Supporting Information

Structure based drug design of novel potent and selective tetrahydropyrazolo[1,5-a]pyrazines as ATR inhibitors

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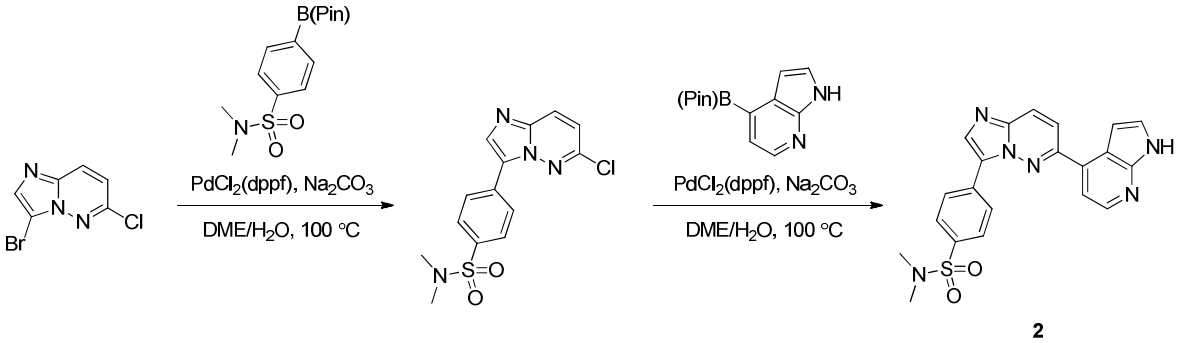
TABLE OF CONTENTS

I. Supporting Information: Chemistry

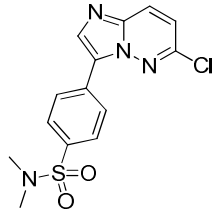
A. General Methods

All reagents and solvents were of commercial quality and used without further purification. Column chromatography was performed using Merck silica gel 60 (230-400 mesh) on either Teledyne Isco CombiFlash Rf or Varian IntelliFlash 310 instruments. The compounds and/or intermediates were characterized by high performance liquid chromatography (HPLC) using a Waters Millenium chromatography system with a 2695 Separation Module (Milford, MA). The analytical columns were reversed phase Phenomenex Luna C18 -5 μ, 4.6 x 50 mm, from Alltech (Deerfield, IL). A gradient elution was used (flow 2.5 mL/min), typically starting with 5% acetonitrile/95% water and progressing to 100% acetonitrile over a period of 10 minutes. All solvents contained 0.1% trifluoroacetic acid (TFA). Mass spectrometric analysis was performed according to two different liquid chromatography / mass spectroscopy (LCMS) methods. Method A employed a Waters System (Alliance HT HPLC and a Micromass ZQ mass spectrometer for the LCMS instrument, an Eclipse XDB-C18, 2.1 x 50 mm for the chromatography column, and a solvent system that was a 5-95% gradient of acetonitrile in water with 0.05% TFA over a 4 min period (flow rate 0.8 mL/min molecular weight range 200-1500; cone Voltage 20 V; column temperature 40°C). Method B employed a Hewlett Packard System (Series 1100 HPLC and a Micromass ZQ mass spectrometer for the LCMS instrument, an Eclipse XDB-C18, 2.1 x 50 mm for the chromatography column, and a solvent system that was a 5-95% gradient of acetonitrile in water with 0.05% TFA over a 4 min period (flow rate 0.8 mL/min molecular weight range 150-850; cone Voltage 50 V; column temperature 30 ºC). All masses were reported as those of the protonated parent ions. ^{1}H and ^{13}C NMR spectra of all compounds were recorded at 400 and 101 MHz, respectively. ^{1}H shifts are referenced to the residual protonated solvent signal (e.g. δ 2.50 for d6-DMSO), and ^{13}C shifts are referenced to the deuterated solvent signal (δ 39.5 for d6-DMSO).

B. Synthesis of compound 2

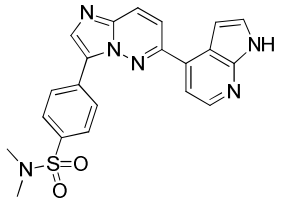


4-(6-chloroimidazo[1,2-b]pyridazin-3-yl)-N,N-dimethylbenzenesulfonamide:



A mixture of 3-bromo-6-chloroimidazo[1,2-b]pyridazine (116 mg, 0.5 mmol), N,N-dimethyl-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)benzenesulfonamide (156 mg, 0.5 mmol), $PdCl_2(dppf)\cdot CH_2Cl_2$ (20.4 mg, 0.025 mmol) and 2 M aqueous sodium carbonate (1 mL, 2.00 mmol) in DME (2 mL) was sealed in a microwave vial and irradiated at 100°C for 10 min and then cooled. The layers were separated, and the aqueous layer was extracted two times with EtOAc. The combined organic layers were dried over $MgSO_4$, filtered and concentrated. The residue was purified by preparatory HPLC to provide the product (70 mg, 0.208 mmol, 42 % yield) as the corresponding TFA salt. LCMS (m/z): 336.9 (M+H+).

