Discovery of Novel, Orally Bioavailable Azaindole Inhibitors of Influenza PB2

Luc J. Farmer,[1*] Michael P. Clark,[2]* Michael J. Boyd,[2] Emanuele Perola,[2] Steven M. Jones,[3] Alice Tsai,[2] Marc D. Jacobs,[2] Upul K. Bandarage,[2] Mark W. Ledeboer,[4] Tiansheng Wang,[2] Hongbo Deng,[2] Brian Ledford,[2] Wenxin Gu,[2] John Duffy,[2] Randy S. Bethiel,[2] Dean Shannon,[5] Randal A. Byrn,[2] Joshua R. Leeman,[2] Rene Rijnbrand,[6] Hamilton B. Bennett,[7] Colleen O'Brien,[2] Christine Memmott,[2] Kwame Nti-Addae,[2] Youssef L. Bennani,[7] Paul S. Charifson.[2]

[1]Paraza Pharma. Inc., 7171 Frederick-Banting, Montreal, Quebec H4S 1Z9, Canada

[2]Vertex Pharmaceuticals Inc., 50 Northern Ave, Boston, Massachusetts 02210, United States

[3]Contrafect Corporation, 28 Wells Ave, 3rd floor, Yonkers, New York 10701, United States

[4]Goldfinch Bio., 215 First St, 4th floor, Cambridge, MA 02142, United States

[5]DE Synthetics Inc., 30 Dineen Drive, Fredericton, New Brunswick E3B 5A3, Canada

[6]Arbutus Biopharma Inc., 3805 Old Easton Road, Doylestown, Pennsylvania 18902, United States

[7]Moderna Therapeutics, Inc. 200 Technology Square, Cambridge, Massachusetts, 02139, United States

[8]Vertex Pharmaceuticals (Canada) Inc., 275 Armand-Frappier, Laval, Quebec H7V 4A7, Canada

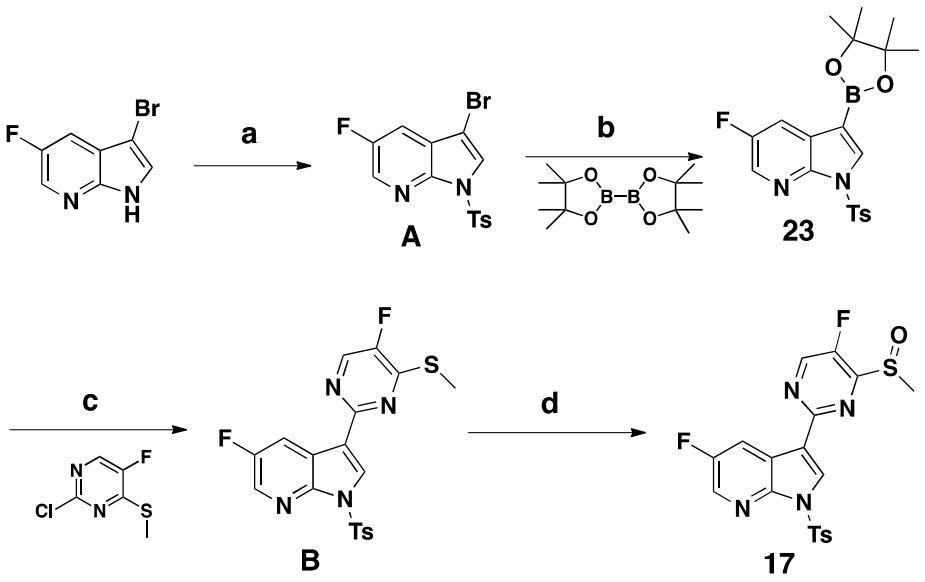
[*] To whom correspondence should be addressed:

Telephone: (781) 530-6954. Fax: 450 978-7972. E-mail: michael_clark@vrtx.com;

All commercially available reagents and anhydrous solvents were used without further purification. Purity assessment for final compounds was based on analytical HPLC: 4.6 x 50 mm Waters YMC Pro-C18 column, 5 μ m, 120Å. Mobile phases are as follows: A, H_2O with 0.2% formic acid; B, CH_3CN with 0.2% formic acid; gradient, 10−90% B in 3 min with 5 min run time at a flow rate of 1.5 mL/min. Unless specified otherwise, all compounds were ≥95% pure. Mass samples were analyzed on a Micro Mass ZQ, ZMD, Quattro LC, or Quatro II mass spectrometer operated in a single MS mode with electrospray ionization. Samples were introduced into the mass spectrometer using flow injection (FIA) or chromatography. The mobile phase for all mass analysis consisted of CH_3CN −water mixtures with either 0.2% formic acid or ammonium formate. High-resolution mass spectra (HRMS) were collected by direct infusion on a Thermo QExactive. The samples were dissolved in MeOH at a concentration of approximately 0.2 mg/mL and infused with a flow rate of 5 μL/min. Electrospray ionization in positive ion mode was employed with a spray voltage of 4.0 kV. The mass resolution was set to 35,000. ^{1}H NMR spectra were recorded using either a Bruker Avance 400 (400 MHz) or a Bruker Avance II-300 (300 MHz) instrument. Preparative column chromatography was performed using Teledyne ISCO RediSep normal phase (35−70 μm) or RediSep Gold normal phase (25−40 μm) silica flash columns using a Teledyne ISCO Combiflash Companion or Combiflash Rf purification system. Preparative HPLC was performed on a Gilson HPLC system equipped a UV−VIS 156 Gilson detector. Separations were accomplished on an Agilent Zorbax SB-C18 column (21.2 x 100 mm) eluted with a linear gradient from 10% to 90% CH_3CN in H_2O over 10 min (0.1% TFA) at a flow rate of 20 mL/min.

Abbreviations: dimethylformamide (DMF), tetrahydrofuran (THF), trifluoroacetic acid (TFA), triethylamine (TEA), methanol (MeOH), dimethoxyethane (DME), dimethylsulfoxide (DMSO), acetonitrile (ACN), lithium diisopropylamide (LDA), 2-dicyclohexylphosphino-2′,4′,6′-triisopropylbiphenyl (XPhos).

Synthetic Scheme 1: 5-fluoro-3-(5-fluoro-4-methylsulfinyl-pyrimidin-2-yl)-1-(*p*-tolylsulfonyl)pyrrolo[2,3-b]pyridine (17)



(a) NaH, Tosyl Chloride, DMF; (b) KOAc, $PdCl_2$(dppf), dioxane, water (c) $Pd(PPh_3)_4$, Na_2CO_3, DME, water; (d) morpholine-4-carbonyl chloride, $^iPr_2NEt$, CH_2Cl_2;

Formation of 3-bromo-5-fluoro-1-(*p*-tolylsulfonyl)pyrrolo[2,3-b]pyridine (A)

3-bromo-5-fluoro-1*H*-pyrrolo[2,3-b]pyridine (5.0 g, 23.3 mmol) was dissolved in DMF (37.5 mL) and cooled to 0 °C. Sodium hydride (1.5 g, 37.2 mmol) was added and the reaction mixture was

stirred for 10 minutes and then treated with tosyl chloride (6.6 g, 34.9 mmol). The mixture was stirred for 30 minutes at 0 °C and then at room temperature for another 90 minutes. The reaction mixture was poured into water (100 mL) and the resulting solid was collected, washed with water and hexanes three times and dried *in vacuo* to afford 8.26 g of 3-bromo-5-fluoro-1-(*p*-tolylsulfonyl)pyrrolo[2,3-b]pyridine, **A:** ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.48 (s, 1H), 8.31 (s, 1H), 8.01 (d, J = 8.3 Hz, 2H), 7.92 (dd, J = 8.4, 2.7 Hz, 1H), 7.44 (d, J = 8.5 Hz, 2H), 2.35 (s, 3H).

Formation of 5-fluoro-1-(*p*-tolylsulfonyl)-3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)pyrrolo[2,3-b]pyridine (23)

3-bromo-5-fluoro-1-(*p*-tolylsulfonyl)pyrrolo[2,3-b]pyridine, **A**, (4.0 g, 10.8 mmol), 4,4,5,5-tetramethyl-2-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1,3,2-dioxaborolane (8.3 g, 32.5 mmol) and potassium acetate (3.2 g, 32.5 mmol) were taken in dioxane (40 mL) containing a few drops of water. After purging with nitrogen for 30 minutes, $PdCl_2$(dppf) (0.8 g, 1.1 mmol) was added. Nitrogen purging was continued for an additional 40 minutes, then the reaction mixture was heated to reflux overnight. After cooling down, the mixture was filtered through Florisil (60g), washed with dichloromethane (220 mL) and concentrated *in vacuo* to provide a brown oil. The crude product was taken into hexane (40 mL) and TBME (14 mL) and heated to reflux. After cooling to room temperature, the resulting suspension was filtered to provide 2.6 g of the desired product as a white solid: ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.42 (dd, J = 2.7, 1.4 Hz, 1H), 8.14 (s, 1H), 8.06 (d, J = 8.4 Hz, 2H), 7.85 (dd, J = 8.6, 2.8 Hz, 1H), 7.44 (d, J = 8.3 Hz, 2H), 2.36 (s, 3H), 1.32 (s, 12H).

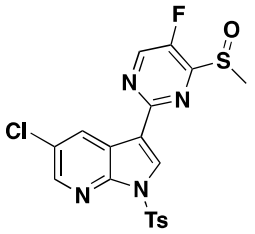
Formation of 5-fluoro-3-(5-fluoro-4-methylsulfanyl-pyrimidin-2-yl)-1-(*p*-tolylsulfonyl)pyrrolo[2,3-b]pyridine (B)

2-chloro-5-fluoro-4-methylsulfanyl-pyrimidine (1.6 g, 9.0 mmol), 5-fluoro-1-(*p*-tolylsulfonyl)-3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)pyrrolo[2,3-b]pyridine, **23**, (2.5 g, 6.0 mmol) and Na_2CO_3 (1.9 g, 18.0 mmol) were dissolved in DME (37.5 mL) and water (7.5 mL). The mixture was purged with nitrogen for 20 minutes, treated with $Pd(PPh_3)_4$, purged with nitrogen for another 20 minutes and heated to reflux overnight. After cooling to room temperature, water (35 mL) was added and the resulting suspension was stirred for 30 minutes. The precipitate was collected by filtration, washed with water and acetonitrile and dried overnight at 50 °C, affording 2.3 g (88.5%) of the desired product as a white solid: ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.70 – 8.57 (m, 2H), 8.55 – 8.42 (m, 2H), 8.09 (d, J = 8.4 Hz, 2H), 7.45 (d, J = 8.4 Hz, 2H), 2.76 (s, 3H), 2.36 (s, 3H).

Formation of 5-fluoro-3-(5-fluoro-4-methylsulfinyl-pyrimidin-2-yl)-1-(*p*-tolylsulfonyl)pyrrolo[2,3-b]pyridine (17)

5-fluoro-3-(5-fluoro-4-methylsulfanyl-pyrimidin-2-yl)-1-(*p*-tolylsulfonyl)pyrrolo[2,3-b]pyridine, **B**, (2.30 g, 5.32 mmol) was dissolved in dichloromethane (107 mL) and treated portionwise with 3-chloroperbenzoic acid (1.19 g, 5.30 mmol), keeping the temperature below 20°C. After stirring for 2 hours, another portion of 3-chloroperbenzoic acid (0.18 g, 0.80 mmol) was added, and stirring was continued for another hour. A third portion of 3-chloroperbenzoic acid (0.07 g, 0.05 mmol) was added and stirring was continued for 30 minutes. The reaction mixture was treated with an aqueous 15% K_2CO_3 solution (30 mL) and the layers were separated. The organic layer was washed with 15% K_2CO_3 and brine, dried (Na_2SO_4), filtered and concentrated *in vacuo* to afford 2.3 g (96%) of the desired product as a yellow solid, which was used without further purification: ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.12 (d, J = 1.5 Hz, 1H), 8.70 (s, 1H), 8.67 (dd, J = 9.1, 2.8 Hz, 1H), 8.53 (d, J = 1.5 Hz, 1H), 8.11 (d, J = 8.4 Hz, 2H), 7.46 (d, J = 8.2 Hz, 2H), 3.05 (s, 3H), 2.36 (s, 3H).

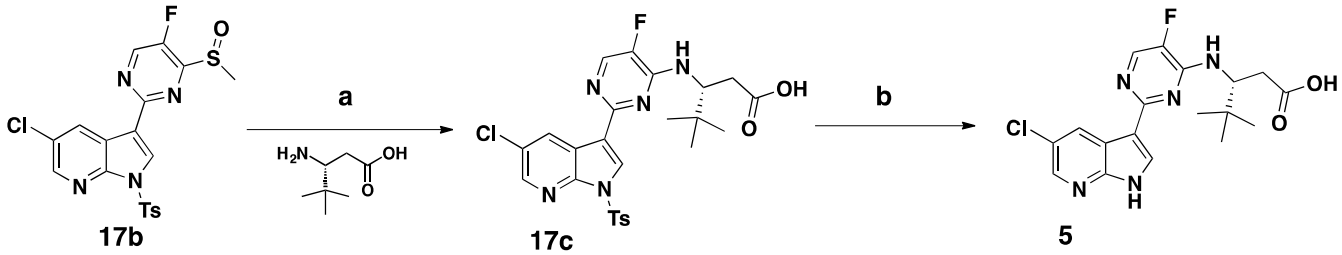
The following analog was prepared in the same fashion as sulfoxide, **17**:



17b

5-chloro-3-(5-fluoro-4-(methylsulfinyl)pyrimidin-2-yl)-1-tosyl-1*H*-pyrrolo[2,3-b]pyridine (17b)
^{1}H NMR (300 MHz, *d*6-DMSO) 9.12 (d, *J* = 1.3 Hz, 1H), 8.90 (d, *J* = 2.4 Hz, 1H), 8.68 (s, 1H), 8.53 (d, *J* = 2.4 Hz, 1H), 8.12 (*d*, J = 8.4 Hz, 2H), 7.46 (d, *J* = 8.4 Hz, 2H), 2.54 - 2.48 (m, 3H), 2.36 (s, 3H).

