeOH in EtOAc) to give 7.78 g (68%) of the desired racemic product as a yellow oil that solidified under high vac over several days. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.82 (dd, J = 1.77, 4.80 Hz, 1H), 7.39 (dd, J = 1.64, 7.20 Hz, 1H), 6.53 (dd, J = 4.80, 7.33 Hz, 1H), 5.70 (s, 2H), 5.24 (d, J = 4.29 Hz, 1H), 4.66 - 4.75 (m, 1H), 1.30 (d, J = 6.32 Hz, 3H).

Resolution of the racemic mixture was accomplished by chiral HPLC using SFC with a Chiralcel OD-H column (20 x 250 mm) and an isocratic mobile phase (10% EtOH/0.1% isopropylamine in heptane/0.1% isopropylamine) to give both enantiomers in >98% ee. Enantiomer **A**: RT = 13.1 min, $[\alpha]_D = -0.5^o$ (ethanol, c = 1.00). Enantiomer **B**: RT = 15.6 min, $[\alpha]_D = +1.1^o$ (ethanol, c = 1.00). Enantiomer **A** was used to prepare compound **8b** and GSK923295 (**1**) as described above.