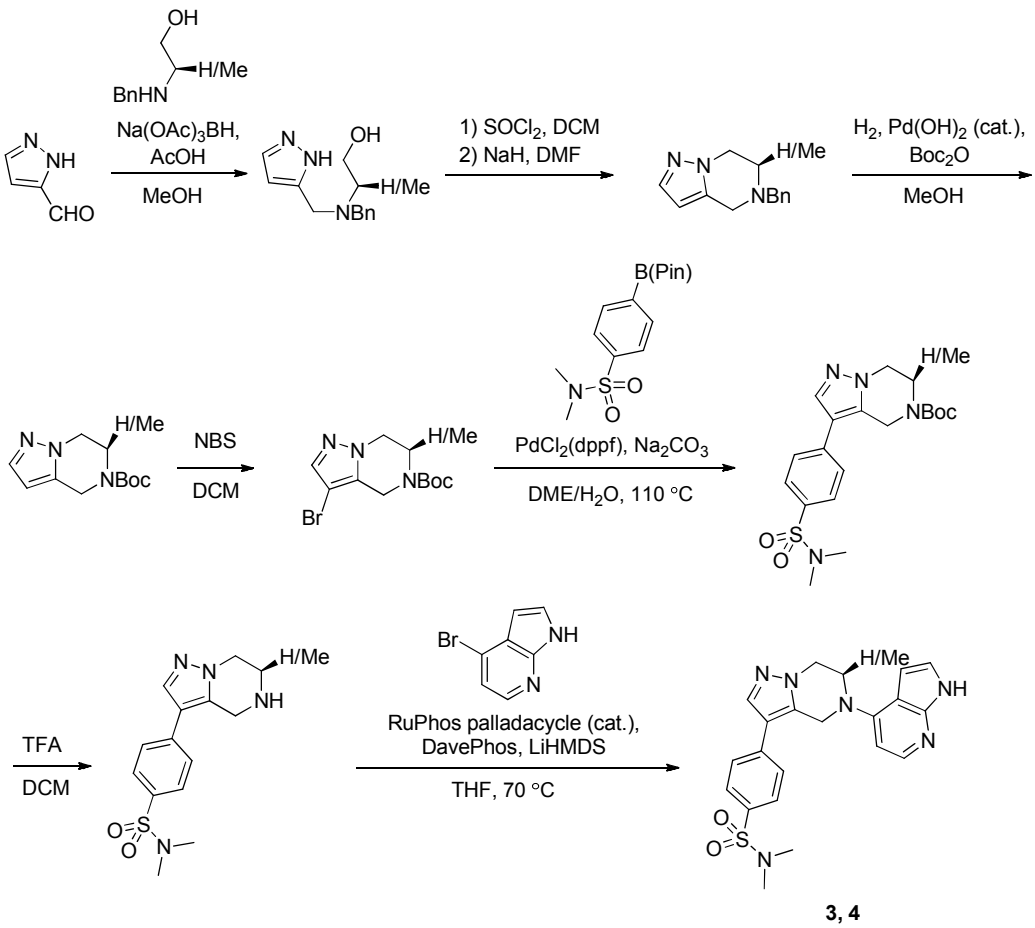
4-(6-(1H-pyrrolo[2,3-b]pyridin-4-yl)imidazo[1,2-b]pyridazin-3-yl)-N,N-dimethylbenzenesulfonamide (2):



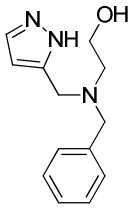
2

A mixture of 4-(6-chloroimidazo[1,2-b]pyridazin-3-yl)-N,N-dimethylbenzenesulfonamide (60 mg, 0.178 mmol), 4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1H-pyrrolo[2,3-b]pyridine (65.2 mg, 0.267 mmol), $PdCl_2(dppf)\cdot CH_2Cl_2$ (14.6 mg, 0.018 mmol) and 2 M aqueous sodium carbonate (0.6 mL, 1.20 mmol) in DME (2 mL) was sealed in a microwave vial and irradiated at 110°C for 10 min and then cooled. The layers were separated, and the aqueous layer was extracted two times with EtOAc. The combined organic layers were dried over $MgSO_4$, filtered and concentrated. The residue was purified by preparatory HPLC to provide the product (8 mg, 0.019 mmol, 11 % yield) as the corresponding TFA salt. LCMS (m/z): 419.0 (M+H+).

C. General method for the synthesis of compounds 3 and 4

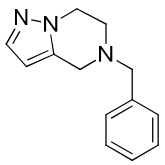


2-(((1H-pyrazol-5-yl)methyl)(benzyl)amino)ethanol :



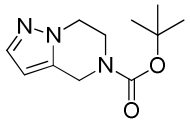
A suspension of 1H-pyrazole-5-carbaldehyde (800 mg, 8.33 mmol) and 2-(benzylamino)ethanol (1.182 ml, 8.33 mmol) in MeOH (40 mL) was stirred at RT for 1 hours. To the mixture was then added sodium sodium triacetoxyborohydride (5.29 g, 24.98 mmol) followed by AcOH (0.953 ml, 16.65 mmol). The reaction mixture was stirred for additional 4 hours at RT. The reaction mixture was quenched with 2 mL of water and then concentrated. The residue was partitioned between saturated aqueous $NaHCO_3$ and EtOAc. The organic layer was dried over $MgSO_4$, filtered and concentrated. The crude product (1.90 g, 8.21 mmol, 99 % yield) was used to next step directly without further purification. LCMS (m/z): 232.0 (M+H+).

5-benzyl-4,5,6,7-tetrahydropyrazolo[1,5-a]pyrazine:



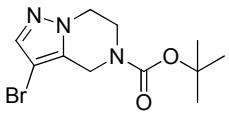
To a solution of 2-(((1H-pyrazol-5-yl)methyl)(benzyl)amino)ethanol (1.93 g, 8.33 mmol) in DCM (40 mL) at 0°C was added thionyl chloride (4.26 mL, 58.3 mmol), and the mixture was warmed to RT and stirred overnight. The solvents were removed under reduced pressure. The residue was re-dissolved in DMF (25 mL), NaH (60% in mineral oil, 2.0 g, 50.0 mmol) was added, and the reaction mixture was stirred for 1 h at RT. The reaction mixture was diluted with EtAOc and washed with brine. The organic layer was dried over $MgSO_4$, filtered and concentrated. The residue was purified on a silica gel column (heptane:EtOAc 1:1) to provide the product (0.876 g, 4.11 mmol, 50 % yield). LCMS (m/z): 214.0 (M+H+).