Synthetic Scheme 2: (*R*)-3-(2-(5-Chloro-1*H*-pyrrolo[2,3-*b*]pyridin-3-yl)-5-fluoropyrimidin-4-ylamino)-4,4-dimethylpentanoic acid (5)



(a) Na_2CO_3, THF, CH_3CN, microwave, 135 ^oC; (b) NaOMe, MeOH, 0 ^oC;

Formation of (*R*)-3-(2-(5-chloro-1-tosyl-1*H*-pyrrolo[2,3-*b*]pyridin-3-yl)-5-fluoro-pyrimidin-4-ylamino)-4,4-dimethylpentanoic acid (17c)

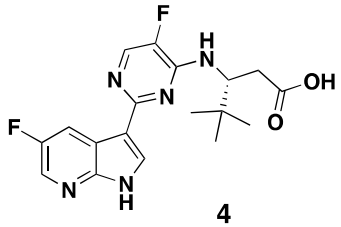
To a solution of 5-chloro-3-(5-fluoro-4-methylsulfinyl-pyrimidin-2-yl)-1-(*p*-tolylsulfonyl)pyrrolo[2,3-b]pyridine, **17b,** (0.100 g, 0.215 mmol) and (*R*)-3-amino-4,4-dimethylpentanoic acid (0.031 g, 0.215 mmol) in tetrahydrofuran (1.66 mL) was added freshly ground Na_2CO_3 (0.068 g, 0.645 mmol) followed by acetonitrile (0.331 mL). The reaction mixture was heated to 135 ^oC for 30 minutes in a microwave reactor. The reaction mixture was slowly poured into 75 mL of 1N HCl. The pH of final solution was adjusted to 1. The aqueous was extracted with EtOAc (3 X 5 mL), washed with brine, dried over Na_2SO_4 and filtered to obtain a crude solid residue. The crude residue was purified via silica gel chromatography (0-10% MeOH-CH_2Cl_2 gradient) afforded 78 mg of the desired product **17c**: LCMS Gradient 10-90%, 0.1% formic acid, 5 minutes, C18/ACN, RT = 3.9 minutes (M+H) 546.22.

(*R*)-3-(2-(5-Chloro-1*H*-pyrrolo[2,3-*b*]pyridin-3-yl)-5-fluoropyrimidin-4-ylamino)-4,4-dimethylpentanoic acid (5)

To a cold (0 °C) solution of (*R*)-3-(2-(5-chloro-1-tosyl-1*H*-pyrrolo[2,3-*b*]pyridin-3-yl)-5-fluoro-pyrimidin-4-ylamino)-4,4-dimethylpentanoic acid, **17c,** (0.08 g, 0.14 mmol) in MeOH (2.6 mL) was added sodium methanolate (2.91 mL of 25 %w/v, 13.46 mmol). The reaction was stirred at room temperature for 30 min and then quenched by dilution into aqueous saturated ammonium chloride solution. The MeOH was evaporated *in vacuo* and the resulting aqueous phase diluted with EtOAc, then extracted with EtOAc (3X). The organics were dried (Na_2SO_4), filtered and concentrated *in vacuo*. Recrystalization from MeOH provided 52 mg of the desired product **5** as a white powder: ^{1}H NMR (*d*6-DMSO) 12.25 (s, 1H): 12.0 (bs, 1H): 8.8 (s, 1H): 8.3 (s, 1H): 8.25 (s, 1H); 8.1 (s, 1H): 7.45 (d, 1H);

4.75 (t, 1H); 2.5 (m, 2H), 1.0 (s, 9H); LCMS Gradient 10-90%, 0.1% formic acid, 5 minutes, C18/ACN, RT = 2.06 minutes (M+H) 392.21; HRMS (ESI) of $C_{18}H_{19}ClFN_5O_2$ [M+H] calcd, 392.12841; found 392.12812. Chiral HPLC >98% ee.

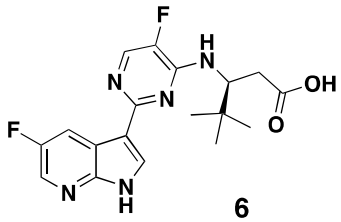
The following analog was prepared in the same fashion as compound **5**:



4

Formation (*R*)-3-((5-fluoro-2-(5-fluoro-1*H*-pyrrolo[2,3-b]pyridin-3-yl)pyrimidin-4-yl)amino)-4,4-dimethylpentanoic acid (4).

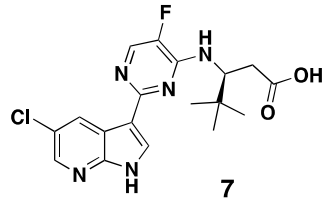
^{1}H NMR (400 MHz, DMSO-d_6) δ 12.23 (s, 1H), 12.03 (s, 1H), 8.68 – 8.52 (m, 1H), 8.27 (s, 1H), 8.19 (d, J = 2.5 Hz, 1H), 8.13 (d, J = 4.0 Hz, 1H), 7.39 (d, J = 9.2 Hz, 1H), 4.83 (t, J = 9.3 Hz, 1H), 2.71 – 2.51 (m, 2H), 0.97 (s, 9H); LCMS Gradient 10-90%, 0.1% formic acid, 5min, C18/ACN, RT = 1.96 minutes (M+H) 377.02; HRMS (ESI) of $C_{18}H_{19}F_2N_5O_2$ [M+H] calcd, 376.15796; found 376.15775. Chiral HPLC shows >99% ee.



6

(*S*)-3-((5-fluoro-2-(5-fluoro-1*H*-pyrrolo[2,3-b]pyridin-3-yl)pyrimidin-4-yl)amino)-4,4-dimethylpentanoic acid (6).

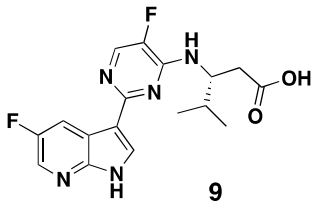
LCMS Gradient 10-90%, 0.1% formic acid, 5 minutes, C18/ACN, Retention Time = 1.93 minutes (M+H) 376.21; HRMS (ESI) of $C_{18}H_{19}F_2N_5O_2$ [M+H] calcd, 392.12841; found 392.12832. Chiral HPLC >98% ee.



7

(*S*)-3-((2-(5-chloro-1*H*-pyrrolo[2,3-b]pyridin-3-yl)-5-fluoropyrimidin-4-yl)amino)-4,4-dimethylpentanoic acid (7).

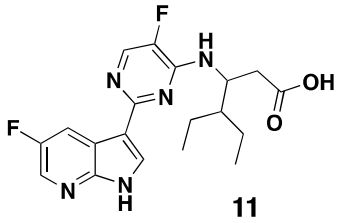
LCMS Gradient 10-90%, 0.1% formic acid, 5 minutes, C18/ACN, Retention Time = 2.06 minutes (M+H) 392.21; HRMS (ESI) of $C_{18}H_{19}ClFN_5O_2$ [M+H] calcd, 376.15796; found 376.15795. Chiral HPLC >98% ee.



9

(*R*)-3-((5-fluoro-2-(5-fluoro-1*H*-pyrrolo[2,3-*b*]pyridin-3-yl)pyrimidin-4-yl)amino)-4-methylpentanoic acid (9).

^{1}H NMR (d6-DMSO) d 12.75 (bs, 1H); 8.5 (d, 1H); 8.4 (m, 4H); 4.7 (bs, 1H); 2.7 (m, 2H); 2.05 (m, 1H); 0.95 (m, 6H); LCMS Gradient 10-90%, 0.1% formic acid, 5 minutes, C18/ACN, Retention Time = 1.83 minutes (M+H) 362.27; HRMS (ESI) of $C_{17}H_{17}F_2N_5O_2$ [M+H] calcd, 362.14231; found 362.14224.

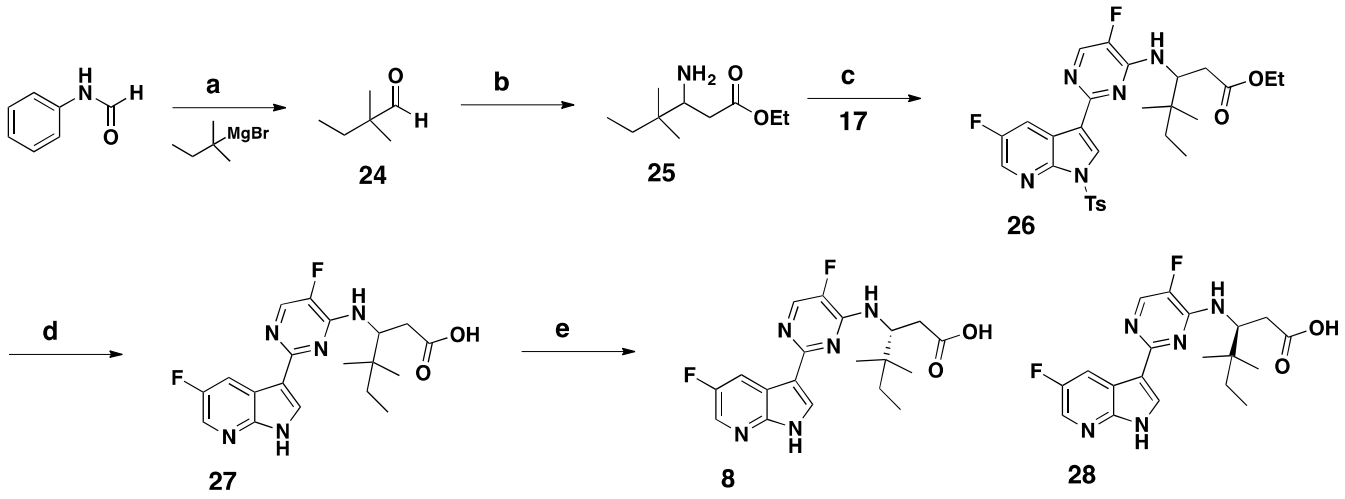


11

(+/-) 4-ethyl-3-((5-fluoro-2-(5-fluoro-1*H*-pyrrolo[2,3-*b*]pyridin-3-yl)pyrimidin-4-yl)amino)hexanoic acid (11).

LCMS Gradient 10-90%, 0.1% formic acid, 5 minutes, C18/ACN, Retention Time = 2.15 minutes (M+H) 390.16; HRMS (ESI) of $C_{19}H_{21}F_2N_5O_2$ [M+H] calcd, 390.17361; found 390.17367.

Synthetic Scheme 3: Formation of 3-((5-fluoro-2-(5-fluoro-1*H*-pyrrolo[2,3-b]pyridin-3-yl)pyrimidin-4-yl)amino)-4,4-dimethylhexanoic acid (8)



a) Et_2O; b) malonic acid, ammonium acetate, ethanol, 80 ^oC; c) 5-fluoro-3-(5-fluoro-4-(methylsulfinyl)pyrimidin-2-yl)-1-tosyl-1*H*-pyrrolo[2,3-b]pyridine, **17**, $^iPr_2NEt$, THF, 80 ^oC; (d) LiOH, THF- H_2O (3:1), 130 °C microwave; e) SFC chiral separation

Formation of 2,2-dimethylbutanal (24)

To a solution of 1,1-dimethylpropyl magnesium chloride (20.0 mL of 1 M, 20.0 mmol) in ether (25 mL) was added *N*-methyl-*N*-phenyl formamide (5.26 mL, 20.0 mmol) in one portion (exothermic). The yellow solution was gently refluxed for two hours and stirred at room temperature for three hours. At the end of this period the Grignard complex was quenched by pouring onto 500 g of crushed ice and 20 ml. of concentrated sulfuric acid. The ether layer was separated and the aqueous phase extracted three times with 50 mL portions of ether. The combined ether extracts were dried ($MgSO_4$) and concentrated *in vacuo*. The crude residue was purified by short-path distillation to afford 1.0 g of pure 2,2-dimethylbutanal as a colorless oil: ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.17 (q, *J* = 7.1 Hz, 2H), 3.03 (dd, *J* = 10.9, 2.3 Hz, 1H), 2.53 (dd, *J* = 15.3, 2.3 Hz, 1H), 2.15 (dd, *J* = 15.3, 10.9 Hz, 1H), 1.50 – 1.33 (m, 3H), 1.28 (dd, *J* = 9.0, 5.3 Hz, 3H), 1.26 – 1.17 (m, 1H), 0.85 (d, *J* = 5.8 Hz, 6H).

Formation of ethyl 3-amino-4,4-dimethylhexanoate (25)

A mixture of 2,2-dimethylbutanal, **24**, (3.00 g, 26.75 mmol), malonic acid (2.08 g, 1.29 mL, 20.00 mmol), ammonium acetate (3.08 g, 40.00 mmol) in ethanol (5 mL) was refluxed for three hours. The precipitate was removed by filtration and washed with ethanol. The solution was used without further purification.

