

Chemistry Methods

General Methods:

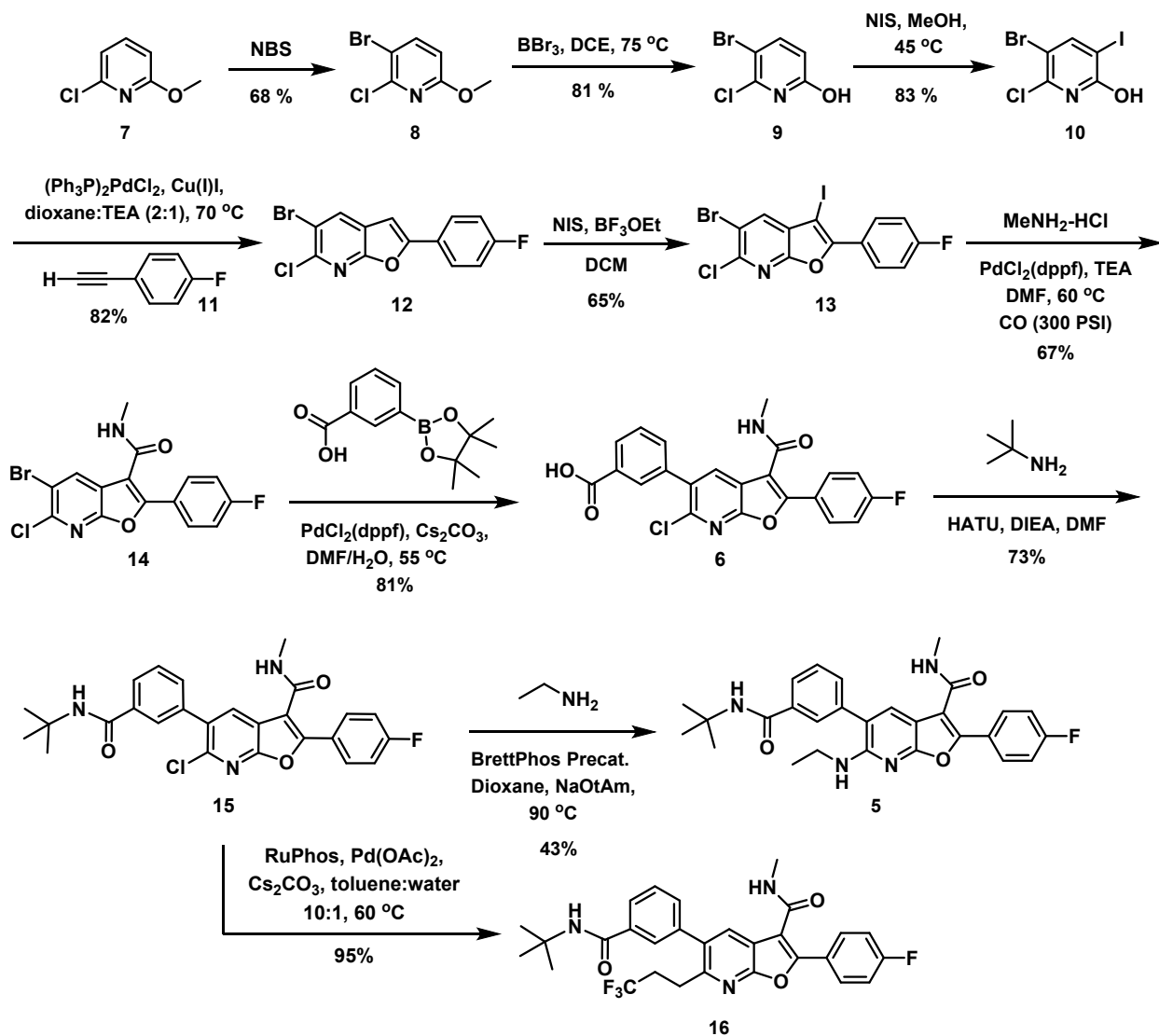
LCMS Method A: LC data was recorded on a Shimadzu LC-10AS liquid chromatograph equipped with a Phenomenex-Luna 10u C18 3.0x50 mm column using a SPD-10AV UV-Vis detector at a detector wave length of 220 nm. The elution conditions employed a flow rate of 5 ml/min , a gradient of 100% solvent A / 0% solvent B to 0% solvent A / 100% solvent B, a gradient time of 2 min, a hold time of 1 min, and an analysis time of 3 min where solvent A was 10% MeOH / 90% H₂O / 0.1% trifluoroacetic acid and solvent B was 10% H₂O / 90% MeOH / 0.1% trifluoroacetic acid. MS data was determined using a Micromass Platform for LC in electrospray mode.

LCMS Method B: LC data was recorded on a Shimadzu LC-10AS liquid chromatograph equipped with a Phenomenex-Luna 3u C18 2.0x30 mm column using a SPD-10AV UV-Vis detector at a detector wave length of 220 nm. The elution conditions employed a flow rate of 1 ml/min , a gradient of 100% solvent A / 0% solvent B to 0% solvent A / 100% solvent B, a gradient time of 2 min, a hold time of 1 min, and an analysis time of 3 min where solvent A was 10% Acetonitrile / 90% H₂O / 0.1% trifluoroacetic acid and solvent B was 10% H₂O / 90% Acetonitrile / 0.1% trifluoroacetic acid. MS data was determined using a Micromass Platform for LC in electrospray mode.

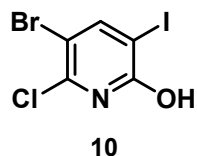
LCMS Method C: LC data was recorded on a Shimadzu LC-10AS liquid chromatograph equipped with a Phenomenex-Luna 3u C18 2.0x50 mm column using a SPD-10AV UV-Vis detector at a detector wave length of 220 nm. The elution conditions employed a flow rate of 1 ml/min , a gradient of 100% solvent A / 0% solvent B to 0% solvent A / 100% solvent B, a gradient time of 4 min, a hold time of 1 min, and an analysis time of 5 min where solvent A was 10% Acetonitrile / 90% H₂O / 0.1% trifluoroacetic acid and solvent B was 10% H₂O / 90% Acetonitrile / 0.1% trifluoroacetic acid. MS data was determined using a Micromass Platform for LC in electrospray mode.

LCMS Method D: LC data was recorded on a Shimadzu LC-10AS liquid chromatograph equipped with a Phenomenex-Luna 3u C18 2.0x30 mm column using a SPD-10AV UV-Vis detector at a detector wave length of 220 nM. The elution conditions employed a flow rate of 1 ml/min , a gradient of 100% solvent A / 0% solvent B to 0% solvent A / 100% solvent B, a gradient time of 4 min, a hold time of 1 min, and an analysis time of 5 min where solvent A was 10% Acetonitrile / 90% H₂O / 10 mM ammonium acetate and solvent B was 10% H₂O / 90% Acetonitrile / 10 mM ammonium acetate. MS data was determined using a Micromass Platform for LC in electrospray mode.

LCMS Method E: LC data was recorded on a Shimadzu LC-10AS liquid chromatograph equipped with a Phenomenex-Luna 3u C18 2.0x30 mm column using a SPD-10AV UV-Vis detector at a detector wave length of 220 nM. The elution conditions employed a flow rate of 1 ml/min , a gradient of 100% solvent A / 0% solvent B to 0% solvent A / 100% solvent B, a gradient time of 4 min, a hold time of 1 min, and an analysis time of 5 min where solvent A was 5% methanol / 95% H₂O / 10 mM ammonium acetate and solvent B was 5% H₂O / 95% methanol / 10 mM ammonium acetate. MS data was determined using a Micromass Platform for LC in electrospray mode.

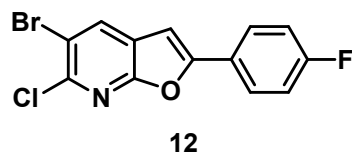


Intermediates:

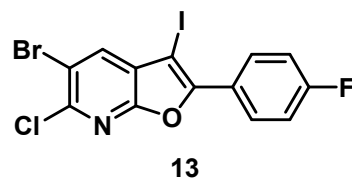


NIS (7.38 g, 32.8 mmol) was added to a stirring solution of commercially available 5-bromo-6-chloropyridin-2-ol (5.70 g, 27.3 mmol) in MeOH (50 ml) at 45 °C. The reaction was allowed to stir at 45 °C for 1 h. The amber colored solution was cooled to rt and then concentrated in vacuo. The resulting yellow solids were diluted with 25 mL DCM, triturated for 30 min, then collected by filtration rinsing with minimal DCM to give 5-bromo-6-chloro-3-iodopyridin-2-ol (7.6 g, 23

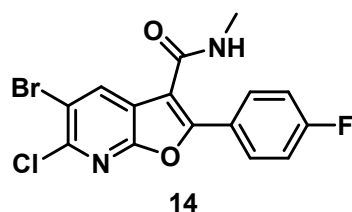
mmol, 83% yield) as a white solid consistent by LCMS and NMR. LC-MS Method A: retention time: 2.62 min; m/z (MH⁺): 335. ¹H NMR (500 MHz, MeOD) δ ppm 8.26 (s, 1H).



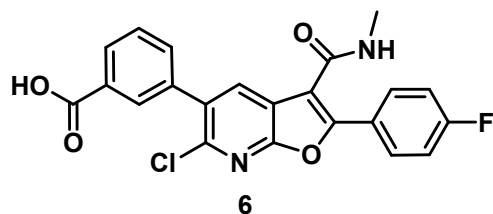
5-bromo-6-chloro-3-iodopyridin-2-ol (200g, 598 mmol) was taken up in 1,4-Dioxane (2 L). N₂ was purged through the solution for 10 min. Triethylamine (800 mL) was added and the solution was purged with N₂ for 5min. Then, copper(I) iodide (39.9 g, 209 mmol), Bis(triphenylphosphine)palladium(II) chloride (21 g, 30 mmol) and 1-ethynyl-4-fluorobenzene (71.9 g, 598 mmol) were added to the reaction and the mixture was heated to 70 °C for 2 h. The reaction was diluted with 2 L of water and stirred for 5 min. The mixture was filtered through Celite, washing with 2 L of ethyl acetate. The organic layer was separated and washed with 500 mL of a brine solution, dried over sodium sulphate and concentrated to afford the expected product 5-bromo-6-chloro-2-(4-fluorophenyl)furo[2,3-b]pyridine (160 g, 82% yield).



BF₃·OEt₂ (2.3 mL, 18 mmol) was added to a stirring mixture of NIS (5.2 g, 23 mmol) and 5-bromo-6-chloro-2-(4-fluorophenyl)furo[2,3-b]pyridine (5.0 g, 15 mmol) in DCM (153 mL) at rt. The reaction was allowed to stir for 16 h. LCMS showed starting material still remained. Another 0.5 equivalents of NIS was added followed by another 0.4 equivalents of BF₃·OEt₂. The reaction was allowed to stir 16 additional h. The reaction was adsorbed onto Celite and was purified on silica gel (Biotage, Hex/DCM gradient, fraction collection at λ = 254 nm) to give the expected product 5-bromo-6-chloro-2-(4-fluorophenyl)-3-iodofuro[2,3-b]pyridine (4.5 g, 10 mmol, 65% yield) consistent by LCMS and NMR. LC-MS Method B: retention time: 2.59 min; m/z (MH⁺): 453.8. ¹H NMR (400 MHz, CHLOROFORM-d) δ 8.24 - 8.15 (m, 2H), 8.00 (s, 1H), 7.26 - 7.19 (m, 2H).

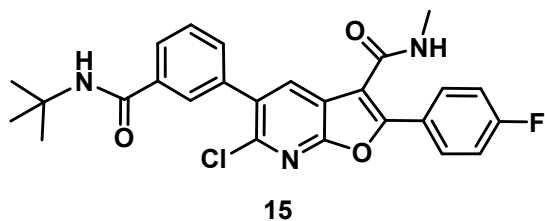


5-bromo-6-chloro-2-(4-fluorophenyl)-3-iodofuro[2,3-b]pyridine (1.00 g, 2.21 mmol), PdCl₂(dppf) (81 mg, 0.11 mmol), methanamine hydrochloride (1.49 g, 22.1 mmol), triethylamine (3.85 mL, 27.6 mmol) were combined in DMF (11 mL) at rt. The reaction was placed in a Parr hydrogenation apparatus “bomb” and charged with 300 PSI of CO (g) and allowed to stir overnight at 55 °C. The reaction was concentrated and adsorbed onto Celite and then purified on silica gel (Biotage, MeOH/DCM gradient, fraction collection at $\lambda = 254$ nm) to give the expected product 5-bromo-6-chloro-2-(4-fluorophenyl)-N-methylfuro[2,3-b]pyridine-3-carboxamide (567 mg, 1.48 mmol, 66.9% yield) consistent by LCMS and NMR. LC-MS Method B: retention time: 1.72 min; m/z (MH⁺): 383,385. ¹H NMR (400 MHz, DMSO-d₆) δ 8.55 (s, 1H), 8.54 - 8.48 (m, 1H), 8.07 - 7.99 (m, 2H), 7.46 - 7.37 (m, 2H), 2.84 (d, J=4.5 Hz, 3H).



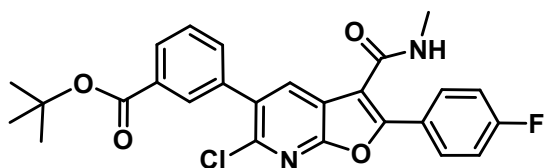
A mixture of 5-bromo-6-chloro-2-(4-fluorophenyl)-N-methylfuro[2,3-b]pyridine-3-carboxamide (400 mg, 1.04 mmol), 3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)benzoic acid (285 mg, 1.15 mmol), PdCl₂(dppf) (76 mg, 0.10 mmol) and cesium carbonate (510 mg, 1.56 mmol) was degassed, charged with N₂ and then diluted with water (1.3 ml) and DMF (13 ml). The mixture was again degassed, charged with N₂ and then heated to 65 °C under an atmosphere of N₂. The reaction mixture was allowed to stir at 65 °C overnight. The mixture was diluted with EtOAc (25 mL) and washed with 1M HCl (50 mL), and sat aq NaCl (50 mL). The organic phase was dried over sodium sulfate, filtered and concentrated. The residue was triturated with DCM to give the expected product 3-(6-chloro-2-(4-fluorophenyl)-3-(methylcarbamoyl)furo[2,3-b]pyridin-5-yl)benzoic acid (380 mg, 0.890 mmol, 85% yield) consistent by LCMS and NMR. LC-MS

Method B: retention time: 1.57 min; m/z (MH⁺): 425. ¹H NMR (400 MHz, DMSO-d₆) δ 8.60 - 8.52 (m, 1H), 8.21 (s, 1H), 8.12 - 8.03 (m, 4H), 7.82 - 7.79 (m, 1H), 7.69 - 7.64 (m, 1H), 7.47 - 7.40 (m, 2H), 2.82 (d, J=4.8 Hz, 3H).



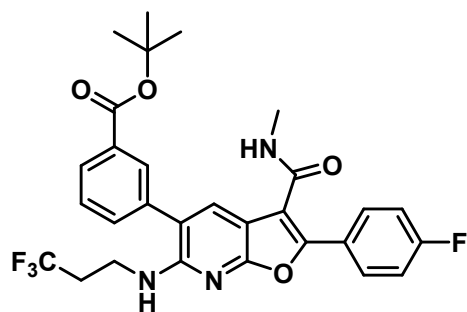
HATU (223 mg, 0.586 mmol) was added to a stirring solution of 3-(6-chloro-2-(4-fluorophenyl)-3-(methylcarbamoyl)furo[2,3-b]pyridin-5-yl)benzoic acid (166 mg, 0.391 mmol), Hünig's base (205 μl, 1.17 mmol), 2-Amino-2-methylpropane (73 μl, 0.78 mmol) in DMF (4 ml) at rt. The reaction was allowed to stir for 1 h. The reaction was then concentrated and was purified on silica gel (Biotage, EtOAc/hexanes gradient, fraction collection at λ = 254 nm) to give the expected product 5-(3-(tert-butylcarbamoyl)phenyl)-6-chloro-2-(4-fluorophenyl)-N-methylfuro[2,3-b]pyridine-3-carboxamide (129 mg, 0.269 mmol, 68.8% yield). LC-MS Method A: retention time: 2.22 min; m/z (MH⁺): 480. ¹H NMR (500 MHz, METHANOL-d₄) δ 8.13 (s, 1H), 8.02 (t, J=6.6 Hz, 2H), 7.90 - 7.79 (m, 3H), 7.67 (dt, J=7.9, 1.3 Hz, 1H), 7.57 (t, J=7.6 Hz, 1H), 7.30 (t, J=7.9 Hz, 2H), 2.94 (s, 3H), 1.48 (s, 9H).

- NOTE – In several cases (mostly with oxadiazole analogues) the order of operations was reversed, whereby C6 was first fixed and the northwestern aryl acid was subsequently modified. For those cases, the intermediates and their preparation are as follows:

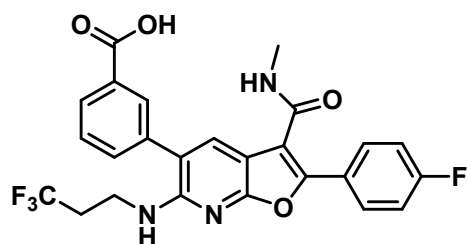


A mixture of 5-bromo-6-chloro-2-(4-fluorophenyl)-N-methylfuro[2,3-b]pyridine-3-carboxamide (5.0 g, 13 mmol), (3-(tert-butoxycarbonyl)phenyl)boronic acid (2.75 g, 12.4 mmol), Pd(Ph₃P)₄ (2.26 g, 1.96 mmol) and cesium carbonate (8.49 g, 26.1 mmol) was degassed/charged with N₂ and diluted with water (22 ml)/DMF (220 ml). The resultant mixture was again degassed, charged with N₂, heated to an internal temperature of 65 °C and allowed to stir under N₂

atmosphere for 16 h. The reaction mixture was cooled to rt then diluted with EtOAc and 1M HCl. The layers were separated and the aq layer was extracted with EtOAc (3 x 10 mL). The combined organic extracts were washed with water, brine, dried over Na₂SO₄ filtered and concentrated. The resultant solid was then purified on SiO₂ eluting with a 0 - 100 % EtOAc in hexanes gradient over 16 CV to give tert-butyl 3-(6-chloro-2-(4-fluorophenyl)-3-(methylcarbamoyl)furo[2,3-b]pyridin-5-yl)benzoate (5.2 g, 11 mmol, 83% yield) as a slightly yellow solid contaminated with the bis-coupled product di-tert-butyl 3,3'-(2-(4-fluorophenyl)-3-(methylcarbamoyl)furo[2,3-b]pyridine-5,6-diyl)dibenzoate. ¹H NMR (500MHz, CHLOROFORM-d) δ 8.18 (s, 1H), 8.08 - 8.02 (m, 2H), 7.95 - 7.89 (m, 2H), 7.63 (dt, *J*=7.6, 1.5 Hz, 1H), 7.52 (t, *J*=7.6 Hz, 1H), 7.24 - 7.18 (m, 2H), 6.03 (d, *J*=4.3 Hz, 1H), 2.99 (d, *J*=4.9 Hz, 3H), 1.62 (s, 9H).