Tert-butyl 6,7-dihydropyrazolo[1,5-a]pyrazine-5(4H)-carboxylate:



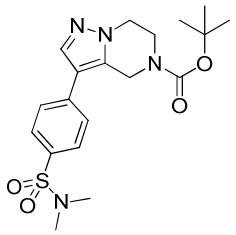
To a solution of 5-benzyl-4,5,6,7-tetrahydropyrazolo[1,5-a]pyrazine (876 mg, 4.11 mmol) in MeOH (20 mL) at RT was added Pearlman's catalyst (346 mg, 0.493 mmol and Boc_2O (2.77 ml, 11.91 mmol). The reaction mixture was stirred under an atmosphere of H_2 for 3 hours. More Pearlman's catalyst (346 mg, 0.493 mmol) was added and the reaction was vigorously stirred under the H_2 atmosphere for another 3 hours. The reaction mixture was filtered through Celite, and the filtrate was concentrated. The residue was purified on a silica gel column (heptane:EtOAc 1:1) to provide the product (916 mg, 4.10 mmol, 100 % yield). LCMS (m/z): 224.1 (M+H+).

Tert-butyl 3-bromo-6,7-dihydropyrazolo[1,5-a]pyrazine-5(4H)-carboxylate:



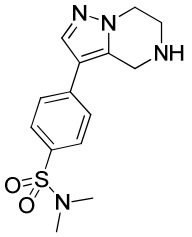
To a stirred solution of tert-butyl 6,7-dihydropyrazolo[1,5-a]pyrazine-5(4H)-carboxylate (480 mg, 2.150 mmol) in DCM (15 mL) at RT was added NBS (383 mg, 2.150 mmol) and the reaction was stirred for 2 h. The mixture was diluted with EtOAc and washed with brine. The organic layer was dried over $MgSO_4$, filtered and concentrated. The crude product was used without further purification. LCMS (m/z): 301.9/303.9 (M+H+.

Tert-butyl 3-(4-(N,N-dimethylsulfamoyl)phenyl)-6,7-dihydropyrazolo[1,5-a]pyrazine-5(4H)-carboxylate:



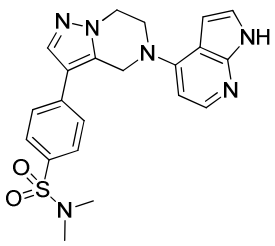
A mixture of tert-butyl 3-bromo-6,7-dihydropyrazolo[1,5-a]pyrazine-5(4H)-carboxylate (432 mg, 1.43 mmol), N,N-dimethyl-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)benzenesulfonamide (668 mg, 2.15 mmol), $PdCl_2(dppf)\cdot CH_2Cl_2$ (117 mg, 0.143 mmol) and 2 M aqueous sodium carbonate (3 mL, 6.00 mmol) in DME (6 mL) was sealed in a microwave vial and irradiated at 110°C for 10 min and then cooled. The layers were separated, and the aqueous layer was extracted two times with EtOAc. The combined organic layers were dried over $MgSO_4$, filtered and concentrated. The residue was purified on a silica gel column (heptane:EtOAc 1:1) to provide the product (406 mg, 0.999 mmol, 70% yield). LCMS (m/z): 407.1 (M+H+).

N,N-dimethyl-4-(4,5,6,7-tetrahydropyrazolo[1,5-a]pyrazin-3-yl)benzenesulfonamide:



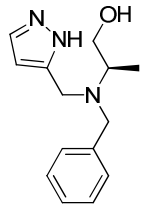
To a stirred solution of tert-butyl 3-(4-(N,N-dimethylsulfamoyl)phenyl)-6,7-dihydropyrazolo[1,5-a]pyrazine-5(4H)-carboxylate (406 mg, 1.00 mmol) in DCM (5 mL) at RT was added TFA (1.16 mL, 15.00 mmol) and the mixture was stirred for 1 hour. The mixture was concentrated, the residue was mixed with saturated aqueous $NaHCO_3$, stirred for 5 min, and then extracted thee times with EtOAc. The combined organics were washed with brine, dried over $MgSO_4$, filtered and concentrated. The crude residue was used without further purification. LCMS (m/z): 306.9 (M+H+).

4-(5-(1H-pyrrolo[2,3-b]pyridin-4-yl)-4,5,6,7-tetrahydropyrazolo[1,5-a]pyrazin-3-yl)-N,N-dimethylbenzenesulfonamide (3):



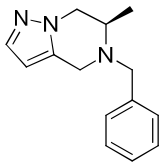
A microwave vial was charged with N,N-dimethyl-4-(4,5,6,7-tetrahydropyrazolo[1,5-a]pyrazin-3-yl)benzenesulfonamide (302 mg, 0.986 mmol), 4-bromo-1H-pyrrolo[2,3-b]pyridine (185 mg, 0.939 mmol), RuPhos palladacycle (41 mg, 0.056 mmol), and DavePhos (22.2 mg, 0.056 mmol). The vial was sealed and purged with Ar. THF (3 mL) was then added followed by LiHMDS (1 M in THF, 7.51 mL, 7.51 mmol). The resulting mixture was heated at 70°C for 1 hour and then cooled to RT. The mixture was quenched with 1 mL of 1 M HCl, stirred for 10 min, and then poured onto saturated aqueous $NaHCO_3$ and extracted three times with EtOAc. The combined organics were dried over $MgSO_4$, filtered, and concentrated. The residue was purified by prep HPLC to provide the TFA salt (140 mg, 0.331 mmol, 35 % yield). HPLC purity >99%. LCMS (m/z): 423.1 (M+H+). ^{1}H NMR (of the corresponding free base) (400 MHz, <cd3cn>) δ ppm 2.68 (s, 6 H) 4.08 (t, *J*=5.48 Hz, 2 H) 4.39 (t, *J*=5.28 Hz, 2 H) 4.87 (s, 2 H) 5.45 (s, 1 H) 6.57 (d, *J*=3.52 Hz, 1 H) 6.62 (d, *J*=5.48 Hz, 1 H) 7.25 (d, *J*=3.52 Hz, 1 H) 7.70 (d, *J*=8.61 Hz, 2 H) 7.76 - 7.82 (m, 2 H) 7.87 (s, 1 H) 8.07 (d, *J*=5.48 Hz, 1 H). ^{13}C NMR (126 MHz, DMSO) δ 149.90, 149.05, 143.79, 137.69, 137.28, 133.89, 131.51, 128.30, 126.43, 123.06, 115.63, 109.78, 101.92, 99.11, 47.30, 46.47, 46.09, 37.60. HRMS calculated for $C_{21}H_{23}N_6O_2S$ 423.1603 Da, measured 423.1605 Da