Sulfuric acid (1.962 g, 1.066 mL, 20.00 mmol) was added to above ethanol solution and the resulting mixture was heated to reflux for two hours. The solvent was removed under reduced pressure. Water (20 mL) and ether (10 mL) were added to the crude residue. The aqueous layer was separated and washed with ether (10 mL). The organic layers were discarded. The aqueous solution was neutralized with sodium hydroxide solution (6N) and saturated sodium bicarbonate solution to basic, and extracted with ethyl acetate (3 x 10 mL). The combined organic layers were washed with water (10 mL), brine (10mL), filtered, dried ($MgSO_4$), filtered and concentrated *in vacuo* to give 0.5 g of the desired product as a light yellow sticky oil, which turned into solid upon standing. The crude product was used without further purification: ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.17 (q, *J* = 7.1 Hz, 2H), 3.03 (dd, *J* = 10.9, 2.3 Hz, 1H), 2.53 (dd, *J* = 15.3, 2.3 Hz, 1H), 2.15 (dd, *J* = 15.3, 10.9 Hz, 1H), 1.50 – 1.33 (m, 3H), 1.28 (dd, *J* = 9.0, 5.3 Hz, 3H), 1.26 – 1.17 (m, 1H), 0.85 (d, *J* = 5.8 Hz, 6H).

Formation of ethyl 3-((5-fluoro-2-(5-fluoro-1-tosyl-1*H*-pyrrolo[2,3-b]pyridin-3-yl)pyrimidin-4-yl)amino)-4,4-dimethylhexanoate (26)

To a suspension of ethyl 3-amino-4,4-dimethylhexanoate, **25**, (0.19 g. 1.00 mmol) and 5-fluoro-3-(5-fluoro-4-methylsulfinyl-pyrimidin-2-yl)-1-(*p*-tolylsulfonyl)pyrrolo[2,3-b]pyridine, **17**, (0.54 g, 1.20 mmol) in THF (14.4 mL) was added *N,N*-diisopropylethylamine (0.26 mL, 1.50 mmol). The mixture was refluxed at 80 °C overnight. After removing the solvents under reduced pressure, the crude product was purified by silica gel chromatography (0-50% EtOAc/Hexane gradient) to afford 155 mg of the desired product as a light yellow solid: ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.61 (dd, *J* = 9.0, 2.9 Hz, 1H), 8.56 (s, 1H), 8.33 (dd, *J* = 2.7, 1.0 Hz, 1H), 8.11 (d, *J* = 8.4 Hz, 2H), 7.30 (d, *J* = 8.2 Hz, 2H), 5.19 (dd, *J* = 10.1, 2.2 Hz, 1H), 4.94 (td, *J* = 10.0, 3.7 Hz, 1H), 3.99 (dt, *J* = 13.7, 6.8 Hz, 2H), 2.40 (s, 3H), 1.42 (dt, *J* = 14.1, 6.9 Hz, 2H), 1.05 (t, *J* = 7.1 Hz, 3H), 1.01 – 0.94 (m, 8H); ^{19}F NMR (282 MHz, $CDCl_3$) δ -130.39 -133.75 (dd, *J* = 9.0, 1.1 Hz, 1F), -158.56 (s, 1F); LCMS Gradient 10-90%, 0.1% formic acid, 5 minutes, C18/ACN, RT = 4.18 minutes (M+H) 572.07.

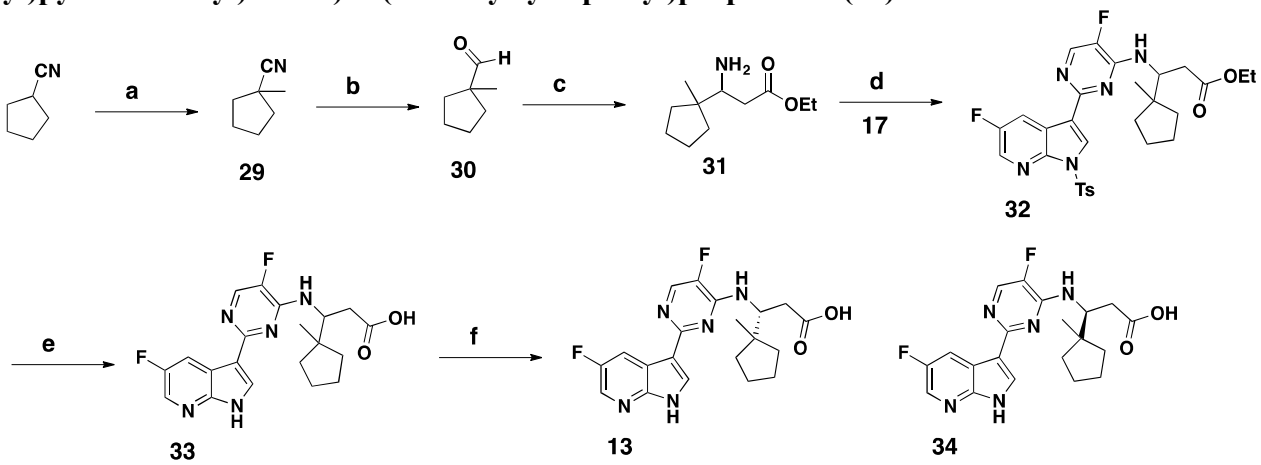
Formation of 3-((5-fluoro-2-(5-fluoro-1*H*-pyrrolo[2,3-b]pyridin-3-yl)pyrimidin-4-yl)amino)-4,4-dimethylhexanoic acid (8).

To a solution of ethyl 3-((5-fluoro-2-(5-fluoro-1-tosyl-1*H*-pyrrolo[2,3-*b*]pyridin-3-yl)pyrimidin-4-yl)amino)-4,4-dimethylhexanoate, **26**, (0.16 g, 0.27 mmol) in THF (6 mL) was added LiOH (1.50 mL of 1 M solution, 1.50mmol). The reaction mixture was heated in a microwave reactor at 130 °C for thirty minutes. The reaction was quenched by the addition of aqueous saturated NH_4Cl solution. The resulting white precipitate was collected and washed with water, acetonitrile and ether. The combined organic phases were then concentrated *in vacuo* to give pure desired carboxylic acid, **27**, as a solid. The solid was diluted with hydrochloric acid (2 mL of 1N solution) and lyophilized to give 110 mg of the desired racemic product as a hydrochloride salt (light yellow powder): ^{1}H NMR (300 MHz, MeOD) δ

8.73 (d, J = 9.5 Hz, 1H), 8.16 (s, 1H), 8.15 – 8.10 (m, 1H), 7.93 (d, J = 4.0 Hz, 1H), 5.02 (d, J = 6.4 Hz, 1H), 3.75 (ddd, J = 6.7, 4.2, 2.5 Hz, 3H), 2.66 (d, J = 11.2 Hz, 1H), 2.45 (dd, J = 14.0, 9.9 Hz, 1H), 1.93 – 1.83 (m, 3H), 1.46 (d, J = 7.5 Hz, 2H), 1.05 – 0.93 (m, 9H); ^{19}F NMR (282 MHz, MeOD) δ -139.17 (s, 1F), -160.86 (s, 1F); LCMS Gradient 10-90%, 0.1% formic acid, 5 minutes, C18/ACN, RT = 2.04 minutes (M+H) 390.23.

The racemic mixture was submitted to SFC chiral separation (15% EtOH 5mL min overnight ASH 4.6*100, 100 bar) to give the individual enantiomers, (R)-isomer **8**, and (S)-isomer **28**. HRMS data for (R)-isomer **8** (ESI) of $C_{19}H_{21}F_2N_5O_2$ [M+H] calcd, 390.17361; found 390.17347.

Synthetic Scheme 4: (*R*)-ethyl 3-((5-fluoro-2-(5-fluoro-1-tosyl-1*H*-pyrrolo[2,3-b]pyridin-3-yl)pyrimidin-4-yl)amino)-3-(1-methylcyclopentyl)propanoate (13).



a) LiHMDS, MeI, THF -78 ^{o}C; b) diisobutylaluminum hydride, CH_2Cl_2, -78 ^{o}C; c) malonic acid, ammonium acetate, ethanol, 80 ^{o}C; d) $^{i}Pr_2NEt$, **17**, THF, 80 ^{o}C; e) LiOH, THF- H_2O (3:1), 130 °C, microwave; f) SFC chiral separation

Formation of 1-methylcyclopentanecarbonitrile (29)

To a cold (-78 °C) solution of lithium bis(trimethylsilylsamide) (48.0 mL of 1 M solution in tetrahydrofuran, 48.0 mmol) in tetrahydrofuran was added dropwise a solution of cyclopentanecarbonitrile (3.81 g, 40.0 mmol) in tetrahydrofuran (10 mL) over a 5 minute period. After stirring at -78 °C for thirty minutes, methyl iodide (3.74 mL, 60.00 mmol) was added in one portion. The reaction was allowed to warm to room temperature overnight. The solution was cooled to 0 °C, ethyl acetate (50 mL) and aqueous saturated ammonium chloride solution (20 mL) was added. Additional water (10 mL) was added to dissolve the solid. The organic layer was separated and washed with aqueous saturated ammonium chloride (20 mL). The aqueous layer was extracted with ethyl acetate (2 X 20 mL). The combined organic phases were washed with brine, dried ($MgSO_4$), filtered and concentrated *in vacuo* to give a 4.7g of a yellow oil that was used without further purification: ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.04 - 1.93 (m, 2H), 1.77- 1.65 (m, 2H), 1.66 - 1.55 (m, 2H), 1.54 (m, 2H), 1.25 (s, 3H).

Formation of 1-methylcyclopentanecarbaldehyde (30)

To a cold (-78 °C) solution of diisobutylaluminum hydride (100.0 mL of 1 M solution, 100.0 mmol) in dichloromethane was added dropwise a solution of 1-methylcyclopentanecarbonitrile, **29**, (4.3 g, 40.0 mmol) in dichloromethane (5 mL). The reaction was kept at -78 °C for thirty minutes. The dry-ice bath was removed and methanol (1 mL) was added to quench the reaction. Potassium sodium tartrate solution (30 mL, 10% solution) was added and the mixture stirred vigorously. The organic layer

was separated and the aqueous layer was extracted with dichloromethane (3 X 20 mL). The combined organic phases were washed with brine, dried over sodium sulfate, filtered and concentrated *in vacuo* to give 3 g of a light yellow oil that was used without further purification: ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.04 - 1.93 (m, 2H), 1.77- 1.65 (m, 2H), 1.66 - 1.55 (m, *2*H), 1.54 (m, 2H), 1.25 (s, 3H).

Formation of ethyl 3-amino-3-(1-methylcyclopentyl)propanoate (31)

A mixture of 1-methylcyclopentanecarbaldehyde, **30**, (3.00 g, 26.75 mmol), malonic acid (1.29 mL, 20.00 mmol) and ammonium acetate (3.08 g, 40.00 mmol) in ethanol (5 mL) was refluxed for 12 hours. The precipitate was removed by filtration and washed with ethanol. The filtrate was used without further purification.

Sulfuric acid (1.07 mL, 20.00 mmol) was added to the above ethanol solution and heated to reflux for 2h. The solvent was removed under reduced pressure. The residue was diluted with water (20 mL) and ether (10 mL). The aqueous layer was separated and washed with ether (10 mL). The organic layers were discarded. The aqueous solution was neutralized with sodium hydroxide solution (6N) to basic, and extracted with ethyl acetate (3 x 10 mL). The combined organic layers were washed with water (10 mL), brine (10 mL), filtered, dried ($MgSO_4$), filtered and concentrated *in vacuo* to give 1.5 g of a light yellow sticky oil that turned into solid upon standing. The crude product was used without further purification: ^{1}H NMR (400 MHz, CDCl3) δ 4.25 – 4.14 (q, 2H), 3.40 (bs, 2H), 3.20 – 3.09 (m, 1H), 2.48 (ddd, *J* = 26.2, 16.0, 6.6 Hz, 2H), 1.77- 1.58 (m, 4H), 1.52 (m, 2H), 1.47 – 1.32 (m, 2H), 1.25 (m, 3H), 0.94 (s, 3H).

Formation of ethyl 3-((5-fluoro-2-(5-fluoro-1-tosyl-1*H*-pyrrolo[2,3-b]pyridin-3-yl)pyrimidin-4-yl)amino)-3-(1-methylcyclopentyl)propanoate (32)

A suspension of ethyl 3-amino-3-(1-methylcyclopentyl)propanoate, **31**, (0.20 g, 1.00 mmol), 5-fluoro-3-(5-fluoro-4-methylsulfinyl-pyrimidin-2-yl)-1-(*p*-tolylsulfonyl)-pyrrolo[2,3-b]pyridine, **17** (0.54 g, 1.20 mmol), and *N,N*-diisopropylethylamine (0.26 mL, 1.50 mmol) in THF (14.4 mL) was refluxed at 80 °C overnight. After removing the solvent *in vacuo*, the crude product was purified by silica gel chromatography (0-50% EtOAc/Hexanes gradient) to afford 300 mg of the desired product as a light yellow solid: ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.49 (dd, *J* = 9.0, 2.8 Hz, 1H), 8.46 (s, 1H), 8.23 (d, *J* = 1.5 Hz, 1H), 8.02 (d, *J* = 8.3 Hz, 2H), 7.99 (d, *J* = 3.1 Hz, 1H), 7.20 (d, *J* = 7.8 Hz, 2H), 5.23 (d, *J* = 8.9 Hz, 1H), 4.80 (td, *J* = 9.7, 3.6 Hz, 1H), 4.04 (q, *J* = 7.1 Hz, 1H), 3.91 (q, *J* = 7.1 Hz, 2H), 2.73 – 2.58 (m, 1H), 2.44 (dd, *J* = 14.7, 9.6 Hz, 1H), 2.33 – 2.21 (m, 3H), 1.72 – 1.46 (m, 7H), 1.42 – 1.31 (m, 1H), 1.28 (t, *J* = 6.1 Hz, 1H), 1.17 (dd, *J* = 13.4, 6.2 Hz, 2H), 0.98 (t, *J* = 7.1 Hz, 6H); LCMS Gradient 10-90%, 0.1% formic acid, 5 minutes, C18/ACN, RT = 4.25 minutes (M+H) 584.29.