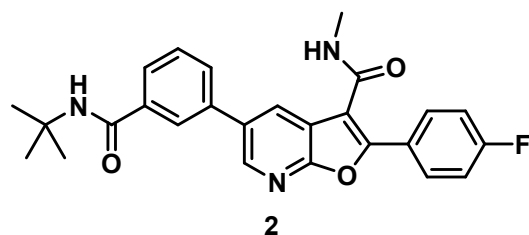


Sodium 2-methylbutan-2-olate (229 mg, 2.08 mmol), tert-butyl 3-(6-chloro-2-(4-fluorophenyl)-3-(methylcarbamoyl)furo[2,3-b]pyridin-5-yl)benzoate (200 mg, 0.416 mmol), 2,2,2-trifluoroethanamine (206 mg, 2.08 mmol), Chloro[2-(dicyclohexylphosphino)-3,6-dimethoxy-2'-4'-6'-tri-*i*-propyl-1,1'-biphenyl][2-(2-aminoethyl)phenyl]palladium(II) (33 mg, 0.042 mmol) was combined, degassed, and taken up in dioxane (8.3 ml) at rt and then was heated to 80 °C. LCMS at 30 min showed major peak with M+H matching that of the expected product. The mixture was diluted with EtOAc and washed with 1M HCl aq, and sat aq NaCl. The organic phase was concentrated and triturated with DCM to give the expected product tert-butyl 3-(2-(4-fluorophenyl)-3-(methylcarbamoyl)-6-((2,2,2-trifluoroethyl)amino)furo[2,3-b]pyridin-5-yl)benzoate (130 mg, 0.239 mmol, 58% yield) consistent by LCMS. LCMS Method B: LC-MS retention time: 2.13 min; m/z (MH⁺): 544.



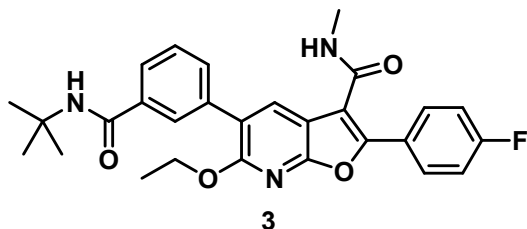
TFA (921 μ l, 11.9 mmol) was added to a stirring solution of tert-butyl 3-(2-(4-fluorophenyl)-3-(methylcarbamoyl)-6-((2,2,2-trifluoroethyl)amino)furo[2,3-b]pyridin-5-yl)benzoate (130 mg, 0.239 mmol) in DCM (2.4 ml) at rt. The reaction was allowed to stir for 2 h then concentrated azeotroping with toluene to dryness. The resulting solid was triturated with DCM to give the expected product 3-(2-(4-fluorophenyl)-3-(methylcarbamoyl)-6-((2,2,2-trifluoroethyl)amino)furo[2,3-b]pyridin-5-yl)benzoic acid (97 mg, 0.20 mmol, 83% yield). LCMS Method B: LC-MS retention time: 1.73 min; m/z (MH^+): 488. 1H NMR (400MHz, DMSO- d_6) δ 13.07 (br. s, 1H), 8.37 - 8.30 (m, $J=4.5$ Hz, 1H), 8.05 - 7.94 (m, 4H), 7.70 - 7.66 (m, 3H), 7.34 (t, $J=8.9$ Hz, 2H), 6.67 (s, 1H), 4.23 - 4.10 (m, $J=3.0$ Hz, 2H), 2.80 (d, $J=4.8$ Hz, 3H).

Examples:

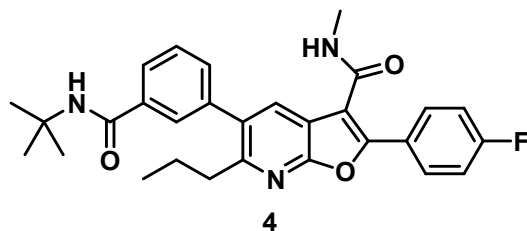


Hünig's base (30.9 μ L, 0.177 mmol) was added to a stirring solution of 3-(2-(4-fluorophenyl)-3-(methylcarbamoyl)furo[2,3-b]pyridin-5-yl)benzoic acid (23 mg, 0.059 mmol), 2-methylpropan-2-amine (20 μ L, 0.059 mmol), and HATU (27 mg, 0.071 mmol) in DMF (589 μ L) at rt. The mixture was allowed to stir for 2 h. The reaction mixture was then diluted with EtOAc (20 mL) and washed with 1M HCl (50 mL), and sat aq NaCl (50 mL). The organic phase was dried over sodium sulfate, filtered and concentrated to give the expected product 5-(3-(tert-butylcarbamoyl)phenyl)-2-(4-fluorophenyl)-N-methylfuro[2,3-b]pyridine-3-carboxamide (7.9 mg, 0.017 mmol, 29.5 % yield). LC-MS Method A: retention time: 1.68 min; m/z (MH^+): 446. 1H NMR (500 MHz, DMSO- d_6) δ ppm 8.74 (1 H, d, $J=2.14$ Hz), 8.55 - 8.61 (1 H, m), 8.39 (1 H,

d, $J=2.44$ Hz), 8.16 (1 H, s), 8.07 (2 H, dd, $J=8.85$, 5.19 Hz), 7.94 (1 H, s), 7.92 (1 H, d, $J=7.63$ Hz), 7.86 (1 H, d, $J=7.93$ Hz), 7.59 (1 H, t, $J=7.63$ Hz), 7.43 (2 H, t, $J=8.85$ Hz), 2.87 (3 H, d, $J=4.58$ Hz), 1.42 (9 H, s).



Sodium ethanolate (0.52 ml, 1.3 mmol) in ethanol 21% was added to a mixture of Chloro[(dicyclohexylphosphino)-3,6-dimethoxy-2',4',6'-triisopropyl-1,1'-biphenyl][2-(2-aminoethyl)phenyl]palladium(II) (3 mg, 4 μ mol), 5-(3-(tert-butylcarbamoyl)phenyl)-6-chloro-2-(4-fluorophenyl)-N-methylfuro[2,3-b]pyridine-3-carboxamide (20 mg, 0.042 mmol) at 100 °C. The reaction was stirred for 30 min and then was purified by preparative reverse phase HPLC on a C18 column using a suitably buffered H₂O/CH₃CN gradient, and fractions containing the expected product were concentrated to afford 5-(3-(tert-butylcarbamoyl)phenyl)-6-ethoxy-2-(4-fluorophenyl)-N-methylfuro[2,3-b]pyridine-3-carboxamide (2 mg, 9% yield). LC-MS Method B: retention time: 1.76 min; m/z (MH⁺): 490. ¹H NMR (500 MHz, METHANOL-*d*₄) δ 8.03 (s, 1H), 7.98 - 7.92 (m, 3H), 7.74 (t, $J=8.3$ Hz, 2H), 7.50 (t, $J=7.7$ Hz, 1H), 7.26 (t, $J=7.8$ Hz, 2H), 4.49 (q, $J=7.1$ Hz, 2H), 2.97 - 2.93 (m, 3H), 1.48 (s, 9H), 1.43 - 1.34 (m, 3H).



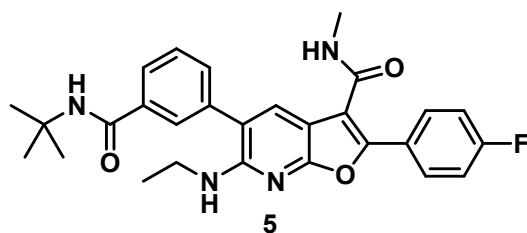
Step 1:

5-(3-(tert-butylcarbamoyl)phenyl)-6-chloro-2-(4-fluorophenyl)-N-methylfuro[2,3-b]pyridine-3-carboxamide (36 mg, 0.075 mmol), (E)-prop-1-en-1-ylboronic acid (64 mg, 0.750 mmol), sodium 6-(dicyclohexylphosphino)-2',6'-dimethoxy-[1,1'-biphenyl]-3-sulfonate (15 mg, 0.030 mmol), diacetoxypalladium (3.4 mg, 0.015 mmol), cesium carbonate (37 mg, 0.11 mmol) were combined, degassed and backfilled with N₂ then dissolved in DMF (1.2 ml) and water (0.25 ml) at rt then heated at 80 °C for 2 h. The reaction was concentrated to dryness by azeotroping with

toluene (3 x 30 mL) and the crude residue was purified on silica gel (Biotage, EtOAc/hexanes gradient, fraction collection at $\lambda = 254$ nm) to give the expected product 1 (E)-5-(3-(tert-butylcarbamoyl)phenyl)-2-(4-fluorophenyl)-N-methyl-6-(prop-1-en-1-yl)furo[2,3-b]pyridine-3-carboxamide (25 mg, 0.051 mmol, 69% yield) consistent by LCMS. LC-MS Method B: retention time:2.28 min; m/z (MH⁺): 486.

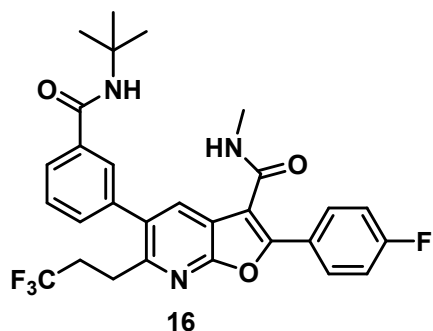
Step 2:

10% Pd/C (16 mg, 0.015 mmol) was added to a stirring solution of (E)-5-(3-(tert-butylcarbamoyl)phenyl)-2-(4-fluorophenyl)-N-methyl-6-(prop-1-en-1-yl)furo[2,3-b]pyridine-3-carboxamide (25 mg, 0.051 mmol) in EtOH (1 ml) at rt. The vessel was evacuated and backfilled with N₂ 2x and then filled with H₂ (g) balloon. The reaction was stirred overnight. Celite was added and the reaction mixture was filtered through a pad of Celite washing with EtOAc. The filtrate was concentrated to dryness. The reaction was then purified by preparative reverse phase HPLC on a C18 column using a suitably buffered H₂O/CH₃CN gradient, and concentrated to give the expected product 5-(3-(tert-butylcarbamoyl)phenyl)-2-(4-fluorophenyl)-N-methyl-6-propylfuro[2,3-b]pyridine-3-carboxamide (10 mg, 0.019 mmol, 38 % yield) consistent by LCMS and NMR. LC-MS Method B: retention time:1.87 min; m/z (MH⁺): 488. ¹H NMR (500 MHz, METHANOL-d₄) δ 8.00 (t, J=6.6 Hz, 2H), 7.93 (s, 1H), 7.81 (dt, J=7.3, 1.7 Hz, 1H), 7.76 (s, 1H), 7.58 - 7.51 (m, 2H), 7.31 - 7.25 (m, 2H), 2.93 (s, 3H), 2.82 - 2.74 (m, 2H), 1.69 (sxt, J=7.5 Hz, 2H), 1.47 (s, 9H), 0.84 (t, J=7.3 Hz, 3H).

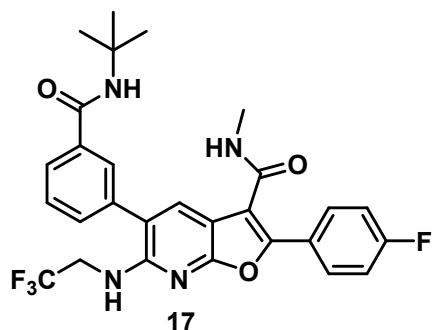


Chloro[2-(dicyclohexylphosphino)-3,6-dimethoxy-2',4', 6'-triisopropyl-1,1'-biphenyl][2-(2-aminoethyl)phenyl]palladium(II) (12 mg, 0.016 mmol) sodium 2-methylbutan-2-olate (344 mg, 3.13 mmol), ethanamine hydrochloride (510 mg, 6.25 mmol), 5-(3-(tert-butylcarbamoyl)phenyl)-6-chloro-2-(4-fluorophenyl)-N-methylfuro[2,3-b]pyridine-3-carboxamide (150 mg, 0.313 mmol) were combined, degassed and taken up in dioxane (6.3 ml) at 100 °C. The reaction was stirred for 1 h. LCMS at 1 h and then was purified by preparative

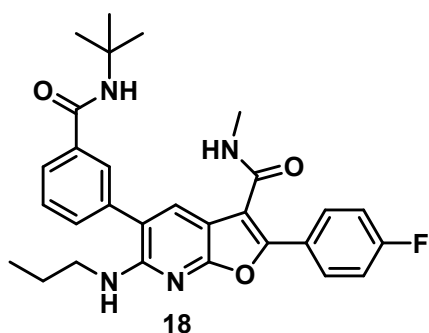
reverse phase HPLC on a C18 column using a suitably buffered H₂O/CH₃CN gradient, and concentrated to give the expected product expected product 5-(3-(tert-butylcarbamoyl)phenyl)-6-(ethylamino)-2-(4-fluorophenyl)-N-methylfuro[2,3-b]pyridine-3-carboxamide (69 mg, 0.134 mmol, 42.9 % yield). LC-MS Method B: retention time: 1.79 min; m/z (MH⁺): 489. ¹H NMR (400 MHz, METHANOL-d₄) δ 7.96 - 7.83 (m, 2H), 7.83 - 7.74 (m, 2H), 7.63 - 7.54 (m, 3H), 7.22 (t, *J*=8.2 Hz, 2H), 3.50 - 3.33 (m, 2H), 2.91 (s, 3H), 1.47 (s, 9H), 1.19 (t, *J*=7.2 Hz, 3H).



A mixture of 5-(3-(tert-butylcarbamoyl)phenyl)-6-chloro-2-(4-fluorophenyl)-N-methylfuro[2,3-b]pyridine-3-carboxamide (30 mg, 0.063 mmol) (3,3,3-trifluoropropyl)-trifluoroborate potassium salt (64 mg, 0.31 mmol), PdCl₂(dppf) (7 mg, 9 μmol) and Cs₂CO₃ (92 mg, 0.28 mmol) at rt under N₂ was added toluene (1.2 mL) and water (0.4 mL). The mixture was flushed with N₂ and then stirred at 100 °C for 4 h. The mixture was diluted with EtOAc (20 mL) and washed with 1M HCl (15 mL), and sat aq NaCl (15 mL). The organic phase was dried over Na₂SO₄, filtered, concentrated and was then purified by preparative reverse phase HPLC on a C18 column using a suitably buffered H₂O/CH₃CN gradient, and concentrated to give the expected product 5-(3-(tert-butylcarbamoyl)phenyl)-2-(4-fluorophenyl)-N-methyl-6-(3,3,3-trifluoropropyl)furo[2,3-b]pyridine-3-carboxamide (4 mg, 11% yield) consistent by LCMS and NMR. LC-MS Method B: retention time: 1.95 min; m/z (MH⁺): 542. ¹H NMR (400MHz, CHLOROFORM-d) δ 8.08 - 7.96 (m, 3H), 7.82 - 7.75 (m, 1H), 7.70 (br. s., 1H), 7.57 - 7.51 (m, 1H), 7.48 - 7.41 (m, 1H), 7.25 - 7.19 (m, 2H), 6.02 (br. s., 1H), 5.87 (br. s., 1H), 3.10 - 2.96 (m, 5H), 2.77 - 2.60 (m, 2H), 1.51 (s, 9H).

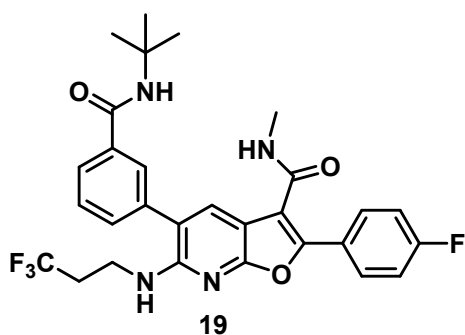


Sodium 2-methylbutan-2-olate (115 mg, 1.04 mmol), 5-(3-(tert-butylcarbamoyl)phenyl)-6-chloro-2-(4-fluorophenyl)-N-methylfuro[2,3-b]pyridine-3-carboxamide (50 mg, 0.10 mmol), 2,2,2-trifluoroethanamine (155 mg, 1.56 mmol), Chloro[2-(dicyclohexylphosphino)-3,6-dimethoxy-2',4', 6'-triisopropyl-1,1'-biphenyl][2-(2-aminoethyl)phenyl]palladium(II) (8 mg, 10 μ mol) were combined, degassed, charged with N₂, taken up in dioxane (2 ml) and then stirred at 100 °C. The reaction was stirred for 1.5 h and then was purified by preparative reverse phase HPLC on a C18 column using a suitably buffered H₂O/CH₃CN gradient, and concentrated to give the expected product 5-(3-(tert-butylcarbamoyl)phenyl)-2-(4-fluorophenyl)-N-methyl-6-((2,2,2-trifluoroethyl)amino)furo[2,3-b]pyridine-3-carboxamide (6 mg, 11 μ mol, 10% yield) consistent by LCMS and NMR. LC-MS Method C: retention time: 3.46 min; m/z (MH⁺): 543. ¹H NMR (400 MHz, CHLOROFORM-d) δ 7.96 - 7.85 (m, 3H), 7.84 - 7.77 (m, 1H), 7.75 (s, 1H), 7.61 - 7.52 (m, 2H), 7.25 - 7.15 (m, 2H), 6.04 (br s, 1H), 5.88 (br s, 1H), 4.88 (br t, J=6.4 Hz, 1H), 4.30 - 4.19 (m, 2H), 2.97 (d, J=4.8 Hz, 3H), 1.50 (s, 9H).