(R)-2-(((1H-pyrazol-5-yl)methyl)(benzyl)amino)propan-1-ol:



A suspension of 1H-pyrazole-5-carbaldehyde (961 mg, 10.0 mmol) and (R)-2-(benzylamino)propan-1-ol (1.65 g, 10.0 mmol) in MeOH (45 mL) was stirred at RT for 1 hours. To the mixture was then added sodium sodium triacetoxyborohydride (6.36 g, 30.0 mmol) followed by AcOH (1.15 mL, 20.0 mmol). The reaction mixture was stirred for additional 4 hours at RT. The reaction mixture was quenched with 2 mL of water and then concentrated. The residue was partitioned between saturated aqueous $NaHCO_3$ and EtOAc. The organic layer was dried over $MgSO_4$, filtered and concentrated. The crude product was used to next step directly without further purification. LCMS (m/z): 246.2 (M+H+).

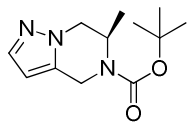
(R)-N-((1H-pyrazol-5-yl)methyl)-N-benzyl-1-chloropropan-2-amine:



To a solution of (R)-2-(((1H-pyrazol-5-yl)methyl)(benzyl)amino)propan-1-ol (2.45 g, 10.0 mmol) in DCM (40 mL) at 25 °C was added thionyl chloride (2.92 mL, 40.0 mmol), and the mixture was

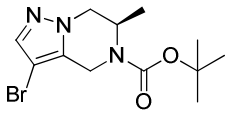
warmed to RT and stirred overnight. The solvents were removed under reduced pressure. The residue was re-dissolved in DMF (7 mL), NaH (60% in mineral oil, 0.6 g, 15.0 mmol) was slowly added, and the reaction mixture was stirred for 1 h at RT. The reaction mixture was diluted with EtAOc and washed with brine. The organic layer was dried over $MgSO_4$, filtered and concentrated. The residue was purified on a silica gel column (heptane:EtOAc 1:0 to 3:2) to provide the product (0.360 g, 1.58 mmol, 49% yield over three steps). LCMS (m/z): 228.2 (M+H+).

(R)-tert-butyl 6-methyl-6,7-dihydropyrazolo[1,5-a]pyrazine-5(4H)-carboxylate:



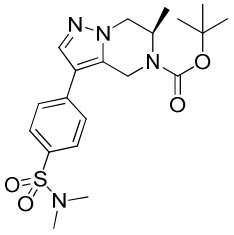
To a solution of (R)-N-((1H-pyrazol-5-yl)methyl)-N-benzyl-1-chloropropan-2-amine (360 mg, 4.11 mmol) in MeOH (12 mL) at RT was added Pearlman's catalyst (222 mg, 1.58 mmol) and Boc_2O (1.04 g, 4.75 mmol). The reaction mixture was stirred under an atmosphere of H_2 for 3 hours. More Pearlman's catalyst (346 mg, 0.493 mmol) was added and the reaction was vigorously stirred under the H_2 atmosphere for another 3 hours. The reaction mixture was filtered through Celite, and the filtrate was concentrated. The residue was purified on a silica gel column (heptane:EtOAc 10:1 to 1:1) to provide the product (330 mg, 1.39 mmol, 88 % yield). LCMS (m/z): 238.3 (M+H+).

(R)-tert-butyl 3-bromo-6-methyl-6,7-dihydropyrazolo[1,5-a]pyrazine-5(4H)-carboxylate:



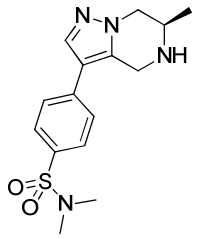
To a stirred solution of (R)-tert-butyl 6-methyl-6,7-dihydropyrazolo[1,5-a]pyrazine-5(4H)-carboxylate (330 mg, 1.39 mmol) in DCM (8 mL) at RT was added NBS (248 mg, 1.391 mmol) and the reaction was stirred for 2 h. The mixture was diluted with EtOAc and washed with brine. The organic layer was dried over $MgSO_4$, filtered and concentrated. The crude product was used without further purification. LCMS (m/z): 316.1/318.1 (M+H+).

(R)-tert-butyl 3-(4-(N,N-dimethylsulfamoyl)phenyl)-6-methyl-6,7-dihydropyrazolo[1,5-a]pyrazine-5(4H)-carboxylate:



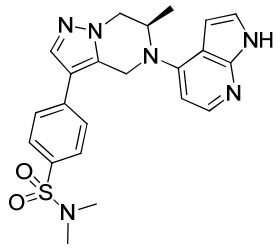
A mixture of (R)-tert-butyl 3-bromo-6-methyl-6,7-dihydropyrazolo[1,5-a]pyrazine-5(4H)-carboxylate (440 mg, 1.39 mmol), N,N-dimethyl-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)benzenesulfonamide (649 mg, 2.09 mmol), $PdCl_2(dppf)\cdot CH_2Cl_2$ (114 mg, 0.139 mmol) and 2 M aqueous sodium carbonate (2.5 mL, 5.00 mmol) in DME (5 mL) was sealed in a microwave vial and irradiated at 110°C for 10 min and then cooled. The layers were separated, and the aqueous layer was extracted two times with EtOAc. The combined organic layers were dried over $MgSO_4$, filtered and concentrated. The residue was purified on a silica gel column (heptane:EtOAc 1:1) to provide the product (421 mg, 1.00 mmol, 72% yield). LCMS (m/z): 421.3 (M+H+).

(R)-N,N-dimethyl-4-(6-methyl-4,5,6,7-tetrahydropyrazolo[1,5-a]pyrazin-3-yl)benzenesulfonamide:



To a stirred solution of (R)-tert-butyl 3-(4-(N,N-dimethylsulfamoyl)phenyl)-6-methyl-6,7-dihydropyrazolo[1,5-a]pyrazine-5(4H)-carboxylate (421 mg, 1.00 mmol) in DCM (5 mL) at RT was added TFA (1.54 mL, 20.02 mmol) and the mixture was stirred for 1 hour. The mixture was concentrated, the residue was mixed with saturated aqueous $NaHCO_3$, stirred for 5 min, and then extracted thee times with EtOAc. The combined organics were washed with brine, dried over $MgSO_4$, filtered and concentrated. The crude residue was used without further purification. LCMS (m/z): 321.3 (M+H+).