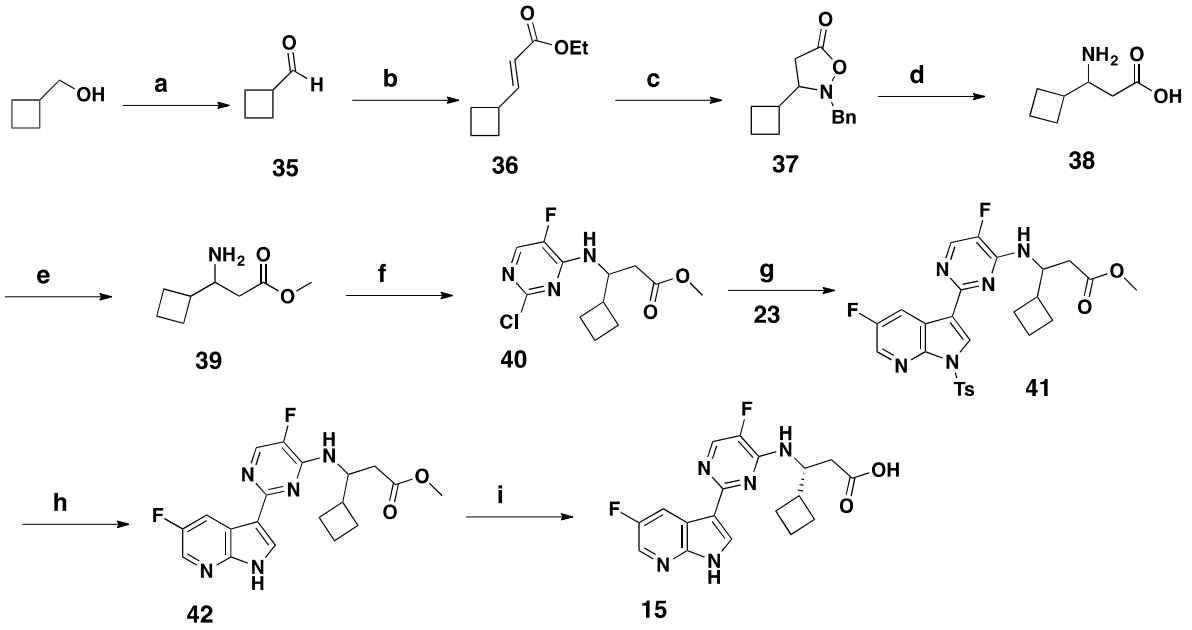
Formation of (*R*)-ethyl 3-((5-fluoro-2-(5-fluoro-1-tosyl-1*H*-pyrrolo[2,3-b]pyridin-3-yl)pyrimidin-4-yl)amino)-3-(1-methylcyclopentyl)propanoate (13).

To a solution of ethyl 3-((5-fluoro-2-(5-fluoro-1-tosyl-1*H*-pyrrolo[2,3-*b*]pyridin-3-yl)pyrimidin-4-yl)amino)-3-(1-methylcyclopentyl)propanoate, **32** (0.16 g, 0.27 mmol) in THF (6 mL) was added LiOH (1.50 mL of 1 M solution, 1.50 mmol). The reaction mixture was irradiated in a microwave reactor for 30 minutes at 130 °C. Aqueous saturated NH_4Cl solution was added to acidify the mixture. The resulting white precipitate was collected and washed with water, acetonitrile and ether. The solid was then dried *in vacuo* to give pure desired acid. To the solid was added hydrochloric acid (2 mL of 1N solution) and the mixture was lyophilized to give 120 mg of the carboxylic acid **33** as a hydrochloride salt (light yellow powder): ^{1}H NMR (400 MHz, MeOD) δ 8.64 (d, *J* = 9.3 Hz, 1H), 8.14 (d, *J* = 8.3 Hz, 2H), 7.97 (d, *J* = 3.6 Hz, 1H), 4.99 (d, *J* = 6.3 Hz, 1H), 3.37 (s, 1H), 2.75 (dd, *J* = 14.9, 3.6 Hz, 1H), 2.55 (dd, *J* = 14.8, 9.7 Hz, 1H), 1.83 – 1.57 (m, 6H), 1.54 – 1.42 (m, 1H), 1.37 (dd, *J* = 11.9, 5.6 Hz, 1H), 1.11 (d, *J* = 19.2 Hz, 3H); LCMS Gradient 10-90%, 0.1% formic acid, 5 minutes, C18/ACN, RT = 2.10 minutes (M+H) 401.94.

The racemic mixture of carboxylic acids was submitted to SFC chiral separation (15% EtOH 5mL min overnight ASH 4.6*100, 100 bar) to give the individual enantiomers, (R)-isomer **13**, and (S)-isomer

34. HRMS data for (R)-isomer **13** (ESI) of $C_{20}H_{21}F_2N_5O_2$ [M+H] calcd, 402.17361; found 402.17360. Chiral HPLC data for (R)-isomer **13** >98% ee; (S)-isomer **34** >98% ee.

Synthetic scheme 5: Formation of (+/-)-3-cyclobutyl-3-((5-fluoro-2-(5-fluoro-1*H*-pyrrolo[2,3-*b*]pyridin-3-yl)pyrimidin-4-yl)amino)propanoic acid (15).



(a) Pyridinium chlorochromate, CH_2Cl_2; (b) ethyl-2-triphenyl-phosphoranylideneacetate, CH_2Cl_2; (c) *N*-benzylhydroxylamine, Et_3N, CH_2Cl_2 (d) H_2, palladium hydroxide, EtOH (e) diazomethyl-trimethylsilane, MeOH, benzene (f) 2,4-dichloro-5-fluoro-pyrimidine, Et_3N, THF, EtOH (g) 5-fluoro-3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1-tosyl-1*H*-pyrrolo[2,3-b]pyridine, **23**, Aq. K_3PO_4, 2-Me-THF, H_2O, X-Phos, $Pd_2\{dba\}_3$; (h) MeOH, NaOMe (i) aq. NaOH, THF, MeOH

Formation of cyclobutanecarbaldehyde (35)

To a stirred suspension of pyridinium chlorochromate (14.9 g, 69.1 mmol) in dichloromethane (150 mL) was added a solution of cyclobutylmethanol (4.0 g, 46.4 mmol) in dichloromethane (60 mL). The reaction mixture turned black within a few minutes and was allowed to stir at room temperature for 1 hour. The mixture was diluted with diethyl ether (500 mL) and filtered through a bed of florisil (100-200 mesh). The crude material was used without further purification. Note: the product is volatile, the solvent was carried with the product onto the next step.

Formation of (*E*)-ethyl 3-cyclobutylacrylate (36)

Ethyl 2-triphenylphosphoranylideneacetate (9.32 g, 26.74 mmol) was added to a solution of cyclobutanecarbaldehyde, **35**, (1.50 g, 17.83 mmol) in dichloromethane (30 mL). The reaction mixture was briefly purged with nitrogen and capped allowed to stir at room temperature overnight. All volatiles were removed at reduced pressure and the residue was dissolved in Et_2O (100 mL) and hexanes (25mL). The resulting pink precipitate was filtered off and discarded. The solvent was removed from the filtrate at reduced pressure. The crude product was purified via silica gel chromatography (0-20% EtOAc/Hexanes gradient) to afford 646 mg (23%) of the desired product: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.05 (dd, J = 15.6, 6.8 Hz, 1H), 5.73 (dd, J = 15.6, 1.4 Hz, 1H), 4.29 – 4.09 (m, 2H), 3.20 – 2.98 (m, 1H), 2.28 – 2.09 (m, 2H), 2.04 – 1.78 (m, 4H), 1.36 – 1.18 (m, 3H).

Formation of 2-benzyl-3-cyclobutylisoxazolidin-5-one (37)

N-benzylhydroxylamine hydrochloride (0.77 g, 4.82 mmol) and triethylamine (0.76 mL, 5.45 mmol) were successively added to a solution of (*E*)-ethyl 3-cyclobutylacrylate, **36**, (0.65 g, 4.19 mmol) in dry dichloromethane (23.5 mL). The reaction mixture was allowed to stir at room temperature under an atmosphere of nitrogen for 3 days. The mixture was diluted with 75 mL of water and the layers were separated. The aqueous phase was reextracted twice more with dichloromethane (50 mL). The combined organic phases were dried over $MgSO_4$, filtered and evaporated to dryness. The residue was purified via silica gel chromatography (0-100% EtOAc/Hexanes gradient) to afford 834 mg (86%) of the desired product: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.38 – 7.26 (m, 5H), 4.64 (s, 1H), 3.82 (q, J = 13.5 Hz, 2H), 3.37 – 3.18 (m, 1H), 2.80 – 2.52 (m, 2H), 2.33 (dd, *J* = 14.5, 5.1 Hz, 1H), 2.22 – 2.09 (m, 1H), 2.01 – 1.68 (m, 5H).

Formation of (+/-)-3-amino-3-cyclobutylpropanoic acid (38)

Dihydroxypalladium (0.252 g, 1.794 mmol) was charged into a flask and flushed with nitrogen. Ethanol (30 mL) was added followed by a solution of 2-benzyl-3-cyclobutyl-isoxazolidin-5-one, **37**, (0.834 g, 3.605 mmol) in approximately 90 mL of ethanol. The reaction mixture was subjected to 50 psi of hydrogen for 4 hours. The pressure was vented and the catalyst was filtered off. All volatiles were removed at reduced pressure. ^{1}H NMR shows the presence of starting material, **37**. The mixture was dissolved in approximately 100 mL of MeOH and added to 83 mg of 10%Pd/C that had been wet with 20 mL of MeOH. The mixture was subjected to 50 psi of H_2 overnight. The pressure was vented and the catalyst was filtered off. All volatiles were removed at reduced pressure to afford 340 mg of product. The resulting crude residue was used without further purification: ^{1}H NMR (400 MHz, *d*6-DMSO) δ 3.06 – 2.83 (m, 1H), 2.28 (ddd, *J* = 23.7, 11.8, 7.7 Hz, 1H), 2.19 – 1.99 (m, 2H), 1.99 – 1.56 (m, 6H).

Formation of (+/-)-methyl 3-amino-3-cyclobutylpropanoate (39)

To a solution of racemic 3-amino-3-cyclobutyl-propanoic acid, **38**, (0.34 g, 2.38 mmol) in MeOH (10.2 mL) and benzene (10.2 mL) was added diazomethyltrimethyl-silane (3.56 mL of 2 M solution, 7.13 mmol) and the reaction mixture was allowed to stir at room temperature under a nitrogen atmosphere overnight. The mixture was diluted with EtOAc and brine. The layers were separated and the organic phase was dried ($MgSO_4$), filtered and concentrated *in vacuo* to afford 354 mg (95%) of crude product that was used without further purification: ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.71 – 3.66 (m, 3H), 3.18 – 2.98 (m, 1H), 2.46 – 2.32 (m, 2H), 2.27 – 1.63 (m, 10H).

Formation of methyl (+/-)-3-((2-chloro-5-fluoropyrimidin-4-yl)amino)-3-cyclobutylpropanoate (40)

To a racemic solution of methyl 3-amino-3-cyclobutylpropanoate, **39**, (0.354 g, 2.252 mmol) and 2,4-dichloro-5-fluoro-pyrimidine (0.414 g, 2.477 mmol) in THF (10 mL) and ethanol (1 mL) was added triethylamine (0.628 mL, 4.504 mmol). The reaction mixture was heated and stirred at 70 ^{o}C for 5 hours. The mixture was filtered and the filtrate was concentrated *in vacuo* to approximately 5 mL final volume. The crude residue was purified via silica gel chromatography (0 -100% EtOAC/hexanes gradient) to afford 289 mg (45%) of the desired product: ^{1}H NMR (300 MHz, $CDCl^3$) δ 7.87 (s, 1H), 5.80 (s, 1H), 4.71 – 4.38 (m, 1H), 3.68 (s, 3H), 2.84 – 2.37 (m, 3H), 2.23 – 1.67 (m, 6H); LCMS Gradient 10-90%, 0.1% formic acid, 5 minutes, C18/ACN, RT = 3.08 minutes (M+H) 287.98.

