Supporting Information

Design of Next-Generation DGAT2 Inhibitor PF-07202954 with Longer Predicted Half-Life

Kevin J. Filipski,[*,†] David J. Edmonds,[†] Michelle R. Garnsey,[†,‡] Daniel J. Smaltz,[†,‡] Karen Coffman,[‡] Kentaro Futatsugi,[†] Jack Lee,[‡] Steven V. O'Neil,[‡] Ann Wright,[‡] Deane Nason,[‡] James R. Gosset,[†] Christine C. Orozco,[‡] Dan Blackler,[†] Guila Fakhoury,[†] Jemy A. Gutierrez,[†] Sylvie Perez,[†] Trenton Ross,[†] Ingrid Stock,[†] Gregory Tesz,[†] and Robert Dullea[†]

[†]Pfizer Worldwide Research, Development, & Medical, 1 Portland Street, Cambridge, Massachusetts 02139, United States

[‡]Pfizer Worldwide Research, Development, & Medical, 558 Eastern Point Road, Groton, Connecticut 06340, United States

Contents:

Synthetic Procedures.

General Experimental Methods. All chemicals, reagents, and solvents were purchased from commercial sources and were used without further purification. ^{1}H NMR data are reported relative to residual solvent signals and are reported as follows: chemical shift (ppm), multiplicity, coupling constant (Hz), and integration. The multiplicities are denoted as: s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; br s, broad singlet. Silica gel chromatography was performed using Biotage or ISCO purification systems with pre-packaged columns. Concentration under reduced pressure was performed on a rotary evaporator with a water bath temperature not exceeding 60 ˚C. Purity of final compounds was assessed by HPLC with UV detection at 215 or 254 nm; all tested compounds showed >90% purity. No unexpected or unusually high-risk safety hazards were encountered.

The synthesis of ervogastat and 2-(5-((3-ethoxypyridin-2-yl)oxy)pyridin-3-yl)pyrimidine-5-carboxylic acid (**14**) has been previously published.[1] Compounds **1-13** were synthesized by an amidation on carboxylic acid **14** followed by a deprotection as detailed in the synthetic procedures below.

Scheme S1. Synthesis of (+)-*tert*-butyl 3-amino-4,4-difluoropiperidine-1-carboxylate (S3)

***tert*-butyl 3-(((benzyloxy)carbonyl)amino)-4-oxopiperidine-1-carboxylate (S1)**

To a solution of *rac-trans-tert*-butyl 3-(((benzyloxy)carbonyl)amino)-4-hydroxypiperidine-1-carboxylate (13.5 g, 38.5 mmol) in dichloromethane (300 mL) was added Dess-Martin periodinane (24.5 g, 57.8 mmol), and the suspension was stirred at 15 °C for 16 h. The suspension was diluted with dichloromethane (200 mL) and filtered. The filtrate was washed with saturated aq. $NaHCO_3$ (2 x 250 mL). The organic layer was dried over anhydrous Na_2SO_4, filtered and concentrated *in vacuo* to afford **S1** as a colorless oil. The crude product was purified by silica gel chromatography (80 g, petroleum ether/ethyl acetate = 5/1) to afford **S1** (13.2 g, 90%) as a white solid. ^{1}H NMR (400 MHz, chloroform-*d*), δ 7.37-7.30 (m, 5H), 5.73 (br.s, 1H), 5.15 – 5.12 (m, 2H), 4.83 (br.s, 1H), 4.46 – 4.31 (m, 2H), 3.08 – 2.95 (m, 1H), 2.70 – 2.48 (m, 3H), 1.51 (s, 9H).

(+)-*tert*-butyl 3-(((benzyloxy)carbonyl)amino)-4,4-difluoropiperidine-1-carboxylate (S2)

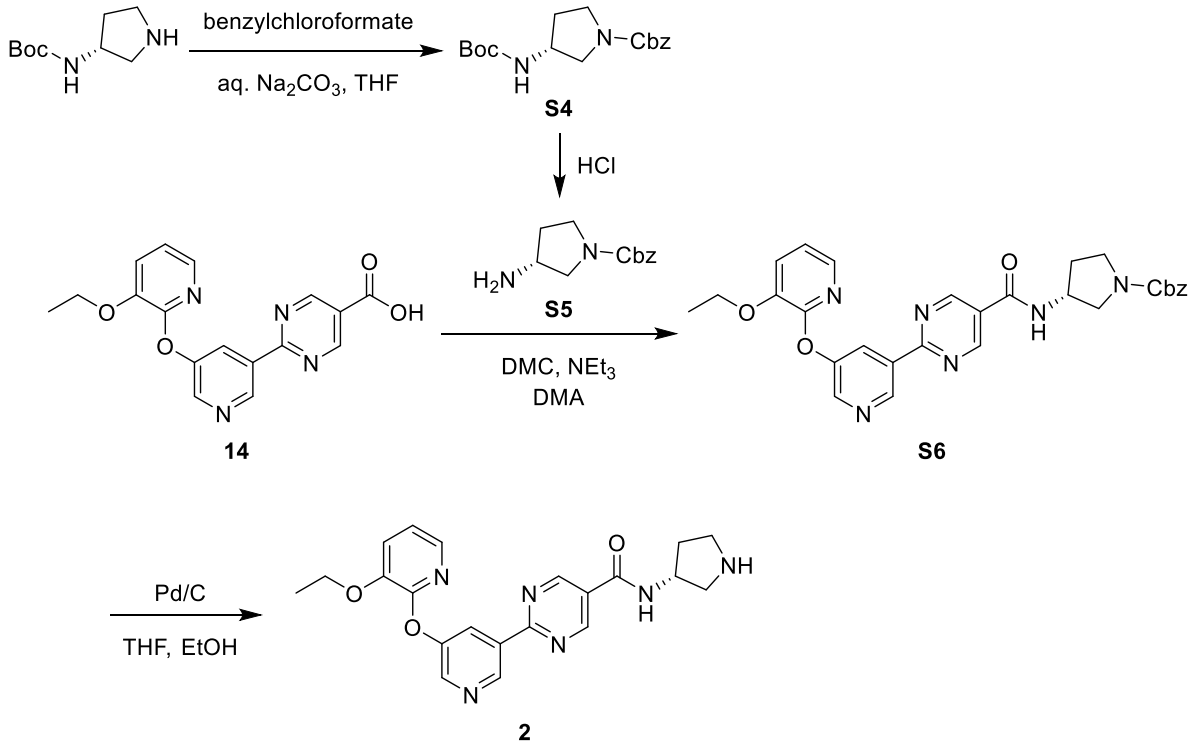
To a solution of **S1** (8.3 g, 23.8 mmol) in dichloromethane (250 mL) was added diethylaminosulfur trifluoride (DAST Reagent) (7.68 g, 47.6 mmol) at 0 °C, and the solution was stirred at 15 °C for 16 h. The yellow solution was diluted with dichloromethane (100 mL) and washed with saturated aq. $NaHCO_3$ (2 x 200 mL). The organic layer was dried over anhydrous Na_2SO_4, filtered and concentrated *in vacuo* to afford *rac*-**S2** as a yellow oil. The crude product was combined with another batch and purified via silica gel chromatography (120 g, petroleum ether/ethyl acetate = 20/1 to 10/1) to afford *rac*-**S2** (3.6 g, combined yield: 37%) as a white solid. The material was further purified via chiral SFC: [Column: Chiral Technologies Chiralpak AD, 10 µm; Mobile phase: 85:15 carbon dioxide / (ethanol containing 0.15% ammonium hydroxide)]. The first-eluting enantiomer had a retention time of 2.73 minutes (Column: Chiral Technologies Chiralcel OJ-H, 4.6 x 250 mm, 5 µm; Mobile phase A: carbon dioxide; Mobile phase B: ethanol containing 0.05% diethylamine; Gradient: 5% to 40% B over 5 minutes; Flow rate: 2.5 mL/minute). The second-eluting enantiomer exhibited a retention time of 3.08 minutes under the same conditions and a positive rotation to give **S2** as a light yellow solid (*ee* = 99.0%, positive optical rotation). ^{1}H NMR (400 MHz, chloroform-*d*), δ 7.37-7.31 (m, 5H), 5.17 – 5.10 (m, 3H), 4.03 – 3.91 (m, 2H), 3.15 – 3.10 (m, 1H), 3.00 – 2.94 (m, 1H), 2.12 – 1.91 (m, 2H), 1.46 (s, 9H).

(+)-*tert*-butyl (*R* or *S*)-3-(((benzyloxy)carbonyl)amino)-4,4-difluoropiperidine-1-carboxylate (S3)

To a solution of **S2** (4.1 g, 11.1 mmol) in MeOH (50 mL) was added $Pd(OH)_2$/C (1.5 g), and the suspension was stirred at 50 °C under H_2 atmosphere (50 Psi) for 16 h. The suspension was filtered and the filtrate was concentrated *in vacuo* to afford **S3** (2.5 g, 94%) as a white solid. ^{1}H NMR (400

MHz, chloroform-*d*) δ 1.31 (2H, br s), 1.46 (9H, s), 1.74-1.93 (1H, m), 2.07-2.24 (1H, m), 2.86-3.11 (2H, m), 3.16-3.30 (1H, m), 3.65-4.00 (2H, m); GCMS *m/z* calc for $C_{10}H_{18}F_2N_2O_2$: 237.13; found: 237.13 $[M+H]^+$. SFC- 99.5% ee%, (Column: Chiral Technologies Chiralcel OJ-H, 4.6 x 250 mm, 5 µm; Mobile phase A: carbon dioxide; Mobile phase B: ethanol containing 0.05% diethylamine; Gradient: 5% to 40% B over 5 minutes; Flow rate: 2.5 mL/minute, retention time = 3.08 min) positive optical rotation.

Scheme S2. Synthesis of (*R*)-2-(5-((3-ethoxypyridin-2-yl)oxy)pyridin-3-yl)-*N*-(pyrrolidin-3-yl)pyrimidine-5-carboxamide (2)



benzyl (*R*)-3-((*tert*-butoxycarbonyl)amino)pyrrolidine-1-carboxylate (S4)

Benzyl chloroformate (4.53 mL, 32.2 mmol) was added to a 0 °C mixture of *tert*-butyl (*R*)-pyrrolidin-3-ylcarbamate (5.00 g, 26.9 mmol) and 1 M aqueous sodium carbonate (53.7 mL, 53.7 mmol) in tetrahydrofuran (150 mL), and the reaction mixture was stirred at 15 °C for 2 d. It was then treated with water (100 mL) and extracted with ethyl acetate (2 x 100 mL). The combined organic layers were washed with saturated aqueous sodium chloride solution (100 mL), dried over sodium sulfate, filtered, and concentrated *in vacuo* to afford **S4** as a yellow solid (8.6 g crude). This material contained impurities by NMR but was used in the next step without additional purification. ^{1}H NMR (400 MHz, chloroform-*d*), characteristic peaks: δ 7.40 – 7.30 (m, 5H), 5.13 (s, 2H), 4.67 – 4.59 (m, 1H), 4.20-4.10 (m, 1H), 3.67 – 3.22 (m, 4H), 2.14 – 2.10 (m, 1H), 1.85 – 1.80 (m, 1H), 1.44 (s, 9H).

benzyl (*R*)-3-aminopyrrolidine-1-carboxylate (S5)

To a solution of **S4** (8.6 g crude, 26.8 mmol theoretical) in methanol (50 mL) was added 4 M HCl in ethyl acetate (50 mL, 200 mmol) dropwise at 19 °C. The yellow solution was stirred at 19 °C for 1 h and then concentrated under reduced pressure to afford crude **S5** (6 g) which was used in the next step without further purification. ^{1}H NMR (400 MHz, DMSO-d_6), characteristic peaks: δ 7.43 – 7.32 (m, 5H), 5.08 (s, 2H), 4.06 – 3.17 (m, 5H), 2.20 – 2.00 (m, 2H).

(*R*)-*N*-(1-benzylpyrrolidin-3-yl)-2-(5-((3-ethoxypyridin-2-yl)oxy)pyridin-3-yl)pyrimidine-5-carboxamide (S6)

To a solution of **14** (6.50 g, 19.2 mmol) in *N*,*N*-dimethylacetamide (150 mL) at 15 °C was added triethylamine (8.03 mL, 57.6 mmol), **S5** (5.92 g, 23.1 mmol) and 2-chloro-1,3-dimethylimidazolinium chloride (DMC) (9.74 g, 57.6 mmol). The resulting mixture was stirred at 50 °C for 1 h. A yellow suspension formed. The mixture was poured into water (500 mL) and extracted with ethyl acetate (3 x 250 mL). The combined organic layers were washed with brine (500 mL), dried over Na_2SO_4, filtered, and concentrated under reduced pressure to give the crude product. This was purified by column chromatography (ethyl acetate in petroleum ether) to yield **S6** (8.5 g, 82%) as a yellow solid. ^{1}H NMR (400 MHz, chloroform-*d*), characteristic peaks: δ 9.49 (s, 1H), 9.21 – 9.18 (m, 2H), 8.65 (d, 1H), 8.55 – 8.54 (m, 1H), 7.72 – 7.71 (m, 1H), 7.33 – 7.06 (m, 7H), 7.26 – 7.05 (m, 1H), 5.16 – 5.07 (m, 2H), 4.73 (m, 1H), 4.22-4.16 (q, 2H), 3.80 – 3.60 (m, 3H), 2.92 – 2.06 (m, 2H), 1.53 – 1.50 (t, 3H). LCMS *m/z* calc for $C_{29}H_{28}N_6O_5$: 541.2; found: 540.9 $[M+H]^+$.

(*R*)-2-(5-((3-ethoxypyridin-2-yl)oxy)pyridin-3-yl)-*N*-(pyrrolidin-3-yl)pyrimidine-5-carboxamide (2)

To a solution of **S6** (6.00 g, 11.1 mmol) in tetrahydrofuran (120 mL) and ethanol (120 mL) was added 10% Pd/C (6 g). The black suspension was degassed and purged with H_2 three times. The mixture was stirred at 15 °C under 15 psi for 5 h. The reaction was filtered through a pad of celite, the filtrate was concentrated under reduced pressure to yield crude product. This was purified by preparatory HPLC (Phenomenex Gemini C18, 250 x 50 mm, 10 µm; water (0.05% ammonium hydroxide) / acetonitrile; flow rate 110 mL/min). The resulting aqueous solution was lyophilized to yield **2** as a yellow solid (2.4 g). ^{1}H NMR (400 MHz, chloroform-*d*), characteristic peaks: δ 9.46 (s, 1H), 9.17 (s, 2H), 8.63 (d, 1H), 8.53 – 8.52 (d, 1H), 7.70 – 7.69 (d, 1H), 7.44 – 7.41 (m, 1H), 7.27 – 7.25 (m, 1H), 7.04 – 7.01 (m, 1H), 4.67 – 4.63 (m, 1H), 4.20 – 4.15 (q, 2H), 3.23 – 3.19 (m, 3H), 3.02 – 2.94 (m, 2H), 2.29 – 2.24 (m, 1H), 1.85 – 1.82 (m, 1H), 1.52 – 1.48 (t, 3H). LCMS *m/z* calc for $C_{21}H_{23}N_6O_3$: 407.2; found: 407.3 $[M+H]^+$, 95.6%. Chiral purity 99.5% (ChiralPak AD-3, 150 x 4.6 mm, 3 µm; flow rate 2.5 mL/min; mobile phase A: CO_2, mobile phase B: ethanol (0.1% ethanolamine), gradient: from 5% to 40% B in 5 min, then hold at 40% for 2.5 min, then 5% B for 2.5 min. Rt = 3.302 min. ^{13}C NMR (DMSO-d_6, 100 MHz) δ = 162.5, 156.9, 152.0, 150.9, 145.3, 144.8, 143.7, 136.9, 133.0, 127.1, 126.4, 121.6, 120.7, 64.1, 52.7, 50.8, 45.3, 32.5, 14.4. HRMS (ESI/QTOF) m/z: $[M+H^+]$ Calc for $C_{21}H_{23}N_6O_3$: 407.1826. Found: 407.1824. $[\alpha]_D^{20}$ 2.7 (*c* 1.3, MeOH).