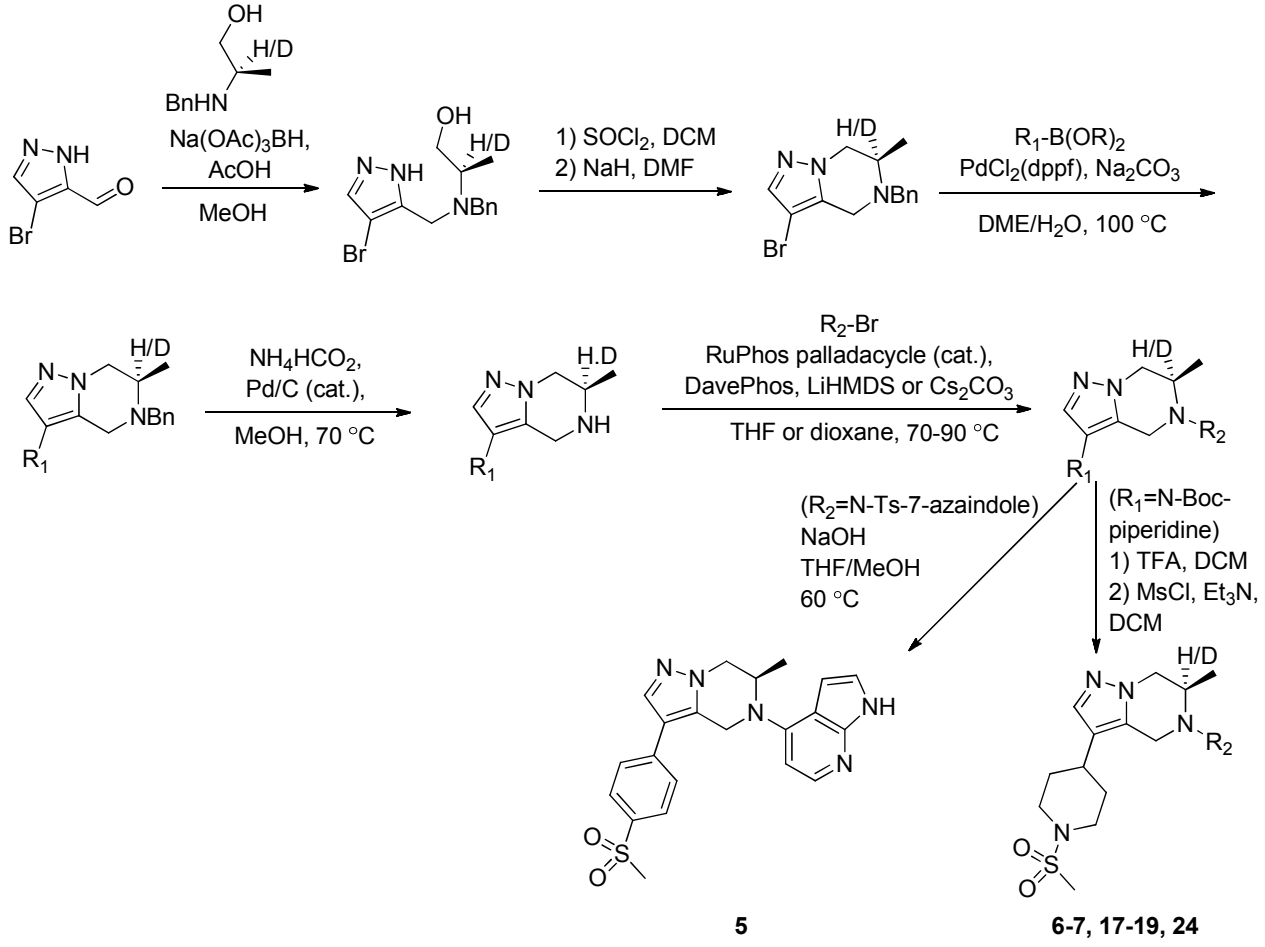
(R)-N,N-dimethyl-4-(6-methyl-5-(1H-pyrrolo[2,3-b]pyridin-4-yl)-4,5,6,7-tetrahydropyrazolo[1,5-a]pyrazin-3-yl)benzenesulfonamide (4):



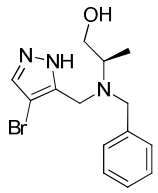
A microwave vial was charged with (R)-N,N-dimethyl-4-(6-methyl-4,5,6,7-tetrahydropyrazolo[1,5-a]pyrazin-3-yl)benzenesulfonamide (180 mg, 0.562 mmol), 4-bromo-1H-pyrrolo[2,3-b]pyridine (111 mg, 0.562 mmol), RuPhos palladacycle (41 mg, 0.056 mmol), and DavePhos (22.2 mg, 0.056 mmol). The vial was sealed and purged with Ar. THF (3 mL) was then added followed by LiHMDS (1 M in THF, 3.93 mL, 3.93 mmol). The resulting mixture was heated at 70°C for 40 min and then cooled to RT. The mixture was quenched with 1 mL of 1 M HCl, stirred for 10 min, and then poured onto