Formation of (+/-)-3-cyclobutyl-3-((5-fluoro-2-(5-fluoro-1-tosyl-1*H*-pyrrolo[2,3-*b*]pyridin-3-yl)pyrimidin-4-yl)amino)propanoate (41)

A solution of tripotassium phosphate (0.640 g, 3.021 mmol) in water (1.735 mL) was added to a solution of racemic methyl 3-[(2-chloro-5-fluoro-pyrimidin-4-yl)amino]-3-cyclobutyl-propanoate, **40**, (0.289 g, 1.005 mmol) in 2-methyltetrahydrofuran (5.782 mL). The mixture was then purged with nitrogen for 20 minutes. 5-fluoro-1-(*p*-tolylsulfonyl)-3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)pyrrolo[2,3-b]pyridine, **23**, (0.460 g, 1.106 mmol) was added and the mixture was purged with nitrogen for an additional 10 minutes. Dicyclohexyl-[2-(2,4,6-triisopropylphenyl)phenyl]phosphane (X-Phos: 0.029 g, 0.060 mmol) and $Pd_2\{dba\}_3$ (0.018 g, 0.020 mmol) were added and the reaction mixture was warmed to 80 °C and stirred at this temperature for 5 hours. The mixture was allowed to cool to room temperature. The reaction mixture was diluted with water and extracted with EtOAc. The layers were separated and the organic phase was washed with brine, dried over $MgSO_4$, filtered and evaporated to dryness. The crude was dissolved in a minimum volume of dichloromethane and purified via silica gel chromatography (0-100%EtOAc/Hexanes).to afford 385 mg (71%) of the desired product: LCMS Gradient 10-90%, 0.1% formic acid, 5 minutes, C18/ACN, RT = 3.68 minutes (M+H) 542.27.

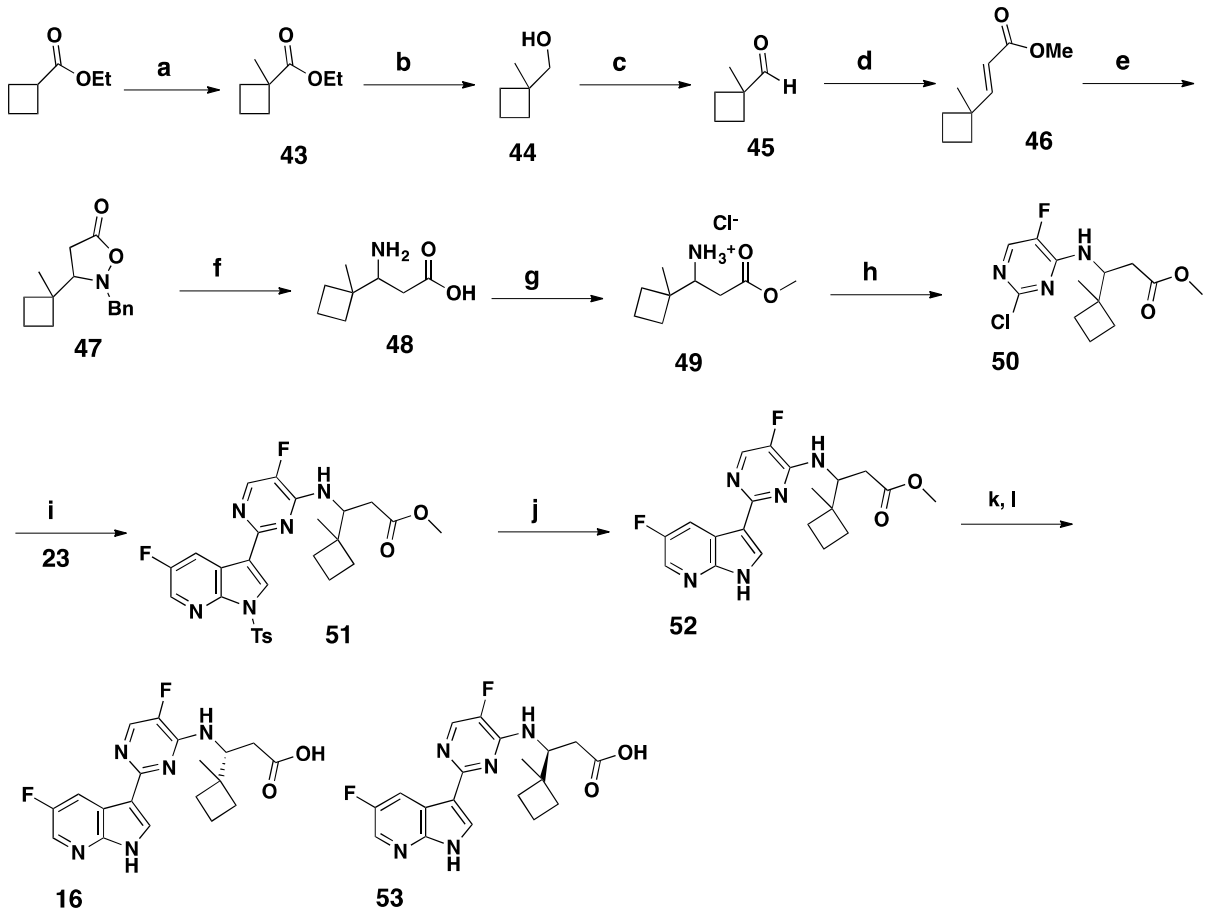
Formation of (+/-)-methyl 3-cyclobutyl-3-((5-fluoro-2-(5-fluoro-1*H*-pyrrolo[2,3-*b*]pyridin-3-yl)pyrimidin-4-yl)amino)propanoate (42)

To a racemic solution of methyl 3-cyclobutyl-3-((5-fluoro-2-(5-fluoro-1-tosyl-1*H*-pyrrolo[2,3-*b*]pyridin-3-yl)pyrimidin-4-yl)amino)propanoate, **39**, (0.151 g, 0.280 mmol) in methanol (1.5 mL) was added NaOMe (1.5 mL of 25 %w/v solution, 6.941 mmol). After stirring the reaction mixture at room temperature for 5 minutes, the mixture was quenched with aqueous saturated NH_4Cl solution and diluted with EtOAc and water. The layers were separated and the organic phase was washed with brine, dried ($MgSO_4$), filtered and evaporated to dryness. The resulting crude residue was dissolved in a minimum volume of dichloromethane and purified via silica gel chromatography (0-100%EtOAc/Hexanes gradient) to afford 108 mg of the desired product: LCMS Gradient 10-90%, 0.1% formic acid, 5 minutes, C18/ACN, RT = 2.29 minutes (M+H) 388.07.

Formation of (+/-)-3-cyclobutyl-3-((5-fluoro-2-(5-fluoro-1*H*-pyrrolo[2,3-*b*]pyridin-3-yl)pyrimidin-4-yl)amino)propanoic acid (15).

To a racemic solution of methyl 3-cyclobutyl-3-((5-fluoro-2-(5-fluoro-1*H*-pyrrolo[2,3-*b*]pyridin-3-yl)pyrimidin-4-yl)amino)propanoate, **42** (0.042 g, 0.109 mmol) in THF (1.5 mL) and MeOH (0.5 mL) was added NaOH (0.300 mL of 2 M solution, 0.600 mmol) and the reaction mixture was warmed to 50 °C. After stirring the reaction mixture for 1 hour, the mixture was diluted with aqueous saturated NH_4Cl solution and EtOAc. The organic layer was dried ($MgSO_4$), filtered and evaporated to dryness to afford 36 mg of the desired product that was used without further purification: ^{1}H NMR (400 MHz, *d*6-DMSO) δ 12.26 (s, 2H), 8.55 (d, *J* = 9.7 Hz, 1H), 8.19 (dd, *J* = 45.1, 15.8 Hz, 3H), 7.48 (d, *J* = 8.1 Hz, 1H), 4.79 (s, 1H), 2.58 (dd, *J* = 20.6, 12.2 Hz, 2H), 1.85 (ddd, *J* = 29.4, 26.5, 21.1 Hz, 7H); LCMS Gradient 10-90%, 0.1% formic acid, 5 minutes, C18/ACN, RT = 2.10 minutes (M+H) 374.02; HRMS data for **14** (ESI) of $C_{18}H_{17}F_2N_5O_2$ [M+H] calcd, 374.14231; found 374.14211.

Synthetic Scheme 6 Formation of (*R*)-3-((5-fluoro-2-(5-fluoro-1*H*-pyrrolo[2,3-*b*]pyridin-3-yl)pyrimidin-4-yl)amino)-3-(1-methylcyclobutyl)propanoic acid (16).



(a) LDA, MeI, THF; (b) $LiAlH_4$, ether; (c) PCC, CH_2Cl_2; (d) 2-(triphenylphosphoran-ylidene)acetate, CH_2Cl_2; (e) *N*-benzylhydroxylamine-HCl, CH_2Cl_2; (f) H_2, Pd/C, MeOH; (g) AcCl, MeOH, reflux; (h) 2,4-dichloro-5-fluoropyrimidine, Et_3N, EtOH, THF, 55 ^oC; (i) 5-fluoro-1-(*p*-tolylsulfonyl)-3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)pyrrolo-[2,3-b]pyridine, **23**, $Pd_2(dba)_3$, XPhos, K_3PO_4, 2-MeTHF, H_2O, 115 ^oC; (j) HCl, dioxane, acetonitrile, 65^oC; (k) LiOH, THF, H_2O, 50^oC; (l) SFC chiral separation

Formation of ethyl 1-methylcyclobutanecarboxylate (43)

A solution of ethyl cyclobutanecarboxylate (20.0 g, 156.0 mmol) in THF (160 mL) was added dropwise to a cold (-78 ^oC) solution of LDA (164 mmol of 2M solution) in THF (40 mL). The solution was warmed to 0 ^oC and then cooled again to -40 ^oC before the addition of iodomethane (10.2 mL, 163.8 mmol). The solution was slowly warmed to room temperature and stirred overnight. The reaction was quenched with an aqueous saturated solution of ammonium chloride and ether was added. The layers were separated and the aqueous layer was washed with ether. The combined organic layers were washed with 1N HCl then dried over $MgSO_4$. The product was purified by distillation: ^1H NMR (400 MHz, MeOD) δ 4.20 – 4.05 (m, 2H), 2.57 – 2.33 (m, 2H), 2.08 – 1.94 (m, 1H), 1.94 – 1.77 (m, 3H), 1.40 (s, 3H), 1.27 (tt, J = 7.1, 1.5 Hz, 3H).

Formation of (1-methylcyclobutyl)methanol (44)

Lithium aluminum hydride (2.1 g, 59.4 mmol) was suspended in ether (150 mL) and cooled to 0 ^oC. A solution of ethyl 1-methylcyclobutanecarboxylate, **43**, (13.0 g, 91.4 mmol) in ether (60 mL) was added dropwise to the $LiAlH_4$ suspension. The mixture was stirred 2 hours in an ice bath then quenched slowly with 1N HCl. The layers were separated and the aqueous layer was washed with ether. The combined organic layers were washed with brine and the volatiles were removed with a gentle stream of nitrogen to afford the desired product that was used without further purification: ^1H NMR (400 MHz,

$CDCl_3$) δ 3.54 – 3.39 (m, 4H), 1.99 – 1.74 (m, 8H), 1.74 – 1.62 (m, 4H), 1.46 – 1.18 (m, 3H), 1.13 (d, *J* = 1.7 Hz, 6H).

Formation of 1-methylcyclobutanecarbaldehyde (45) and methyl 3-(1-methylcyclobutyl)acrylate (46)

A solution of (1-methylcyclobutyl)methanol, **43**, (1.00 g, 9.98 mmol) in dichloromethane (25 mL) was added to a suspension of PCC (2.69 g, 12.50 mmol) and Celite (2.70 g) in dichloromethane (25 mL). The reaction mixture was stirred 2 hours and filtered through a pad of silica gel (eluting with dichloromethane). The solvents were removed with a stream of nitrogen until volume was approximately 20 mL. 2-(triphenyl-phosphoranylidene)acetate (0.98 g, 10.00 mmol) was added in one portion and the mixture was stirred for 7 hours. The volatiles were removed under reduced pressure and a solution of 10% Hexanes/ether was added. The resulting solid was filtered off and discarded. The resulting solution was poured directly on silica gel and eluted with EtOAc/Hexanes to afford the desired product: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.05 (d, *J* = 15.8 Hz, 1H), 5.66 (dd, *J* = 15.8, 1.3 Hz, 1H), 4.21 – 4.00 (m, 2H), 2.12 – 1.73 (m, 7H), 1.29 – 1.17 (m, 6H).

Formation (+/-)-2-benzyl-3-(1-methylcyclobutyl)isoxazolidin-5-one (47)

N-benzylhydroxylamine (hydrochloric acid) (0.28 g, 1.80 mmol) and triethylamine (0.28 mL, 2.00 mmol) were added to a solution of methyl 3-(1-methylcyclobutyl)acrylate, **46**, (0.26 g, 1.50 mmol) in dichloromethane (9.5 mL). The reaction mixture was stirred at 50 ^{o}C overnight. The reaction mixture was cooled to room temperature and the mixture was diluted with dichloromethane and water. The layers were separated with a phase separator and the aqueous layer was washed with dichloromethane. The organic layers were combined and the volatiles removed under reduced pressure. The residue was purified on silica gel (EtOAc/Hexanes) to afford the desired product as a racemic mixture: LCMS Gradient 10-90%, 0.1% formic acid, 5 minutes, C18/ACN, RT = 1.47 minutes (M+H) 246.10.