(*S*)-2-(5-((3-ethoxypyridin-2-yl)oxy)pyridin-3-yl)-*N*-(pyrrolidin-3-yl)pyrimidine-5-carboxamide (1)

1 was synthesized in a comparable manner to **2** starting with the opposite enantiomer, *tert*-butyl (*S*)-pyrrolidin-3-ylcarbamate, to yield **1** as a yellow solid (2.2 g, 32% over two steps). ^{1}H NMR (400 MHz, chloroform-*d*), characteristic peaks: δ 9.50 (s, 1H), 9.17 (s, 2H), 8.66 – 8.65 (d, 1H), 8.56 – 8.55 (d, 1H), 7.72 – 7.71 (d, 1H), 7.29 – 7.26 (m, 1H), 7.06 – 6.99 (m, 2H), 4.67 – 4.63 (m, 1H), 4.22 – 4.17 (q, 2H), 3.25 – 3.14 (m, 2H), 2.99 – 2.96 (m, 2H), 2.32 (br.s, 1 H), 2.30 – 2.27 (m, 1H), 1.81 – 1.80 (m, 1H), 1.53 – 1.48 (t, 3H). LCMS *m/z* calc for $C_{21}H_{23}N_6O_3$: 407.2; found: 407.3 $[M+H]^+$, 93.7%. Chiral purity 99.5% (ChiralPak AD-3, 150 x 4.6 mm, 3 µm; flow rate 2.5 mL/min; mobile phase A: CO_2, mobile phase B: ethanol (0.1% ethanolamine), gradient: from 5% to 40% B in 5 min, then hold at 40% for 2.5 min, then 5% B for 2.5 min. Rt = 2.590 min.

General library protocol for analogs 3-5 and 8

Step 1. To a vial containing amine (150 µmol, 1.5 equiv.) was added a solution of **14** (100 µmol, 1.0 equiv) in dimethylacetamide (0.2 M) at room temperature. Diisopropylethyl amine (52 µL, 300 µmol, 3.0 eq.) was added, followed by a 0.2 M solution of HATU (500 µL, 100 µmol, 1.0 eq.) in dimethylacetamide. The vials were capped and heated to 50 °C for 16 hours. The reaction was concentrated by Speedvac and then ethyl acetate (2 mL) and water (1 mL) were added. The organic layer was removed and the water was further extracted with ethyl acetate (3 x 2 mL). The organic layers were combined and concentrated with Speedvac and the residue was used without further purification.

Step 2. To the vial containing the residue from Step 1 was added a prepared solution of DCM/TFA (v/v = 4/1). The vial was capped and maintained at 30 °C for 16 hours. The mixture was then concentrated by Speedvac and purified by preparatory HPLC to give the final product.

2-{5-[(3-ethoxypyridin-2-yl)oxy]pyridin-3-yl}-*N*-(piperidin-4-yl)pyrimidine-5-carboxamide, trifluoroacetate salt (3)

This analogue was synthesized using the general library protocol above starting from *tert*-butyl 4-aminopiperidine-1-carboxylate. Preparatory HPLC: Column: Boston Green ODS C18 150*30 mm*5 µm; Mobile phase A: 0.225% formic acid in water; Mobile phase B: acetonitrile; Gradient: 9% B to 49% B for 10 minutes Hold time: 2 minutes at 100% B; Flow rate: 30 mL/min; Purity: 99.68%. Analytical HPLC: Column: Waters XBridge C18, 2.1 x 50 mm, 5 µm; Mobile phase A: 0.0375% trifluoroacetic acid in water; Mobile phase B: 0.01875% trifluoroacetic acid in acetonitrile; Gradient: 10% B for 0.50 minutes; 10% to 100% B over 3.5 minutes; Flow rate: 0.8 mL/minute. Rt = 1.91 min; LCMS *m/z* calc for $C_{22}H_{25}N_6O_3$: 421.2; found: 421 $[M+H]^+$.

2-{5-[(3-ethoxypyridin-2-yl)oxy]pyridin-3-yl}-*N*-[(3*S*)-piperidin-3-yl]pyrimidine-5-carboxamide, trifluoroacetate salt (4)

This analogue was synthesized using the general library protocol above starting from *tert*-butyl (*S*)-3-aminopiperidine-1-carboxylate. Preparatory HPLC: Column: Agela Durashell C18 150*25 mm*5 µm; Mobile phase A: 0.225% formic acid in water; Mobile phase B: acetonitrile; Gradient:

0% B to 48% B for 12 minutes Hold time: 2 minutes at 100% B; Flow rate: 25 mL/min; Purity: 100%. Conditions for analytical HPLC. Column: Waters XBridge C18, 2.1 x 50 mm, 5 µm; Mobile phase A: 0.0375% trifluoroacetic acid in water; Mobile phase B: 0.01875% trifluoroacetic acid in acetonitrile; Gradient: 1% to 5% B over 0.6 minutes; 5% to 100% B over 3.4 minutes; Flow rate: 0.8 mL/minute. Rt = 2.29 min; LCMS *m/z* calc for $C_{22}H_{25}N_6O_3$: 421.2; found: 421 $[M+H]^+$.

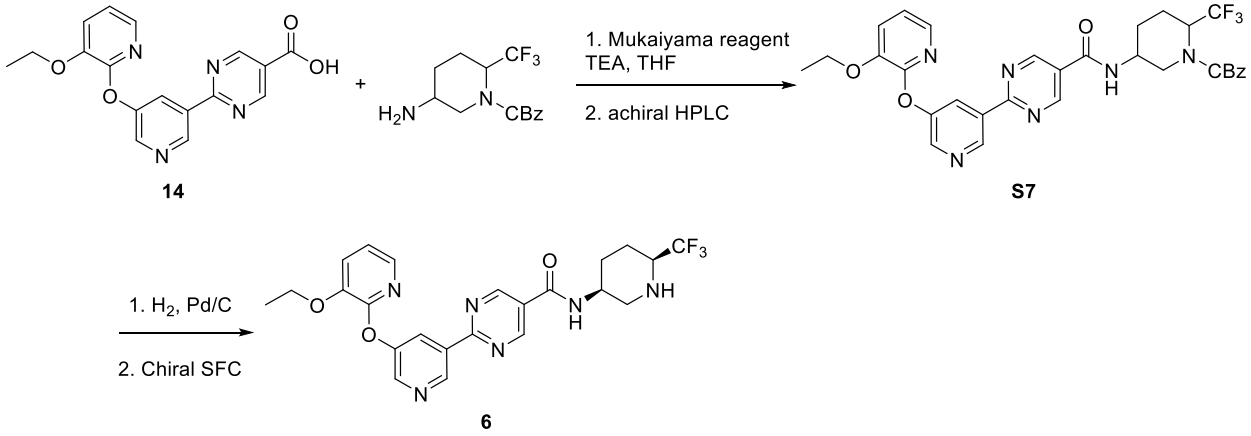
2-{5-[(3-ethoxypyridin-2-yl)oxy]pyridin-3-yl}-*N*-[(3*R*)-piperidin-3-yl]pyrimidine-5-carboxamide, trifluoroacetate salt (5)

This analogue was synthesized using the general library protocol above starting from *tert*-butyl (*R*)-3-aminopiperidine-1-carboxylate. Preparatory HPLC: Column: Agela Durashell C18 150*25 mm*5 µm; Mobile phase A: 0.225% formic acid in water; Mobile phase B: acetonitrile; Gradient: 0% B to 48% B for 12 minutes; Hold time: 2 minutes at 100% B; Flow rate: 25 mL/min; Purity: 99.24%. Conditions for analytical HPLC. Column: Waters XBridge C18, 2.1 x 50 mm, 5 µm; Mobile phase A: 0.0375% trifluoroacetic acid in water; Mobile phase B: 0.01875% trifluoroacetic acid in acetonitrile; Gradient: 1% to 5% B over 0.6 minutes; 5% to 100% B over 3.4 minutes; Flow rate: 0.8 mL/minute. Rt = 2.64 min; LCMS *m/z* calc for $C_{22}H_{25}N_6O_3$: 421.2; found: 421 $[M+H]^+$.

***N*-[(3[*S* or *R*])-4,4-difluoropiperidin-3-yl]-2-{5-[(3-ethoxypyridin-2-yl)oxy]pyridin-3-yl}pyrimidine-5-carboxamide, trifluoroacetate salt (8)**

This analogue was synthesized using the general library protocol above starting from **S3**. Conditions for preparatory HPLC: Column: Agela Durashell C18 150*25 mm*5 µm; Mobile phase A: 0.225% formic acid in water; Mobile phase B: acetonitrile; Gradient: 1% B to 51% B for 12 minutes; Hold time: 2 minutes at 100% B; Flow rate: 25 mL/min; Purity: 100%. Conditions for analytical HPLC: Column: XBridge C18, 2.1 x 50 mm, 5 µm; Mobile phase A: 0.0375% TFA in water; Mobile phase B: 0.01875% TFA in acetonitrile; Gradient: 1% B for 0.6 minute, then 1% to 5% B over 3.4 minutes; Flow rate: 0.8 mL/minute LCMS *m/z* calc for $C_{22}H_{23}F_2N_6O_3$: 457.2; found: 457 $[M+H]^+$, Rt = 2.481 min,

Scheme S3. Synthesis of 2-{5-[(3-ethoxypyridin-2-yl)oxy]pyridin-3-yl}-*N*-[6-(trifluoromethyl)piperidin-3-yl]pyrimidine-5-carboxamide, trifluoroacetate salt, cis enantiomer (6)



benzyl 5-(2-(5-((3-ethoxypyridin-2-yl)oxy)pyridin-3-yl)pyrimidine-5-carboxamido)-2-(trifluoromethyl)piperidine-1-carboxylate (S7)

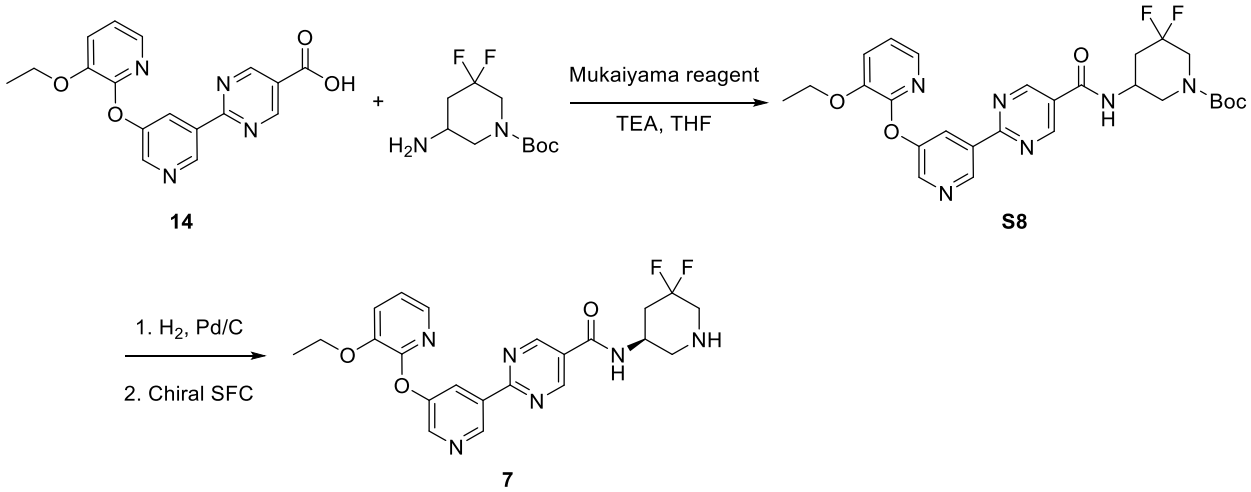
To a solution of **14** (80 mg, 0.236 mmol) in THF (5 mL) was added triethylamine (0.709 mmol, 0.0989 mL), benzyl 5-amino-2-(trifluoromethyl)piperidine-1-carboxylate (150.0 mg, 0.4962 mmol) and 2-chloro-1-methylpyridinium iodide (Mukaiyama reagent, 90.6 mg, 0.355 mmol) at 25 °C. The resulting mixture was stirred at 80 °C for 2 hours. The mixture was combined with another batch (0.06 mmol scale). The combined mixture was diluted with water (20 mL) and extracted with ethyl acetate (2 x 30 mL). The combined organic layer was washed with brine (20 mL), dried over sodium sulfate, filtered and concentrated *in vacuo*. The residue was purified by preparatory TLC (petroleum ether:ethyl acetate = 1:3; UV) to afford **S7** (120.0 mg, 65%) as a yellow solid. LCMS *m/z* calc for $C_{31}H_{30}F_3N_6O_5$: 623.2; found: 623.3 [M+H]+, 97%.