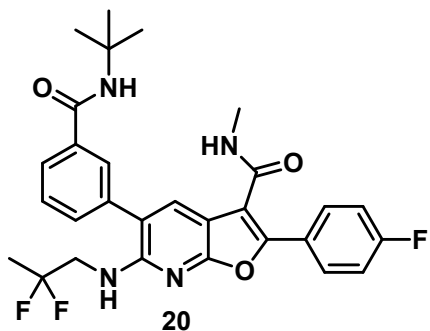
Sodium 2-methylbutan-2-olate (92 mg, 0.83 mmol), 5-(3-(tert-butylcarbamoyl)phenyl)-6-chloro-2-(4-fluorophenyl)-N-methylfuro[2,3-b]pyridine-3-carboxamide (40 mg, 0.083 mmol), propan-1-amine (49.3 mg, 0.83 mmol), Chloro[2-(dicyclohexylphosphino)-3,6-dimethoxy-2',4', 6'-triisopropyl-1,1'-biphenyl][2-(2-aminoethyl)phenyl]palladium(II) (7 mg, 8 μ mol) were

combined, degassed with N₂, and taken up in dioxane (1.7 ml) at 100 °C. The reaction mixture was stirred for 1 h and then was purified by preparative reverse phase HPLC on a C18 column using a suitably buffered H₂O/CH₃CN gradient, and concentrated to give the expected product 5-(3-(tert-butylcarbamoyl)phenyl)-2-(4-fluorophenyl)-N-methyl-6-(propylamino)furo[2,3-b]pyridine-3-carboxamide (20 mg, 0.038 mmol, 45% yield) consistent by LCMS and NMR. LC-MS Method B: retention time: 1.89 min; m/z (MH⁺): 503. ¹H NMR (400 MHz, CHLOROFORM-d) δ 7.94 - 7.88 (m, 2H), 7.80 - 7.73 (m, 3H), 7.58 - 7.52 (m, 2H), 7.17 (t, J=8.1 Hz, 2H), 6.01 (s, 1H), 5.84 (br s, 1H), 3.46 - 3.41 (m, 2H), 2.97 (d, J=4.8 Hz, 3H), 1.60 (dq, J=14.4, 7.4 Hz, 2H), 1.50 (s, 9H), 0.94 (t, J=7.4 Hz, 3H).



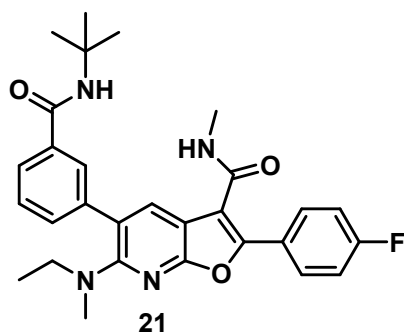
Sodium 2-methylbutan-2-olate (57 mg, 0.52 mmol), 5-(3-(tert-butylcarbamoyl)phenyl)-6-chloro-2-(4-fluorophenyl)-N-methylfuro[2,3-b]pyridine-3-carboxamide (25 mg, 0.052 mmol), 3,3,3-trifluoropropan-1-amine hydrochloride (78 mg, 0.52 mmol), Chloro[2-(dicyclohexylphosphino)-3,6-dimethoxy-2',4', 6'-triisopropyl-1,1'-biphenyl][2-(2-aminoethyl)phenyl]palladium(II) (4 mg, 5 μmol) were combined in dioxane (1 ml) at 100 °C in a seal microwave (MW) vial under inert atmosphere, N₂. The reaction was stirred for 1 h and then was purified by preparative reverse phase HPLC on a C18 column using a suitably buffered H₂O/CH₃CN gradient, and concentrated to give the expected product 5-(3-(tert-butylcarbamoyl)phenyl)-2-(4-fluorophenyl)-N-methyl-6-((3,3,3-trifluoropropyl)amino)furo[2,3-b]pyridine-3-carboxamide (23 mg, 0.039 mmol, 75% yield) consistent by LCMS and NMR. LC-MS Method B: retention time: 2.79 min; m/z (MH⁺): 557. ¹H NMR (500 MHz, CHLOROFORM-d) δ 7.85 (t, J=6.5 Hz, 2H), 7.79 (s, 1H), 7.74 (d, J=7.1 Hz, 1H), 7.69 (s, 1H), 7.62 - 7.49 (m, 2H), 7.20 (t, J=8.2 Hz, 2H), 6.29 (br s, 1H), 6.18 (br d, J=4.7 Hz, 1H), 3.74 (t, J=6.5 Hz, 2H), 2.99 (d, J=4.9 Hz, 3H), 2.57 - 2.46 (m, 2H), 1.51 (s, 9H).

Preparation of 5-(3-(tert-butylcarbamoyl)phenyl)-6-((2,2-difluoropropyl)amino)-2-(4-fluorophenyl)-N-methylfuro[2,3-b]pyridine-3-carboxamide



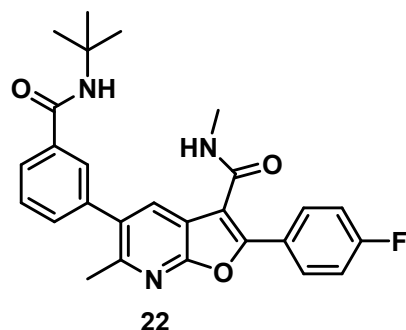
Sodium 2-methylbutan-2-olate (11 mg, 0.10 mmol), 5-(3-(tert-butylcarbamoyl)phenyl)-6-chloro-2-(4-fluorophenyl)-N-methylfuro[2,3-b]pyridine-3-carboxamide (20 mg, 0.042 mmol), 2,2-difluoropropan-1-amine hydrochloride (14 mg, 0.10 mmol), and Chloro[2-(dicyclohexylphosphino)-3,6-dimethoxy-2'-4'-6'-tri-*i*-propyl-1,1'-biphenyl][2-(2-aminoethyl)phenyl]palladium(II) (3.3 mg, 4.2 μ mol) were combined, degassed, and taken up in dioxane (1 ml) at rt and then was heated at 100 °C until LCMS indicated starting material was consumed. The reaction was diluted with EtOAc (15 mL) and washed with 1M HCl (10 mL), and sat aq NaCl (10 mL). The organic phase was concentrated and the crude material was purified via preparative LC/MS with the following conditions: Column: Waters XBridge C18, 19 x 100 mm, 5- μ m particles; Guard Column: Waters XBridge C18, 19 x 10 mm, 5- μ m particles; Mobile Phase A: water; Mobile Phase B: acetonitrile; Buffer: 20-mM ammonium acetate; Gradient: 20-95% B over 10.9 minutes, then a 4.0 minute hold at 95% B; Flow: 25 mL/min. Fractions containing the desired product were combined and dried via centrifugal evaporation. The material was further purified via preparative LC/MS with the following conditions: Column: Waters XBridge C18, 19 x 200 mm, 5- μ m particles; Guard Column: Waters XBridge C18, 19 x 10 mm, 5- μ m particles; Mobile Phase A: water; Mobile Phase B: methanol; Buffer: 20-mM ammonium acetate; Gradient: 40-95% B over 19.5 minutes, then a 14.0 minute hold at 95% B; Flow: 20 mL/min. Fractions containing the desired product were combined and dried via centrifugal evaporation. The yield of the product was 3.6 mg, and its estimated purity by LCMS analysis was 100%. Two analytical LC/MS injections were used to determine the final purity. Injection 1 conditions: Column: Waters BEH C18, 2.0 x 50 mm, 1.7- μ m particles; Mobile Phase

A: 5:95 acetonitrile:water with 10 mM ammonium acetate; Mobile Phase B: 95:5 acetonitrile:water with 10 mM ammonium acetate; Temperature: 40 °C; Gradient: 0.5 min hold at 0%B, 0-100% B over 4 minutes, then a 0.5-minute hold at 100% B; Flow: 1 mL/min. retention time : 3.09 M+H = 539 . Injection 2 conditions: Column: Waters BEH C18, 2.0 x 50 mm, 1.7- μ m particles; Mobile Phase A: 5:95 methanol:water with 10 mM ammonium acetate; Mobile Phase B: 95:5 methanol:water with 10 mM ammonium acetate; Temperature: 40 °C; Gradient: 0.5 min hold at 0%B, 0-100% B over 4 minutes, then a 0.5-minute hold at 100% B; Flow: 0.5 mL/min. retention time : 4.09 M+H = 539. Proton NMR was acquired in deuterated DMSO. ^1H NMR (500MHz, DMSO- d_6) δ 8.40 - 8.34 (m, 1H), 7.96 (dd, J=8.9, 5.5 Hz, 2H), 7.89 (s, 1H), 7.87 - 7.83 (m, 1H), 7.82 (s, 1H), 7.65 (s, 1H), 7.60 - 7.55 (m, 2H), 7.34 (t, J=8.9 Hz, 2H), 6.34 - 6.30 (m, 1H), 3.92 - 3.83 (m, 2H), 2.79 (d, J=4.6 Hz, 3H), 1.64 (t, J=18.9 Hz, 3H), 1.39 (s, 9H).

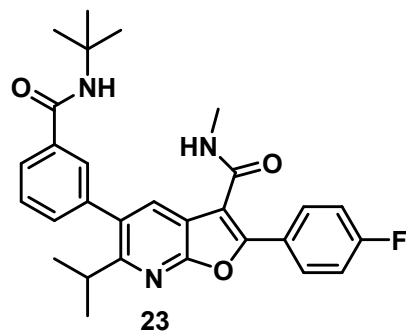


Sodium 2-methylbutan-2-olate (34 mg, 0.31 mmol) , Chloro[2-(dicyclohexylphosphino)-3,6-dimethoxy-2',4', 6'-triiisopropyl-1,1'-biphenyl][2-(2-aminoethyl)phenyl]palladium(II) (2.5 mg, 3.1 μ mol), N-methylethanamine (37 mg, 0.63 mmol), 5-(3-(tert-butylcarbamoyl)phenyl)-6-chloro-2-(4-fluorophenyl)-N-methylfuro[2,3-b]pyridine-3-carboxamide (15 mg, 0.031 mmol) were combined, degassed, charged with with N_2 , taken up in dioxane (1 ml) and stirred at at 85 °C. The reaction was stirred at this temperature for 1 h and then was purified by preparative reverse phase HPLC on a C18 column using a suitably buffered $\text{H}_2\text{O}/\text{CH}_3\text{CN}$ gradient, and concentrated to give the expected product 5-(3-(tert-butylcarbamoyl)phenyl)-6-(ethyl(methyl)amino)-2-(4-fluorophenyl)-N-methylfuro[2,3-b]pyridine-3-carboxamide (3.1 mg, 5.86 μ mol, 18.75 % yield). LC-MS Method B: retention time:1.84 min; m/z (MH $^+$): 503. ^1H NMR (400 MHz, METHANOL- d_4) δ 8.04 - 7.89 (m, 3H), 7.82 (s, 1H), 7.74 (d, J=7.3 Hz, 1H),

7.67 (d, J=7.3 Hz, 1H), 7.56 - 7.48 (m, 1H), 7.25 (t, J=8.2 Hz, 2H), 3.28 - 3.13 (m, 2H), 2.93 (s, 3H), 2.79 (s, 3H), 1.51 - 1.39 (m, 9H), 0.98 (t, J=7.2 Hz, 3H).



5-(3-(tert-butylcarbamoyl)phenyl)-6-chloro-2-(4-fluorophenyl)-N-methylfuro[2,3-b]pyridine-3-carboxamide (20 mg, 0.042 mmol), Trimethylboroxine (58 μ l, 0.42 mmol), sodium 6-(dicyclohexylphosphino)-2',6'-dimethoxy-[1,1'-biphenyl]-3-sulfonate (8.5 mg, 0.017 mmol), diacetoxypalladium (1.9 mg, 8.3 μ mol), cesium carbonate (20 mg, 0.063 mmol) were degassed and backfilled with N_2 then dissolved in DMF (1 ml) and water (0.1 ml) at rt then heated to 80 $^{\circ}$ C. The reaction was stirred at this temperature for 1 h and then was purified by preparative reverse phase HPLC on a C18 column using a suitably buffered H_2O/CH_3CN gradient, and concentrated to give the expected product 5-(3-(tert-butylcarbamoyl)phenyl)-2-(4-fluorophenyl)-N,6-dimethylfuro[2,3-b]pyridine-3-carboxamide (17 mg, 0.035 mmol, 84% yield) consistent by LCMS and NMR. LC-MS Method B: retention time: 1.62 min; m/z (MH⁺): 460. 1H NMR (400 MHz, CHLOROFORM-d) δ 8.00 (s, 1H), 7.97 - 7.88 (m, 2H), 7.81 - 7.65 (m, 2H), 7.56 - 7.42 (m, 2H), 7.23 - 7.16 (m, 2H), 6.46 - 6.33 (m, 1H), 6.30 (s, 1H), 3.00 (d, J=5.0 Hz, 3H), 2.52 (s, 3H), 1.53 - 1.45 (m, 9H).

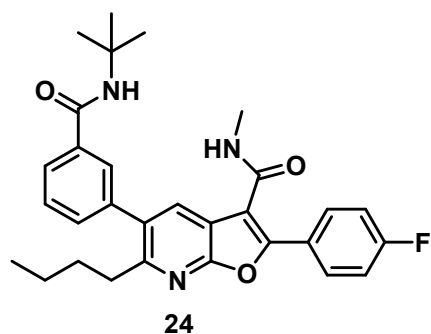


Step 1:

5-(3-(tert-butylcarbamoyl)phenyl)-6-chloro-2-(4-fluorophenyl)-N-methylfuro[2,3-b]pyridine-3-carboxamide (18 mg, 0.038 mmol), K₃PO₄ (60 mg, 0.28 mmol), sodium 6-(dicyclohexylphosphino)-2',6'-dimethoxy-[1,1'-biphenyl]-3-sulfonate (4 mg, 8 μmol), diacetoxypalladium (0.9 mg, 4 μmol), 6-methyl-2-(prop-1-en-2-yl)-1,3,6,2-dioxazaborocane-4,8-dione (15 mg, 0.075 mmol) were combined, degassed and backfilled with N₂ then dissolved in dioxane (0.6 ml) and water (0.1 ml) at rt then heated to 100 °C and stirred at this temp for 1 h. The reaction mixture was purified by preparative reverse phase HPLC on a C18 column using a suitably buffered H₂O/CH₃CN gradient, and concentrated to give the expected product 5-(3-(tert-butylcarbamoyl)phenyl)-2-(4-fluorophenyl)-N-methyl-6-(prop-1-en-2-yl)furo[2,3-b]pyridine-3-carboxamide (7.0 mg, 0.013 mmol, 36% yield) consistent by LCMS. LC-MS Method B: retention time: 3.46 min; m/z (MH⁺): 486.

Step 2:

10% Pd/C (14 mg, 0.014 mmol) was added to a stirring solution of 5-(3-(tert-butylcarbamoyl)phenyl)-2-(4-fluorophenyl)-N-methyl-6-(prop-1-en-2-yl)furo[2,3-b]pyridine-3-carboxamide (33 mg, 0.068 mmol) in MeOH/EtOAc (1 mL/0.5 mL) at rt. A catalytic amount AcOH was added. The vessel was evacuated and backfilled with N₂ 2x and then filled with H₂ (g) balloon. The reaction was stirred overnight. LCMS shows only trace amounts of product. 8 additional mg of 10% Pd/C was added to the reaction mixture and the reaction was placed in a Parr “bomb” and charged with 100 PSI of H₂ (g) and allowed to stir for 4 h. LCMS still shows starting material. An additional 6 mg of 10% Pd/C was added and the reaction and the mixture was again allowed to stir overnight in the Parr bomb under 100 PSI of H₂. The reaction was filtered through a pad of Celite and was purified by preparative reverse phase HPLC on a C18 column using a suitably buffered H₂O/CH₃CN gradient, and concentrated to give the expected product 5-(3-(tert-butylcarbamoyl)phenyl)-2-(4-fluorophenyl)-6-isopropyl-N-methylfuro[2,3-b]pyridine-3-carboxamide (12 mg, 0.023 mmol, 34% yield) consistent by LCMS and NMR. LC-MS Method B: retention time: 1.94 min; m/z (MH⁺): 488. ¹H NMR (400 MHz, CHLOROFORM-d) δ 8.04 - 7.95 (m, 3H), 7.75 (d, J=7.2 Hz, 1H), 7.69 (s, 1H), 7.53 - 7.41 (m, 2H), 7.21 (t, J=8.0 Hz, 2H), 6.06 (s, 1H), 5.93 (br d, J=4.3 Hz, 1H), 3.19 (quin, J=6.7 Hz, 1H), 3.00 (d, J=5.0 Hz, 3H), 1.89 (br s, 3H), 1.50 (s, 9H), 1.27 (d, J=6.8 Hz, 6H).



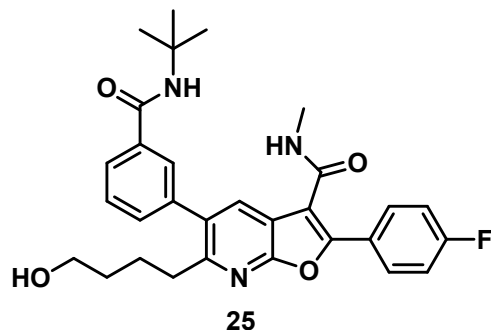
Step 1:

5-(3-(tert-butylcarbamoyl)phenyl)-6-chloro-2-(4-fluorophenyl)-N-methylfuro[2,3-b]pyridine-3-carboxamide (25 mg, 0.052 mmol), (E)-but-1-en-1-ylboronic acid (52 mg, 0.52 mmol), sodium 6-(dicyclohexylphosphino)-2',6'-dimethoxy-[1,1'-biphenyl]-3-sulfonate (11 mg, 0.021 mmol), diacetoxypalladium (2.3 mg, 10 μ mol), cesium carbonate (26 mg, 0.078 mmol) were degassed and backfilled with N₂ then dissolved in DMF (1 ml) and water (0.1 ml) at rt then heated at 80 °C for 1 h. The reaction was purified on silica gel (Biotage, EtOAc/hexanes gradient, fraction collection at $\lambda = 254$ nm) to give the expected product (E)-6-(but-1-en-1-yl)-5-(3-(tert-butylcarbamoyl)phenyl)-2-(4-fluorophenyl)-N-methylfuro[2,3-b]pyridine-3-carboxamide (quant.) consistent by LCMS. LC-MS Method B: retention time: 1.88 min; m/z (MH⁺): 500.

Step 2:

10% Pd/C (23 mg, 0.022 mmol) was added to a stirring solution of (E)-6-(but-1-en-1-yl)-5-(3-(tert-butylcarbamoyl)phenyl)-2-(4-fluorophenyl)-N-methylfuro[2,3-b]pyridine-3-carboxamide (54 mg, 0.11 mmol) in EtOH (1 ml) at rt. The vessel was evacuated and backfilled with N₂ 2x and then filled with H₂ (g) balloon. The reaction was stirred overnight. LCMS shows SM remains. An additional 20 mg of 10% Pd/C and catalytic AcOH was added and the reaction mixture was allowed to stir overnight under a balloon atmosphere of H₂. After stirring overnight LCMS indicted the reaction was complete. The reaction was filtered and purified by preparative reverse phase HPLC on a C18 column using a suitably buffered H₂O/CH₃CN gradient, and concentrated to give the expected product 6-butyl-5-(3-(tert-butylcarbamoyl)phenyl)-2-(4-fluorophenyl)-N-methylfuro[2,3-b]pyridine-3-carboxamide (9.0 mg, 0.017 mmol, 16% yield) consistent by LCMS and NMR. LC-MS Method B: retention time: 1.88 min; m/z (MH⁺): 502.