Formation of (+/-)-3-amino-3-(1-methylcyclobutyl)propanoic acid (48)

A solution of racemic 2-benzyl-3-(1-methylcyclobutyl)isoxazolidin-5-one, **47**, (0.18 g, 1.28 mmol) in MeOH (2.9 mL) was shaken overnight under 50 psi hydrogen in the presence of 50 mg palladium hydroxide catalyst. The mixture was filtered through Celite and the volatiles were removed under reduced pressure to afford the desired product that was used without further purification: ^{1}H NMR (400 MHz, MeOD) δ 3.42 (dd, *J* = 11.0, 1.9 Hz, 1H), 2.26 (ddd, *J* = 27.8, 16.7, 6.5 Hz, 2H), 1.86 (dddd, *J* = 36.9, 26.3, 11.2, 7.6 Hz, 6H), 1.18 (s, 3H).

Formation of (+/-)-methyl 3-((2-chloro-5-fluoropyrimidin-4-yl)amino)-3-(1-methylcyclobutyl)propanoate (50)

Racemic 3-amino-3-(1-methylcyclobutyl)propanoic acid, **48**, (2.3 g, 14.4 mmol) was dissolved in methanol (104 mL). The solution was cooled in an ice bath and acetyl chloride (5.6 g, 71.9 mmol) was added dropwise (Temp kept <10 ^{o}C). The reaction mixture was heated to 65 ^{o}C and stirred at that temperature for 3 hours. The reaction mixture was cooled to room temperature and then flushed with toluene to remove volatiles. Crude racemic 3-methoxy-1-(1-methylcyclobutyl)-3-oxopropan-1-aminium chloride, **49**, was used without further purification.

Racemic 3-methoxy-1-(1-methylcyclobutyl)-3-oxopropan-1-aminium chloride, **49**, (3.3 g, 15.9 mmol) was dissolved in a mixture of 59 mL THF and 6.6 mL EtOH and the solution was cooled in an ice bath. 2,4-Dichloro-5-fluoro-pyrimidine (2.9 g, 18.0 mmol) was added followed by dropwise addition of triethylamine (5.1 g, 51.0 mmol). The reaction mixture was stirred at 55 ^{o}C for 17 hours. The reaction mixture was cooled to room temperature after which water and dichloromethane were added. The phases were separated and the aqueous layer was washed with dichloromethane. The

organic layers were combined and washed with brine. The solvents were removed and the residue was purified via silica gel chromatography (EtOAc/Hexanes) to afford the desired product: LCMS Gradient 10-90%, 0.1% formic acid, 5 minutes, C18/ACN, RT = 3.23 minutes (M+H) 302.35.

Formation of (+/-)-methyl 3-((5-fluoro-2-(5-fluoro-1-tosyl-1*H*-pyrrolo[2,3-b]pyridin-3-yl)pyrimidin-4-yl)amino)-3-(1-methylcyclobutyl)propanoate (51)

A solution of 5-fluoro-1-(*p*-tolylsulfonyl)-3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)pyrrolo[2,3-b]pyridine, **23**, (3.31 g, 7.95 mmol), racemic methyl 3-((2-chloro-5-fluoropyrimidin-4-yl)amino)-3-(1-methylcyclobutyl)propanoate, **50**, (2.00 g, 6.63 mmol) and K_3PO_4 (4.22 g, 20.00 mmol) in 2-MeTHF (253 mL) and water (56 mL) was purged with nitrogen for 0.75 h. 2-dicyclohexylphosphino-2′,4′,6′-triisopropylbiphenyl (XPhos) (0.38 g, 0.80 mmol) and $Pd_2(dba)_3$ (0.15 g, 0.17 mmol) were added and the reaction mixture was stirred at 115 ^oC in a sealed tube for 2 hours. The reaction mixture was cooled and the aqueous phase was removed. The organic phase was filtered through a pad of Celite and the mixture was concentrated to dryness. The residue was purified via silica gel chromatography (EtOAc/Hexanes) to afford the desired product: LCMS Gradient 10-90%, 0.1% formic acid, 5 minutes, C18/ACN, RT = 2.32 minutes (M+H) 556.44.

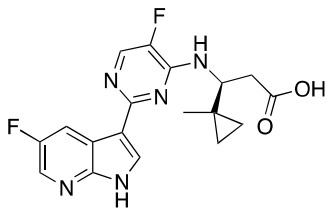
Formation of (+/-)-methyl 3-((5-fluoro-2-(5-fluoro-1*H*-pyrrolo[2,3-b]pyridin-3-yl)pyrimidin-4-yl)amino)-3-(1-methylcyclobutyl)propanoate (52)

To a racemic solution of methyl 3-((5-fluoro-2-(5-fluoro-1-tosyl-1*H*-pyrrolo[2,3-b]pyridin-3-yl)pyrimidin-4-yl)amino)-3-(1-methylcyclobutyl)propanoate**, 51,** (3.3 g, 5.9 mmol) in acetonitrile (25 mL) was added HCl (26 mL of 4N solution in dioxane). The reaction mixture was heated to 65 ^oC for 4 hours. The solution was cooled to room temperature and the solvents were removed under reduced pressure. The mixture was flushed with acetonitrile after which aqueous sodium bicarbonate and ethyl acetate were added. The phases were separated and the aqueous layer washed with ethyl acetate. The combined organic phases were dried with Na_2SO_4, filtered and concentrated *in vacuo*. The resulting residue was purified via silica gel chromatography (EtOAc/Hexanes) to afford the desired product: LCMS Gradient 10-90%, 0.1% formic acid, 5 minutes, C18/ACN, RT = 2.34 minutes (M+H) 403.11.

Formation of 3-((5-fluoro-2-(5-fluoro-1*H*-pyrrolo[2,3-b]pyridin-3-yl)pyrimidin-4-yl)amino)-3-(1-methylcyclobutyl)propanoic acid (16 and 53)

To a solution of methyl 3-((5-fluoro-2-(5-fluoro-1*H*-pyrrolo[2,3-b]pyridin-3-yl)pyrimidin-4-yl)amino)-3-(1-methylcyclobutyl)propanoate, **52,** (1.75 g, 4.36 mmol) in THF (25 mL) was added aqueous 1N LiOH (13.1 mL). The mixture was heated to 50 ^oC for 3.5 hours. The reaction mixture was cooled to room temperature and diluted with water. The THF was removed under reduced pressure and the residue was then flushed twice with hexanes. Ether was added and the layers separated (the ether layer was discarded). The pH was adjusted to 5.5 with 1N HCl and the resulting solid was filtered and washed with water. The solid was flushed with heptanes and dried over P_2O_5 to give the desired product: ^1H NMR (400 MHz, DMSO) δ 12.17 (d, J = 60.2 Hz, 2H), 8.59 (d, J = 8.4 Hz, 1H), 8.39 – 8.05 (m, 3H), 7.52 (s, 1H), 5.00 (s, 1H), 2.23 (d, J = 7.7 Hz, 1H), 2.00 (s, 1H), 1.81 (d, J = 48.3 Hz, 2H), 1.62 (s, 1H), 1.46 (s, 1H), 1.21 (s, 3H); LCMS Gradient 10-90%, 0.1% formic acid, 5 minutes, C18/ACN, RT = 2.08 minutes (M+H) 388.46. The racemic mixture was submitted to SFC chiral separation (15% EtOH 5mL overnight ASH 4.6*100, 100 bar) to obtain the individual enantiomers, **16** and **53**; HRMS data for (R)-isomer **6** (ESI) of $C_{19}H_{19}F_2N_5O_2$ [M+H] calcd, 388.15796; found 388.15801. Chiral HPLC purity: (R)-isomer 99%ee; (S)-isomer > 99%ee.

The following compound **14** was prepared in the same fashion as described above for **16**:

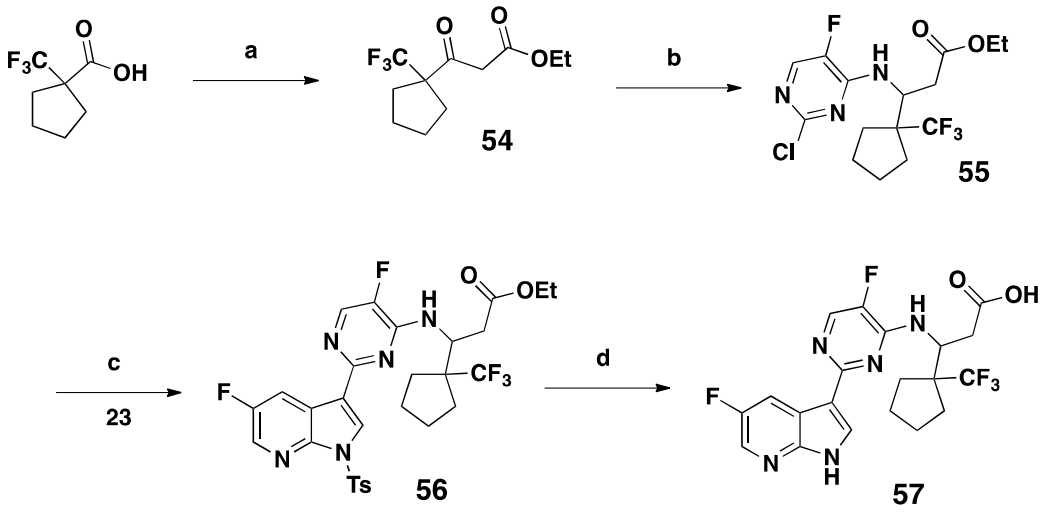


14

(3R)-3-[[5-fluoro-2-(5-fluoro-1H-pyrrolo[2,3,b]pyridin-3-yl)pyrimidin-4-yl]amino]-3-(1-methylcyclopentyl)propanoic acid (14).

Resolved by SFC separation (10% MeOH, 90%CO_2 10mL min, 100 bar) peak 1: 15.29 min; 100% ee. ^{1}H NMR (300 MHz, MeOD) δ 8.59 (dd, J = 9.6, 2.9 Hz, 1H), 8.15 (d, J = 2.7 Hz, 2H), 8.01(d, J = 4.1 Hz, 1H), 4.60 (dd, J = 8.3, 6.0 Hz, 1H), 2.90 – 2.68 (m, 2H), 1.17 (s, 3H), 0.85(dt, J = 9.7, 6.7 Hz, 1H), 0.64 (dt, J = 9.4, 4.9 Hz, 1H), 0.47 – 0.33 (m, 1H), 0.27 (ddd, J =21.3, 12.8, 10.1 Hz, 1H); LCMS $[M + H]^+$ = 374.42; t_R = 1.95 min (10-90% CH_3CN-water with 0.1% formic acid; 5 min); HRMS data for (R)-isomer **14** (ESI) of $C_{18}H_{17}F_2N_5O_2$ [M+H] calcd, 374.14231; found 374.14196.

Synthetic Scheme 7: Formation of (+/-)-3-(5-fluoro-2-(5-fluoro-1*H*-pyrrolo[2,3-*b*]pyridin-3-yl)pyrimidin-4-ylamino)-3-(1-(trifluoromethyl)cyclopentyl)propanoic acid (57).



(a) i. carbonyl diimidazole, CH_2Cl_2; ii. potassium ethyl malonate, $MgCl_2$, dimethylaminopyridine (DMAP), Et_3N, THF, CH_3CN; (b) i. ammonium acetate, EtOH, reflux; ii. sodium cyanoborohydride, AcOH, EtOAc; iii. 2,4-dichloro-5-fluoropyrimidine, $^iPr_2NEt$, EtOH; (c) 5-fluoro-1-(*p*-tolylsulfonyl)-3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)pyrrolo[2,3-b]pyridine, **23**, X-phos, $Pd_2(dba)_3$, K_3PO_4, 2-methyl THF, H_2O, 135 ^oC, microwave; (d) LiOH, MeOH, 65 ^oC.

Formation of ethyl 3-oxo-3-(1-(trifluoromethyl)cyclopentyl)propanoate (54).

To a solution of 1-(trifluoromethyl)cyclopentanecarboxylic acid (1.30 g, 7.14 mmol) in dichloromethane (14 mL) was added carbonyl diimidazole (5.46 g, 33.68 mmol). After stirring 5 hours at room temperature, the reaction was concentrated *in vacuo* to a residue.

In another flask, 3-ethoxy-3-oxo-propanoate (Potassium Ion) (2.03 g, 11.90 mmol) was mixed with dichloromagnesium (1.13 g, 11.90 mmol) and dimethylaminopyridine (72.65 mg, 0.59 mmol) in THF (23.13 mL) and acetonitrile (11.57 mL). After 3 hours, the above crude solution in THF (10 mL) was added, followed by triethylamine (1.66 mL, 11.90 mmol). The reaction was allowed to stir at 25 ^{o}C for 8 hours. The crude product was isolated by extracting into ethyl acetate (2 x 100 mL) vs 1N HCl (100 mL), dried over sodium sulfate and concentrated *in vacuo* to afford 1.0 g of the desired product as a yellow oil: ^{1}H NMR (300 MHz, $CDCl_3$) □ 12.58 (s, H), 5.32 (s, H), 4.27 - 4.18 (m, 2 H), 2.33 - 2.14 (m, 2 H), 2.05 - 1.85 (m, 4 H), 1.77 - 1.69 (m, 2 H) and 1.30 (td, *J* = 7.1, 3.2 Hz, 3 H) ppm.