2-{5-[(3-ethoxypyridin-2-yl)oxy]pyridin-3-yl}-*N*-[6-(trifluoromethyl)piperidin-3-yl]pyrimidine-5-carboxamide, trifluoroacetate salt, cis enantiomer (6)

To a solution of **S7** (45.0 mg, 0.0723 mmol) in ethyl acetate (5 mL) was added wet Pd/C (55 mg) at 25 °C. The resulting black mixture was degassed and purged with H_2 three times. Then the mixture was stirred at 25 °C under a balloon of H_2 atmosphere for 7 hr. The mixture was filtered and the filtrate was combined with another batch. The combined mixture was concentrated *in vacuo* to give the racemic product (80 mg) which was purified by preparatory HPLC: Column: Waters Xbridge Prep OBD C18 150*30 mm*5 µm; Mobile phase A: 0.04% ammonium hydrate in water with 10 mM ammonium carbonate; Mobile phase B: acetonitrile; Gradient: 23% B to 53% B for 10 minutes; Hold time: 2 minutes at 100% B; Flow rate: 25 mL/min. The cis and trans isomers were separated and assigned via NMR. The cis isomeric mixture (*rac*-**6)** was isolated as a white solid (13.9 mg). ^{1}H NMR (400 MHz, $CDCl_3$) δ = 9.55 (br s, 1H), 9.20 (s, 2H), 8.67 (br s, 1H), 8.58 (s, 1H), 7.72 (d, *J* = 3.9 Hz, 1H), 7.26 (s, 1H), 7.21 (br d, *J* = 7.3 Hz, 1H), 7.03 (dd, *J* = 4.9, 7.8 Hz, 1H), 4.41 - 4.34 (m, 1H), 4.19 (q, *J* = 7.0 Hz, 2H), 3.28 - 3.20 (m, 1H), 3.17 (br d, *J* = 12.7 Hz, 1H), 3.02 (dd, *J* = 1.7, 12.0 Hz, 1H), 2.26 - 2.14 (m, 1H), 1.99 - 1.87 (m, 1H), 1.87 - 1.79 (m, 1H), 1.78 - 1.70 (m, 2H), 1.51 (t, *J* = 7.1 Hz, 3H). LCMS *m/z* calc for $C_{22}H_{24}F_3N_6O_3$: 489.2; found: 489.3 [M+H]+, 100%. Then, *rac*-**6** was separated into its component enantiomers using supercritical fluid chromatography {Column: Phenomenex Lux Amylose-1, 5 µm; Mobile phase: 7:3 carbon dioxide / [ethanol containing 0.2% (7 M ammonia in methanol)]}. The first-eluting enantiomer was designated as *ent*-**6**, and the second-eluting enantiomer as **6**. Retention time for *ent*-**6**: 6.48 minutes [Column: Phenomenex Lux Amylose-1, 4.6 x 250 mm, 5 µm; Mobile phase A: carbon dioxide; Mobile phase B: ethanol containing 0.2% (7 M ammonia in methanol); Gradient: 5% B for 1.0 minute, then 5% to 60% B over 8.0 minutes; Flow rate: 3.0 mL/minute; Back pressure: 120 bar]. Achiral LCMS *m/z* calc for $C_{22}H_{24}F_3N_6O_3$: 489.2; found: 489.4 $[M+H]^+$. Rt = 1.91 min, 100%. Retention time for **6**: 6.68 minutes (Analytical conditions identical to those used for *ent*-**6**). ^{1}H NMR (400 MHz, Chloroform-*d*) δ 9.47 (s, 1H), 9.11 (s, 2H), 8.59 (d, *J* = 2.7 Hz, 1H), 8.50 (t, *J* = 2.3 Hz, 1H), 7.65 (dd, *J* = 4.9, 1.5 Hz, 1H), 7.18 (M, 1H) 7.06 (d, *J* = 7.8 Hz, 1H), 6.96 (dd, *J* = 7.9, 4.9 Hz, 1H), 4.30 (m, 1H), 4.12 (q, *J* = 7.0 Hz, 2H), 3.15 (q, *J* = 6.0, 5.3 Hz, 1H), 3.09 – 2.92 (m, 2H), 2.11 (d, *J* = 10.4 Hz, 1H), 1.78 – 1.59 (m, 2H), 1.43 (t, *J* = 7.0 Hz, 3H), 1.30 – 1.19 (m, 2H). Achiral LCMS *m/z* calc for $C_{22}H_{24}F_3N_6O_3$: 489.2; found: 489.4 $[M+H]^+$.

Rt = 1.91 min, 100%. These two compounds are enantiomers of one another, but of undetermined absolute stereochemistry.

Scheme S4. Synthesis of *N*-[(3[*S* or *R*])-5,5-difluoropiperidin-3-yl]-2-{5-[(3-ethoxypyridin-2-yl)oxy]pyridin-3-yl}pyrimidine-5-carboxamide (7)



***tert*-butyl (*S*)-5-(2-(5-((3-ethoxypyridin-2-yl)oxy)pyridin-3-yl)pyrimidine-5-carboxamido)-3,3-difluoropiperidine-1-carboxylate (S8)**

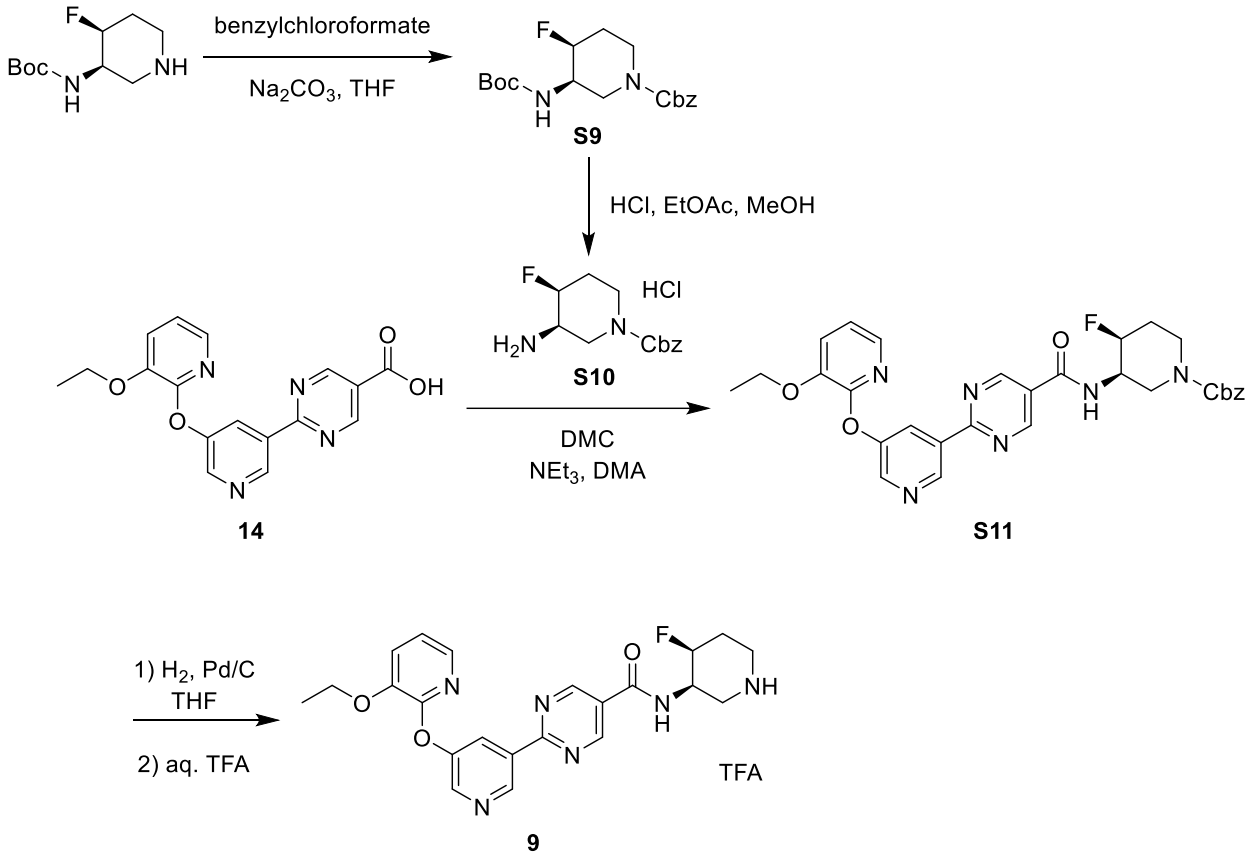
To a solution of **14** (90 mg, 0.266 mmol) in THF (3 mL) was added triethylamine (0.798 mmol, 0.111 mL), *tert*-butyl 5-amino-3,3-difluoropiperidine-1-carboxylate (69.1 mg, 0.293 mmol) and 2-chloro-1-methylpyridinium iodide (Mukaiyama reagent, 102 mg, 0.399 mmol) at 25 °C. The resulting mixture was stirred at 80 °C for 2 hours. The mixture was combined with another batch. The combined mixture was diluted with water (20 mL) and extracted with ethyl acetate (2 x 30 mL). The combined organic layer was washed with brine (20 mL), dried over sodium sulfate, filtered and concentrated *in vacuo*. This was purified via silica gel chromatography (ISCO 4g, 82% ethyl acetate in petroleum ether) to afford the product **S8 (**130.0 mg, ~79%) as a yellow solid. LCMS *m/z* calc for $C_{27}H_{31}F_2N_6O_5$: 557.2; found: 557.3 $[M+H]^+$. Rt = 0.897 min, 98.8%.

***N*-[(3[*S* or *R*])-5,5-difluoropiperidin-3-yl]-2-{5-[(3-ethoxypyridin-2-yl)oxy]pyridin-3-yl}pyrimidine-5-carboxamide (7)**

To a solution of **S8** (100 mg, 0.1797 mmol) in DCM (9 mL) was added TFA (3 mL) at 26 °C and the solution was stirred for 2 hours. The mixture was combined with another batch. The combined mixture was concentrated, adjusted to pH ~ 7 with saturated aqueous sodium bicarbonate and concentrated *in vacuo* to give the crude product which was purified by preparatory HPLC (Column: Waters Xbridge Prep OBD C18 150*30 mm*5 µm; Mobile phase A: 0.04% ammonium hydrate in water with 10 mM ammonium carbonate; Mobile phase B: acetonitrile; Gradient: 21% B to 51% B for 10 minutes; Hold time: 2 minutes at 100% B; Flow rate: 25 mL/min. to yield *rac*-**7** (47.8 mg, ~45%) as a light yellow solid. LCMS *m/z* calc for $C_{22}H_{23}F_2N_6O_3$: 557.2; found: 557.3 $[M+H]^+$. Rt = 0.897 min, 98.8%). *Rac*-**7** was separated into its component enantiomers using supercritical fluid chromatography [Column: Chiral Technologies Chiralpak IC, 5 µm; Mobile

phase: 3:2 carbon dioxide / (ethanol containing 0.1% ammonium hydroxide)]. The first-eluting enantiomer was designated as **7** and gave a retention time of 3.42 minutes ([Column: Chiral Technologies Chiralpak IC, 3 µm; Mobile phase: 3:2 carbon dioxide / (ethanol containing 0.05% diethylamine); Flow rate 2.5 mL/minute]. ^{1}H NMR (400 MHz, chloroform-*d*) δ 9.55 – 9.49 (m, 1H), 9.16 (s, 2H), 8.68 – 8.62 (m, 1H), 8.56 (br s, 1H), 7.71 (br d, *J* = 5.0 Hz, 1H), 7.3 – 7.23 (m, 1H, assumed; partially obscured by solvent peak), 7.02 (dd, *J* = 7.9, 4.9 Hz, 1H), 6.95 (br d, *J* = 8 Hz, 1H), 4.58 – 4.49 (m, 1H), 4.18 (q, *J* = 7.0 Hz, 2H), 3.28 – 3.17 (m, 1H), 3.08 – 2.89 (m, 3H), 2.39 – 2.11 (m, 2H), 1.50 (t, *J* = 7.0 Hz, 3H); LCMS *m/z* calc for $C_{22}H_{23}F_2N_6O_3$: 457.2; found; 457.4 $[M+H]^+$, 100%. The second-eluting enantiomer was designated as *ent*-**7** and gave a retention time of 4.42 minutes (Analytical conditions identical to those used for **7**).

Scheme S5. Synthesis of 2-(5-((3-ethoxypyridin-2-yl) oxy)pyridin-3-yl)-*N*-((3*R*,4*S*)-4-fluoropiperidin-3-yl)pyrimidine-5-carboxamide (9)



benzyl (3*R*,4*S*)-3-[(*tert*-butoxycarbonyl)amino]-4-fluoropiperidine-1-carboxylate (S9)

Benzyl chloroformate (0.116 mL, 0.813 mmol) was added to a 0 °C mixture of *tert*-butyl [(3*R*,4*S*)-4-fluoropiperidin-3-yl]carbamate (150 mg, 0.69 mmol) and sodium carbonate (146 mg, 1.38 mmol) in tetrahydrofuran (8 mL), and the reaction mixture was stirred at 25 °C for three days. It was then treated with water (20 mL) and extracted with ethyl acetate (2 x 20 mL). The combined organic layers were washed with saturated aqueous sodium chloride solution (50 mL), dried over

sodium sulfate, filtered, and concentrated *in vacuo* to afford **S9** as a colorless oil (290 mg). This material contained impurities by ^{1}H NMR analysis, but was used in the next step without additional purification. ^{1}H NMR (400 MHz, chloroform-*d*), characteristic peaks: δ 4.95 – 4.76 (m, 2H), 3.87 – 3.68 (m, 1H), 3.12 – 2.99 (m, 1H), 2.11 – 1.96 (m, 1H), 1.45 (s, 9H).

benzyl (3*R*,4*S*)-3-amino-4-fluoropiperidine-1-carboxylate, hydrochloride salt (S10)

A mixture of **S9** (from the previous step; 290 mg, ≤0.69 mmol) and hydrogen chloride (4 M solution in 1,4-dioxane; 6.0 mL) was stirred at 15 °C for 1 hour, whereupon it was concentrated *in vacuo*, affording **S10** as a white solid. Yield: 200 mg, 0.69 mmol, quantitative over 2 steps. ^{1}H NMR (400 MHz, deuterium oxide) δ 7.48 – 7.33 (m, 5H), 5.14 (s, 2H), 5.11 (br d, J_{HF} = 48 Hz, 1H), 4.11 – 3.94 (m, 1H), 3.88 – 3.28 (m, 4H), 2.14 – 2.01 (m, 1H), 2.01 – 1.81 (m, 1H).

benzyl (3*R*,4*S*)-3-(2-(5-((3-ethoxypyridin-2-yl)oxy)pyridin-3-yl)pyrimidine-5-carboxamido)-4-fluoropiperidine-1-carboxylate (S11)