saturated aqueous $NaHCO_3$ and extracted three times with EtOAc. The combined organics were dried over $MgSO_4$, filtered, and concentrated. The residue was purified by prep HPLC to provide the TFA salt (86 mg, 0.197 mmol, 35 % yield). HPLC purity >99%. LCMS (m/z): 437.2 (M+H+). ^{1}H NMR (400 MHz, <cd3cn>) δ ppm 1.26 (d, *J*=6.65 Hz, 3 H) 2.67 - 2.72 (m, 6 H) 4.37 (d, *J*=13.30 Hz, 1 H) 4.55 (dd, *J*=13.30, 3.91 Hz, 1 H) 5.13 (d, *J*=15.65 Hz, 1 H) 5.17 - 5.27 (m, 1 H) 5.35 (d, *J*=16.04 Hz, 1 H) 6.81 (d, *J*=7.04 Hz, 1 H) 6.90 (dd, *J*=3.33, 1.76 Hz, 1 H) 7.34 - 7.41 (m, 1 H) 7.69 - 7.77 (m, 2 H) 7.83 (d, *J*=8.22 Hz, 2 H) 7.91 (s, 1 H) 7.98 (d, *J*=7.43 Hz, 1 H). ^{13}C NMR (126 MHz, DMSO) δ 152.95, 139.99, 138.45, 137.43, 135.06, 132.27, 131.73, 128.72, 127.27, 124.30, 116.80, 109.03, 103.73, 100.97, 52.27, 50.58, 43.62, 38.08, 16.51. HRMS calculated for $C_{22}H_{25}N_6O_2S$ 453.1709 Da, measured 453.1712 Da.

D. General method for the synthesis of compounds 5-7, 17-19, and 24

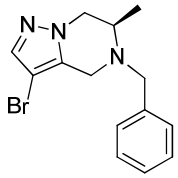


(R)-2-(benzyl((4-bromo-1H-pyrazol-5-yl)methyl)amino)propan-1-ol:



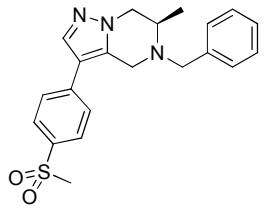
A suspension of 4-bromo-1H-pyrazole-5-carbaldehyde (2.4 g, 13.72 mmol) and (R)-2-(benzylamino)propan-1-ol (2.61 g, 15.77 mmol) in MeOH (60 mL) was stirred at room temperature for 2 h. To this mixture was added sodium triacetoxyborohydride (8.72 g, 41.1 mmol) followed by acetic acid (1.570 mL, 27.4 mmol). The reaction mixture was stirred for additional 3 h at RT. The reaction mixture was quenched with 4 mL of water and concentrated. The residue was partitioned between saturated aqueous $NaHCO_3$ and EtOAc. The organic layer was washed with brine, dried over $MgSO_4$, filtered and concentrated. The residue was purified on a silica gel column (heptane:EtOAc 0:1 to 1:1) to give the product (1.823 g, 5.62 mmol, 41 % yield). LCMS (m/z): 324.1/326.0 (M+H+).

(R)-5-benzyl-3-bromo-6-methyl-4,5,6,7-tetrahydropyrazolo[1,5-a]pyrazine :



To a solution of (R)-2-(benzyl((4-bromo-1H-pyrazol-5-yl)methyl)amino)propan-1-ol (1.8 g, 5.55 mmol) in DCM (10 mL) was added $SOCl_2$ (1.621 mL, 22.21 mmol) dropwise at RT. The resulting solution was stirred at room temperature for 6 h. The reaction mixture was concentrated and dried under high vacuum. The crude residue was taken up in DMF (20 mL) at RT and NaH (60% in mineral oil, 0.133 mg, 5.55 mmol) was slowly added. The reaction was stirred for 1 h and then diluted with EtOAc, washed with brine and water and then dried over $MgSO_4$, filtered and concentrated. The resulting crude residue was used without further purification. LCMS (m/z): 306.1/308.0 (M+H+).

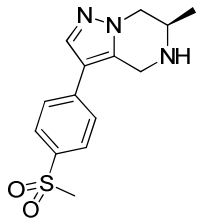
(R)-5-benzyl-6-methyl-3-(4-(methylsulfonyl)phenyl)-4,5,6,7-tetrahydropyrazolo[1,5-a]pyrazine:



A mixture of (R)-5-benzyl-3-bromo-6-methyl-4,5,6,7-tetrahydropyrazolo[1,5-a]pyrazine (1072 mg, 3.50 mmol), 4-(methylsulfonyl)phenylboronic acid (980 mg, 4.90 mmol), $PdCl_2(dppf)\cdot CH_2Cl_2$ (200

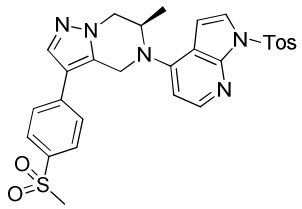
mg, 0.245 mmol) and 2 M aqueous Na_2CO_3 (3.5 mL, 7.00 mmol) in DME (8 ml) was was sealed in a microwave vial and irradiated at 110 °C for 10 min. The residue was diluted with EtOAc and washed with brine. The organic was dried $MgSO_4$, filtered and concentrated. The residue was purified on a silica gel column (heptane:EtOAc 1:1) to give the product (670 mg, 1.76 mmol, 50 % yield). LCMS (m/z): 382.1 (M+H+).

(R)-6-methyl-3-(4-(methylsulfonyl)phenyl)-4,5,6,7-tetrahydropyrazolo[1,5-a]pyrazine:



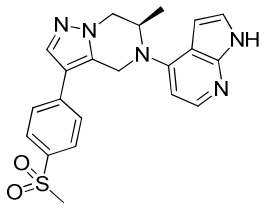
A mixture of (R)-5-benzyl-6-methyl-3-(4-(methylsulfonyl)phenyl)-4,5,6,7-tetrahydropyrazolo[1,5-a]pyrazine (500 mg, 1.311 mmol), ammonium formate (331 mg, 5.24 mmol) and 10% Pd/C (279 mg, 2.62 mmol) in MeOH (10 mL) was stirred at 72 °C for 40 min in a sealed microwave vial and then cooled. The reation mixture was filtered and the filtrate was concentrated, diluted with EtOAc, washed with saturated aqueous $NaHCO_3$ and brine, and then dried over $MgSO_4$, filtered, and concentrated. The crude product (240 mg, 0.824 mmol, 63% yield) was used without further purification. LCMS (m/z): 292.0 (M+H+).