¹H NMR (400 MHz, CHLOROFORM-d) δ 8.03 - 7.96 (m, 3H), 7.76 (d, J=7.2 Hz, 1H), 7.71 - 7.68 (m, 1H), 7.55 - 7.43 (m, 2H), 7.21 (t, J=8.1 Hz, 2H), 6.03 (s, 1H), 5.92 (br d, J=4.5 Hz, 1H), 3.00 (d, J=5.0 Hz, 3H), 2.82 - 2.74 (m, 2H), 1.74 - 1.68 (m, 2H), 1.50 (s, 9H), 1.31 - 1.19 (m, 2H), 0.83 (t, J=7.4 Hz, 3H).



Step 1:

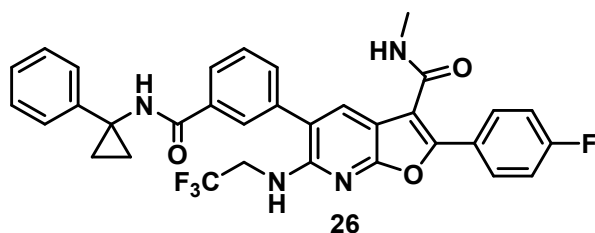
5-(3-(tert-butylcarbamoyl)phenyl)-6-chloro-2-(4-fluorophenyl)-N-methylfuro[2,3-b]pyridine-3-carboxamide (58 mg, 0.12 mmol), (E)-tert-butyldimethyl((4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)but-3-en-1-yl)oxy)silane (64 μ l, 0.18 mmol), sodium 6-(dicyclohexylphosphino)-2',6'-dimethoxy-[1,1'-biphenyl]-3-sulfonate (25 mg, 0.048 mmol), diacetoxypalladium (5.4 mg, 0.024 mmol), cesium carbonate (59 mg, 0.18 mmol) were combined, degassed and backfilled with N₂ then dissolved in DMF (1 ml) and water (0.2 ml) at rt then heated to 100°C and stirred at this temperature for 1 h. The reaction mixture was concentrated and was purified on silica gel (Biotage, EtOAc/hexanes gradient, fraction collection at $\lambda = 254$ nm) to give product 1 (E)-5-(3-(tert-butylcarbamoyl)phenyl)-6-(4-((tert-butyldimethylsilyl)oxy)but-1-en-1-yl)-2-(4-fluorophenyl)-N-methylfuro[2,3-b]pyridine-3-carboxamide (53 mg, 0.084 mmol, 70% yield) consistent by LCMS. LC-MS Method A: retention time:2.53 min; m/z (MH⁺): 630.

Step 2:

10% Pd/C (12 mg, 0.012 mmol) was added to a stirring solution of (E)-5-(3-(tert-butylcarbamoyl)phenyl)-6-(4-((tert-butyldimethylsilyl)oxy)but-1-en-1-yl)-2-(4-fluorophenyl)-N-methylfuro[2,3-b]pyridine-3-carboxamide (25 mg, 0.040 mmol) in EtOH (1 ml) at rt. The vessel was evacuated and backfilled with N₂ 2x and then filled with H₂ (g) balloon. The reaction was stirred overnight. Celite was added to the reaction and it was filtered through a pad of Celite

washing with EtOAc. The filtrate was concentrated to dryness and diluted with 1 mL of THF and treated with .1 mL 4N HCl in dioxane and allowed to stir at 60 °C for 10 min. The reaction was then purified by preparative reverse phase HPLC on a C18 column using a suitably buffered H₂O/CH₃CN gradient, and concentrated to give the expected product 5-(3-(tert-butylcarbamoyl)phenyl)-2-(4-fluorophenyl)-6-(4-hydroxybutyl)-N-methylfuro[2,3-b]pyridine-3-carboxamide (9.3 mg, 0.017 mmol, 43% yield). LC-MS Method B: retention time: 1.52 min; m/z (MH⁺): 518. ¹H NMR (500 MHz, METHANOL-d₄) δ 8.00 (t, J=6.7 Hz, 2H), 7.93 (s, 1H), 7.82 (d, J=7.2 Hz, 1H), 7.77 (s, 1H), 7.60 - 7.51 (m, 2H), 7.33 - 7.24 (m, 2H), 3.45 (t, J=6.6 Hz, 2H), 2.99 - 2.91 (m, 3H), 2.89 - 2.79 (m, 2H), 1.82 - 1.65 (m, 2H), 1.49 - 1.42 (m, 11H).

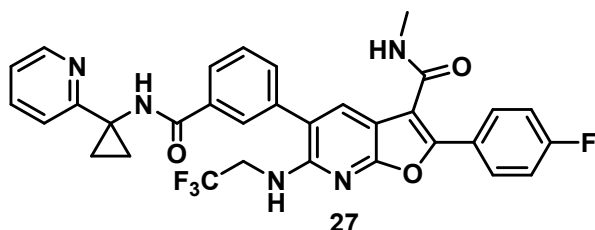
Preparation of 2-(4-fluorophenyl)-N-methyl-5-(3-((1-phenylcyclopropyl)carbamoyl)phenyl)-6-((2,2,2-trifluoroethyl)amino)furo[2,3-b]pyridine-3-carboxamide



2-(3H-[1,2,3]triazolo[4,5-b]pyridin-3-yl)-1,1,3,3-tetramethylisouronium hexafluorophosphate(V) (23 mg, 0.062 mmol) was added to stirring solution of 3-(2-(4-fluorophenyl)-3-(methylcarbamoyl)-6-((2,2,2-trifluoroethyl)amino)furo[2,3-b]pyridin-5-yl)benzoic acid (20 mg, 0.041 mmol), N-ethyl-N-isopropylpropan-2-amine (0.022 mL, 0.12 mmol) and 1-phenylcyclopropanamine hydrochloride (10 mg, 0.062 mmol) in DMF (1 mL) at rt. The mixture was allowed to stir at rt for 1 h. LCMS shows complete reaction. The solution was filtered and then the crude material was purified via preparative LC/MS with the following conditions: Column: Waters XBridge C18, 19 x 200 mm, 5-µm particles; Mobile Phase A: water with 20-mM ammonium acetate; Mobile Phase B: 95:5 acetonitrile:water with 20-mM ammonium acetate; Gradient: 20-100% B over 20 minutes, then a 5-minute hold at 100% B; Flow: 20 mL/min. Fractions containing the desired product were combined and dried via centrifugal evaporation. The yield of the product was 7.4 mg, and its estimated purity by LCMS analysis

was 100%. Two analytical LC/MS injections were used to determine the final purity. Injection 1 conditions: Column: Waters Acquity UPLC BEH C18, 2.1 x 50 mm, 1.7- μ m particles; Mobile Phase A: 5:95 acetonitrile:water with 10 mM ammonium acetate; Mobile Phase B: 95:5 acetonitrile:water with 10 mM ammonium acetate; Temperature: 50 °C; Gradient: 0-100% B over 3 minutes, then a 0.75-minute hold at 100% B; Flow: 1.11 mL/min. Retention time: 3.67 min; M+H = 603. Injection 2 conditions: Column: Waters Acquity UPLC BEH C18, 2.1 x 50 mm, 1.7- μ m particles; Mobile Phase A: 5:95 acetonitrile:water with 0.05% TFA; Mobile Phase B: 95:5 acetonitrile:water with 0.1% TFA; Temperature: 50 °C; Gradient: 0-100% B over 3 minutes, then a 0.75-minute hold at 100% B; Flow: 1.11 mL/min. Retention time: 4.16 min; M+H = 603. ¹H NMR (500 MHz, DMSO-d₆) δ 9.28 (br. s., 1H), 8.45 - 8.34 (m, 1H), 8.04 - 7.92 (m, 4H), 7.70 (d, *J*=2.4 Hz, 1H), 7.66 - 7.55 (m, 2H), 7.34 (t, *J*=8.9 Hz, 2H), 7.31 - 7.10 (m, 5H), 6.69 - 6.60 (m, 1H), 4.26 - 4.11 (m, 2H), 2.83 - 2.76 (m, 3H), 1.32 - 1.24 (m, 4H).

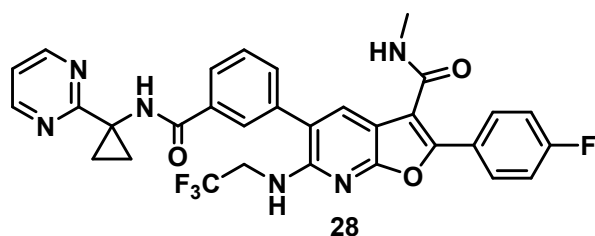
Preparation of 2-(4-fluorophenyl)-N-methyl-5-(3-((1-(pyridin-2-yl)cyclopropyl)carbamoyl)phenyl)-6-((2,2,2-trifluoroethyl)amino)furo[2,3-b]pyridine-3-carboxamide



3-(2-(4-fluorophenyl)-3-(methylcarbamoyl)-6-((2,2,2-trifluoroethyl)amino)furo[2,3-b]pyridin-5-yl)benzoic acid (20 mg, 0.041 mmol) was taken up in DMF (0.5 ml) and treated with N-ethyl-N-isopropylpropan-2-amine (21 μ l, 0.12 mmol), 1-(pyridin-2-yl)cyclopropanamine hydrochloride (18 mg, 0.10 mmol) followed by 2-(3H-[1,2,3]triazolo[4,5-b]pyridin-3-yl)-1,1,3,3-tetramethylisouronium hexafluorophosphate(V) (23 mg, 0.062 mmol). The reaction mixture was allowed to stir for 1 h, then was diluted with MeOH the crude material was purified via preparative LC/MS with the following conditions: Column: Waters XBridge C18, 19 x 200 mm, 5- μ m particles; Guard Column: Waters XBridge C18, 19 x 10 mm, 5- μ m particles; Mobile Phase A: water with 20-mM ammonium acetate; Mobile Phase B: 95:5 acetonitrile:water with 20-mM ammonium acetate; Gradient: 40-80% B over 20 minutes, then a 5-minute hold at 100% B;

Flow: 20 mL/min. Fractions containing the desired product were combined and dried via centrifugal evaporation. The yield of the product was 13.2 mg, and its estimated purity by LCMS analysis was 100%. Two analytical LC/MS injections were used to determine the final purity. Injection 1 conditions: Column: Waters BEH C18, 2.0 x 50 mm, 1.7- μ m particles; Mobile Phase A: 5:95 acetonitrile:water with 10 mM ammonium acetate; Mobile Phase B: 95:5 acetonitrile:water with 10 mM ammonium acetate; Temperature: 40°C; Gradient: 0.5 min hold at 0% B, 0-100% B over 4 minutes, then a 0.5-minute hold at 100% B; Flow: 1 mL/min. Retention time: 2.93 min; M+H = 604. Injection 2 conditions: Column: Waters BEH C18, 2.0 x 50 mm, 1.7- μ m particles; Mobile Phase A: 5:95 methanol:water with 10 mM ammonium acetate; Mobile Phase B: 95:5 methanol:water with 10 mM ammonium acetate; Temperature: 40 °C; Gradient: 0.5 min hold at 0%B, 0-100% B over 4 minutes, then a 0.5-minute hold at 100% B; Flow: 0.5 mL/min. Retention time: 3.97 min; M+H = 604. ¹H NMR (500MHz, DMSO-d₆) δ 9.33 (s, 1H), 8.47 - 8.33 (m, 2H), 8.04 - 7.88 (m, 4H), 7.73 - 7.58 (m, 4H), 7.40 - 7.29 (m, 3H), 7.19 - 7.10 (m, 1H), 6.68 - 6.59 (m, 1H), 4.25 - 4.12 (m, 2H), 2.79 (d, J=4.0 Hz, 3H), 1.62 - 1.49 (m, 2H), 1.31 - 1.20 (m, 2H).

Preparation of 2-(4-fluorophenyl)-N-methyl-5-(3-((1-(pyrimidin-2-yl)cyclopropyl)carbamoyl)phenyl)-6-((2,2,2-trifluoroethyl)amino)furo[2,3-b]pyridine-3-carboxamide



Step 1:

3-(6-chloro-2-(4-fluorophenyl)-3-(methylcarbamoyl)furo[2,3-b]pyridin-5-yl)benzoic acid (50 mg, 0.12 mmol) was taken up in DMF (1 ml) and treated with N-ethyl-N-isopropylpropan-2-amine (62 μ l, 0.35 mmol), 1-(pyrimidin-2-yl)cyclopropanamine dihydrochloride (49 mg, 0.24 mmol) followed by 2-(3H-[1,2,3]triazolo[4,5-b]pyridin-3-yl)-1,1,3,3-tetramethylisouronium hexafluorophosphate(V) (67 mg, 0.18 mmol). The reaction was allowed to stir for 1 h then concentrated and was purified on silica gel (Biotage, MeOH/DCM gradient, fraction collection at

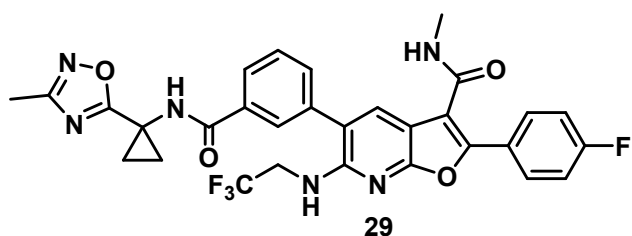
$\lambda = 254$ nm) to give the expected product 2-(6-chloro-2-(4-fluorophenyl)-N-methyl-5-(3-((1-(pyrimidin-2-yl)cyclopropyl)carbamoyl)phenyl)furo[2,3-b]pyridine-3-carboxamide (quant. yield). LCMS Method E, retention time: 2.84. M+H = 542.

Step 2:

Sodium 2-methylbutan-2-olate (36 mg, 0.32 mmol), 6-chloro-2-(4-fluorophenyl)-N-methyl-5-(3-((1-(pyrimidin-2-yl)cyclopropyl)carbamoyl)phenyl)furo[2,3-b]pyridine-3-carboxamide (35 mg, 0.065 mmol), 2,2,2-trifluoroethanamine (32 mg, 0.32 mmol), and Chloro[2-(dicyclohexylphosphino)-3,6-dimethoxy-2'-4'-6'-tri-*i*-propyl-1,1'-biphenyl][2-(2-aminoethyl)phenyl]palladium(II) (5 mg, 6 μ mol) were combined, degassed, and taken up in dioxane (1.3 mL) at rt and then was heated to 90 °C. LCMS at 15 min showed major peak with M+H matching that of the expected product. The mixture was diluted with EtOAc and washed with sat aq NaCl. The organic phase was concentrated and the crude material was purified via preparative LC/MS with the following conditions: Column: Waters XBridge C18, 19 x 200 mm, 5- μ m particles; Guard Column: Waters XBridge C18, 19 x 10 mm, 5- μ m particles; Mobile Phase A: water with 20-mM ammonium acetate; Mobile Phase B: 95:5 methanol:water with 20-mM ammonium acetate; Gradient: 50-90% B over 20 minutes, then a 4-minute hold at 100% B; Flow: 20 mL/min. Fractions containing the desired product were combined and dried via centrifugal evaporation. The yield of the product was 19.3 mg, and its estimated purity by LCMS analysis was 100%. Two analytical LC/MS injections were used to determine the final purity. Injection 1 conditions: Column: Waters BEH C18, 2.0 x 50 mm, 1.7- μ m particles; Mobile Phase A: 5:95 acetonitrile:water with 10 mM ammonium acetate; Mobile Phase B: 95:5 acetonitrile:water with 10 mM ammonium acetate; Temperature: 40 °C; Gradient: 0.5 min hold at 0%B, 0-100% B over 4 minutes, then a 0.5-minute hold at 100% B; Flow: 1 mL/min. retention time: 2.81, M+H = 605. Injection 2 conditions: Column: Waters BEH C18, 2.0 x 50 mm, 1.7- μ m particles; Mobile Phase A: 5:95 methanol:water with 10 mM ammonium acetate; Mobile Phase B: 95:5 methanol:water with 10 mM ammonium acetate; Temperature: 40 °C; Gradient: 0.5 min hold at 0% B, 0-100% B over 4 minutes, then a 0.5-minute hold at 100% B; Flow: 0.5 mL/min. retention time: 3.83, M+H = 605. ¹H NMR (500MHz, DMSO-*d*₆) δ 9.26 (s, 1H), 8.64 (d, J=4.9 Hz, 2H), 8.43 - 8.34 (m, 1H), 8.02 - 7.87 (m, 4H), 7.67 (s, 1H), 7.64 - 7.56 (m, 2H), 7.32 (t,

J=8.7 Hz, 2H), 7.25 (t, J=4.7 Hz, 1H), 6.58 (t, J=6.3 Hz, 1H), 4.26 - 4.12 (m, 2H), 2.78 (d, J=4.3 Hz, 3H), 1.66 - 1.53 (m, 2H), 1.40 - 1.30 (m, 2H).