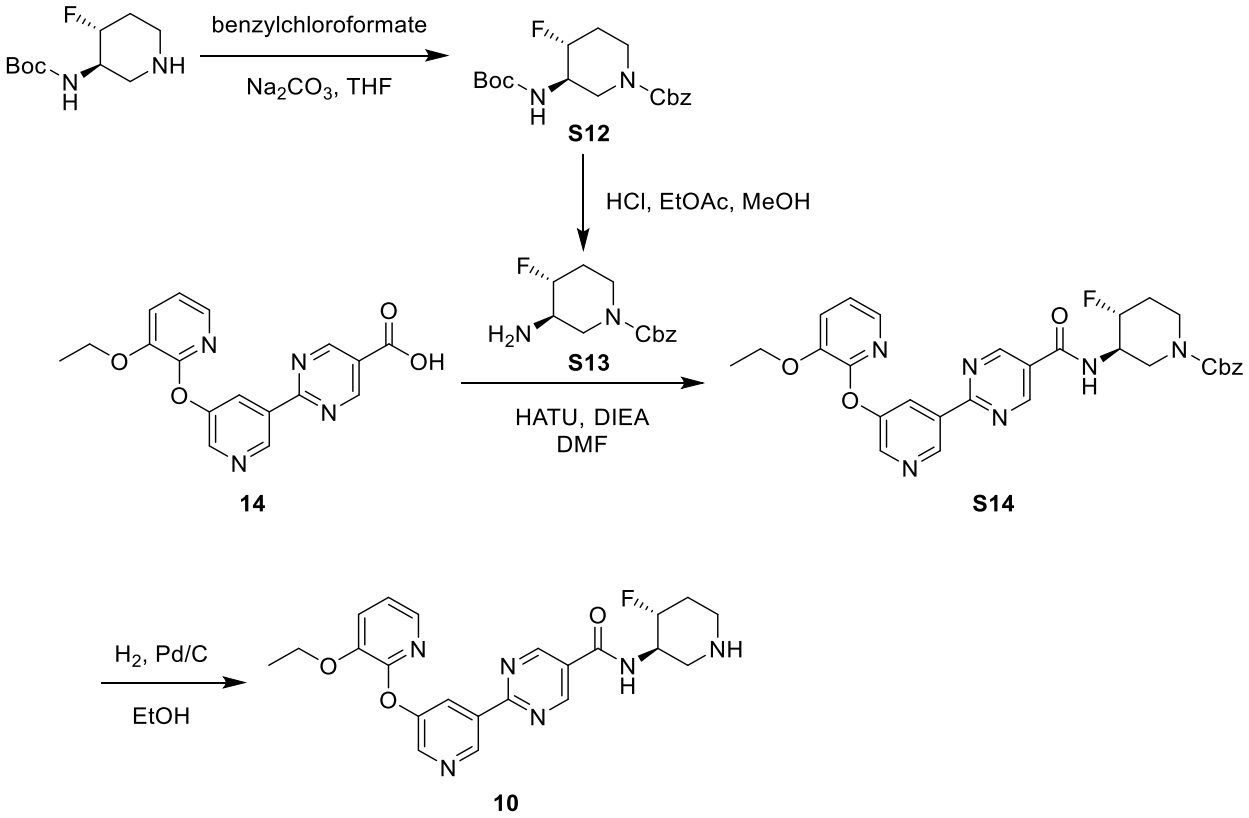
To a 25 °C solution of **14** (50.0 mg, 0.148 mmol) in *N*,*N*-dimethylacetamide (3.0 mL) was added **S10** (51.2 mg, 0.177 mmol), 2-chloro-1,3-dimethyl-4,5-dihydro-1*H*-imidazol-3-ium chloride (75.0 mg, 0.444 mmol), and triethylamine (61.8 µL, 0.443 mmol). The reaction mixture was stirred at 50 °C for 1 hour, whereupon water (20 mL) was added, and the resulting mixture was extracted with ethyl acetate (20 mL). The organic layer was washed with saturated aqueous sodium chloride solution (20 mL), dried over sodium sulfate, filtered, concentrated *in vacuo*, and subjected to silica gel chromatography (Eluent: 4:1 ethyl acetate / petroleum ether), affording **S11** (80.0 mg, 0.140 mmol, 95%) as a yellow solid. LCMS *m/z* calc for $C_{30}H_{30}FN_6O_5$: 573.2; found: 573.2 $[M+H]^+$.

2-(5-((3-ethoxypyridin-2-yl) oxy)pyridin-3-yl)-*N*-((3*R*,4*S*)-4-fluoropiperidin-3-yl)pyrimidine-5-carboxamide, trifluoroacetate salt (9)

To a solution of **S11** (60.0 mg, 0.105 mmol) in tetrahydrofuran (10 mL) was added 10% palladium on carbon (60.0 mg), whereupon the mixture was degassed under vacuum and then purged with hydrogen; this evacuation-purge cycle was carried out a total of three times. The reaction mixture was stirred under a balloon of hydrogen for 5 hours at 25 °C and then combined with a similar reaction carried out using **S11** (20.0 mg, 34.9 µmol). After this mixture had been filtered, the filtrate was concentrated *in vacuo* and purified using reversed-phase HPLC (Column: YMC-Actus Triart C18, 5 µm; Mobile phase A: water containing 0.05% ammonium hydroxide; Mobile phase B: acetonitrile; Gradient: 24% to 64% B). The free base of **9** (12 mg, 27 µmol, 19%) was obtained as a white solid. ^{1}H NMR (400 MHz, chloroform-*d*) δ 9.52 (d, *J* = 1.8 Hz, 1H), 9.17 (s, 2H), 8.65 (d, *J* = 2.7 Hz, 1H), 8.58 – 8.54 (m, 1H), 7.70 (dd, *J* = 4.9, 1.5 Hz, 1H), 7.27 – 7.23 (m, 1H, assumed; partially obscured by solvent peak), 7.02 (dd, *J* = 7.9, 4.9 Hz, 1H), 6.68 (br d, *J* = 8.8 Hz, 1H), 4.93 (br d, J_{HF} = 49 Hz, 1H), 4.48 – 4.31 (m, 1H), 4.18 (q, *J* = 7.0 Hz, 2H), 3.10 (dd, *J* = 12.1, 4.5 Hz, 1H), 3.00 – 2.80 (m, 3H), 2.15 – 2.01 (m, 1H), 1.97 – 1.77 (m, 1H), 1.50 (t, *J* = 7.0 Hz, 3H). LCMS *m/z* calc for $C_{22}H_{24}FN_6O_3$: 439.2; found: 439.1 $[M+H]^+$. The free base of **9** (12 mg, 27 µmol) was dissolved in an aqueous solution of trifluoroacetic acid (0.1% trifluoroacetic acid in water; 12 mL) and then lyophilized for 16 hours to provide **9** (12.9 mg, 19.4 µmol, 14%) as a yellow gum. ^{1}H NMR (400 MHz, chloroform-*d*) δ 10.56 – 10.28 (br s, 1H), 9.80 – 9.55 (br s,

1H), 9.71 (s, 1H), 9.24 (s, 2H), 9.09 (br s, 1H), 8.86 (br d, *J* = 7.9 Hz, 1H), 8.77 (d, *J* = 2.5 Hz, 1H), 7.72 (dd, *J* = 4.9, 1.5 Hz, 1H), 7.32 (dd, *J* = 8.1, 1.5 Hz, 1H), 7.14 (dd, *J* = 8.0, 4.9 Hz, 1H), 4.97 (br d, J_{HF} = 49 Hz, 1H), 4.96 – 4.78 (m, 1H), 4.18 (q, *J* = 7.0 Hz, 2H), 3.83 – 3.72 (m, 1H), 3.5 – 3.2 (m, 3H), 2.42 – 2.11 (m, 2H), 1.50 (t, *J* = 7.0 Hz, 3H). LCMS *m/z* calc for $C_{22}H_{24}FN_6O_3$: 439.2; found: 439.4 $[M+H]^+$, 96.0%.

Scheme S6. Synthesis of 2-{5-[(3-ethoxypyridin-2-yl)oxy]pyridin-3-yl}-*N*-[(3*R*,4*R*)-4-fluoropiperidin-3-yl]pyrimidine-5-carboxamide (10)



benzyl (3*R*,4*R*)-3-[(*tert*-butoxycarbonyl)amino]-4-fluoropiperidine-1-carboxylate (S12)

Benzyl chloroformate (258 mg, 1.51 mmol) was added to a 0 °C mixture of *tert*-butyl [(3*R*,4*R*)-4-fluoropiperidin-3-yl]carbamate (300 mg, 1.37 mmol) in tetrahydrofuran (15 mL) and aqueous sodium carbonate solution (1 M; 2.75 mL, 2.75 mmol). After the reaction mixture had been stirred at 15 °C for 16 hours, water (20 mL) was added, and the resulting mixture was extracted with ethyl acetate (2 x 30 mL). The combined organic layers were washed with saturated aqueous sodium chloride solution (50 mL), dried over sodium sulfate, filtered, and concentrated *in vacuo* to provide **S12** (485 mg, 1.38 mmol, quantitative) as a white solid. ^{1}H NMR (400 MHz, chloroform-*d*) δ 7.43 – 7.28 (m, 5H), 5.14 (AB quartet, J_{AB} = 12.3 Hz, $\Delta\nu_{AB}$ = 14.2 Hz, 2H; downfield doublet is broadened), 4.83 – 4.53 (m, 2H), 3.87 – 3.33 (m, 4H), 2.06 – 1.75 (m, 2H), 1.44 (s, 9H).

benzyl (3*R*,4*R*)-3-amino-4-fluoropiperidine-1-carboxylate, hydrochloride salt (S13)

A solution of **S12** (485 mg, 1.38 mmol) in methanol (6 mL) was treated with hydrogen chloride (solution in ethyl acetate; 12 mL). After the reaction mixture had been stirred at 20 °C for 1 hour, it was concentrated *in vacuo*, affording **S13** (370 mg, 1.28 mmol, 93%) as a white solid. ^{1}H NMR (400 MHz, deuterium oxide) δ 7.50 – 7.39 (m, 5H), 5.17 (s, 2H), 4.93 – 4.71 (m, 1H, assumed; partially obscured by solvent peak), 4.42 – 4.27 (m, 1H), 4.24 – 3.98 (m, 1H), 3.51 – 3.39 (m, 1H), 3.21 – 2.99 (m, 2H), 2.29 – 2.16 (m, 1H), 1.86 – 1.71 (m, 1H).

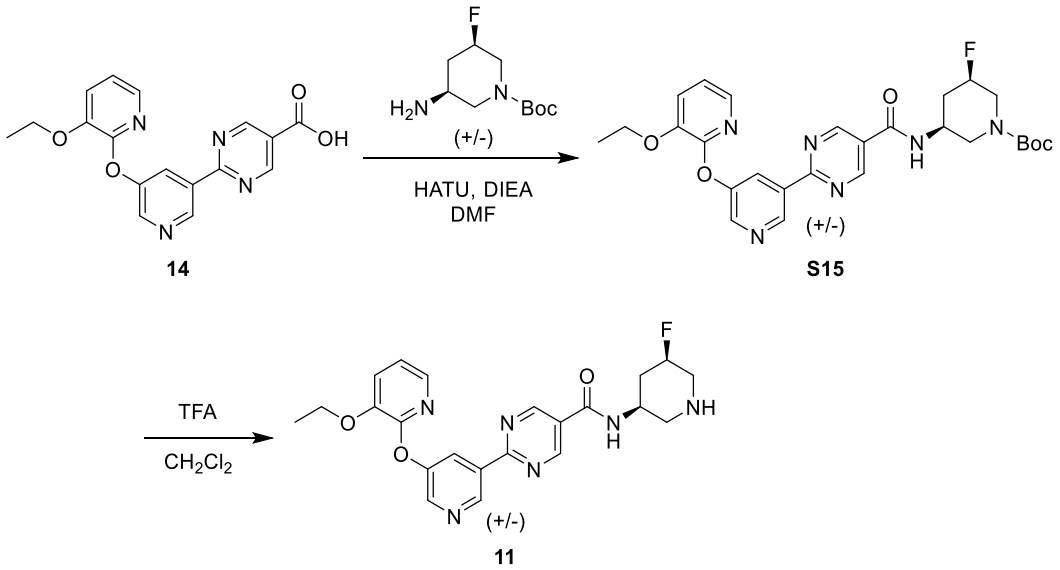
benzyl (3*R*,4*R*)-3-{[(2-{5-[(3-ethoxypyridin-2-yl)oxy]pyridin-3-yl}pyrimidin-5-yl)carbonyl]amino}-4-fluoropiperidine-1-carboxylate (S14)

To a mixture of **14** (170 mg, 0.502 mmol), **S13** (145 mg, 0.502 mmol), and *N*,*N*-diisopropylethylamine (0.263 mL, 1.51 mmol) in *N*,*N*-dimethylformamide (8 mL) was added *O*-(7-azabenzotriazol-1-yl)-*N,N,N',N'*-tetramethyluronium hexafluorophosphate (HATU; 287 mg, 0.755 mmol). The reaction mixture was stirred at 18 °C for 2 hours, whereupon it was combined with two similar reactions carried out using **S13** (42.7 mg, 0.148 mmol and 171 mg, 0.592 mmol), diluted with water (50 mL), and extracted with ethyl acetate (30 mL). The organic layer was washed with saturated aqueous sodium chloride solution (50 mL), dried over sodium sulfate, filtered, and concentrated under reduced pressure. Upon silica gel chromatography (Gradient: 0% to 100% ethyl acetate in petroleum ether), **S14** (Combined yield: 540 mg, 0.943 mmol, 76%) was obtained as a yellow solid. LCMS *m/z* calc for $C_{30}H_{30}FN_6O_5$: 573.2; found: 573.1 $[M+H]^+$.

2-{5-[(3-ethoxypyridin-2-yl)oxy]pyridin-3-yl}-*N*-[(3*R*,4*R*)-4-fluoropiperidin-3-yl]pyrimidine-5-carboxamide (10)

A mixture of **S14** (300 mg, 0.524 mmol) and 10% palladium on carbon (300 mg) in ethanol (20 mL) was stirred under a balloon of hydrogen for 2 hours at 15 °C. After the reaction mixture had been combined with two similar reactions carried out using **S14** (200 mg, 0.349 mmol and 40 mg, 70 µmol), it was filtered through a pad of diatomaceous earth. The filtrate was concentrated, and the residue was purified using reversed-phase HPLC (Column: Agela Durashell C18, 5 µm; Mobile phase A: 0.05% ammonium hydroxide in water; Mobile phase B: acetonitrile; Gradient: 30% to 50% B), affording **10** (Combined yield: 174 mg, 0.397 mmol, 42%) as a white solid. ^{1}H NMR (400 MHz, chloroform-*d*) δ 9.52 (d, *J* = 1.8 Hz, 1H), 9.20 (s, 2H), 8.65 (d, *J* = 2.8 Hz, 1H), 8.56 (dd, *J* = 2.7, 1.8 Hz, 1H), 7.71 (dd, *J* = 4.9, 1.5 Hz, 1H), 7.28 – 7.23 (m, 1H, assumed; partially obscured by solvent peak), 7.17 (br d, *J* = 8 Hz, 1H), 7.02 (dd, *J* = 7.9, 4.9 Hz, 1H), 4.86 – 4.66 (m, 1H), 4.37 – 4.26 (m, 1H), 4.18 (q, *J* = 7.0 Hz, 2H), 3.36 (ddd, *J* = 12.3, 3.4, 3.4 Hz, 1H), 3.09 – 2.99 (m, 1H), 2.86 – 2.75 (m, 2H), 2.11 – 1.81 (m, 2H), 1.50 (t, *J* = 7.0 Hz, 3H). LCMS *m/z* calc for $C_{22}H_{24}FN_6O_3$: 439.2; found: 439.2 $[M+H]^+$, 100%.