(R)-6-methyl-3-(4-(methylsulfonyl)phenyl)-5-(1-tosyl-1H-pyrrolo[2,3-b]pyridin-4-yl)-4,5,6,7-tetrahydropyrazolo[1,5-a]pyrazine:



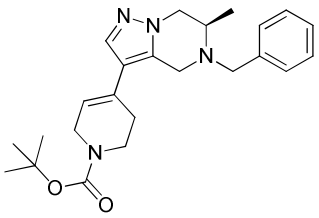
A microwave vial was charged with (R)-6-methyl-3-(4-(methylsulfonyl)phenyl)-4,5,6,7-tetrahydropyrazolo[1,5-a]pyrazine (200 mg, 0.686 mmol), 4-bromo-1-tosyl-1H-pyrrolo[2,3-b]pyridine (265 mg, 0.755 mmol), RuPhos palladacycle (50 mg, 69 µmol), DavePhos (27 mg, 69 µmol) and Cs_2CO_3 (688 mg, 4.12 mmol). The vial was sealed and purged with Ar. 1,4-dioxane (6 mL) was then added, and the resulting mixture was heated at 85 °C for 2 hours and then cooled to RT. The mixture was diluted with ethyl acetate, filtered, and the filtrate was concentrated. The residue was purified by prep HPLC to provide the product as the TFA salt (130 mg, 0.231 mmol, 34 % yield). LCMS (m/z): 562.2 (M+H+).

(R)-6-methyl-3-(4-(methylsulfonyl)phenyl)-5-(1H-pyrrolo[2,3-b]pyridin-4-yl)-4,5,6,7-tetrahydropyrazolo[1,5-a]pyrazine (5):



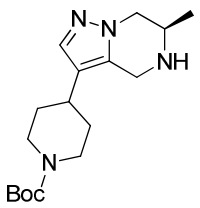
A microwave vial was charged with (R)-6-methyl-3-(4-(methylsulfonyl)phenyl)-5-(1-tosyl-1H-pyrrolo[2,3-b]pyridin-4-yl)-4,5,6,7-tetrahydropyrazolo[1,5-a]pyrazine (130 mg, 0.231 mmol), 10 M aqueous NaOH (0.3 mL , 3.0 mmol), MeOH (3 mL) and THF (1 mL) . The mixture was stirred at 60 °C overnight and then cooled to RT. The mixture was poured into a mixture of EtOAc and brine. The organic layer was separated, dried over $MgSO_4$, filtered and concentrated. The residue was purified by prep HPLC to provide the product as the corresponding TFA salt (62 mg, 0.152 mmol, 66 % yield). HPLC purity >99%. LCMS (m/z): 408.1 (M+H+). ^{1}H NMR (of the corresponding free base) (400 MHz, <dmso>) δ ppm 1.15 (d, *J*=6.65 Hz, 3 H) 3.23 (s, 3 H) 4.24 (d, *J*=12.91 Hz, 1 H) 4.56 (dd, *J*=12.91, 4.30 Hz, 1 H) 4.88 (d, *J*=3.52 Hz, 2 H) 4.94 - 5.03 (m, 1 H) 6.62 (br. s., 1 H) 6.67 (d, *J*=5.48 Hz, 1 H) 7.32 (d, *J*=2.35 Hz, 1 H) 7.85 (d, *J*=8.22 Hz, 2 H) 7.91 - 7.97 (m, 2 H) 8.04 (d, *J*=5.48 Hz, 1 H) 8.07 (s, 1 H) 11.45 - 11.66 (m, 1 H). ^{13}C NMR (126 MHz, DMSO) δ 149.65, 148.67, 143.49, 137.93, 137.89, 137.62, 132.84, 127.66, 126.56, 122.91, 115.58, 109.76, 102.37, 99.55, 52.25, 49.48, 43.67, 41.66, 13.87.HRMS calculated for $C_{21}H_{22}N_5O_2S$ 408.1494 Da, measured 408.1489 Da.

(R)-tert-butyl 4-(5-benzyl-6-methyl-4,5,6,7-tetrahydropyrazolo[1,5-a]pyrazin-3-yl)-5,6-dihydropyridine-1(2H)-carboxylate:



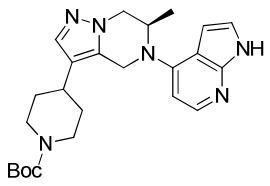
(R)-5-benzyl-3-bromo-6-methyl-4,5,6,7-tetrahydropyrazolo[1,5-a]pyrazine (476 mg, 1.555 mmol), tert-butyl 4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-5,6-dihydropyridine-1(2H)-carboxylate (673 mg, 2.176 mmol), $PdCl_2(dppf)\cdot CH_2Cl_2$ (127 mg, 0.155 mmol) and 2 M aq. sodium carbonate (2.332 mL, 4.66 mmol) were mixed in DME (7 mL) in a microwave tube. The mixture was purged with argon for 15 min. The tube was sealed and heated to 90 ºC for 4 h. The reaction mixture was poured onto water and extracted three times with EtOAc. The combined organics were washed with brine, dried over $MgSO_4$, filtered and concentrated. The residue was purified on a silica gel column (heptane:EtOAc 1:0 to 1:3) to give the product (466 mg, 1.141 mmol, 73.4 % yield) as a tan foam. LCMS (m/z): 409.3 (M+H+).

(R)-tert-butyl 4-(6-methyl-4,5,6,7-tetrahydropyrazolo[1,5-a]pyrazin-3-yl)piperidine-1-carboxylate:



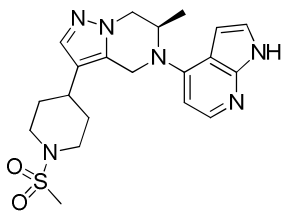
(R)-tert-butyl 4-(5-benzyl-6-methyl-4,5,6,7-tetrahydropyrazolo[1,5-a]pyrazin-3-yl)-5,6-dihydropyridine-1(2H)-carboxylate (112 mg, 0.274 mmol), ammonium formate (121 mg, 1.919 mmol), and 10% Pd/C (58.3 mg, 0.055 mmol) was taken up in MeOH (3 mL), sealed in a microwave vial, and heated to 70 °C for 1.5 h. The mixture was filtered through a pad of Celite, washing with EtOAc, and then partitioned between EtOAc and satuated aqueous $NaHCO_3$. The aqeous layer was extracted with more EtOAc, and the combined organics were washed with brine, dried over $MgSO_4$, filtered and concentrated. The crude product (80 mg, 0.250 mmol, 91 % yield) was isolated as a colorless oil which was used without further purification. LCMS (m/z): 321.2 (M+H+).