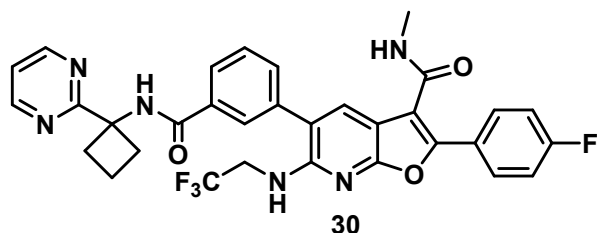
Preparation of 2-(4-fluorophenyl)-N-methyl-5-(3-((1-(3-methyl-1,2,4-oxadiazol-5-yl)cyclopropyl)carbamoyl)phenyl)-6-((2,2,2-trifluoroethyl)amino)furo[2,3-b]pyridine-3-carboxamide



3-(2-(4-fluorophenyl)-3-(methylcarbamoyl)-6-((2,2,2-trifluoroethyl)amino)furo[2,3-b]pyridin-5-yl)benzoic acid (40 mg, 0.082 mmol) was taken up in DMF (1 ml) and treated with N-ethyl-N-isopropylpropan-2-amine (43 μ l, 0.25 mmol), 1-(3-methyl-1,2,4-oxadiazol-5-yl)cyclopropanamine hydrochloride (17 mg, 0.098 mmol) followed by 2-(3H-[1,2,3]triazolo[4,5-b]pyridin-3-yl)-1,1,3,3-tetramethylisouronium hexafluorophosphate(V) (47 mg, 0.12 mmol). The reaction was allowed to stir for 1 h. Then the reaction was quenched with MeOH (1 mL) and the crude material was purified via preparative LC/MS with the following conditions: Column: Waters XBridge C18, 19 x 200 mm, 5- μ m particles; Guard Column: Waters XBridge C18, 19 x 10 mm, 5- μ m particles; Mobile Phase A: water with 20-mM ammonium acetate; Mobile Phase B: 95:5 acetonitrile:water with 20-mM ammonium acetate; Gradient: 20-100% B over 20 minutes, then a 5-minute hold at 100% B; Flow: 20 mL/min. Fractions containing the desired product were combined and dried via centrifugal evaporation. The yield of the product was 15.4 mg, and its estimated purity by LCMS analysis was 98%. Two analytical LC/MS injections were used to determine the final purity. Injection 1 conditions: Column: Waters BEH C18, 2.0 x 50 mm, 1.7- μ m particles; Mobile Phase A: 5:95 acetonitrile:water with 10 mM ammonium acetate; Mobile Phase B: 95:5 acetonitrile:water with 10 mM ammonium acetate; Temperature: 40 $^{\circ}$ C; Gradient: 0.5 min hold at 0% B, 0-100% B over 4 minutes, then a 0.5-minute hold at 100% B; Flow: 1 mL/min. Injection 2 conditions: Column: Waters BEH C18, 2.0 x 50 mm, 1.7- μ m particles; Mobile Phase A: 5:95 methanol:water with 10 mM ammonium acetate; Mobile Phase B: 95:5 methanol:water with 10 mM ammonium acetate; Temperature: 40 $^{\circ}$ C; Gradient: 0.5 min

hold at 0% B, 0-100% B over 4 minutes, then a 0.5-minute hold at 100% B; Flow: 0.5 mL/min. ¹H NMR (500MHz, DMSO-d₆) δ 9.54 (s, 1H), 8.42 - 8.32 (m, 1H), 7.98 - 7.92 (m, 4H), 7.69 - 7.61 (m, 3H), 7.34 (t, J=8.9 Hz, 2H), 6.61 (t, J=6.3 Hz, 1H), 4.21 - 4.14 (m, 2H), 2.79 (d, J=4.6 Hz, 3H), 2.26 (s, 3H), 1.67 - 1.63 (m, 2H), 1.52 - 1.47 (m, 2H).

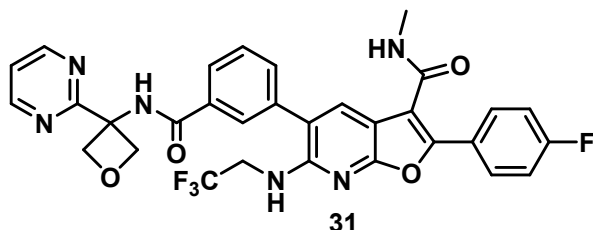
Preparation of 2-(4-fluorophenyl)-N-methyl-5-(3-((1-(pyrimidin-2-yl)cyclobutyl)carbamoyl)phenyl)-6-((2,2,2-trifluoroethyl)amino)furo[2,3-b]pyridine-3-carboxamide



3-(2-(4-fluorophenyl)-3-(methylcarbamoyl)-6-((2,2,2-trifluoroethyl)amino)furo[2,3-b]pyridin-5-yl)benzoic acid (15 mg, 0.031 mmol) was taken up in DMF (0.3 ml) and treated with N-ethyl-N-isopropylpropan-2-amine (16 μl, 0.092 mmol), 1-(pyrimidin-2-yl)cyclobutanamine hydrochloride (29 mg, 0.15 mmol) followed by 2-(3H-[1,2,3]triazolo[4,5-b]pyridin-3-yl)-1,1,3,3-tetramethylisouronium hexafluorophosphate(V) (18 mg, 0.046 mmol). The reaction was allowed to stir for 1 h, then was diluted with MeOH. The crude material was purified via preparative LC/MS with the following conditions: Column: Waters XBridge C18, 19 x 200 mm, 5-μm particles; Guard Column: Waters XBridge C18, 19 x 10 mm, 5-μm particles; Mobile Phase A: water with 20-mM ammonium acetate; Mobile Phase B: 95:5 acetonitrile:water with 20-mM ammonium acetate; Gradient: 20-100% B over 20 minutes, then a 5-minute hold at 100% B; Flow: 20 mL/min. Fractions containing the desired product were combined and dried via centrifugal evaporation. The yield of the product was 4.1 mg, and its estimated purity by LCMS analysis was 97%. Two analytical LC/MS injections were used to determine the final purity. Injection 1 conditions: Column: Waters BEH C18, 2.0 x 50 mm, 1.7-μm particles; Mobile Phase A: 5:95 acetonitrile:water with 10 mM ammonium acetate; Mobile Phase B: 95:5 acetonitrile:water with 10 mM ammonium acetate; Temperature: 40 °C; Gradient: 0.5 min hold at 0% B, 0-100% B over 4 minutes, then a 0.5-minute hold at 100% B; Flow: 1 mL/min. Retention time: 2.92 min; M+H = 619. Injection 2 conditions: Column: Waters BEH C18, 2.0 x

50 mm, 1.7- μ m particles; Mobile Phase A: 5:95 methanol:water with 10 mM ammonium acetate; Mobile Phase B: 95:5 methanol:water with 10 mM ammonium acetate; Temperature: 40 °C; Gradient: 0.5 min hold at 0% B, 0-100% B over 4 minutes, then a 0.5-minute hold at 100% B; Flow: 0.5 mL/min. Retention time: 3.98 min; M+H = 619. ¹H NMR (500MHz, DMSO-d₆) δ 9.19 (br. s., 1H), 8.82 - 8.71 (m, 2H), 8.42 - 8.32 (m, 1H), 8.02 - 7.90 (m, 4H), 7.69 (s, 1H), 7.65 - 7.54 (m, 2H), 7.39 - 7.27 (m, 3H), 6.67 - 6.58 (m, 1H), 4.24 - 4.13 (m, 2H), 2.83 - 2.69 (m, 5H), 2.55 (d, J=8.5 Hz, 2H), 2.12 - 1.96 (m, 2H).

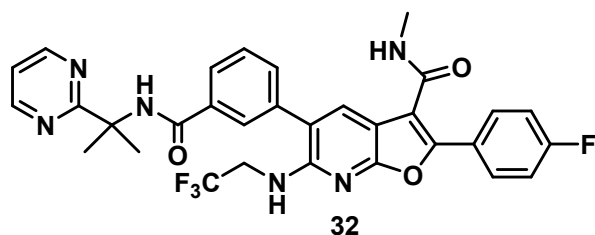
Preparation of 2-(4-fluorophenyl)-N-methyl-5-(3-((3-(pyrimidin-2-yl)oxetan-3-yl)carbamoyl)phenyl)-6-((2,2,2-trifluoroethyl)amino)furo[2,3-b]pyridine-3-carboxamide



3-(2-(4-fluorophenyl)-3-(methylcarbamoyl)-6-((2,2,2-trifluoroethyl)amino)furo[2,3-b]pyridin-5-yl)benzoic acid (30 mg, 0.062 mmol) was taken up in DMF (1 ml) and treated with N-ethyl-N-isopropylpropan-2-amine (32 μ l, 0.19 mmol), 3-(pyrimidin-2-yl)oxetan-3-amine hydrochloride (29 mg, 0.15 mmol) followed by 2-(3H-[1,2,3]triazolo[4,5-b]pyridin-3-yl)-1,1,3,3-tetramethylisouronium hexafluorophosphate(V) (35 mg, 0.092 mmol). The reaction mixture was allowed to stir for 1 h. The reaction mixture was diluted with MeOH and was purified via preparative LC/MS with the following conditions: Column: Waters XBridge C18, 19 x 200 mm, 5- μ m particles; Guard Column: Waters XBridge C18, 19 x 10 mm, 5- μ m particles; Mobile Phase A: water with 20-mM ammonium acetate; Mobile Phase B: 95:5 acetonitrile:water with 20-mM ammonium acetate; Gradient: 10-100% B over 20 minutes, then a 5-minute hold at 100% B; Flow: 20 mL/min. Fractions containing the desired product were combined and dried via centrifugal evaporation. The yield of the product was 7.3 mg, and its estimated purity by LCMS analysis was 93%. Two analytical LC/MS injections were used to determine the final purity. Injection 1 conditions: Column: Waters BEH C18, 2.0 x 50 mm, 1.7- μ m particles; Mobile Phase A: 5:95 acetonitrile:water with 10 mM ammonium acetate; Mobile Phase B: 95:5

acetonitrile:water with 10 mM ammonium acetate; Temperature: 40 °C; Gradient: 0.5 min hold at 0% B, 0-100% B over 4 minutes, then a 0.5-minute hold at 100% B; Flow: 1 mL/min. Retention time: 2.70 min; M+H = 621. Injection 2 conditions: Column: Waters BEH C18, 2.0 x 50 mm, 1.7- μ m particles; Mobile Phase A: 5:95 methanol:water with 10 mM ammonium acetate; Mobile Phase B: 95:5 methanol:water with 10 mM ammonium acetate; Temperature: 40 °C; Gradient: 0.5 min hold at 0% B, 0-100% B over 4 minutes, then a 0.5-minute hold at 100% B; Flow: 0.5 mL/min. retention time: 2.70 min; M+H = 621. ¹H NMR (500MHz, DMSO-d₆) δ 9.73 (s, 1H), 8.89 - 8.78 (m, 2H), 8.45 - 8.30 (m, 1H), 8.05 - 7.89 (m, 4H), 7.72 - 7.57 (m, 3H), 7.43 (br. s., 1H), 7.34 (t, J=7.9 Hz, 2H), 6.69 - 6.58 (m, 1H), 5.12 - 5.03 (m, 2H), 4.96 - 4.86 (m, 2H), 4.25 - 4.09 (m, 2H), 2.84 - 2.72 (m, 3H).

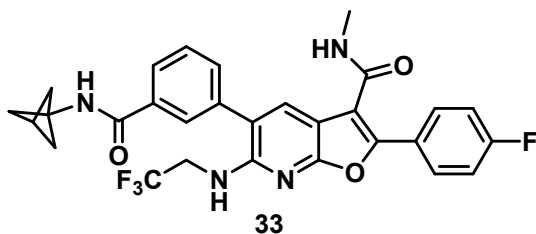
Preparation of 2-(4-fluorophenyl)-N-methyl-5-(3-((2-(pyrimidin-2-yl)propan-2-yl)carbamoyl)phenyl)-6-((2,2,2-trifluoroethyl)amino)furo[2,3-b]pyridine-3-carboxamide



3-(2-(4-fluorophenyl)-3-(methylcarbamoyl)-6-((2,2,2-trifluoroethyl)amino)furo[2,3-b]pyridin-5-yl)benzoic acid (15 mg, 0.031 mmol) was taken up in DMF (0.3 ml) and treated with N-ethyl-N-isopropylpropan-2-amine (16 μ l, 0.092 mmol), 2-(pyrimidin-2-yl)propan-2-amine hydrochloride (53 mg, 0.31 mmol) followed by 2-(3H-[1,2,3]triazolo[4,5-b]pyridin-3-yl)-1,1,3,3-tetramethylisouronium hexafluorophosphate(V) (18 mg, 0.046 mmol). The reaction was allowed to stir for 1 h. The crude material was purified via preparative LC/MS with the following conditions: Column: Waters XBridge C18, 19 x 200 mm, 5- μ m particles; Guard Column: Waters XBridge C18, 19 x 10 mm, 5- μ m particles; Mobile Phase A: water with 20-mM ammonium acetate; Mobile Phase B: 95:5 acetonitrile:water with 20-mM ammonium acetate; Gradient: 35-75% B over 20 minutes, then a 5-minute hold at 100% B; Flow: 20 mL/min. Fractions containing the desired product were combined and dried via centrifugal evaporation. The yield of the product was 4.7 mg, and its estimated purity by LCMS analysis was 100%. Two analytical

LC/MS injections were used to determine the final purity. Injection 1 conditions: Column: Waters BEH C18, 2.0 x 50 mm, 1.7- μ m particles; Mobile Phase A: 5:95 acetonitrile:water with 10 mM ammonium acetate; Mobile Phase B: 95:5 acetonitrile:water with 10 mM ammonium acetate; Temperature: 40 °C; Gradient: 0.5 min hold at 0% B, 0-100% B over 4 minutes, then a 0.5-minute hold at 100% B; Flow: 1 mL/min. retention time: 2.91 M+H = 607. Injection 2 conditions: Column: Waters BEH C18, 2.0 x 50 mm, 1.7- μ m particles; Mobile Phase A: 5:95 methanol:water with 10 mM ammonium acetate; Mobile Phase B: 95:5 methanol:water with 10 mM ammonium acetate; Temperature: 40 °C; Gradient: 0.5 min hold at 0% B, 0-100% B over 4 minutes, then a 0.5-minute hold at 100% B; Flow: 0.5 mL/min. retention time: 3.97 M+H = 607. ¹H NMR (500MHz, DMSO-d₆) δ 8.74 (d, J=4.6 Hz, 2H), 8.68 (s, 1H), 8.42 - 8.33 (m, 1H), 8.00 - 7.90 (m, 3H), 7.86 (d, J=6.4 Hz, 1H), 7.69 (s, 1H), 7.63 - 7.52 (m, 2H), 7.39 - 7.28 (m, 3H), 6.68 - 6.60 (m, 1H), 4.25 - 4.09 (m, 2H), 2.79 (d, J=4.3 Hz, 3H), 1.71 (s, 6H).

Preparation of 5-(3-(bicyclo[1.1.1]pentan-1-ylcarbamoyl)phenyl)-2-(4-fluorophenyl)-N-methyl-6-((2,2,2-trifluoroethyl)amino)furo[2,3-b]pyridine-3-carboxamide



Step 1:

N-ethyl-N-isopropylpropan-2-amine (0.40 mL, 2.3 mmol) was added to stirring solution of 3-(6-chloro-2-(4-fluorophenyl)-3-(methylcarbamoyl)furo[2,3-b]pyridin-5-yl)benzoic acid (120 mg, 0.28 mmol), 2-(3H-[1,2,3]triazolo[4,5-b]pyridin-3-yl)-1,1,3,3-tetramethylisouronium hexafluorophosphate(V) (160 mg, 0.42 mmol) and bicyclo[1.1.1]pentan-1-amine hydrochloride (34 mg, 0.28 mmol) in DMF (1 mL) at rt. The mixture was allowed to stir at rt for 16 h. The reaction mixture was then diluted with EtOAc (15 mL) and NH₄Cl. The layers were separated and the aq layer was extracted with EtOAc (2 x 10 mL). The combined organic extracts were washed with water, brine, dried over Na₂SO₄, filtered and concentrated to give a brown residue which was adsorbed on Celite and purified by flash column chromatography on silica gel eluting 0 - 100 % EtOAc in hexanes gradient over 15 CV to give 5-(3-(bicyclo[1.1.1]pentan-1-

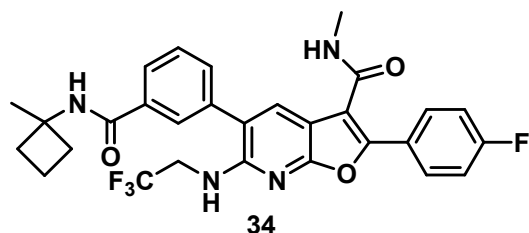
ylcarbamoyl)phenyl)-6-chloro-2-(4-fluorophenyl)-N-methylfuro[2,3-b]pyridine-3-carboxamide (0.110 g, 0.230 mmol, 79% yield) as a white solid. ¹H NMR (400MHz, DMSO-d₆) δ 9.07 (s, 1H), 8.58 - 8.52 (m, 1H), 8.19 (s, 1H), 8.09 - 8.03 (m, 2H), 7.99 - 7.89 (m, 2H), 7.69 (dt, *J*=7.9, 1.3 Hz, 1H), 7.64 - 7.56 (m, 1H), 7.48 - 7.39 (m, 2H), 2.82 (d, *J*=4.5 Hz, 3H), 2.47 (s, 1H), 2.10 (s, 6H).

Step 2:

Sodium 2-methylbutan-2-olate (34 mg, 0.31 mmol), 5-(3-(bicyclo[1.1.1]pentan-1-ylcarbamoyl)phenyl)-6-chloro-2-(4-fluorophenyl)-N-methylfuro[2,3-b]pyridine-3-carboxamide (30 mg, 0.061 mmol), 2,2,2-trifluoroethanamine (30 mg, 0.31 mmol), Chloro[2-(dicyclohexylphosphino)-3,6-dimethoxy-2',4', 6'-triisopropyl-1,1'-biphenyl][2-(2-aminoethyl)phenyl]palladium(II) (5 mg, 6 μmol) were combined, degassed, and taken up in dioxane (1.2 ml) at rt and then was heated to 90 °C for 15 min. The reaction mixture was then diluted with EtOAc and washed with 1M HCl aq, and sat aq NaCl aq. The organic phase was concentrated and the crude material was purified via preparative LC/MS with the following conditions: Column: Waters XBridge C18, 19 x 200 mm, 5-μm particles; Guard Column: Waters XBridge C18, 19 x 10 mm, 5-μm particles; Mobile Phase A: water with 20-mM ammonium acetate; Mobile Phase B: 95:5 acetonitrile:water with 20-mM ammonium acetate; Gradient: 50-90% B over 12 minutes, then a 5-minute hold at 100% B; Flow: 20 mL/min. Fractions containing the desired product were combined and dried via centrifugal evaporation. The yield of the product was 16.2 mg, and its estimated purity by LCMS analysis was 98%. Two analytical LC/MS injections were used to determine the final purity. Injection 1 conditions: Column: Waters BEH C18, 2.0 x 50 mm, 1.7-μm particles; Mobile Phase A: 5:95 acetonitrile:water with 10 mM ammonium acetate; Mobile Phase B: 95:5 acetonitrile:water with 10 mM ammonium acetate; Temperature: 40 °C; Gradient: 0.5 min hold at 0% B, 0-100% B over 4 minutes, then a 0.5-minute hold at 100% B; Flow: 1 mL/min. Injection 2 conditions: Column: Waters BEH C18, 2.0 x 50 mm, 1.7-μm particles; Mobile Phase A: 5:95 methanol:water with 10 mM ammonium acetate; Mobile Phase B: 95:5 methanol:water with 10 mM ammonium acetate; Temperature: 40 °C; Gradient: 0.5 min hold at 0% B, 0-100% B over 4 minutes, then a 0.5-minute hold at 100% B; Flow: 0.5 mL/min. Proton NMR was acquired in deuterated DMSO. ¹H NMR (500MHz, DMSO-d₆) δ 9.04 (s, 1H), 8.38 (d, *J*=4.6 Hz, 1H), 7.94 (dd, *J*=8.9, 5.5 Hz, 2H), 7.87 (m, 2H),

7.65 (s, 1H), 7.60 - 7.53 (m, 2H), 7.33 (t, J=8.9 Hz, 2H), 6.57 (t, J=6.3 Hz, 1H), 4.25 - 4.08 (m, 2H), 2.78 (d, J=4.6 Hz, 3H), 2.45 (s, 1H), 2.08 (s, 6H).