Scheme S7. Synthesis of *rac-cis*-2-{5-[(3-ethoxypyridin-2-yl)oxy]pyridin-3-yl}-*N*-[5-fluoropiperidin-3-yl]pyrimidine-5-carboxamide, trifluoroacetate salt (11)



(+/-)-*cis*-*tert*-butyl 3-(2-(5-((3-ethoxypyridin-2-yl)oxy)pyridin-3-yl)pyrimidine-5-carboxamido)-5-fluoropiperidine-1-carboxylate (S15)

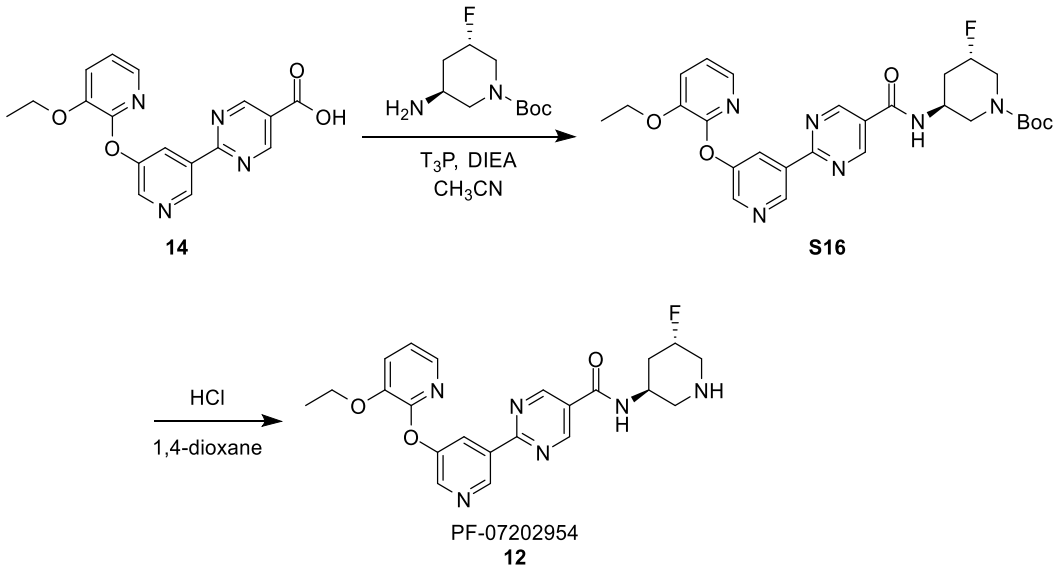
To a mixture of **14** (160 mg, 0.499 mmol), (+/-)-*cis*-1,1-dimethylethyl-3-amino-5-fluoro-1-piperidinecarboxylate (109 mg, 0.499 mmol), and *N*,*N*-diisopropylethylamine (0.269 mL, 1.50 mmol) in *N*,*N*-dimethylformamide (1.7 mL) was added *O*-(7-azabenzotriazol-1-yl)-*N,N,N',N'*-tetramethyluronium hexafluorophosphate (HATU; 218 mg, 0.574 mmol). The reaction mixture was stirred at 80 °C for 5 hours, diluted with water (50 mL), and extracted with ethyl acetate (30 mL). The organic layer was washed with saturated aqueous sodium chloride solution (50 mL), dried over magnesium sulfate, filtered, and concentrated under reduced pressure. Upon silica gel chromatography (Gradient: 0% to 10% methanol in dichloromethane), **S15** was obtained and used directly in the next step. LCMS *m/z* calc for $C_{27}H_{32}FN_6O_5$: 539.2; found: 539.2 $[M+H]^+$, calc'd 539.2, Rt = 0.91 min, 100% ELSD.

(+/-)-*cis*-2-{5-[(3-ethoxypyridin-2-yl)oxy]pyridin-3-yl}-*N*-[5-fluoropiperidin-3-yl]pyrimidine-5-carboxamide, trifluoroacetate salt (11)

Trifluoroacetic acid (0.382 mL, 4.99 mmol) was added to a vial containing **S15** (theoretical 0.499 mmol) dissolved in dichloromethane (2.50 mL). After stirring at room temperature for 4 hours, additional trifluoroacetic acid was added (0.2 mL). After stirring for another 16 h, the reaction was concentrated to give a yellow oil. Trituration with heptane/ethyl acetate (1:1, 5 mL) and filtration yielded **11** as a yellow solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.40 (d, J = 1.8 Hz, 1H), 9.27 (s, 2H), 9.15 – 8.93 (br s, 2H), 8.79 (br d, J = 6.9 Hz, 1H), 8.65 (d, J = 2.7 Hz, 1H), 8.38 (dd, J = 2.7, 1.8 Hz, 1H), 7.68 (dd, J = 4.9, 1.5 Hz, 1H), 7.58 (dd, J = 8.0, 1.5 Hz, 1H), 7.18 (dd, J = 8.0, 4.8

Hz, 1H), 5.01 (br d, J_{HF} = 45.8 Hz, 1H), 4.36 – 4.25 (m, 1H), 4.18 (q, *J* = 7.0 Hz, 2H), 3.57 – 3.45 (m, 1H), 3.39 (br d, *J* = 12.4 Hz, 1H), 3.31 – 3.19 (m, 1H), 3.06 (dd, *J* = 12.4, 8.1 Hz, 1H), 2.43 – 2.29 (m, 1H), 2.08 – 1.95 (m, 1H), 1.37 (t, *J* = 7.0 Hz, 3H); LCMS *m/z* calc for $C_{22}H_{24}FN_6O_3$ 439.2; found: 439.2 $[M+H]^+$, Rt = 0.57 min, 100%.

Scheme S8. Synthesis of 2-{5-[(3-ethoxypyridin-2-yl) oxy]pyridin-3-yl}-*N*-[(3*S*,5*S*)-5-fluoropiperidin-3-yl]pyrimidine-5-carboxamide (12)



***tert*-butyl (3*S*,5*S*)-3-{[(2-{5-[(3-ethoxypyridin-2-yl)oxy]pyridin-3-yl}pyrimidin-5-yl)carbonyl]amino}-5-fluoropiperidine-1-carboxylate (S16)**

N,*N*-Diisopropylethylamine (53.1 mL, 305 mmol) and *tert*-butyl (3*S*,5*S*)-3-amino-5-fluoropiperidine-1-carboxylate (9.50 g, 43.5 mol) were added to a solution of **14** (14.7 g, 43.4 mmol) in acetonitrile (210 mL). The mixture was cooled to 0 °C, and then 2,4,6-tripropyl-1,3,5,2,4,6-trioxatriphosphinane 2,4,6-trioxide (T3P; 50% solution in ethyl acetate; 30.5 mL, 51.2 mmol) was added via syringe, over approximately 4 minutes. After the reaction mixture was stirred at 0 °C for 45 minutes, the ice bath was removed and the reaction mixture was allowed to come to room temperature and stir for 17 hours. It was then concentrated *in vacuo*, the residue was partitioned between water and ethyl acetate, and the aqueous layer was extracted twice with ethyl acetate. The combined organic layers were washed sequentially with saturated aqueous sodium bicarbonate solution and saturated aqueous sodium chloride solution; a precipitate that appeared during the saturated aqueous sodium chloride wash was removed via filtration and discarded. The saturated aqueous sodium chloride layer was extracted once with ethyl acetate, and the combined organic layers were concentrated *in vacuo*. The residue was dissolved in a mixture of methylene chloride and methanol and pre-absorbed onto silica gel. Silica gel chromatography (Gradient: 30% to 100% ethyl acetate in heptane) was carried out to afford **S16** (19.1 g, 35.5 mmol, 82%) as an off-white solid. ^{1}H NMR (400 MHz, chloroform-*d*) δ 9.48 (d, *J* = 1.8 Hz, 1H), 9.12 (s, 2H), 8.63 (d, *J* = 2.6 Hz, 1H), 8.55 (dd, *J* = 2.7, 1.8 Hz, 1H), 7.70 (dd, *J* = 4.9, 1.5 Hz, 1H), 7.26 (dd, *J* = 7.8, 1.5 Hz, 1H), 7.03 (dd, *J* = 7.9, 4.9 Hz, 1H), 6.97 – 6.37 (v br m, 1H), 4.78 (br d, J_{HF} = 46.7 Hz,

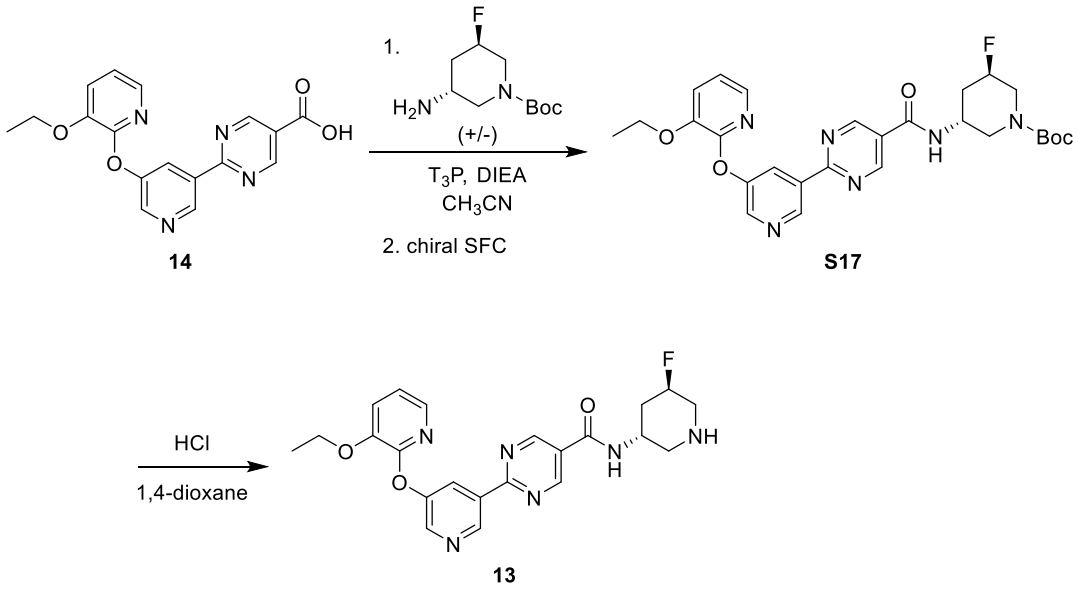
1H), 4.46 – 4.33 (m, 1H), 4.18 (q, *J* = 7.0 Hz, 2H), 4.08 – 3.05 (v br m, 4H), 2.41 – 2.11 (m, 1H), 2.02 – 1.79 (m, 1H), 1.49 (t, *J* = 7.0 Hz, 3H), 1.49 (s, 9H). LCMS *m/z* calc for $C_{27}H_{32}FN_6O_5$: 539.2; found: 539.3 $[M+H]^+$.

2-{5-[(3-ethoxypyridin-2-yl)oxy]pyridin-3-yl}-*N*-[(3*S*,5*S*)-5-fluoropiperidin-3-yl]pyrimidine-5-carboxamide (12)

A solution of hydrogen chloride in 1,4-dioxane (4 M; 520 mL, 2.1 mol) was added over 10 minutes to a room temperature solution of **S16** (159 g, 295 mmol) in tetrahydrofuran (850 mL); the reaction temperature increased from 35 °C to 40 °C, and this temperature was maintained using a heating mantle. After the addition was completed, the reaction mixture was stirred for 3 hours at 40 °C. LCMS analysis indicated that 20% of the starting material remained, so a solution of hydrogen chloride in 1,4-dioxane (4 M; 150 mL, 600 mmol) was again added to the reaction mixture, and stirring was continued at 40 °C for 30 minutes. At this point, the reaction mixture was treated with a solution of hydrogen chloride in 1,4-dioxane (4 M; 60 mL, 240 mmol). After an additional 45 minutes at 40 °C, the reaction mixture was concentrated *in vacuo*, and the resulting solid was dissolved in water (1 L). This solution was treated with aqueous sodium hydroxide solution (1 M; 900 mL, 900 mmol) and then diluted with water (400 mL) to facilitate stirring; after 15 minutes at room temperature, the precipitate was collected via filtration and washed with water (4 x 250 mL). This solid was brought to a total volume of 800 mL by addition of water, and then slurried with methanol (800 mL) at room temperature for 2 hours, using an overhead stirrer. The slurry was filtered, and the filter cake was washed with a mixture of methanol and water (1:1, 1 L). This solid was combined with the product from several similar reactions carried out using **S16** (≤946 mmol); the combined batches were suspended in ethyl acetate (1.1 L) and stirred for 1 hour at room temperature using a mechanical stirrer. After the solid had been collected by filtration, it was washed with ethyl acetate to afford **12 (**330 g, 753 mmol, 61% over 2 steps) as an off-white solid.

^{1}H NMR (400 MHz, DMSO-d_6) δ 9.38 (d, *J* = 1.8 Hz, 1H), 9.26 (s, 2H), 8.64 (d, *J* = 2.7 Hz, 1H), 8.60 (br d, *J* = 7.9 Hz, 1H), 8.36 (dd, *J* = 2.7, 1.8 Hz, 1H), 7.68 (dd, *J* = 4.9, 1.5 Hz, 1H), 7.56 (dd, *J* = 8.1, 1.5 Hz, 1H), 7.17 (dd, *J* = 8.0, 4.8 Hz, 1H), 4.81 (br d, J_{HF} = 48.3 Hz, 1H), 4.22 – 4.07 (m, 1H), 4.17 (q, *J* = 7.0 Hz, 2H), 3.02 – 2.86 (m, 2H), 2.69 (br dd, *J* = 35.1, 14.2 Hz, 1H), 2.5 – 2.38 (m, 2H, assumed; partially obscured by solvent peak), 2.19 – 2.07 (m, 1H), 1.91 – 1.72 (m, 1H), 1.37 (t, *J* = 7.0 Hz, 3H). qNMR purity = 99.53%. ^{13}C NMR (DMSO-d_6, 100 MHz) δ = 162.6, 162.4, 156.9, 152.0, 150.9, 145.3, 144.8, 143.7, 136.9, 133.0, 127.1, 126.4, 121.6, 120.7, 88.1 (d, *J* = 168.0 Hz), 64.2, 49.6, 48.6 (d, *J* = 19.8 Hz), 43.9, 34.9 (d, *J* = 22.0 Hz), 14.4. HRMS (ESI/QTOF) m/z: $[M+H^+]$ Calc for $C_{22}H_{23}FN_6O_3$: 439.1888. Found: 439.1893. LCMS *m/z* calc for $C_{22}H_{24}FN_6O_3$: 439.2; found: 439.3 $[M+H]^+$. $[\alpha]_D^{20}$ -8.0 (*c* 1.3, MeOH).