Formation of (+/-)-ethyl 3-(2-chloro-5-fluoropyrimidin-4-ylamino)-3-(1-(trifluoromethyl)-cyclopentyl)propanoate (55)

A solution of ethyl 3-oxo-3-(1-(trifluoromethyl)cyclopentyl)propanoate, **54**, (0.500 g, 1.982 mmol) and ammonium acetate (0.458 g, 5.946 mmol) in EtOH (20 mL) was warmed to reflux for 3 hours. The crude reaction was concentrated *in vacuo* to a residue and redissolved in EtOAc (20 mL). The new mixture was cooled to 0 ^{o}C, and acetic acid (0.338 mL, 5.946 mmol) and sodium cyanoborohydride (0.498 g, 7.928 mmol, 4 equiv) were added to the mixture. The reaction was allowed to warm to room temperature and stirred overnight. The reaction was quenched with aqueous saturated sodium bicarbonate solution (10 mL) and extracted with ethyl acetate (2 x 20 mL). The organic phase was concentrated *in vacuo* and redissolved in EtOH (20 mL). To the solution was added 2,4-dichloro-5-fluoro-pyrimidine (0.496 g, 2.973 mmol) and *N,N*-diisopropylethylamine base (2.0 mL). The reaction was refluxed for 12 hours and then concentrated *in vacuo*. The residue was purified by silica gel chromatography (EtOAc) yielding 84 mg of the desired product as a yellow oil: LCMS Gradient 10-90%, 0.1% formic acid, 5 minutes, C18/ACN, Retention Time = 3.54 minutes (M+H) 384.40.

Formation of (+/-)-ethyl 3-(5-fluoro-2-(5-fluoro-1-tosyl-1*H*-pyrrolo[2,3-*b*]pyridin-3-yl)pyrimidin-4-ylamino)-3-(1-(trifluoromethyl)cyclopentyl)propanoate (56)

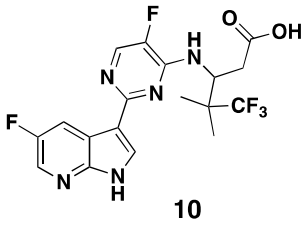
To a solution of racemic ethyl 3-(2-chloro-5-fluoropyrimidin-4-ylamino)-3-(1-(trifluoromethyl)cyclopentyl)propanoate, **55**, (0.084 g, 0.219 mmol) in THF (10 mL) and water (1 mL) was added 5-fluoro-1-(*p*-tolylsulfonyl)-3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)pyrrolo[2,3-b]pyridine, **23**, (0.137 g, 0.328 mmol) and potassium phosphate (0.140 g, 0.657 mmol). The resulting mixture was degassed under a stream of nitrogen for 10 minutes. To the reaction was then added X-Phos (0.010 g, 0.021 mmol) and $Pd_2(dba)_3$ (0.010 g, 0.011 mmol). The reaction was irradiated for 15 minutes at 135 °C in a microwave. The resulting mixture was concentrated *in vacuo* to a brown oil which was purified by silica gel chromatography ($EtOAc/CH_2Cl_2$) to afford 80 mg of the desired product as a pale yellow solid: LCMS Gradient 10-90%, 0.1% formic acid, 5 minutes, C18/ACN, Retention Time = 4.22 minutes (M+H) 638.42.

Formation of (+/-)-3-(5-fluoro-2-(5-fluoro-1*H*-pyrrolo[2,3-*b*]pyridin-3-yl)pyrimidin-4-ylamino)-3-(1-(trifluoromethyl)cyclopentyl)propanoic acid (57)

To a solution of racemic ethyl 3-(5-fluoro-2-(5-fluoro-1-tosyl-1*H*-pyrrolo[2,3-*b*]pyridin-3-yl)pyrimidin-4-ylamino)-3-(1-(trifluoromethyl)cyclopentyl)propanoate, **56**, (0.080 g, 0.120 mmol) in THF (10 mL) was added lithium hydroxide (2 mL of 2N solution). The reaction was refluxed for 3 hours and cooled to room temperature. The non aqueous solvent was removed under reduced pressure

and the aqueous layer was adjusted to pH 4. The aqueous layer was extracted with ethyl acetate (2 x 20 mL). The combined organic phases concentrated in vacuo to afford 16 mg of the desired product as a pale yellow solid: ^{1}H NMR (300 MHz, *d*6-DMSO) □ 8.51 (s, H), 8.25 - 7.97 (m, 2 H), 7.58 - 7.42 (m, 2 H), 7.12 (d, *J* = 7.5 Hz, H), 4.35 (m, H), 2.85 (m, 2 H) and 1.27 - 0.70 (m, 8 H) ppm; LCMS Gradient 10-90%, 0.1% formic acid, 5 minutes, C18/ACN, Retention Time = 2.55 minutes (M+H) 456.45.

The following analogs can be prepared in a similar fashion as the procedure described above:

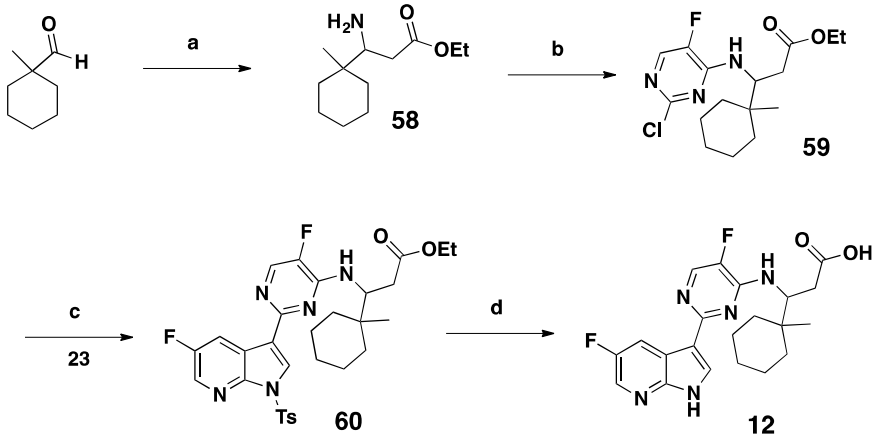


10

(+/-)-5,5,5-Trifluoro-3-(5-fluoro-2-(5-fluoro-1*H*-pyrrolo[2,3-*b*]pyridin-3-yl)pyrimidin-4-ylamino)-4,4-dimethylpentanoic acid (10)

^{1}H NMR (300 MHz, MeOD) □ 8.66 (d, *J* = 8.9 Hz, H), 8.29 (s, H), 8.22 - 8.18 (m, 2 H), 4.16 - 4.06 (m, H), 2.97 (s, H), 2.92 (s, H), and 1.27 - 1.21 (m, 6 H) ppm; LCMS Gradient 10-90%, 0.1% formic acid, 5 minutes, C18/ACN, Retention Time = 2.22 minutes (M+H) 430.41; HRMS (ESI) of $C_{18}H_{16}F_2N_5O_2$ [M+H] calcd, 430.12969; found 430.12978.

Synthetic Scheme 8: (+/-) 3-(5-fluoro-2-(5-fluoro-1*H*-pyrrolo[2,3-*b*]pyridin-3-yl)pyrimidin-4-ylamino)-3-(1-methylcyclohexyl)propanoic acid (12)



(a) NH_4OAc, malonic acid, EtOH, reflux; (b) 2,4-dichloro-5-fluoropyrimidine, ^{i}Pr$_2$NEt, THF, MeOH, 95 ^{o}C; (c) 5-fluoro-1-(*p*-tolylsulfonyl)-3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)pyrrolo[2,3-b]pyridine (**23**) K_3PO_4 X-Phos, $Pd_2(dba)_3$, 2-MeTHF, water, 120 ^{o}C; (d) 4N HCl, CH_3CN, 65 ^{o}C; (e) LiOH, water, THF.

Formation of (+/-)-ethyl-3-amino-3-(1-methylcyclohexyl)propanoate (58)

A solution of 1-methylcyclohexanecarbaldehyde (2.75 g, 21.79 mmol), malonic acid (2.27 g, 21.79 mmol) and ammonium acetate (3.36 g, 43.58 mmol) in absolute ethanol (5 mL) was heated at

reflux for 4 hours. The solid was filtered and washed with ethanol (10 mL). The filtrate was concentrated *in vacuo* to give a thick oil that was diluted with CH_2Cl_2 (50 mL). The precipitated solid was filtered and the filtrate was concentrated *in vacuo* to afford 4.3 grams of a yellow oil. Concentrated sulfuric acid (1.16 mL, 21.79 mmol) was added to a solution of the crude material in absolute ethanol (25 mL) and the mixture was refluxed for 12 hours. The solution was cooled to room temperature and concentrated *in vacuo* to give a thick oil. Water (10 mL) was added and the solution was neutralized with 2N NaOH. The aqueous layer was extracted with EtOAc (3x 25 mL), dried ($MgSO_4$), filtered and concentrated in vacuo to afford 2.4 grams of desired product: LCMS Gradient 10-90%, 0.1% formic acid, 5 minutes, C18/ACN, Retention Time = 1.54 minutes (M+H) 214.14.

Formation of (+/-)-ethyl 3-(2-chloro-5-fluorropyrimidin-4-ylamino)-3-(1-methylcyclohexyl) propanoate (59)

A mixture of 2,4-dichloro-5-fluoro-pyrimidine (1.83 g, 85.33 mmol), racemic ethyl-3-amino-3-(1-methylcyclohexyl)propanoate, **58,** (2.34 g, 11.0 mmol) and *N,N*-diisopropylethylamine (4.79 g, 27.50 mmol) in THF (40 mL) and methanol (10 mL) was heated at 95 ^oC for 3 hours. The solution was cooled to room temperature and the solvent was evaporated under reduced pressure. The crude residue was purified by silica gel chromatography (0-60% EtOAc/Hexanes gradient) to afford 620 mg of the desired product as a white foamy solid: ^1H NMR (400 MHz, $CDCl_3$) δ 7.80 (d, J = 2.6 Hz, 1H), 5.37 (m, 1H), 4.59 (m, 1H), 4.00 (q, 7.2 Hz, 2H), 2.62 (dd, J = 14.7, 3.8 Hz, 1H), 1.67(m,1H),1.17 (m, 10H), 1.10 (t, J = 7.1 Hz, 3H), 0.85 (s, 3H); LCMS Gradient 10-90%, 0.1% formic acid, 5 minutes, C18/ACN, Retention Time = 3.69 minutes (M+H) 344.39.

Formation of (+/-)-ethyl 3-(5-fluoro-2-(5-fluoro-1-tosyl-1*H*-pyrrolo[2,3-*b*]pyridin-3-yl)pyrimidin-4ylamino)-3-(1- methylcyclohexyl)propanoate (60)

A solution of 5-fluoro-1-(*p*-tolylsulfonyl)-3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)pyrrolo[2,3-b]pyridine, **23**, (0.51 g, 1.22 mmol), racemic ethyl 3-(2-chloro-5-fluorropyrimidin-4-ylamino)-3-(1-methylcyclohexyl)propanoate, **59,** (0.35 g, 1.02 mmol) and K_3PO_4 (0.52 g, 2.44 mmol) in 2-methyl THF (8 mL) and water (2 mL) was degassed under a stream of nitrogen for 30 minutes. X-Phos (0.03 g, 0.07 mmol) and $Pd_2(dba)_3$ (0.02 g, 0.02 mmol) were added and the resulting mixture was heated at 115 ^oC in a pressure vial for 4 hours. The reaction mixture was cooled to room temperature, filtered and concentrated *in vacuo*. The residue was dissolved in EtOAc (50 mL) and washed with water. The organic layer was dried ($MgSO_4$), filtered and concentrated *in vacuo*. The crude residue was purified via silica gel chromatography (0-35% EtOAc/Hexanes gradient) to afford 486 mg of the desired product as a white solid: ^1H NMR (400 MHz, $CDCl_3$) δ 8.50 (m, 1H), 8.48 (s, 1H), 8.24 (d, J = 1.7 Hz, 1H), 8.01 (m, 3H), 7.20(m, 2H), 5.12 (m, 1H), 4.88 (m, 1H), 3.89(q, J= 7.4 Hz, 2H), 2.71 (dd, J = 14.5, 3.8 Hz, 1H), 2.39 ? 2.32 (m, 1H), 2.31 (s, 3H), 1.60-1.32 (m 10H), 0.95 (t, J =7.4 3H). 0.87 (s, 3H); LCMS Gradient 60-98%, 0.1% formic acid, 7 minutes, C18/ACN, Retention Time = 2.81 minutes (M+H) 599.19.

Formation of (+/-)-ethyl 3-(5-fluoro-2-(5-fluoro-1*H*-pyrrolo[2,3-*b*]pyridin-3-yl)pyrimidin-4-ylamino)-3-(1-methylcyclohexyl)propanoate (61)

To a solution of ethyl 3-(5-fluoro-2-(5-fluoro-1-tosyl-1*H*-pyrrolo[2,3-*b*]pyridin-3-yl)pyrimidin-4ylamino)-3-(1- methylcyclohexyl)propanoate, **59**, (0.49 mg, 0.81 mmol) in CH_3CN (3 mL) was added HCl (2.0 mL of 4M solution in dioxane, 8.1 mmol). The solution was heated at 70 ^oC for 3 hours and then cooled to room temperature. The solvent was removed under reduced pressure and the product was neutralized with aqueous saturated $NaHCO_3$ solution. The precipitate was extracted with EtOAc (3x10 mL). The solvent was dried ($MgSO_4$), filtered and concentrated *in vacuo*. The crude residue was purified by silica gel chromatography (0-70%EtOAc/Hexanes gradient) to afford 230 mg of the desired product as an off-white solid: ^1H NMR (400 MHz, $CDCl_3$) δ 9.55 (s, 1H), 8.58 (dd, J = 9.3, 2.5 Hz,

1H), 8.18 (s, 2H), 8.00 (d, *J* = 2.7 Hz, 1H), 5.13 (brs, 1H), 4.95 (t, *J* = 8.2 Hz, 1H), 3.84 (m, 2H), 2.72 (m, 1H), 2.38 (m, 1H), 1.67 - 1.15 (m, 10H), 0.94 (m, 3H); LCMS Gradient 10-90%, 0.1% formic acid, 5 minutes, C18/ACN, Retention Time = 2.77 minutes (M+H) 444.36.