Preparation of 2-(4-fluorophenyl)-N-methyl-5-(3-((1-methylcyclobutyl)carbamoyl)phenyl)-6-((2,2,2-trifluoroethyl)amino)furo[2,3-b]pyridine-3-carboxamide



Step 1:

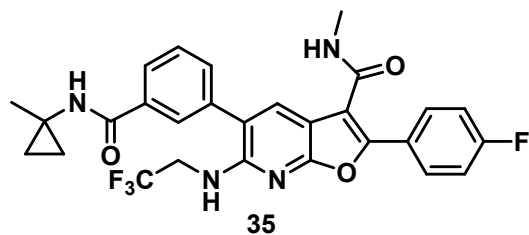
3-(6-chloro-2-(4-fluorophenyl)-3-(methylcarbamoyl)furo[2,3-b]pyridin-5-yl)benzoic acid (100 mg, 0.235 mmol) was taken up in DMF (2.4 ml) and treated with N-ethyl-N-isopropylpropan-2-amine (123 μ l, 0.706 mmol), 1-methylcyclobutanamine hydrochloride (34 mg, 0.28 mmol) followed by 2-(3H-[1,2,3]triazolo[4,5-b]pyridin-3-yl)-1,1,3,3-tetramethylisouronium hexafluorophosphate(V) (134 mg, 0.353 mmol). The reaction was allowed to stir for 1 h. The reaction mixture was concentrated and purified on silica gel (Biotage, EtOAc/hexanes gradient, fraction collection at $\lambda = 254$ nm) to give the expected product 6-chloro-2-(4-fluorophenyl)-N-methyl-5-(3-((1-methylcyclobutyl)carbamoyl)phenyl)furo[2,3-b]pyridine-3-carboxamide (70 mg, 0.14 mmol, 60% yield) consistent by LCMS. LCMS Method D, retention time: 2.99. M+H = 492.

Step 2:

sodium 2-methylbutan-2-olate (39 mg, 0.37 mmol), 6-chloro-2-(4-fluorophenyl)-N-methyl-5-(3-((1-methylcyclobutyl)carbamoyl)phenyl)furo[2,3-b]pyridine-3-carboxamide (35 mg, 0.071 mmol), 2,2,2-trifluoroethanamine (35 mg, 0.36 mmol), and Chloro[2-(dicyclohexylphosphino)-3,6-dimethoxy-2'-4'-6'-tri-i-propyl-1,1'-biphenyl][2-(2-aminoethyl)phenyl]palladium(II) (6 mg, 7 μ mol) were combined, degassed, and taken up in dioxane (1.4 ml) at rt and then was heated to 90 °C. LCMS at 15 min showed major peak with M+H matching that of the expected product. The mixture was diluted with EtOAc and washed with sat aq NaCl. The organic phase was concentrated. The crude material was purified via preparative LC/MS with the following

conditions: Column: Waters XBridge C18, 19 x 200 mm, 5- μ m particles; Guard Column: Waters XBridge C18, 19 x 10 mm, 5- μ m particles; Mobile Phase A: water with 20-mM ammonium acetate; Mobile Phase B: 95:5 acetonitrile:water with 20-mM ammonium acetate; Gradient: 10-100% B over 20 minutes, then a 4-minute hold at 100% B; Flow: 20 mL/min. Fractions containing the desired product were combined and dried via centrifugal evaporation. The yield of the product was 16.7 mg, and its estimated purity by LCMS analysis was 100%. Two analytical LC/MS injections were used to determine the final purity. Injection 1 conditions: Column: Waters BEH C18, 2.0 x 50 mm, 1.7- μ m particles; Mobile Phase A: 5:95 acetonitrile:water with 10 mM ammonium acetate; Mobile Phase B: 95:5 acetonitrile:water with 10 mM ammonium acetate; Temperature: 40 °C; Gradient: 0.5 min hold at 0% B, 0-100% B over 4 minutes, then a 0.5-minute hold at 100% B; Flow: 1 mL/min. retention time: 4.09, M+H = 555. Injection 2 conditions: Column: Waters BEH C18, 2.0 x 50 mm, 1.7- μ m particles; Mobile Phase A: 5:95 methanol:water with 10 mM ammonium acetate; Mobile Phase B: 95:5 methanol:water with 10 mM ammonium acetate; Temperature: 40 °C; Gradient: 0.5 min hold at 0% B, 0-100% B over 4 minutes, then a 0.5-minute hold at 100% B; Flow: 0.5 mL/min. retention time: 4.09 M+H = 555. Proton NMR was acquired in deuterated DMSO. ^1H NMR (500MHz, DMSO- d_6) δ 8.44 (s, 1H), 8.41 - 8.33 (m, 1H), 7.94 (dd, J=8.7, 5.3 Hz, 2H), 7.90 - 7.85 (m, 2H), 7.66 (s, 1H), 7.62 - 7.53 (m, 2H), 7.33 (t, J=8.7 Hz, 2H), 6.57 (t, J=6.3 Hz, 1H), 4.25 - 4.12 (m, 2H), 2.78 (d, J=4.6 Hz, 3H), 2.39 - 2.26 (m, 2H), 2.04 - 1.94 (m, 2H), 1.86 - 1.74 (m, 2H), 1.47 (s, 3H).

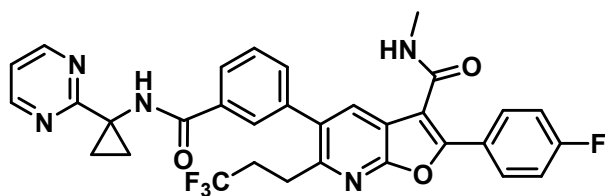
Preparation of 2-(4-fluorophenyl)-N-methyl-5-(3-((1-methylcyclopropyl)carbamoyl)phenyl)-6-((2,2,2-trifluoroethyl)amino)furo[2,3-b]pyridine-3-carboxamide



N-ethyl-N-isopropylpropan-2-amine (0.043 mL, 0.25 mmol) was added to stirring solution of 3-(2-(4-fluorophenyl)-3-(methylcarbamoyl)-6-((2,2,2-trifluoroethyl)amino)furo[2,3-b]pyridin-5-yl)benzoic acid (15 mg, 0.031 mmol), 2-(3H-[1,2,3]triazolo[4,5-b]pyridin-3-yl)-1,1,3,3-

tetramethylisouronium hexafluorophosphate(V) (17.55 mg, 0.046 mmol) and cyclopropyl amine (5.0 mg, 0.046 mmol) in DMF (1 mL) at rt. The mixture was allowed to stir at rt for 1 h. LCMS shows complete reaction. The solution was filtered and the crude material was purified via preparative LC/MS with the following conditions: Column: Waters XBridge C18, 19 x 200 mm, 5- μ m particles; Guard Column: Waters XBridge C18, 19 x 10 mm, 5- μ m particles; Mobile Phase A: water with 20-mM ammonium acetate; Mobile Phase B: 95:5 acetonitrile:water with 20-mM ammonium acetate; Gradient: 35-65% B over 20 minutes, then a 5-minute hold at 100% B; Flow: 20 mL/min. Fractions containing the desired product were combined and dried via centrifugal evaporation. To give 10.2 mg of 2-(4-fluorophenyl)-N-methyl-5-(3-((1-methylcyclopropyl)carbamoyl)phenyl)-6-((2,2,2-trifluoroethyl)amino)furo[2,3-b]pyridine-3-carboxamide, and its estimated purity by LCMS analysis was 100%. Two analytical LC/MS injections were used to determine the final purity. Injection 1 conditions: Column: Waters BEH C18, 2.0 x 50 mm, 1.7- μ m particles; Mobile Phase A: 5:95 acetonitrile:water with 10 mM ammonium acetate; Mobile Phase B: 95:5 acetonitrile:water with 10 mM ammonium acetate; Temperature: 40 °C; Gradient: 0.5 min hold at 0% B, 0-100% B over 4 minutes, then a 0.5-minute hold at 100% B; Flow: 1 mL/min. Injection 2 conditions: Column: Waters BEH C18, 2.0 x 50 mm, 1.7- μ m particles; Mobile Phase A: 5:95 methanol:water with 10 mM ammonium acetate; Mobile Phase B: 95:5 methanol:water with 10 mM ammonium acetate; Temperature: 40 °C; Gradient: 0.5 min hold at 0% B, 0-100% B over 4 minutes, then a 0.5-minute hold at 100% B; Flow: 0.5 mL/min. Retention time: 4.00 M+H = 541. Proton NMR was acquired in deuterated DMSO. ¹H NMR (500 MHz, DMSO-d₆) δ 7.82 (br s, 1H), 7.47 (br s, 1H), 7.09 - 7.01 (m, 2H), 6.97 (br s, 2H), 6.76 (s, 1H), 6.71 - 6.61 (m, 2H), 6.44 (br t, J=8.7 Hz, 2H), 5.67 (br d, J=5.2 Hz, 1H), 3.32 - 3.22 (m, 2H), 2.59 - 2.49 (m, 97H), 2.41 - 2.21 (m, 5H), 1.89 (br s, 3H), 1.60 (br s, 4H), 0.48 (s, 3H), -0.16 (br s, 2H), -0.28 (br s, 2H).

Preparation of: *tert*-butyl 3-(6-chloro-2-(4-fluorophenyl)-3-(methylcarbamoyl)furo[2,3-b]pyridin-5-yl)benzoate



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Step 1: preparation of 6-chloro-2-(4-fluorophenyl)-N-methyl-5-(3-((1-(pyrimidin-2-yl)cyclopropyl)carbamoyl)phenyl)furo[2,3-b]pyridine-3-carboxamide

3-(6-chloro-2-(4-fluorophenyl)-3-(methylcarbamoyl)furo[2,3-b]pyridin-5-yl)benzoic acid (50 mg, 0.120 mmol) was taken up in DMF (1.2 ml) and treated with N-ethyl-N-isopropylpropan-2-amine (62 μ l, 0.35 mmol), 1-(pyrimidin-2-yl)cyclopropanamine dihydrochloride (49 mg, 0.24 mmol) followed by 2-(3H-[1,2,3]triazolo[4,5-b]pyridin-3-yl)-1,1,3,3-tetramethylisouronium hexafluorophosphate(V) (67 mg, 0.18 mmol). The reaction was allowed to stir for 1 h then concentrated and was purified on silica gel (Biotage, MeOH/DCM gradient, fraction collection at $\lambda = 254$ nm) to give the expected product 6-chloro-2-(4-fluorophenyl)-N-methyl-5-(3-((1-(pyrimidin-2-yl)cyclopropyl)carbamoyl)phenyl)furo[2,3-b]pyridine-3-carboxamide (quant yield) LCMS Method E, retention time: 2.84 min. M+H = 542.

Step 2: preparation of the titled compound:

6-chloro-2-(4-fluorophenyl)-N-methyl-5-(3-((1-(pyrimidin-2-yl)cyclopropyl)carbamoyl)phenyl)furo[2,3-b]pyridine-3-carboxamide (38 mg, 0.070 mmol), 3,3,3-trifluoropropane-1-trifluoroborate (72 mg, 0.35 mmol), dicyclohexyl(2',6'-diisopropoxy-[1,1'-biphenyl]-2-yl)phosphine (13 mg, 0.028 mmol), PdOAc₂ (3.1 mg, 0.014 mmol), cesium carbonate (69 mg, 0.21 mmol) were degassed and backfilled with N₂ then dissolved in toluene (4.2 ml) and water (0.42 ml) at rt then heated at 80 °C. The reaction was allowed to stir 16 h. The mixture was diluted with EtOAc and washed with 1M HCl, and sat aq NaCl. The organic phase was concentrated and was diluted with DMF/MeOH and the crude material was purified via preparative LC/MS with the following conditions: Column: Waters XBridge C18, 19 x 200 mm, 5- μ m particles; Guard Column: Waters XBridge C18, 19 x 10 mm, 5- μ m particles; Mobile Phase A: water with 20-mM ammonium acetate; Mobile Phase B: 95:5 acetonitrile:water with 20-mM ammonium acetate; Gradient: 20-100% B over 20 minutes, then a 4-minute hold at 100% B;

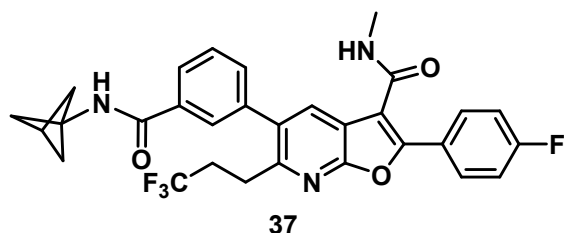
Flow: 20 mL/min. Fractions containing the desired product were combined and dried via centrifugal evaporation. The yield of the product was 13.7 mg, and its estimated purity by LCMS analysis was 98%. Two analytical LC/MS injections were used to determine the final purity.

Injection 1 conditions: Column: Waters BEH C18, 2.0 x 50 mm, 1.7- μ m particles; Mobile Phase A: 5:95 acetonitrile:water with 10 mM ammonium acetate; Mobile Phase B: 95:5 acetonitrile:water with 10 mM ammonium acetate; Temperature: 40 °C; Gradient: 0.5 min hold at 0% B, 0-100% B over 4 minutes, then a 0.5-minute hold at 100% B; Flow: 1 mL/min.

Retention time: 2.87. M+H = 604. Injection 2 conditions: Column: Waters BEH C18, 2.0 x 50 mm, 1.7- μ m particles; Mobile Phase A: 5:95 methanol:water with 10 mM ammonium acetate; Mobile Phase B: 95:5 methanol:water with 10 mM ammonium acetate; Temperature: 40 °C; Gradient: 0.5 min hold at 0% B, 0-100% B over 4 minutes, then a 0.5-minute hold at 100% B; Flow: 0.5 mL/min. Retention time: 4.02. M+H = 604.

^1H NMR (500MHz, DMSO- d_6) δ 9.30 (s, 1H), 8.68 (d, J=4.6 Hz, 2H), 8.52 (q, J=4.7 Hz, 1H), 8.06 (dd, J=8.5, 5.5 Hz, 2H), 8.01 (d, J=7.3 Hz, 1H), 7.99 - 7.95 (m, 2H), 7.68 - 7.61 (m, 2H), 7.42 (t, J=8.7 Hz, 2H), 7.28 (t, J=4.9 Hz, 1H), 3.07 - 3.00 (m, 2H), 2.85 - 2.73 (m, 5H), 1.64 - 1.58 (m, 2H), 1.39 - 1.33 (m, 2H)

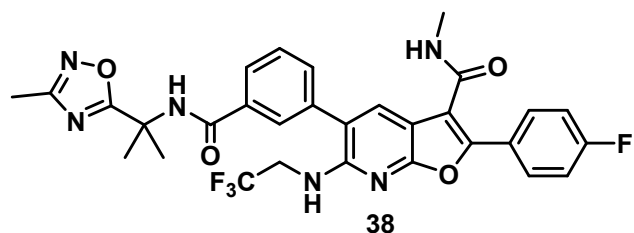
Preparation of 5-(3-(bicyclo[1.1.1]pentan-1-ylcarbamoyl)phenyl)-2-(4-fluorophenyl)-N-methyl-6-(3,3,3-trifluoropropyl)furo[2,3-b]pyridine-3-carboxamide



5-(3-(bicyclo[1.1.1]pentan-1-ylcarbamoyl)phenyl)-6-chloro-2-(4-fluorophenyl)-N-methylfuro[2,3-b]pyridine-3-carboxamide (30 mg, 0.061 mmol), potassium trifluoro borate salt (62.4 mg, 0.306 mmol), dicyclohexyl(2',6'-diisopropoxy-[1,1'-biphenyl]-2-yl)phosphine (23 mg, 0.049 mmol), PdOAc₂ (5.50 mg, 0.024 mmol), cesium carbonate (60 mg, 0.18 mmol) were degassed and backfilled with N₂ then dissolved in toluene (3711 μ l) and water (371 μ l) at rt then heated at 95 °C. The reaction mixture was allowed to stir at this temp for 16 h. The solution was diluted with EtOAc and 1 M HCl. The layers were separated and the aq layer was extracted

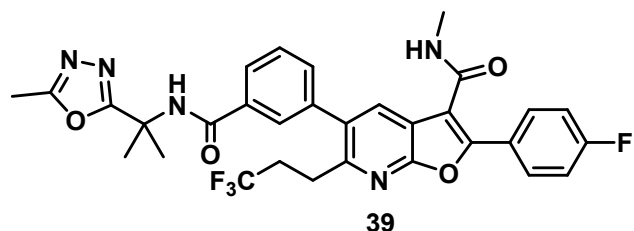
with EtOAc (2 x 10 mL). The combined organic extracts were washed with water, brine, dried over sodium sulfate, filtered and concentrated to give a brown residue. This residue was purified via preparative LC/MS with the following conditions: Column: Waters XBridge C18, 19 x 200 mm, 5- μ m particles; Guard Column: Waters XBridge C18, 19 x 10 mm, 5- μ m particles; Mobile Phase A: water with 20-mM ammonium acetate; Mobile Phase B: 95:5 acetonitrile:water with 20-mM ammonium acetate; Gradient: 55-95% B over 12 minutes, then a 5-minute hold at 100% B; Flow: 20 mL/min. Fractions containing the desired product were combined and dried via centrifugal evaporation to give 5-(3-(bicyclo[1.1.1]pentan-1-ylcarbamoyl)phenyl)-2-(4-fluorophenyl)-N-methyl-6-(3,3,3-trifluoropropyl)furo[2,3-b]pyridine-3-carboxamide 17.9 mg (53% yield), with an estimated purity by LCMS analysis was 97%. Two analytical LC/MS injections were used to determine the final purity. Injection 1 conditions: Column: Waters BEH C18, 2.0 x 50 mm, 1.7- μ m particles; Mobile Phase A: 5:95 acetonitrile:water with 10 mM ammonium acetate; Mobile Phase B: 95:5 acetonitrile:water with 10 mM ammonium acetate; Temperature: 40 °C; Gradient: 0.5 min hold at 0% B, 0-100% B over 4 minutes, then a 0.5-minute hold at 100% B; Flow: 1 mL/min. Injection 2 conditions: Column: Waters BEH C18, 2.0 x 50 mm, 1.7- μ m particles; Mobile Phase A: 5:95 methanol:water with 10 mM ammonium acetate; Mobile Phase B: 95:5 methanol:water with 10 mM ammonium acetate; Temperature: 40 °C; Gradient: 0.5 min hold at 0% B, 0-100% B over 4 minutes, then a 0.5-minute hold at 100% B; Flow: 0.5 mL/min. Retention time: 3.23 M+H = 552. Proton NMR was acquired in deuterated DMSO. ¹H NMR (500 MHz, DMSO-d₆) δ 8.23 (s, 1H), 7.70 - 7.66 (m, 1H), 7.23 (t, J=6.8 Hz, 1H), 7.14 - 7.05 (m, 1H), 6.81 - 6.76 (m, 1H), 6.59 (t, J=8.4 Hz, 1H), 2.50 (s, 11H), 2.18 (dd, J=9.0, 6.9 Hz, 1H), 1.99 (d, J=4.6 Hz, 1H), 1.97 - 1.87 (m, 1H), 1.69 - 1.64 (m, 5H), 1.27 (s, 3H).