Scheme S9. Synthesis of 2-{5-[(3-ethoxypyridin-2-yl)oxy]pyridin-3-yl}-*N*-[(3*R*,5*R*)-5-fluoropiperidin-3-yl]pyrimidine-5-carboxamide, trifluoroacetate salt (13)



***tert*-butyl (3*R*,5*R*)-3-(2-(5-((3-ethoxypyridin-2-yl)oxy)pyridin-3-yl)pyrimidine-5-carboxamido)-5-fluoropiperidine-1-carboxylate (S17)**

To a mixture of **14** (100 mg, 0.296 mmol), *rac*-*tert*-butyl-3-amino-5-fluoropiperidine-1-carboxylate (77.4 mg, 0.355 mmol), and *N*,*N*-diisopropylethylamine (0.360 mL, 2.07 mmol) in *N*,*N*-dimethylformamide (2 mL) was added *O*-(7-azabenzotriazol-1-yl)-*N,N,N',N'*-tetramethyluronium hexafluorophosphate (HATU; 229 mg, 0.591 mmol). The reaction mixture was stirred at room temperature for 1 hour, diluted with water (20 mL), and extracted with ethyl acetate (2 x 30 mL). The organic layer was washed with saturated aqueous sodium chloride solution (20 mL), dried over sodium sulfate, filtered, and concentrated under reduced pressure. Upon silica gel chromatography (Gradient: 30% to 100% ethyl acetate in heptane), **S17** was obtained. Chiral HPLC purification (Column: Lux Amylose-1 250 mm x 21.2 mm x 5 µm, Mobile phase A: carbon dioxide; Mobile phase B: 0.2% 7 N ammonia in methanol in 2-propanol; Isocratic flow: 80% A; Flow rate: 80 mL/min; back pressure: 120 Bar) was performed to separate the enantiomers. LCMS *m/z* calc for $C_{27}H_{32}FN_6O_5$: 539.2; found: 540.3 $[M+H]^+$, Rt = 0.91 min, 100% ELSD.

2-{5-[(3-ethoxypyridin-2-yl)oxy]pyridin-3-yl}-*N*-[(3*R*,5*R*)-5-fluoropiperidin-3-yl]pyrimidine-5-carboxamide, trifluoroacetate salt (13)

Trifluoroacetic acid (1.0 mL) was added to a vial containing **S17** (40 mg, 0.074 mmol) suspended in dichloromethane (1 mL). After stirring at room temperature for 3 hours, the reaction was concentrated to give **13** as a yellow oil. HPLC purification (Column: Sunfire C18 19 x 100 mm x 5 µm; Mobile phase A: 0.05% TFA in water (v/v); Mobile phase B: 0.05% TFA in acetonitrile

(v/v); Gradient: 5% to 35% B over 8.5 minutes; 35% to 95% B in 30 seconds; hold for 10 minutes; flow rate: 25 mL/min). Conditions for analytical HPLC: Column: Waters Atlantis dC18, 4.6 x 50 mm, 5 µm; Mobile phase A: 0.05% trifluoroacetic acid in water (v/v); Mobile phase B: 0.05% trifluoroacetic acid in acetonitrile (v/v); Gradient: 5.0% to 95% B, linear over 4.0 minutes; Flow rate: 2 mL/minute. Rt = 1.82 minutes. LCMS *m/z* calc for $C_{22}H_{24}FN_6O_3$: 439.2; found: 439.1 $[M+H]^+$, 100%.

Table S1 Cerep Bioprint Off-Target Panel:

Target	Format	Single Point Compound Test Concentration (uM) or IC_{50}/EC_{50}	PF-07202954 % activity
Abl Kinase	Kinase Inhibition	10	1
Acetylcholinesterase	Enzyme Inhibition	10	12
Adenosine A1 Receptor	Agonism	10	-14
Adenosine A2a Receptor	Agonism	10	1
Adrenergic Alpha 1a Adrenoceptor	Agonism	10	-2
Adrenergic Alpha 1a Adrenoceptor	Antagonism	10	-8
Adrenergic Alpha 2a receptor	Agonism	10	-1
Adrenergic Alpha 2b Adrenoceptor	Agonism	10	-1
Adrenergic Alpha 2b Adrenoceptor	Antagonism	10	2
Adrenergic Beta 1 Adrenoceptor	Agonism	10	1
Adrenergic Beta 1 Adrenoceptor	Antagonism	10	-21
Adrenergic Beta 2 Adrenoceptor	Agonism	10	-4
Adrenergic Beta 2 Adrenoceptor	Antagonism	10	8
AMPA Receptor	Ion Channel Binding	10	7
Androgen Receptor	NHR Binding	10	-5
Angiotensin 1	Agonism	10	-4
Angiotensin 1	Antagonism	10	1
Angiotensin Converting Enzyme	Enzyme Inhibition	10	-9
Aurora A Kinase	Kinase Inhibition	10	-22
Cannabinoid CB1	Agonism	10	-40
Cannabinoid CB1	Antagonism	10	3
Cholecystokinin 2	Agonism	10	-1
Choline Transporter	Transporter Binding	10	6
Corticotrophin Releasing Factor 1 (CRF1) Receptor	Agonism	10	1
Corticotrophin Releasing Factor 1 (CRF1) Receptor	Antagonism	10	-43
Cyclooxygenase 2	Enzyme Inhibition	10	22
Delta Opioid Receptor	Agonism	10	-2
Dopamine 2S	Agonism	10	7
Dopamine 2S	Antagonism	10	6
Dopamine D1	Agonism	10	1
Dopamine D1	Antagonism	10	9
Dopamine Transporter	Transporter Binding	10	11
EGFR Kinase	Kinase Inhibition	10	-1
Endothelin A	Agonism	10	-3
GABA Transporter	Transporter Binding	10	1
GABAa (benzodiazepine site)	Ion Channel Binding	10	-6
GABAa (Cl Channel)	Ion Channel Binding	10	5
GABAa1 Receptor	Ion Channel Binding	10	-14

Glucocorticoid Receptor	NHR Binding	10	-6
Histamine H1	Agonism	10	-4
Histamine H1	Antagonism	10	-4
Histamine H2	Agonism	10	1
Histamine H3	Agonism	10	-24
Kappa Opioid	Agonism	10	-9
Kdr Kinase (VEGFR2)	Kinase Inhibition	10	-2
LCK Kinase	Kinase Inhibition	10	-3
L-Type Ca2+ Channel (nifedipine site)	Ion Channel Binding	10	15
L-Type Ca2+ Channel (diltiazem site)	Ion Channel Binding	10	28
L-Type Ca2+ Channel (verapamil site)	Ion Channel Binding	10	3
Melanocortin MC2 Receptor	Agonism	10	1
Melanocortin MC2 Receptor	Antagonism	10	-2
Monoamine Oxidase A	Enzyme Inhibition	10	19
Mu Opioid	Antagonism	10	6
Mu Opioid	Agonism	10	1
Muscarinic 1	Agonism	10	-5
Muscarinic 1	Antagonism	10	-11
Muscarinic 2	Agonism	10	-27
Muscarinic 2	Antagonism	10	17
Muscarinic 3	Agonism	10	-2
Muscarinic 3	Antagonism	10	-13
Na+ Channel (site 2)	Ion Channel Binding	10	1
Neurokinin Nk1	Agonism	10	-1
Nicotinic Acetylcholine Receptor (muscle-type)	Ion Channel Binding	10	111
Nicotinic Acetylcholine Receptor (muscle-type)	Ion Channel Binding	IC_{50}	>10 uM
Nicotinic Acetylcholine Receptor (alpha4, beta2 subunit containing)	Ion Channel Binding	10	-1
NMDA Receptor (glutamate site)	Ion Channel Binding	10	-16
NMDA Receptor (PCP binding site)	Ion Channel Binding	10	-19
Norepinephrine Transporter	Transporter Binding	10	-4
P38alpha MAP Kinase	Kinase Inhibition	10	-5
Phosphodiesterase 3B	Enzyme Inhibition	10	-11
Phosphodiesterase 4D2	Enzyme Inhibition	10	30
PPAR-gamma	NHR Binding	10	-15
Serotonin 5-HT1a Receptor	Agonism	10	-1
Serotonin 5-HT1b Receptor	Agonism	10	-17
Serotonin 5-HT2a	Agonism	10	2
Serotonin 5-HT2b	Agonism	10	4
Serotonin 5-HT3	Ion Channel Binding	10	7
Serotonin 5-HT4e	Agonism	10	-0
Serotonin Transporter	Transporter Binding	10	-4
Src Kinase	Kinase Inhibition	10	3
Thyrotopin Releasing Hormone 1 (TRH1) Receptor	Agonism	10	-2
Thyrotopin Releasing Hormone 1 (TRH1) Receptor	Antagonism	10	-19
Vasopressin 1a	Agonism	10	-10

PF-07202954 was tested in single point at 10 µM against a broad off-target panel to determine selectivity for DGAT2. The only target (shaded in table) that showed >50% activity @ 10 µM was followed up with dose response curve and demonstrated an IC_{50} >10 µM.

In Vivo Studies.

8-Day Treatment of Western Diet-fed Rats with PF-07202954

In Vivo Study Design

Sprague Dawley rats weighing 180-200 grams were ordered from Charles River Laboratories (Boston, MA) and acclimated on PicoLab® Rodent Diet 20 chow for 72 hours upon arrival. At the end of acclimation, a subset of rats was switched to a Western diet (D12079b, Research Diets, New Brunswick, NJ) for 21 days. In addition to Western Diet fed animals, a control study cohort was maintained on PicoLab® Rodent Diet 20 for the duration of the study. Animals were randomized according to their housing location and assigned into dose groups; vehicle administered chow diet, vehicle administered Western diet and 3 mg/kg PF-07202954, 10 mg/kg PF-07202954, 30 mg/kg PF-07202954 or 100 mg/kg PF-07202954 fed Western diet. Each dose group contained 8 animals. Animals were maintained on the Western diet for 14 days and then administered PF-07202954 twice daily for the final 7 days of the study, a total of 21 days on Western Diet. On the final day of the study, rats were administered PF-07202954 in the morning at approximately 9 am. Two hours post-dose, approximately 500 µL of blood was collected by conscious tail bleeding into K2-EDTA tubes, mixed and placed on ice for measurement of plasma PF-07202954 concentrations and TG levels. Following the collection of tail blood, rats were euthanized with CO_2 and livers were promptly removed and flash frozen. Terminal blood was collected by cardiac stick and transferred to K2-EDTA tubes. Plasma was separated according to manufacturer's protocol.

Triglyceride Analysis

Liver tissue was placed in aluminum foil and was snap-frozen in a liquid nitrogen bath. The tissue was stored at -80 °C until processing. On the day of processing, liver tissue, wrapped in aluminum foil, was pulverized with a hammer, on an aluminum heat block in a liquid nitrogen bath. Pulverization of the liver tissue produced a homogeneous powder. Homogenization buffer, Tris pH 7.4, 98.9 mL 0.9% NaCl and 100 µL of Triton X 100, was mixed on a stir plate for 10 minutes prior to using. Sample weights of approximately 100 mg of homogenous liver tissue were weighed and placed in Lysing Matrix D tube with 1 mL of homogenization buffer. All samples were then placed in the FastPrep FP120 for 2 minutes or until tissue was properly homogenized. All samples were then spun for 30 seconds at 10,000 × g, to clear foam from the homogenates. Following sample homogenization, 50 µL of sample was transferred to a sterile mixing plate with 450 µL of DPBS to create a 1:10 dilution. Samples were re-suspended and transferred to sampling tubes for analysis on the Siemens Advia XPT Clinical Analyzer.

For plasma TG, plasma was isolated using the K2-EDTA tubes according to the manufacturer's protocol. Approximately 100 µL was transferred to sampling tubes for the Siemens Advia XPT Clinical Analyzer. Siemens Advia Triglyceride assay (NC0956353) was run according to manufacturer's protocol.

For plasma, TGs are reported as mg/dL. For hepatic TGs, the mg/dL reading was normalized per gram of tissue. A tissue density of 1 is assumed in the calculation.

Gene Expression Profiling

The left lateral lobe of the liver was crushed into a fine powder by pulverizing on a block, bathed in liquid nitrogen. Approximately 25 mg was used to prepare total RNA using the Qiagen RNeasy kit. The quantity and quality of the RNA was determined using a Nanodrop 8000, by measuring absorbance ratios at 260 nm/230 nm and 260 nm/280 nm. cDNA was synthesized from RNA by the High-Capacity cDNA Reverse Transcription kit. For each sample, 25 µL Master Mix was combined with 25 µL of reaction buffer containing 2 µg of RNA into a single well of a 96-well plate. Reverse transcription was performed using a SimpliAmp Thermal Cycler. The reaction cycles were as follows: 25 °C for 10 minutes, 37 °C for 2 hours and 85 °C for 5 minutes. The cycled samples were maintained at 4 °C and then re-suspended with 150 µL of nuclease free H_2O. cDNA samples were stored at -20 °C. Gene expression was performed in a 384 well plate. Each well contained 2 µL of cDNA (~20 ng) and 8 µL of TaqMan Gene expression Master Mix containing TaqMan probes and nuclease free H_2O. Table S2 shows the probes used during this assay, all for the *Rattus norvegicus* genome.

Statistical Analysis

After testing for normality the data was log transformed and an ANOVA was performed taking account for unequal variance with a Dunnett post hoc test then applied to adjust for multiple comparisons against the western diet vehicle treated group. Statistical analysis was performed using GraphPad Prism version 8.4.2 for Windows, GraphPad Software (La Jolla, CA, USA).