Formation of (+/-)-3-(5-fluoro-2-(5-fluoro-1*H*-pyrrolo[2,3-*b*]pyridin-3-yl)pyrimidin-4-ylamino)-3-(1-methylcyclohexyl)propanoic acid (12)

LiOH (0.118 mg, 4.927 mmol) was added to a solution of ethyl 3-(5-fluoro-2-(5-fluoro-1*H*-pyrrolo[2,3-*b*]pyridin-3-yl)pyrimidin-4-ylamino)-3-(1-methylcyclohexyl)-propanoate, **61**, (0.23 g, 0.49 mmol) in water (5 mL) and THF (5 mL). The solution was stirred at 95 ^{o}C for 18 hours and then cooled to room temperature. The solvent was removed under reduced pressure. The residue was diluted with water (10 mL) and neutralized with 2N HCl. The resulting precipitate was extracted with EtOAc (3x10 mL). The organic phase was dried ($MgSO_4$), filtered and concentrated *in vacuo* to afford 210 mg of the desired product as an off-white solid: ^{1}H NMR (400 MHz, CD_3OD) δ 8.78 (dd, *J* = 9.7, 2.7 Hz, 1H), 8.16 (s, 2H), 7.99 (d, *J* = 4.1 Hz, 1H), 5.20 (d, *J* = 9.9 Hz, 1H), 2.86 - 2.69 (m, 1H), 2.53 (dd, *J* = 14.7, 11.0 Hz, 1H), 1.76 - 1.56 (m, 2H), 1.53 (m, 4H), 1.29 (m, 4H), 1.02 (s, 3H); LCMS Gradient 10-90%, 0.1% formic acid, 5 minutes, C18/ACN, Retention Time = 2.20 minutes (M+H) 416.27; HRMS (ESI) of $C_{21}H_{23}F_2N_5O_2$ [M+H] calcd, 416.18926; found 416.18921.

Materials Used

Martin-Darby Canine Kidney (MDCK, CCL-34) cells obtained from American Type Culture Collection (ATCC) were maintained in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 2 mM L-glutamine, 1,000U/ml penicillin, 1,000 ug/ml streptomycin, 10 mM HEPES, and 10% fetal bovine medium. Antiviral assays were performed in supplemented DMEM with no FBS. Influenza virus strain A/PR/8/34 (tissue culture adapted) was obtained from ATCC (VR-1469). Low-passage virus stocks were prepared in MDCK cells using standard methods (WHO Manual on Animal Influenza Diagnosis and Surveillance, 2002).

PB2 Binding Assay: FP assay

The binding affinity for compounds to the cap binding domain of PB2 was determined using a competition binding fluorescence polarization (FP) assay (Memmott *et al*, manuscript in press). PB2 binding was examined using a 165 amino acid fragment of PB2 that had been identified as the cap binding domain portion of the full-length protein (Guilligay *et al*, Nat Struct Mol Biol, 15:500-506, 2008). This PB2 domain was incubated with test compounds and with a 5'-FITC-labeled probe for 60 minutes at room temperature to reach equilibrium. Values for probe only wells were used as background. PB2 Kd values were determined by fitting the background subtracted data to an equation for competitive displacement of a fluorescent probe (Wang, FEBS letters, 360:111-114, 1995) using Prism (GraphPad).

Influenza Antiviral Assay: bDNA assay

A cell-based antiviral assay was developed that depends on the multiplication of virus-specific RNA molecules in the infected cells, with negative strand RNA levels being directly measured using the branched-chain DNA (bDNA), hybridization method (Wagaman *et al*, J. Virol Meth, 105:105-114, 2002). Cells were initially infected at an MOI of 0.2 in 96-well microtiter plates and incubated in the presence of test compound for approximately 20 hours. Viral replication was quantified by determination of negative strand HA RNA levels by bDNA assay. Dose response curves were analyzed using 4- parameter curve fitting methods. The concentration of test compound resulting in viral RNA levels equal to that of 50% of the control wells were reported as EC50.

Methods for Influenza Efficacy in Mice

For efficacy studies, Balb/c mice (4-5 weeks of age) were challenged with 5x103 TCID50 in a total volume of 50 µl by intranasal by intranasal instillation (25 µl/nostril) under general anesthesia (Ketamine/Xylazine). Uninfected controls were challenged with tissue culture media (DMEM, 50 µl total volume). For this treatment study, compounds **4** (10, 30 and 60 mg/kg) and **16** (1, 3, 10 and 30 mg/kg) or vehicle only (0.5% Methylcellulose/0.5% Tween 80), were administered by oral gavage 48 hours post infection and continued twice daily for 10 days. Animals were monitored for survival for 21 days and Kaplan Meier plots.

Crystallization and X-ray Analysis

Crystals of the PB2 cap-binding domain (residues R318-M483) were grown by the vapor diffusion method at approximately 20 °C. A mixture of 1 µL protein solution (2.8 mg/ml protein, 50 mM Tris buffer pH 8, 200 mM sodium chloride, 2 mM dithiothreitol, 1 mM anthraquinone-2,6-disulfonic acid disodium salt, 7.5 mM GTP) and 0.4 µL well solution (approximately 1.5 M sodium formate, 100 mM sodium citrate buffer pH 4.7, 10 mM dithiothreitol) was suspended over 1 mL of well solution. The crystals were transferred to a soaking solution (3.25 M sodium formate, 100 mM sodium citrate buffer pH 4.7) containing 1 mM JNJ872 (compound **4**). Crystals were incubated approximately 15 hours at room temperature, and then transferred to a cryo-preservative solution (soaking solution with 25 % v/v glycerol) prior to freezing in liquid nitrogen.

X-ray data were collected at the Stanford Synchrotron Radiation Lightsource (BL7-1). The intensities were integrated and scaled using autoPROC (Global Phasing Inc, Cambridge, UK). The protein model was built using COOT (CCP4) and refined using autoBUSTER (Global Phasing Inc, Cambridge, UK).

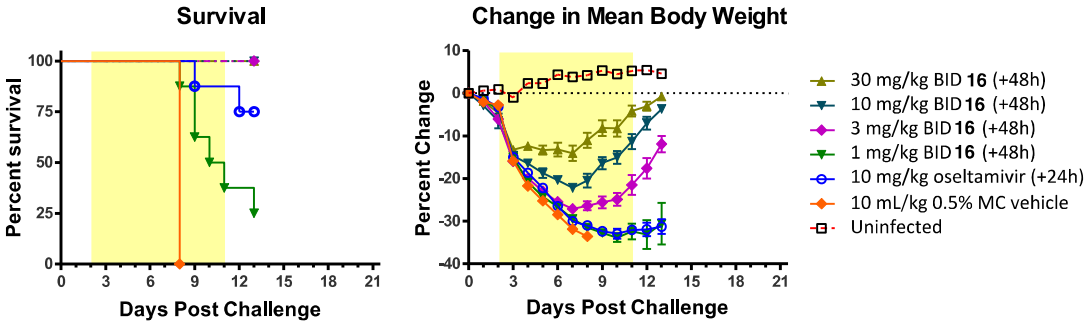


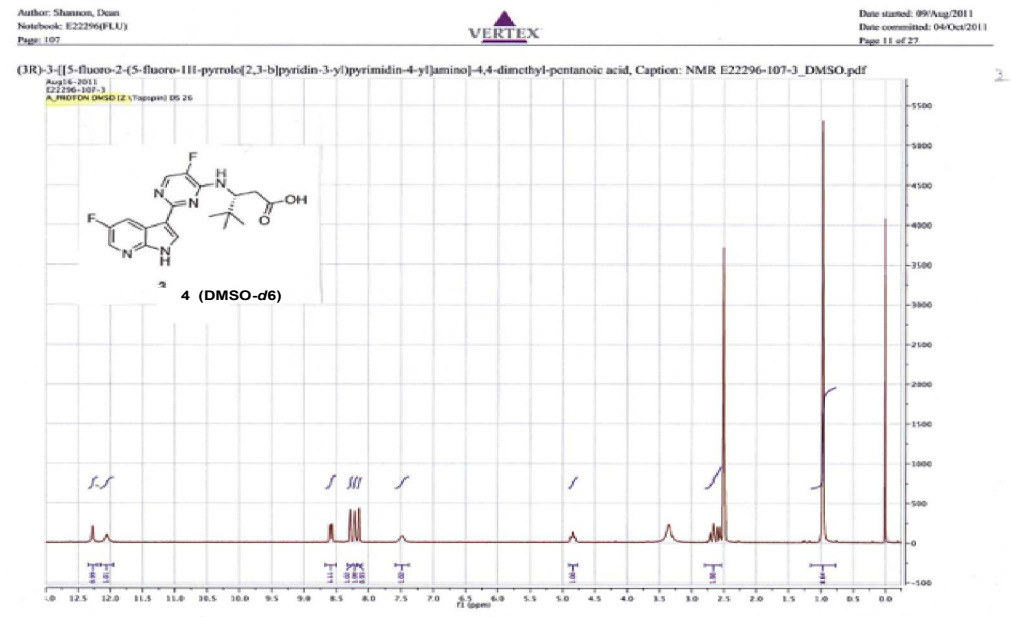
Figure 6. *In-vivo* activity of **16** and oseltamivir in mouse influenza A model when administered 48h post infection. Survival and body weight curves of male BALB/C mice (8 mice/group) inoculated with mouse-adapted influenza viruses A/PR/8/34 (5e3 $TCID_{50}$/mouse) by intranasal instillation

Supplemental. Table 4. Compound 4 Activity Against Oseltamivir Sensitive and Resistant Influenza A Viruses

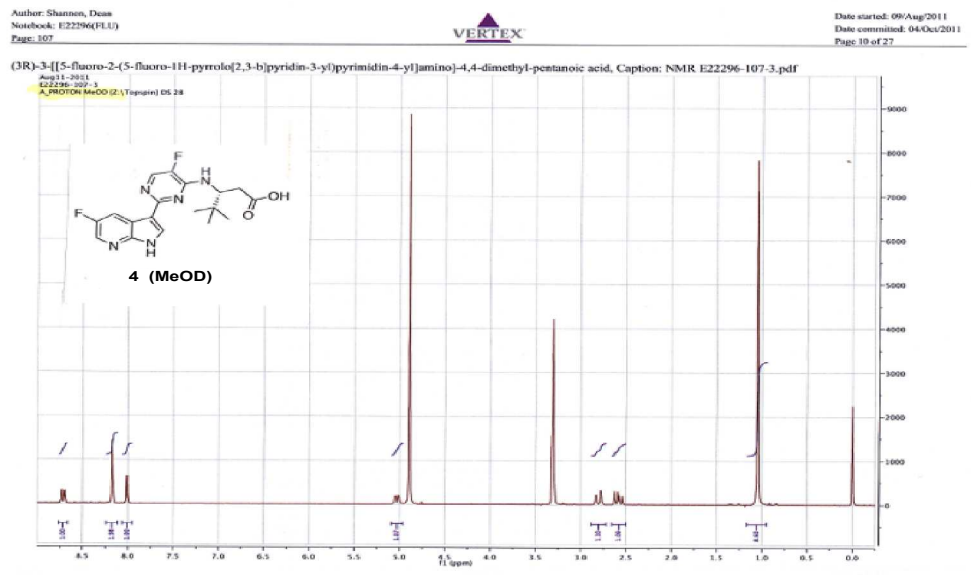
Virus Name	Type/Subtype	NAIR/S	Oseltamivir Carboxylate Mean IC_{50}, µM	Zanamivir Mean IC_{50}, µM	VRT-1038353 CP EC_{50}, µM
A/Georgia/17/2006	A (H1N1); Seasonal	S	0.00045	0.00049	0.0091
A/Georgia/20/2006	A (H1N1); Seasonal	R	>0.066	0.00080	0.0017
A/Henan/Jinshui/147/2007	A (H3N2); Seasonal	S	0.00039	0.00081	0.019
A/Texas/12/2007	A (H3N2); Seasonal	R	0.0047	0.00062	0.030
A/California/07/2009	A (H1N1); Pandemic	S	0.00031	0.00034	0.0040
A/Texas/48/2009	A (H1N1); Pandemic	R	0.028	0.00045	0.0012

CP: cell protection; EC50: effective concentration at which ATP is half the maximum in the CPE-based assay; IC50: effective concentration at which neuraminidase enzyme activity is 50% inhibited; NAI: neuraminidase inhibitors; R, NAI resistant; S, NAI sensitive.

Note: Shown are the averages of three independent chemiluminescent neuraminidase enzyme inhibition assays.



Supplemental Figure 1. ^{1}H NMR Spectrum of **4** (300 MHz, DMSO-d6).



Supplemental Figure 2. ^{1}H NMR Spectrum of **4** (300 MHz, MeOD).