Preparation of 2-(4-fluorophenyl)-N-methyl-5-(3-((2-(3-methyl-1,2,4-oxadiazol-5-yl)propan-2-yl)carbamoyl)phenyl)-6-((2,2,2-trifluoroethyl)amino)furo[2,3-b]pyridine-3-carboxamide



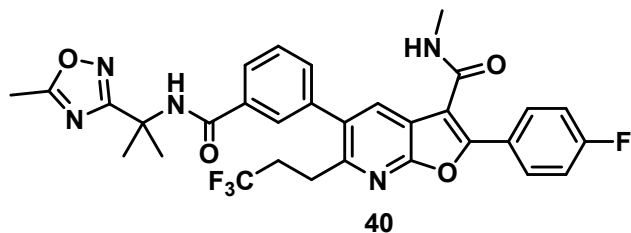
3-(2-(4-fluorophenyl)-3-(methylcarbamoyl)-6-((2,2,2-trifluoroethyl)amino)furo[2,3-b]pyridin-5-yl)benzoic acid (40 mg, 0.082 mmol) was taken up in DMF (1 ml) and treated with N-ethyl-N-isopropylpropan-2-amine (43 μ l, 0.25 mmol), 2-(3-methyl-1,2,4-oxadiazol-5-yl)propan-2-amine hydrochloride (17 mg, 0.098 mmol) followed by 2-(3H-[1,2,3]triazolo[4,5-b]pyridin-3-yl)-1,1,3,3-tetramethylisouronium hexafluorophosphate(V) (47 mg, 0.12 mmol). The reaction was allowed to stir for 1 h then the mixture was diluted with MeOH (1 mL) and the crude material was purified via preparative LC/MS with the following conditions: Column: Waters XBridge C18, 19 x 200 mm, 5- μ m particles; Guard Column: Waters XBridge C18, 19 x 10 mm, 5- μ m particles; Mobile Phase A: water with 20-mM ammonium acetate; Mobile Phase B: 95:5 methanol:water with 20-mM ammonium acetate; Gradient: 50-90% B over 40 minutes, then a 4-minute hold at 100% B; Flow: 20 mL/min. Fractions containing the desired product were combined and dried via centrifugal evaporation. The yield of the product was 17.0 mg, and its estimated purity by LCMS analysis was 96%. Two analytical LC/MS injections were used to determine the final purity. Injection 1 conditions: Column: Waters BEH C18, 2.0 x 50 mm, 1.7- μ m particles; Mobile Phase A: 5:95 acetonitrile:water with 10 mM ammonium acetate; Mobile Phase B: 95:5 acetonitrile:water with 10 mM ammonium acetate; Temperature: 40 $^{\circ}$ C; Gradient: 0.5 min hold at 0% B, 0-100% B over 4 minutes, then a 0.5-minute hold at 100% B; Flow: 1 mL/min. Injection 2 conditions: Column: Waters BEH C18, 2.0 x 50 mm, 1.7- μ m particles; Mobile Phase A: 5:95 methanol:water with 10 mM ammonium acetate; Mobile Phase B: 95:5 methanol:water with 10 mM ammonium acetate; Temperature: 40 $^{\circ}$ C; Gradient: 0.5 min hold at 0% B, 0-100% B over 4 minutes, then a 0.5-minute hold at 100% B; Flow: 0.5 mL/min. 1 H NMR (500MHz, DMSO- d_6) δ 8.99 (s, 1H), 8.41 - 8.33 (m, 1H), 8.01 - 7.88 (m, 4H), 7.68 (s, 1H), 7.65 - 7.57 (m, 2H), 7.34 (t, J=8.7 Hz, 2H), 6.62 (t, J=6.3 Hz, 1H), 4.24 - 4.09 (m, 2H), 2.79 (d, J=4.6 Hz, 3H), 2.31 (s, 3H), 1.70 (s, 6H).

Preparation of methyl 3-(2-(4-fluorophenyl)-3-(methylcarbamoyl)-6-((2,2,2-trifluoroethyl)amino)furo[2,3-b]pyridin-5-yl)benzoate



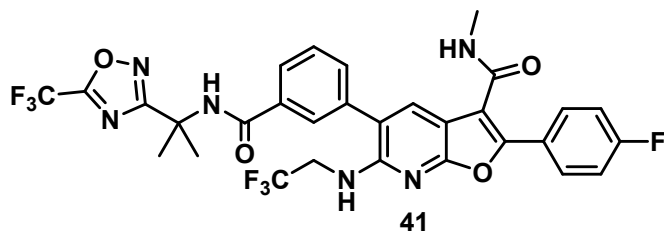
N-ethyl-N-isopropylpropan-2-amine (0.043 mL, 0.25 mmol) was added to stirring solution of 3-(2-(4-fluorophenyl)-3-(methylcarbamoyl)-6-((2,2,2-trifluoroethyl)amino)furo[2,3-b]pyridin-5-yl)benzoic acid (15 mg, 0.031 mmol), 2-(3H-[1,2,3]triazolo[4,5-b]pyridin-3-yl)-1,1,3,3-tetramethylisouronium hexafluorophosphate(V) (18 mg, 0.046 mmol) and 2-(5-methyl-1,3,4-oxadiazol-2-yl)propan-2-amine (4.3 mg, 0.031 mmol) in DMF (1 mL) at rt. The mixture was allowed to stir at rt for 1 h. LCMS shows complete reaction. The solution was filtered and crude material was purified via preparative LC/MS with the following conditions: Column: Waters XBridge C18, 19 x 200 mm, 5- μ m particles; Mobile Phase A: water with 20-mM ammonium acetate; Mobile Phase B: 95:5 acetonitrile:water with 20-mM ammonium acetate; Gradient: 30-100% B over 20 minutes, then a 5-minute hold at 100% B; Flow: 20 mL/min. Fractions containing the desired product were combined and dried via centrifugal evaporation to give methyl 3-(2-(4-fluorophenyl)-3-(methylcarbamoyl)-6-((2,2,2-trifluoroethyl)amino)furo[2,3-b]pyridin-5-yl)benzoate 7.6 mg (40% yield), with an estimated purity by LCMS analysis of 95%. Two analytical LC/MS injections were used to determine the final purity. Injection 1 conditions: Column: Waters BEH C18, 2.0 x 50 mm, 1.7- μ m particles; Mobile Phase A: 5:95 acetonitrile:water with 10 mM ammonium acetate; Mobile Phase B: 95:5 acetonitrile:water with 10 mM ammonium acetate; Temperature: 40 °C; Gradient: 0.5 min hold at 0% B, 0-100% B over 4 minutes, then a 0.5-minute hold at 100% B; Flow: 1 mL/min. Injection 2 conditions: Column: Waters BEH C18, 2.0 x 50 mm, 1.7- μ m particles; Mobile Phase A: 5:95 methanol:water with 10 mM ammonium acetate; Mobile Phase B: 95:5 methanol:water with 10 mM ammonium acetate; Temperature: 40 °C; Gradient: 0.5 min hold at 0% B, 0-100% B over 4 minutes, then a 0.5-minute hold at 100% B; Flow: 0.5 mL/min. Retention time: 2.85 M+H = 611.

Preparation of 2-(4-fluorophenyl)-N-methyl-5-(3-((2-(5-methyl-1,2,4-oxadiazol-3-yl)propan-2-yl)carbamoyl)phenyl)-6-((2,2,2-trifluoroethyl)amino)furo[2,3-b]pyridine-3-carboxamide



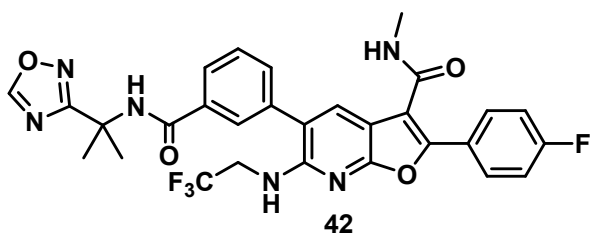
N-ethyl-N-isopropylpropan-2-amine (0.043 mL, 0.25 mmol) was added to stirring solution of 3-(2-(4-fluorophenyl)-3-(methylcarbamoyl)-6-((2,2,2-trifluoroethyl)amino)furo[2,3-b]pyridin-5-yl)benzoic acid (15 mg, 0.031 mmol), 2-(3H-[1,2,3]triazolo[4,5-b]pyridin-3-yl)-1,1,3,3-tetramethylisouronium hexafluorophosphate(V) (18 mg, 0.046 mmol) and oxadiazole (5.5 mg, 0.031 mmol) in DMF (1 mL) at rt. The mixture was allowed to stir at rt for 1 h. LCMS shows complete reaction. The solution was filtered and the crude material was purified via preparative LC/MS with the following conditions: Column: Waters XBridge C18, 19 x 100 mm, 5- μ m particles; Guard Column: Waters XBridge C18, 19 x 10 mm, 5- μ m particles; Mobile Phase A: water; Mobile Phase B: acetonitrile; Buffer: 20-mM ammonium acetate; Gradient: 20-95% B over 10.9 minutes, then a 4.0 minute hold at 95% B; Flow: 25 mL/min. Fractions containing the desired product were combined and dried via centrifugal evaporation to give Preparation of 2-(4-fluorophenyl)-N-methyl-5-(3-((2-(5-methyl-1,2,4-oxadiazol-3-yl)propan-2-yl)carbamoyl)phenyl)-6-((2,2,2-trifluoroethyl)amino)furo[2,3-b]pyridine-3-carboxamide 2.3 mg (12% yield), with an estimated purity by LCMS analysis of 100%. Two analytical LC/MS injections were used to determine the final purity. Injection 1 conditions: Column: Waters BEH C18, 2.0 x 50 mm, 1.7- μ m particles; Mobile Phase A: 5:95 acetonitrile:water with 10 mM ammonium acetate; Mobile Phase B: 95:5 acetonitrile:water with 10 mM ammonium acetate; Temperature: 40 °C; Gradient: 0.5 min hold at 0% B, 0-100% B over 4 minutes, then a 0.5-minute hold at 100% B; Flow: 1 mL/min. Injection 2 conditions: Column: Waters BEH C18, 2.0 x 50 mm, 1.7- μ m particles; Mobile Phase A: 5:95 methanol:water with 10 mM ammonium acetate; Mobile Phase B: 95:5 methanol:water with 10 mM ammonium acetate; Temperature: 40 °C; Gradient: 0.5 min hold at 0% B, 0-100% B over 4 minutes, then a 0.5-minute hold at 100% B; Flow: 0.5 mL/min. Retention time: 2.88 M+H = 611. Proton NMR was acquired in deuterated DMSO. ¹H NMR (500 MHz, DMSO-d₆) δ 8.65 (br s, 1H), 8.38 (br d, J=4.3 Hz, 1H), 8.05 - 7.85 (m, 2H), 7.69 (s, 1H), 7.65 - 7.57 (m, 1H), 7.35 (t, J=7.7 Hz, 1H), 6.64 (br s, 1H), 4.26 - 4.06 (m, 1H), 3.41 (br d, J=2.1 Hz, 75H), 2.80 (br d, J=4.3 Hz, 2H), 2.57 - 2.52 (m, 2H), 1.68 (s, 3H).

Preparation of 2-(4-fluorophenyl)-N-methyl-6-((2,2,2-trifluoroethyl)amino)-5-(3-((2-(5-(trifluoromethyl)-1,2,4-oxadiazol-3-yl)propan-2-yl)carbamoyl)phenyl)furo[2,3-b]pyridine-3-carboxamide



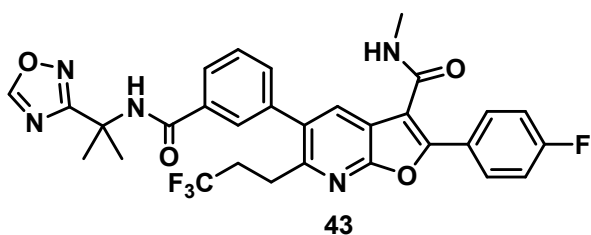
2-(3H-[1,2,3]triazolo[4,5-b]pyridin-3-yl)-1,1,3,3-tetramethylisouronium hexafluorophosphate(V) (18 mg, 0.046 mmol) was added to stirring solution of 3-(2-(4-fluorophenyl)-3-(methylcarbamoyl)-6-((2,2,2-trifluoroethyl)amino)furo[2,3-b]pyridin-5-yl)benzoic acid (15 mg, 0.031 mmol), N-ethyl-N-isopropylpropan-2-amine (0.016 mL, 0.092 mmol) and 2-(5-(trifluoromethyl)-1,2,4-oxadiazol-3-yl)propan-2-amine hydrochloride (11 mg, 0.046 mmol) in DMF (1 mL) at rt. The mixture was allowed to stir at rt for 1 h. LCMS shows complete reaction. The solution was filtered and then the crude material was purified via preparative LC/MS with the following conditions: Column: Waters XBridge C18, 19 x 200 mm, 5- μ m particles; Mobile Phase A: water with 20-mM ammonium acetate; Mobile Phase B: 95:5 acetonitrile:water with 20-mM ammonium acetate; Gradient: 40-80% B over 20 minutes, then a 5-minute hold at 100% B; Flow: 20 mL/min. Fractions containing the desired product were combined and dried via centrifugal evaporation. The yield of the product was 2.9 mg, and its estimated purity by LCMS analysis was 100%. Two analytical LC/MS injections were used to determine the final purity. Injection 1 conditions: Column: Waters BEH C18, 2.0 x 50 mm, 1.7- μ m particles; Mobile Phase A: 5:95 acetonitrile:water with 10 mM ammonium acetate; Mobile Phase B: 95:5 acetonitrile:water with 10 mM ammonium acetate; Temperature: 40 °C; Gradient: 0.5 min hold at 0%B, 0-100% B over 4 minutes, then a 0.5-minute hold at 100% B; Flow: 1 mL/min. Retention time: 3.31 min; m/z (MH⁺): 665. Injection 2 conditions: Column: Waters BEH C18, 2.0 x 50 mm, 1.7- μ m particles; Mobile Phase A: 5:95 methanol:water with 10 mM ammonium acetate; Mobile Phase B: 95:5 methanol:water with 10 mM ammonium acetate; Temperature: 40 °C; Gradient: 0.5 min hold at 0% B, 0-100% B over 4 minutes, then a 0.5-minute hold at 100% B; Flow: 0.5 mL/min. Retention time: 4.18 min; m/z (MH⁺): 665. ¹H NMR (500MHz, DMSO-d₆) δ 8.90 (s, 1H), 8.40 - 8.33 (m, 1H), 8.02 - 7.92 (m, 3H), 7.92 - 7.86 (m, 1H), 7.69 (s, 1H), 7.65 - 7.58 (m, 2H), 7.34 (t, J=8.9 Hz, 2H), 6.69 - 6.60 (m, 1H), 4.24 - 4.13 (m, 2H), 2.84 - 2.77 (m, 3H), 1.74 (s, 6H).

Preparation of 5-(3-((2-(1,2,4-oxadiazol-3-yl)propan-2-yl)carbamoyl)phenyl)-2-(4-fluorophenyl)-N-methyl-6-((2,2,2-trifluoroethyl)amino)furo[2,3-b]pyridine-3-carboxamide



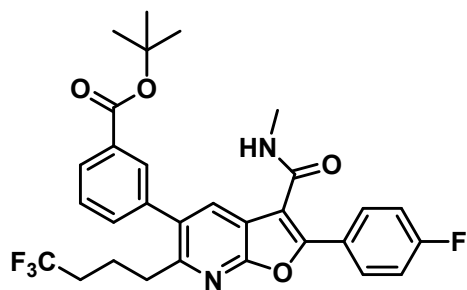
2-(3H-[1,2,3]triazolo[4,5-b]pyridin-3-yl)-1,1,3,3-tetramethylisouronium hexafluorophosphate(V) (117 mg, 0.308 mmol) was added to stirring solution of 3-(2-(4-fluorophenyl)-3-(methylcarbamoyl)-6-((2,2,2-trifluoroethyl)amino)furo[2,3-b]pyridin-5-yl)benzoic acid (100 mg, 0.205 mmol), N-ethyl-N-isopropylpropan-2-amine (0.11 mL, 0.62 mmol) and 2-(1,2,4-oxadiazol-3-yl)propan-2-amine hydrochloride (50 mg, 0.31 mmol) in DMF (1 mL) at rt. The mixture was allowed to stir at rt for 1 h. The reaction was then purified by preparative reverse phase HPLC on a C18 column using a suitably buffered H₂O/CH₃CN gradient, and concentrated to give the expected product 5-(3-((2-(1,2,4-oxadiazol-3-yl)propan-2-yl)carbamoyl)phenyl)-2-(4-fluorophenyl)-N-methyl-6-((2,2,2-trifluoroethyl)amino)furo[2,3-b]pyridine-3-carboxamide (61 mg, 0.100 mmol, 49 % yield). LCMS Method B: LC-MS retention time: 1.73 min; m/z (MH⁺): 597. ¹H NMR (400MHz, DMSO-d₆) δ 9.43 (s, 1H), 8.70 (s, 1H), 8.40 - 8.32 (m, 1H), 8.01 - 7.94 (m, 2H), 7.93 (s, 1H), 7.90 - 7.85 (m, 1H), 7.69 (s, 1H), 7.64 - 7.57 (m, 2H), 7.38 - 7.31 (m, 2H), 6.63 (t, J=6.4 Hz, 1H), 4.26 - 4.12 (m, 2H), 2.80 (d, J=4.8 Hz, 3H), 1.70 (s, 6H).