Table S2. Expression of Hepatic Genes Normalized to Vehicle Western Diet-Fed Animals

Gene Symbol	Western Diet (D12079b)[a]			
	Thermo Fisher Catalog Number	Vehicle	PF-07202954 10 mg/kg BID	PF-07202954 100 mg/kg BID
Abcg5	Rn00587092_m1	1 ± 0.30	0.51 ± 0.14	0.38 ± 0.08
Abcg8	Rn00590367_m1	1 ± 0.35	1.67 ± 0.84	1.75 ± 0.45
Acaca	Rn00573474_m1	1 ± 0.07	0.98 ± 0.15	0.59 ± 0.06**
Acot2	Rn02133135_m1	1 ± 0.17	1.12 ± 0.07	0.89 ± 0.08
Acot4	Rn04219152_m1	1 ± 0.15	1.35 ± 0.12	0.85 ± 0.10
Cyp51	Rn01526553_m1	1 ± 0.05	0.99 ± 0.06	0.62 ± 0.08**
Fads2	Rn00580220_m1	1 ± 0.10	0.78 ± 0.06	0.34 ± 0.05****
Fasn	Rn01463550_m1	1 ± 0.18	0.78 ± 0.23	0.22 ± 0.06**
Fdps	Rn00821389_g1	1 ± 0.08	0.83 ± 0.03	0.47 ± 0.06***
Insig1	Rn00574380_m1	1 ± 0.17	1.84 ± 0.95	0.28 ± 0.03***
Insig2	Rn00710111_m1	1 ± 0.08	0.99 ± 0.10	0.79 ± 0.05
Pcsk9	Rn01416753_m1	1 ± 0.08	0.54 ± 0.06**	0.36 ± 0.09**
Pmvk	Rn01462515_m1	1 ± 0.09	0.91 ± 0.05	0.56 ± 0.07**
Pnpla3	Rn01502361_m1	1 ± 0.31	0.72 ± 0.29	0.05 ± 0.02**

Scd1	Rn00594894_g1	1 ± 0.09	0.84 ± 0.09	0.44 ± 0.05***
Srebp1	Rn01495769_m1	1 ± 0.10	0.59 ± 0.09*	0.66 ± 0.09*

**** = p<0.0001 versus western diet vehicle-treated animals; *** = p<0.001 versus western diet vehicle-treated animals; ** = p<0.01 versus western diet vehicle-treated animals; * = p<0.05 versus western diet vehicle-treated animals; Abcg5 = ATP Binding Cassette Subfamily G Member 5; Abcg8 = ATP Binding Cassette Subfamily G Member 8; Acaca = Acetyl-CoA Carboxylase Alpha; Acot2 = Acyl-CoA thioesterase 2; Acot4 = Acyl-CoA thioesterase 4; Cyp51 = Cytochrome P450, family 51, subfamily A; Fads2 = Fatty acid desaturase 2; Fasn = Fatty acid synthase; Fdps = Farnesyl diphosphate synthase; Insig1 = Insulin induced gene 1; Insig2 = Insulin induced gene 2; p = Probability value; Pcsk9 = Proprotein convertase subtilisin/ kexin type 9; Pmvk = Phosphomevalonate kinase; Pnpla3 = Patatin-like phospholipase domain-containing protein 3; Scd1 = Stearoyl-Coenzyme A desaturase 1; SREBP1 = Sterol-response element binding protein.
a. Values are reported as fold change compared to vehicle ± Standard error of the mean.

Single Dose Treatment of Sucrose Diet-fed Rats with PF-07202954

In Vivo Study Design

Sprague Dawley rats weighing 180-200 grams were ordered from Charles River Laboratories (Boston, MA). Rats were acclimated for 72 hours, during which they were fed PicoLab® Rodent Diet 20 (5053). At the end of acclimation, animals were switched to TD03045 high sucrose diet (Harlan Tekland) for 48 hours. Animals were randomized into dose groups as follows; vehicle, or PF-07202954 at 10 mg/kg and 100 mg/kg for both 2 and 4 hours of compound treatment with 8 animals per group. On the day of the experiment, animals were fasted between 6:00-6:30 a.m. and orally dosed (10 mL/kg) with either vehicle or a selected dose of PF-07202954 approximately 4 hours later. Animals were consciously bled via the tail vein (500 µL blood) into tubes containing K_2-EDTA at 2 and 4 hours post-dose to assess pharmacokinetic exposure of PF-07202954. Following the collection of tail blood, animals were euthanized with CO_2 and livers were promptly removed and flash frozen. Terminal blood was collected by cardiac puncture and plasma was separated from whole blood collected in K_2-EDTA containing tubes, according to manufacturer's protocol.

Triglyceride Analysis

Liver tissue was placed in aluminum foil and was snap-frozen in a liquid nitrogen bath. The tissue was stored at -80 °C until processing. On the day of processing, liver tissue, wrapped in aluminum foil, was pulverized with a hammer, on an aluminum heat block in a liquid nitrogen bath. Pulverization of the liver tissue produced a homogeneous powder. Homogenization buffer, Tris pH 7.4, 98.9 mL 0.9% NaCl and 100 µL of Triton X 100, was mixed on a stir plate for 10 minutes prior to using. Sample weights of approximately 100 mg of homogenous liver tissue were weighed and placed in Lysing Matrix D tube with 1 mL of homogenization buffer. All samples were then placed in the FastPrep FP120 for 2 minutes or until tissue was properly homogenized. All samples were then spun for 30 seconds at 10,000 × g, to clear foam from the homogenates. Following sample homogenization, 50 µL of sample was transferred to a sterile mixing plate with 450 µL of DPBS to create a 1:10 dilution. Samples were re-suspended and transferred to sampling tubes for analysis on the Siemens Advia XPT Clinical Analyzer.

For plasma TG, plasma was isolated using the K_2-EDTA tubes according to the manufacturer's protocol. Approximately 100 µL was transferred to sampling tubes for the Siemens Advia XPT Clinical Analyzer. Siemens Advia Triglyceride assay (NC0956353) was run according to manufacturer's protocol.

For plasma, TGs are reported as mg/dL. For hepatic TGs, the mg/dL reading was normalized per gram of tissue. A tissue density of 1 is assumed in the calculation.

Gene Expression Profiling

The left lateral lobe of the liver was crushed into a fine powder by pulverizing on a block, bathed in liquid nitrogen. Approximately, 25 mg was used to prepare total RNA using the Qiagen RNeasy kit (Qiagen Inc., Valencia, CA, USA). The quantity and quality of the RNA was determined using a Nanodrop 8000 (Thermo Fisher Scientific Waltham, MA), by measuring absorbance ratios at 260 nm/230 nm and 260 nm/280 nm. Two micrograms of RNA were used to synthesize cDNA By the High-Capacity cDNA Reverse Transcription kit (Thermo Fisher Scientific). For each sample, 25 µL Master Mix was combined with 25 µL of reaction buffer containing 2 µg of RNA, into a single well of a 96-well plate. Reverse transcription was performed using a SimpliAmp Thermal Cycler (Thermo Fisher Scientific). The reaction cycles were as follows: 25 °C for 10 minutes, 37 °C for 2 hours and 85 °C for 5 minutes. The cycled samples were maintained at 4 °C and then re-suspended with 150 µL of nuclease free H_2O. cDNA samples were stored at -20 °C. Gene expression was performed in a 384 well plate. Each well contained 2 µL of sample cDNA (~20 ng) and 8 µL of TAQMAN Gene expression Master Mix (Thermofisher Scientific) containing TAQMAN probes (Thermofisher Scientific) and nuclease free H_2O. Table S3 shows the probes used during this assay, all for the *Rattus Norvegicus* genome.

Statistical Analysis

After testing for normality, the data were log transformed and an ANOVA was performed taking account for unequal variance with a Dunnett post hoc test then applied to adjust for multiple comparisons against the sucrose vehicle treated group. Statistical analysis was performed using GraphPad Prism version 8.0.2 for Windows, GraphPad Software (La Jolla, CA, USA).

Table S3. Gene Expression Relative to Vehicle Treated Control Group at 4 hours

Gene Symbol	Sucrose Diet TD03045[a]			
	ThermoFisher Catalog Number	Vehicle	PF-07202954 10 mg/kg	PF-07202954 100 mg/kg
Abcg5	Rn00587092_m1	1 ± 0.16	4.11 ± 2.83	16.30 ± 4.11****
Abcg8	Rn00590367_m1	1 ± 0.18	11.95 ± 9.87	41.86 ± 11.32****
Acaca	Rn00573474_m1	1 ± 0.10	0.93 ± 0.20	0.18 ± 0.01****
Acot2	Rn02133135_m1	1 ± 0.14	1.00 ± 0.12	1.36 ± 0.13
Acot4	Rn04219152_m1	1 ± 0.22	0.93 ± 0.05	1.55 ± 0.21
Cyp51	Rn01526553_m1	1 ± 0.09	0.89 ± 0.11	0.71 ± 0.11
Fads2	Rn00580220_m1	1 ± 0.08	0.61 ± 0.06**	0.31 ± 0.03****

Fasn	Rn01463550_m1	1 ± 0.19	0.97 ± 0.33	0.05 ± 0.01****
Fdps	Rn00821389_g1	1 ± 0.11	0.74 ± 0.10	0.41 ± 0.05****
Insig1	Rn00574380_m1	1 ± 0.14	0.41 ± 0.08**	0.17 ± 0.03****
Insig2	Rn00710111_m1	1 ± 0.10	0.64 ± 0.06*	0.61 ± 0.05**
Pcsk9	Rn01416753_m1	1 ± 0.12	0.68 ± 0.09	0.25 ± 0.05****
Pmvk	Rn01462515_m1	1 ± 0.08	0.80 ± 0.09	0.59 ± 0.07**
Pnpla3	Rn01502361_m1	1 ± 0.23	0.53 ± 0.26	0.001 ± 0.00****
Scd1	Rn00594894_g1	1 ± 0.07	0.92 ± 0.20	0.10 ± 0.02****
Srebp1	Rn01495769_m1	1 ± 0.12	0.62 ± 0.14	0.14 ± 0.02****

**** = p<0.0001 versus sucrose vehicle-treated animals; ** = p<0.01 versus sucrose vehicle-treated animals; * = p<0.05 versus sucrose vehicle-treated animals; Abcg5 = ATP Binding Cassette Subfamily G Member 5; Abcg8 = ATP Binding Cassette Subfamily G Member 8; Acaca = Acetyl-CoA Carboxylase Alpha; Acot2 = Acyl-CoA thioesterase 2; Acot4 = Acyl-CoA thioesterase 4; Cyp51 = Cytochrome P450, family 51, subfamily A; Fads2 = Fatty acid desaturase 2; Fasn = Fatty acid synthase; Fdps = Farnesyl diphosphate synthase; Insig1 = Insulin induced gene 1; Insig2 = Insulin induced gene 2; Pcsk9 = Proprotein convertase subtilisin/ kexin type 9; Pmvk = Phosphomevalonate kinase; Pnpla3 = Patatin-like phospholipase domain-containing protein 3; Scd1 = Stearoyl-Coenzyme A desaturase 1; SREBP1 = Sterol-response element binding protein.

a. Values are reported as fold change compared to vehicle ± standard error of the mean.

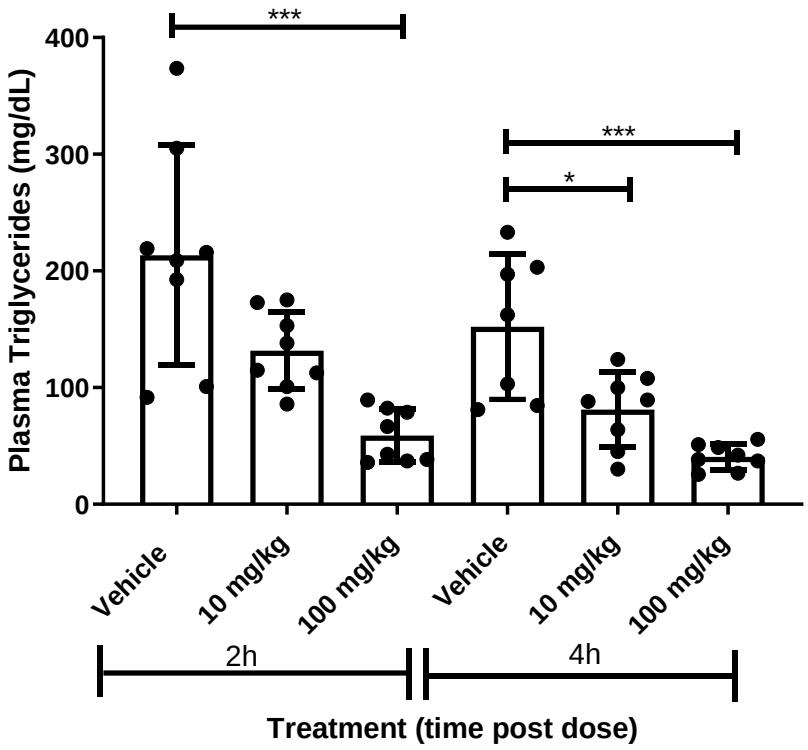
Table S4. Changes in Plasma Triglycerides After Administration of PF-07202954

Treatment	**Time**	**Plasma[a]**		**Liver[a]**	
		Avg TG (mg/dL)	**% decrease**	**Avg TG (μg/mg)**	**% decrease**
Vehicle	2h	213.5	0%	29.6	0%
10 mg/kg	2h	131.6	38%	28.1	5%
100 mg/kg	2h	58.9	72%***	32.4	+9%
Vehicle	4h	152.1	0%	26.0	0%
10 mg/kg	4h	81.1	47%*	20.4	22%
100 mg/kg	4h	40.6	73%***	10.3	60%***

*** = p<0.001 versus time-matched sucrose vehicle-treated animals; * = p<0.05 versus time-matched sucrose vehicle-treated animals; Avg = Average; h = hour; n = Number of replicates; % = Percent; TG = Triglyceride.

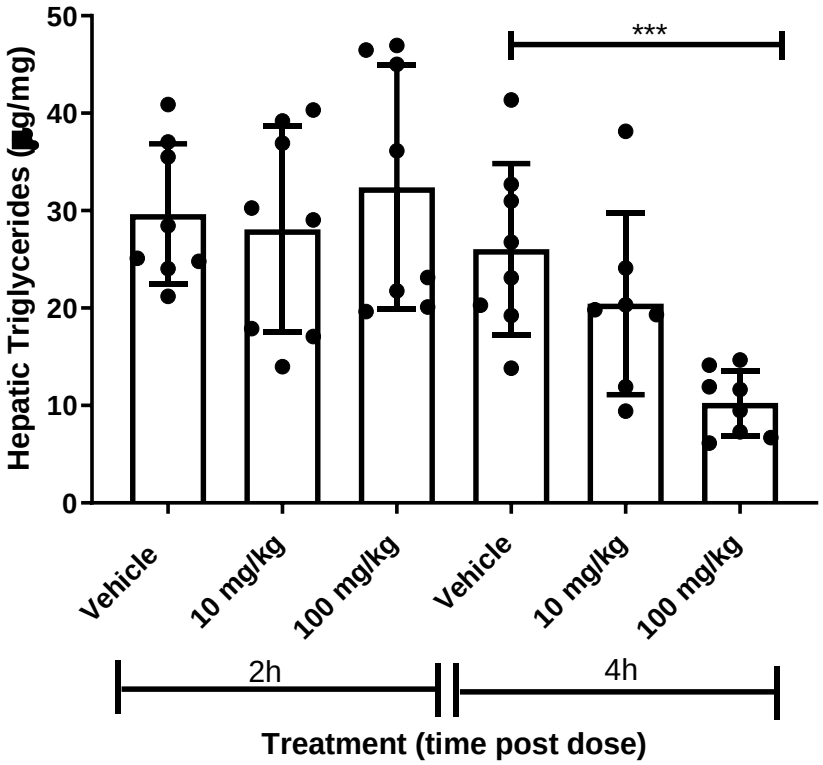
a. Data reported is the mean TG and percent decrease for liver and plasma, with n = 8 per group.

Figure S1. Circulating Triglycerides



Data presented are mean ± standard deviation.
*** = p<0.001 versus time-matched sucrose vehicle-treated animals; * = p<0.05 versus time-matched sucrose vehicle treated animals; h = Hours.

Figure S2. Hepatic Triglycerides



Data presented are mean ± standard error of the mean.
*** = p<0.001 versus time-matched sucrose vehicle-treated animals; h = Hours.

Biochemical Functional Assay to Measure Inhibition of DGAT2

Briefly, 1 µL of serially diluted PF-07202954 was spotted into 384-well polypropylene plates which also contained control wells with either 1 µL DMSO or 1 µL of a proprietary inhibitor specific for the enzyme to be used. Subsequently, 4 µL of a 0.04% (0.4 mg/ mL) BSA solution in HPLC grade water was added to the plate and incubated for 15 min at room temperature. This was followed by addition of 10 µL of solution containing microsome-bound enzyme in assay buffer (100 mM HEPES pH 7.4, 20 mM MgCl2) and methyl arachidonyl fluorophosphonate (MAFP, final concentration of 100 nM). After a preincubation of the enzyme and PF-07202954 at room temperature for 2 h, 4 µL of substrate solution containing ^{14}C decanoyl-CoA (^{14}C dec-CoA) and 1,2-didecanoyl SN glycerol in acetone (2.5% final assay concentration) were added. Plates were covered with a lid and allowed to incubate at room temperature for 40 min. Reaction conditions were optimized for each batch of enzyme to reflect the linear range of enzyme activity with less than 30% substrate conversion. The final assay conditions include 7.5 ng/µL hDGAT2, 5.96 µM ^{14}C dec-CoA, 25 µM substrate.

The enzyme reactions were stopped by adding 5 μL of a 1% solution of phosphoric acid diluted in HPLC grade water. A volume of 45 μL of Microscint-E was then added to each well. The plates were sealed with Top-seal covers and phase partitioning was performed on a Big Bear Shaker (HT-91100 Microplate Orbital Shaker) for 5 min at alternating speeds of 3500 rpm for 15 sec then 500 rpm for 5 sec. Plates were then centrifuged for 5 min at 1000 rpm, and the Top-seal covers were replaced with new ones. After a 3 hr incubation to allow for complete phase partitioning, the plates were read on a Trilux Microbeta instrument.

The raw data from the Trilux Microbeta reader was expressed as corrected counts per minute and was analyzed using proprietary software (Activity Base). The percent effect at each concentration of PF-07202954 was calculated relative to the values for the uninhibited control wells (negative controls) and fully inhibited control wells (positive controls) on each assay plate. The positive control wells contained a saturating concentration of a proprietary compound that produced full enzymatic inhibition while the negative control wells contained DMSO alone and was used to set the baseline response in the assay. IC50 values were determined from the percent effect data using a 4-parameter logistic dose response model. Proprietary reference compounds were tested in each experiment for quality control, and the experiment was deemed acceptable if the IC50 values were within 3-fold of previous data.

Assay to Measure Inhibition of DGAT2 in primary human hepatocytes

Cryopreserved human hepatocytes and culturing reagents were obtained from BioIVT (Hicksville, NY). Geltrex Basement Membrane Matrix (catalog number, A1413302), Williams' E media (catalog number, 12551-032), Dulbecco Modified Eagle Media (DMEM, catalog number, 11995), penicillin-streptomycin (catalog number, 15140122), glutamine (catalog number, 25030081), and Dulbecco's phosphate buffered saline (DPBS, catalog number, 14190094), were purchased from ThermoFisher (Waltham, MA). [^{14}C]glycerol (catalog number, NEC441X) and [^{14}C]oleate (catalog number, NEC317) was obtained from PerkinElmer (Waltham, MA). Thin layer chromatography (TLC) plates (catalog number, Z741119), sodium dodecanoate (catalog number, L9755), isopropyl alcohol (catalog number, 563935), tetrahydrofuran (THF, catalog number, 439215), ethyl acetate (catalog number, 439169), chloroform ($CHCl_3$, catalog number, 372978), methanol (MeOH, catalog number, 322415), potassium chloride (KCl, catalog number, P9541), hexane (catalog number, 296090), diethyl ether (catalog number, 296082), acetic acid (catalog number, 320099) were obtained from Sigma-Aldrich (St. Louis, MO). PF-04620110, a selective DGAT1 inhibitor[2], was synthesized at Pfizer Inc., Groton, CT.

Hepatocytes were plated into collagen-coated 96-well plates on day 1 at a density of 50,000 cells/well. All cells were cultured in a 37 °C, 5% CO_2, 95% air, 95% humidity incubator and hepatocytes were maintained in BioIVT incubation media. On day 2 the media was removed and cells were overlaid with Geltrex Basement Membrane Matrix and fresh culture media added. The morning of the experiment the media was removed and replaced with 150 mL/well Williams' E serum-free medium containing 1X penicillin-streptomycin / glutamine and 400 mM dodecanoate. The plate was returned to the 37 °C incubator for 45 minutes after which 1.58 mL of 300 mM DGAT1 inhibitor, PF-04620110, in 25:75 DMSO:DPBS was added; followed by the addition of 1.58 mL of 100X stocks for each test concentration of DGAT2 inhibitor, also in

DMSO:DPBS. Control wells were given 1.58 mL of 25:75 DMSO:DPBS. This dilution format yielded a final in-well concentration of PF-04620110 of 3 mM and 8 test concentrations of PF-07202954 ranging from 0.3 nM to 1 mM. After 15 minutes 5.0 mL of ^{14}C-glycerol (0.04 mCi/mL) in DPBS was added to all wells. The plate was returned to the incubator for 3 hours incubation period. At the end of the incorporation period the media was removed and the wells washed once with 150 mL DPBS. After removing the wash, 100 mL/well of 90:10 isopropanol:tetrahydofuran was added. The plate was sealed with a foil plate cover and placed on a plate shaker for 15 minutes and then centrifuged 3000 rpm x 10 minutes. In order to separate triglyceride from the other lipid species, 40 mL of the total 100 mL extract/well was applied per lane of the TLC plate and developed in hexane:diethyl ether:acetic acid (75:23:2) until the solvent front reached the plate top. The plate was then dried under N2. In order to quantify the amount of radioactivity in the samples, an unused lane on the TLC plate was spotted with 1 to 10 mL amounts of a ^{14}C-standard (^{14}C-oleate in EtOH). The plate was covered in Saranwrap and placed against a phosphorimaging screen for 18-24 hours. The exposed phosphor screen was scanned using a GE Healthcare Typhoon FLA 9500 Imager (Marlborough, MA) followed by quantification of triglyceride radioactivity using ImageQuant TL software (GE Healthcare, Marlborough, MA) and calibration from the ^{14}C-standard curve. The half maximal inhibitory concentrations (IC_{50}) were determined by plotting percent inhibition relative to DMSO control versus the log-transformed inhibitor concentration using GraphPad Prism (GraphPad Software, Inc., La Jolla, CA).

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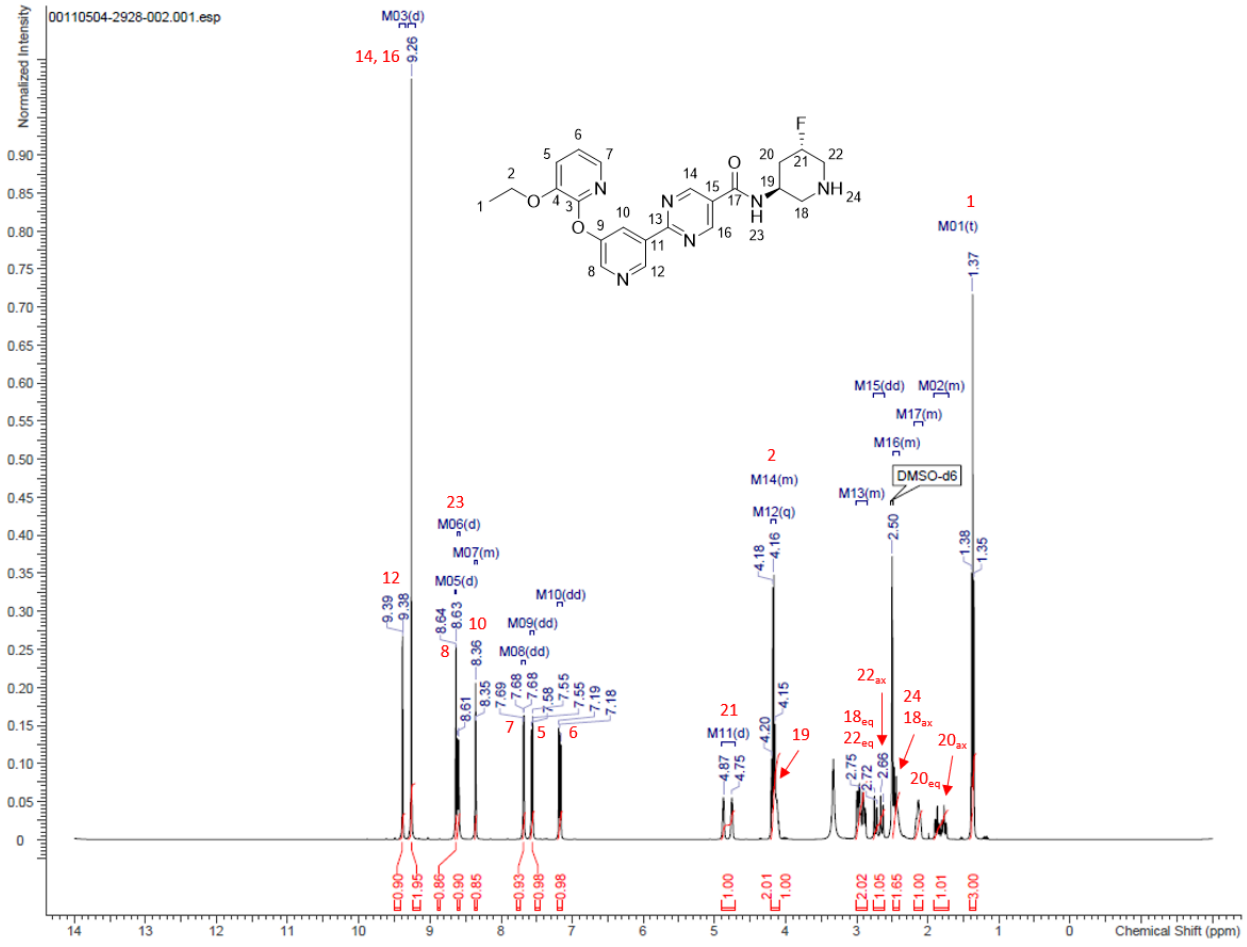
2. Dow, R. L.; Li, J. C.; Pence, M. P.; Gibbs, E. M.; LaPerle, J. L.; Litchfield, J.; Piotrowski, D. W.; Munchhof, M. J.; Manion, T. B.; Zavadoski, W. J.; Walker, G. S.; McPherson, R. K.; Tapley, S.; Sugarman, E.; Guzman-Perez, A.; DaSilva-Jardine, P., Discovery of PF-04620110, a Potent, Selective, and Orally Bioavailable Inhibitor of DGAT-1. *ACS Med Chem Lett* **2011,** *2* (5), 407-12.

^{1}H NMR Spectrum of PF-07202954



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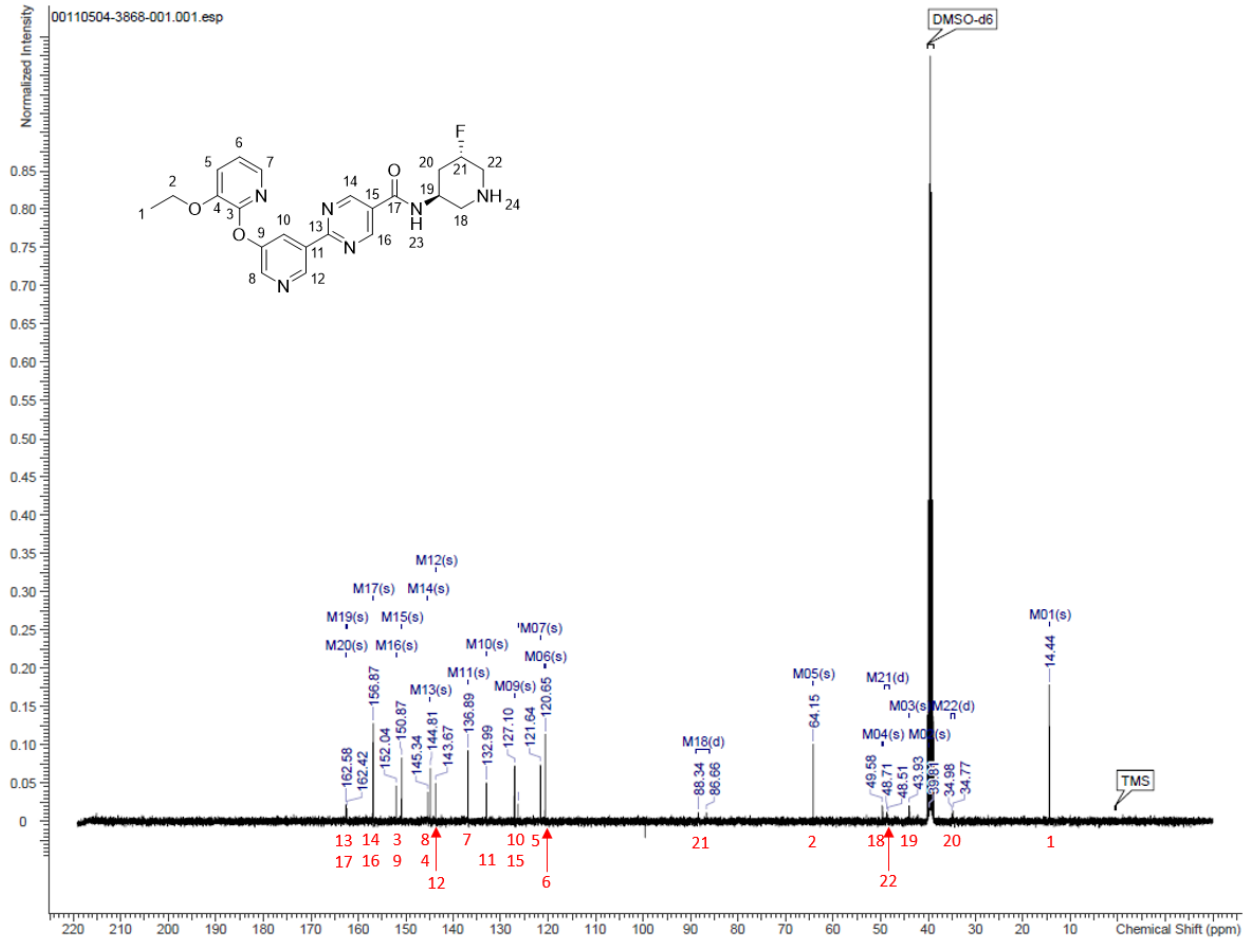
^{13}C NMR Spectrum of PF-07202954



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