Preparation of 5-(3-((2-(1,2,4-oxadiazol-3-yl)propan-2-yl)carbamoyl)phenyl)-2-(4-fluorophenyl)-N-methyl-6-(3,3,3-trifluoropropyl)furo[2,3-b]pyridine-3-carboxamide



Step 1:

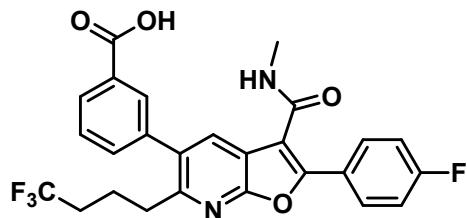
Preparation of: *tert*-butyl 3-(2-(4-fluorophenyl)-3-(methylcarbamoyl)-6-(3,3,3-trifluoropropyl)furo[2,3-*b*]pyridin-5-yl)benzoate



tert-butyl 3-(6-chloro-2-(4-fluorophenyl)-3-(methylcarbamoyl)furo[2,3-*b*]pyridin-5-yl)benzoate (1.50 g, 3.12 mmol), 3,3,3-trifluoropropane-1-trifluoroborate (1.59 g, 7.80 mmol), dicyclohexyl(2',6'-diisopropoxy-[1,1'-biphenyl]-2-yl)phosphine (582 mg, 1.25 mmol), Pd(OAc)₂ (140 mg, 0.624 mmol), cesium carbonate (3.05 g, 9.36 mmol) were combined, degassed, backfilled with N₂ then dissolved in toluene (100 mL) and water (10.0 mL) at rt and then heated at 60 °C. The reaction mixture was allowed to stir 16 h. The reaction mixture was diluted with ethyl acetate and washed with sat NaHCO₃ followed by sat aq NaCl. The organic phase was concentrated and purified on silica gel (Biotage, EtOAc/hexanes gradient, fraction collection at λ = 254 nm) to give the expected product *tert*-butyl 3-(2-(4-fluorophenyl)-3-(methylcarbamoyl)-6-(3,3,3-trifluoropropyl)furo[2,3-*b*]pyridin-5-yl)benzoate (1.6 g, 3.0 mmol, 95% yield). LCMS Method B: LC-MS retention time: 2.20 min; m/z (MH⁺): 543. ¹H NMR (400MHz, DMSO-*d*₆) δ 8.51 - 8.45 (m, 1H), 8.09 - 8.03 (m, 2H), 8.01 - 7.96 (m, 2H), 7.93 (t, J=1.5 Hz, 1H), 7.72 - 7.70 (m, 1H), 7.68 - 7.62 (m, 1H), 7.44 - 7.38 (m, 2H), 2.98 (dd, J=9.2, 6.4 Hz, 2H), 2.85 - 2.71 (m, 5H), 1.60 - 1.54 (m, 9H).

Step 2:

Preparation of : 3-(2-(4-fluorophenyl)-3-(methylcarbamoyl)-6-(3,3,3-trifluoropropyl)furo[2,3-*b*]pyridin-5-yl)benzoic acid



TFA (2.24 ml, 29.0 mmol) was added to a stirring solution of *tert*-butyl 3-(2-(4-fluorophenyl)-3-(methylcarbamoyl)-6-(3,3,3-trifluoropropyl)furo[2,3-*b*]pyridin-5-yl)benzoate (315 mg, 0.581

mmol) in dichloroethane (5.81 ml) at rt. The reaction was allowed to stir for 1 h at rt and then was concentrated to dryness azeotroping with toluene to give the expected product 3-(2-(4-fluorophenyl)-3-(methylcarbamoyl)-6-(3,3,3-trifluoropropyl)furo[2,3-b]pyridin-5-yl)benzoic acid (189 mg, 0.389 mmol, 67% yield) LCMS Method B: LC-MS retention time: 2.89 min; m/z (MH⁺): 487. ¹H NMR (400MHz, DMSO-d₆) δ 13.16 (br. s, 1H), 8.55 - 8.47 (m, 1H), 8.10 - 8.02 (m, 3H), 8.00 - 7.96 (m, 2H), 7.74 (d, J=7.8 Hz, 1H), 7.69 - 7.62 (m, 1H), 7.42 (t, J=8.9 Hz, 2H), 3.05 - 2.96 (m, 2H), 2.85 - 2.70 (m, 5H).

Step 3:

2-(3H-[1,2,3]triazolo[4,5-b]pyridin-3-yl)-1,1,3,3-tetramethylisouronium hexafluorophosphate(V) (879 mg, 2.31 mmol) was added to stirring solution of 3-(2-(4-fluorophenyl)-3-(methylcarbamoyl)-6-(3,3,3-trifluoropropyl)furo[2,3-b]pyridin-5-yl)benzoic acid (750 mg, 1.54 mmol), N-ethyl-N-isopropylpropan-2-amine (0.81 ml, 4.6 mmol) and 2-(1,2,4-oxadiazol-3-yl)propan-2-amine hydrochloride (277 mg, 1.70 mmol) in DMF (10 ml) at rt. The mixture was allowed to stir at rt for 1 h. The reaction was purified on silica gel (Biotage, EtOAc/DCM gradient, fraction collection at λ = 254 nm) to give the expected product 5-(3-((2-(1,2,4-oxadiazol-3-yl)propan-2-yl)carbamoyl)phenyl)-2-(4-fluorophenyl)-N-methyl-6-(3,3,3-trifluoropropyl)furo[2,3-b]pyridine-3-carboxamide (711 mg, 1.17 mmol, 76% yield) LCMS Method B; LC-MS retention time: 1.76 min; m/z (MH⁺): 596. ¹H NMR (500MHz, DMSO-d₆) δ 9.46 - 9.37 (m, 1H), 8.79 - 8.74 (m, 1H), 8.55 - 8.46 (m, 1H), 8.09 - 8.02 (m, 2H), 7.99 - 7.86 (m, 3H), 7.68 - 7.57 (m, 2H), 7.46 - 7.36 (m, 2H), 3.05 - 2.95 (m, 2H), 2.83 - 2.74 (m, 5H), 1.70 (br. s., 6H).

Biology Methods

Cell lines: GT1 and GT2 replicon cells have been described previously^{1,2} and were propagated in DMEM supplemented with 10% FBS, 100 IU/ml penicillin, 100 µg/ml streptomycin, and 0.5 mg/ml G418

Cytotoxicity: To evaluate cytotoxicity, cells were incubated in the presence of serially diluted compounds for 3 days at 37°C and cell viability was quantitated using an Alamar blue assay. All CC₅₀ values were calculated using the median effect equation.

Antiviral activity: To evaluate compound efficacy, HCV replicon cell lines were incubated in 96-well plates in the presence of compound for 3 days. Renilla luciferase activity was then assayed with a Dual-Glo luciferase assay or EnduRen cell substrate (Promega) according to the manufacturer's directions. Plates were read on a TopCount NXT microplate scintillation and luminescence counter (Packard Instrument Company, Meriden, CT). The antiviral activity of test compounds, expressed as the 50% effective concentration (EC_{50}), was determined as previously described.³ To study the effect of human serum (HS) on compound efficacy, the standard 10% FBS in cell culture experiments was supplemented with 40% HS.

Enzyme Assays

Cloning, expression and purification of HCV NS5B protein: The cDNA encoding the open reading frame for HCV NS5B Con 1 C316N, with a C-terminal 18 amino acid truncation, was cloned into a pet21b vector for expression.⁴ The plasmid was used to transform competent BL21(DE3) *E. coli* cells (Novagen) according to the manufacturer's protocol. Untagged NS5B proteins were expressed and isolated to >90% purity using heparin sepharose and polyU sepharose chromatography.⁴ Enzymes were stored at -80C in buffer containing 20 mM Tris-HCL, pH 7.4, 200 mM NaCl, 0.1 mM EDTA, 2 mM DTT, 0.5% Triton X-100, 50% glycerol.

Polymerase activity assays: RNA synthesis was measured by detecting the incorporation of radiolabeled nucleotides. Regardless of the assay format used to measure activity, inhibition by inhibitor was detected as a decrease in the incorporation of radiolabeled nucleotides compared to an untreated control. The half maximal inhibition values (IC_{50}) were determined using seven different inhibitor concentrations [I], and calculated using the formula: $y = y_{min} + [(y_{max} - y_{min}) / (1 + IC_{50}/x)^n]$, where x is the inhibitor concentration and n is the hill coefficient. In all assay formats compounds were serially diluted 1:3 in DMSO and transferred to 96-well assay plates [Corning 3365] for a final DMSO concentration of 2%. In the polyC:pGpG assay format, the newly synthesized RNA product was precipitated by 10% TCA and quantified on the Packard Top Count NXT. The polyA:dT assay format using scintillation proximity assay (SPA) beads, did not require this step. Both assay formats included a 24hr pre-incubation of NS5B with RNA template and compound.

PolyC:pGpG (Di-nucleotide primer assay): NS5B polymerase initiates primer dependent replication in a reaction containing pGpG primer (8.6 μ M), homopolymeric C template (0.35 nM), NS5B enzyme (2.8 nM), 20 mM Tris-HCl (pH 7.5), 5 mM MgCl₂, 2.5 mM KCl, 1 mM DTT, 50 μ g/mL BSA (B6917, Sigma, ST. Louis, MO), 1 μ M GTP, and [33P]-GTP (1 μ Ci., 3000 Ci/mmol, Perkin Elmer NEG-606H). After a \geq 1 h pre-incubation of NS5B polymerase, template and compound, RNA synthesis was initiated by the addition of primer and GTP. Reactions (total volume of 0.06 mL) were incubated at 30°C for 15 min.

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Protein Production

NS5B 1b WT

A pET21b-derived plasmid containing the cDNA encoding the C-terminal 18-AA-deleted NS5B (genotype 1b Con1 strain) was transformed in *Escherichia coli* BL21(DE3) Star cells. The cells were grown overnight at 37 °C in 10 mL LB media containing 100 μ g/mL carbenicillin. The

starter culture was used to inoculate a larger culture (1:100 volume) in TB/autoinduction media, which was grown at 37 °C for 6 h. The temperature was then lowered to 20 °C and growth was continued for another 16 h. Finally, the cells were harvested by centrifugation and washed with PBS, and frozen at -80 °C until purification. To purify the protein, cell paste was thawed and resuspended at 10 mL/g paste, in a lysis buffer comprised of 20 mM Hepes, pH 7.3, 400 mM NaCl, 5 mM DTT, 0.5 mM EDTA, 5 mM MgSO₄, 2 mM ATP, 0.1% β-Octyl glucoside, protease inhibitor tablet (Roche Complete tablet) and Benzonase (10 units/mL, Novagen, 70746). The material was lysed by two passes through a homogenizer operated at a pressure of 800 bar. The lysate was clarified by centrifugation at 10,800 x g. Purification was achieved using cation exchange chromatography (SP-FF Sepharose, GE Healthcare), followed by Orange Dye A affinity chromatography (Sigma, St. Louis, MO) and size exclusion chromatography (Superdex 75, GE Healthcare). A final purity of >95% homogeneity was achieved, as demonstrated by SDS-PAGE. The molecular mass of the protein was confirmed by LC/MS, consistent with the des-Met form. The final protein was stored at -80 °C and pH 7.3 in buffer containing 20 mM Hepes, 250 mM NaCl, 1 mM EDTA, 5 mM DTT, 0.1% β-Octyl Glucoside, and glycerol 10% (v/v).

HCV-NS5B-2A-JFH (1-574)-L30S

A pET21b-derived plasmid was transformed in *Escherichia coli* Rosetta 2 (DE3) cells (Cat No. 71400-1PKG). The cells were grown overnight at 37 °C in 200 mL TB media containing 15 µg/mL Chloramphenicol + 30µg/mL Carbenicillin. This starter culture was used to inoculate 12 L of culture (1:100 volume) in TB/Autoinduction media (Novagen Overnight Express Autoinduction System Media Cat No. 71491-3) containing 15 µg/mL Chloramphenicol + 30

$\mu\text{g/mL}$ Carbenicillin. The culture was grown at 37 °C for approximately four hours when an OD_{600} of 1 was achieved. The temperature was then lowered to 20 °C and growth was continued overnight (approx. 21-24 hours). The cells were harvested by centrifugation and frozen at -80 °C until purification. To purify the protein, 200 g of wet cell paste was thawed and resuspended at 2.5 mL/g paste, in a lysis buffer (pH 7.3) comprised of 25 mM Hepes, 400 mM NaCl, 1 mM MgCl_2 , 10% Glycerol (v/v), 0.1% β -Octyl glucoside, 5 mM DTT, protease inhibitor tablet (Roche Complete tablet), Benzonase (10 units/mL, Sigma, cat# E8263-25KU), and rLysozyme (10 units/mL, Thermo Scientific, cat# 90082). The material was lysed by sonication, and the lysate was clarified by centrifugation at 10 krpm (30 min, 4 °C). Purification was achieved using cation exchange chromatography (SP-FF Sepharose, GE Healthcare), followed by size exclusion chromatography (Superdex 75, GE Healthcare). A final purity of >90% homogeneity was achieved, as demonstrated by SDS-PAGE. The molecular mass of the protein was confirmed by LC/MS, consistent with the des-Met form (observed mass = 63,715.9 Da). The final protein was aliquoted and stored at -80 °C in buffer (pH 7.3) containing 20 mM Hepes, 400 mM NaCl, 5 mM DTT, 0.1% β -Octyl Glucoside, and glycerol 10% (v/v).

Crystallization Methods

NS5B 1b WT

Crystals of NS5B 1b WT were prepared by the hanging drop vapor diffusion method. The protein stock solution consisted of 9.0 mg/mL (0.141 mM based on the calculated MW of 63,878 Da) in 250 mM NaCl, 0.10% Octyl β -D-Glucopyranoside, 10 % Glycerol (v/v), 0.5 mM EDTA, 5.0 mM DTT buffered by 20 mM HEPES at pH 7.3. The protein was complexed with 0.352 mM (2.5 molar excess) of a compound that binds to the NS5B P495 thumb site on ice for 4 h. The mixture was clarified by centrifugation and filtered through a 0.2 μm filter. Crystallization

screens were prepared using a SCREENMAKER 96+8 (Innovadyne, Santa Rosa, CA, USA) on NEURPROBE hanging drop trays. The optimized conditions consisted of a reservoir solution with 1.53 M ammonium sulfate, 150 mM sodium potassium tartrate and 100 mM sodium citrate pH 5.6. Drops were formed from 0.8 μ L of the protein solution and 0.8 μ L of the reservoir solution (total initial volume of 1.6 μ L), mixed, and placed at 20 °C to equilibrate. Crystals appeared within 3 days and grew for an additional 7 days. Co-crystals of NS5B and the P495 thumb site binding compound (PDB compound ID 23E) were soaked within a 2 μ L drop composed of 1 mM compound **5**, 1.75 M (NH₄)₂SO₄, 200 mM sodium potassium tartrate and 0.1 M sodium citrate, pH 5.6. The final volume of DMSO in the mixture was 6.0% (v/v). The soaking was carried out up to 5 days at room temperature. The co-crystals of NS5B/P495 thumb site binding compound (PDB compound ID 23E) were soaked with compound **5** were transferred to a cryo-protectant drop consisting of 25% glycerol (v/v), 1.8 M (NH₄)₂SO₄, 200 mM sodium potassium tartrate, 0.1 M sodium citrate, pH 5.6 and flash-cooled by plunging a looped crystal into liquid nitrogen.

HCV-NS5B-2A-JFH(1-574)-L30S

Crystals of HCV-NS5B-2A-JFH(1-574)-L30S were prepared by the sitting drop vapor diffusion method. The protein stock solution consisted of 10.8 mg/mL (0.169 mM based on the calculated MW of 63,847 Da) in 400 mM NaCl, 10% Glycerol (v/v), 0.1% β -OG, 5 mM DTT, 25 mM HEPES at pH 7.3. The protein was complexed with 0.676 mM (4.0 molar excess) of compound **5** on ice for 4 h. The mixture was clarified by centrifugation. Crystallization screens were prepared using a Mosquito LCP (TTP Labtech, Melbourn, UK) on a 96-well, 2-drop MRC tray. The optimized conditions consisted of a reservoir solution of 25 %w/v PEG4K, 0.2 M (NH₄)₂SO₄, 0.1 M sodium acetate, pH 4.6. Drops were formed from 0.4 μ L of the protein solution and 0.4 μ L of

the reservoir solution (total initial volume of 0.8 μL), mixed, and placed at 20 °C to equilibrate. Crystals appeared within 14 days. The crystals of HCV-NS5B-2A-JFH(1-574)-L30S with compound **5** were transferred to a cryo-protectant drop prepared by mixing 40% PEG 400 (v/v) and 40% glycerol (v/v) diluted with water. 2.5 μL of the 40:40 mixture was combined with 7.5 μL of crystallization growth condition. The cryo-solution was added serially to crystallization drop. Once crystals had been equilibrated in the cryo-solution crystals were flash frozen into liquid nitrogen.

Structure Determination

Data for HCV NS5B 1b WT in complex with compound **5** was collected at the Canadian Light Source beamline 08ID-1 with a Rayonix MX-300 detector (Grochulski et al. 2011). The structure was determined using rigid body refinement with AMoRe starting with PDB 3Q0Z. The data were processed with HKL2000 (Otwinowski Minor 1997). Data for the HCV NS5B 2a L30S in complex with compound **5** was collected at the Advanced Photon Source on beamline 17-ID using a Dectris Pilatus 6M detector. The data were processed using autoPROC (GlobalPhasing, Ltd.), which used XDS for integration (Kabsch, 2010a,b) and SCALA (Evans, 2005) for scaling. Both structures were refined with BUSTER/TNT (GlobalPhasing, Ltd.) and used restraint dictionaries generated with GRADE (GlobalPhasing, Ltd.). The models were manipulated in real space with COOT (Emsley et al., 2010).

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Crystallographic Statistics for complexes of HCV NS5B 2a L30S and HCV NS5B 1b with compound 5.

Protein	HCV NS5B 2a L30S	HCV NS5B 1b
Resolution range (Å)	42.47-1.97 (2.02-1.97)	49.12-3.04 (3.15-3.04)
Completeness (%)	99.1 (90.3)	99.9 (99.7)
σ cutoff	0	0
No. of reflections, working set	42430 (2828)	26804 (2768)
No. of reflections, test set	2257 (149)	1063 (88)
Final Rcryst	0.169 (0.186)	0.188 (0.214)
Final Rfree	0.207 (0.235)	0.222 (0.266)
No. of non-H atoms		
Protein	4322	8374
Ion (SO4)	20	60
PE5 PG4	53	0
Ligand	36	130
Water	383	0
Total	4814	8564
R.m.s. deviations		
Bonds (Å)	0.010	0.010
Angles (°)	1.0	1.1
Average B factors (Å ²)		
Protein	28.5	65.3
Ion (SO4)	45.7	116.4
PE5 PG4	61.6	-
Ligand	19.8	59.8
Water	38.3	-
Ramachandran plot*		
Most favoured	93.3	90.8

Allowed	6.5	8.8
Disallowed	0.0	0.1

Numbers in parentheses represent the highest resolution shell

*as defined in Laskowski, R.A., MacArthur, M.W., Moss, D.S., and Thornton, J.M. (1993).

PROCHECK: a program to check the stereochemical quality of protein structures. *J. Appl. Crystallogr.* **26**, 283–291.