(R)-tert-butyl 4-(6-methyl-5-(1H-pyrrolo[2,3-b]pyridin-4-yl)-4,5,6,7-tetrahydropyrazolo[1,5-a]pyrazin-3-yl)piperidine-1-carboxylate:



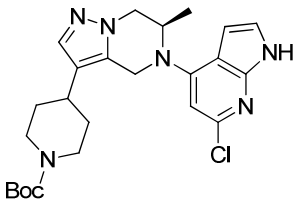
A microwave vial was charged with (R)-tert-butyl 4-(6-methyl-4,5,6,7-tetrahydropyrazolo[1,5-a]pyrazin-3-yl)piperidine-1-carboxylate (205 mg, 0.640 mmol), 4-bromo-1H-pyrrolo[2,3-b]pyridine (132 mg, 0.672 mmol), RuPhos palladacycle (46.6 mg, 0.064 mmol), and DavePhos (25.2 mg, 0.064 mmol). The vial was sealed, vacuum flushed three times with Ar, and then taken up in THF (1 mL). LiHMDS (1.0 M in THF, 3.84 mL, 3.84 mmol) was added, and then the mixture was heated to 70 °C for 1 h. The reaction mixture was cooled to room temperature and partitioned between EtOAc and saturated aqueous $NaHCO_3$. The aqueous layer was extracted with more EtOAc, and the combined organics were washed with brine, dried over $MgSO_4$, filtered and concentrated. The residue was purified on a silica gel column (heptane:EtOAc 9:1 to 3:7) to give the product (187 mg, 0.429 mmol, 67 % yield) as a yellow solid. LCMS (m/z): 437.2 (M+H+).

(R)-6-methyl-3-(1-(methylsulfonyl)piperidin-4-yl)-5-(1H-pyrrolo[2,3-b]pyridin-4-yl)-4,5,6,7-tetrahydropyrazolo[1,5-a]pyrazine (6):



To a stirred solution of (R)-tert-butyl 4-(6-methyl-5-(1H-pyrrolo[2,3-b]pyridin-4-yl)-4,5,6,7-tetrahydropyrazolo[1,5-a]pyrazin-3-yl)piperidine-1-carboxylate (187 mg, 0.428 mmol) in DCM (4 mL) at 25 °C was added TFA (0.66 mL, 8.57 mmol) and the mixture was stirred for 1 h. The reaction mixture was cooled to room temperature and partitioned between EtOAc and saturated aqueous $NaHCO_3$. The aqueous layer was extracted with more EtOAc, and the combined organics were dried over $MgSO_4$, filtered and concentrated. Some of the so-obtained crude residue (20 mg, 0.059 mmol) was taken up in DCM (1 mL) at 25 °C, and Et_3N (0.029 mL, 0.208 mmol) and Mesyl-Cl (9.5 mg, 0.083 mmol) were added. The mixture was allowed to stir for 30 min, and then quenched with a few drops of water and concentrated. The residue was purified by prep HPLC to provide the product as the corresponding TFA salt (3.5 mg, 0.059 mmol, 14.2 % yield). HPLC purity >99%. LCMS (m/z): 415.1 (M+H+). ^{1}H NMR (of the corresponding free base) (400 MHz, DMSO-d_6) δ ppm 1.05 (d, *J*=6.65 Hz, 3 H) 1.54 - 1.70 (m, 2 H) 1.90 (d, *J*=11.35 Hz, 2 H) 2.60 - 2.71 (m, 1 H) 2.79 (t, *J*=12.13 Hz, 2 H) 2.88 (s, 3 H) 3.62 (d, *J*=11.74 Hz, 2 H) 4.09 (d, *J*=12.91 Hz, 1 H) 4.40 (dd, *J*=12.72, 4.50 Hz, 1 H) 4.45 - 4.53 (m, 1 H) 4.58 - 4.67 (m, 1 H) 4.84 - 4.94 (m, 1 H) 6.54 (d, *J*=5.48 Hz, 1 H) 6.56 - 6.59 (m, 1 H) 7.25 - 7.31 (m, 1 H) 7.39 (s, 1 H) 7.99 (d, *J*=5.48 Hz, 1 H) 11.49 (br. s., 1 H). ^{13}C NMR (126 MHz, DMSO) δ 149.91, 148.79, 143.71, 136.33, 130.94, 122.69, 109.60, 102.11, 99.62, 51.88, 49.80, 45.97, 45.93, 40.45, 33.93, 32.09, 31.74, 30.39, 13.94. HRMS calculated for $C_{20}H_{27}N_6O_2S$ 415.1916 Da, measured 415.1916 Da.

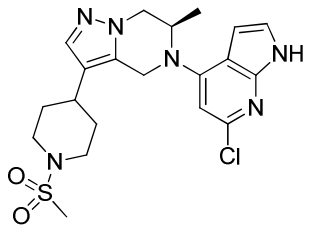
(R)-tert-butyl 4-(5-(6-chloro-1H-pyrrolo[2,3-b]pyridin-4-yl)-6-methyl-4,5,6,7-tetrahydropyrazolo[1,5-a]pyrazin-3-yl)piperidine-1-carboxylate:



A microwave vial was charged with (R)-tert-butyl 4-(6-methyl-4,5,6,7-tetrahydropyrazolo[1,5-a]pyrazin-3-yl)piperidine-1-carboxylate (81 mg, 0.253 mmol), 4-bromo-6-chloro-1H-pyrrolo[2,3-b]pyridine (76 mg, 0.323 mmol), RuPhos palladacycle (27.6 mg, 0.038 mmol), and DavePhos (15 mg, 0.038 mmol). The vial was sealed, vacuum flushed three times with Ar, and then taken up in THF (1 mL). LiHMDS (1.0 M in THF, 1.77 mL, 1.77 mmol) was added, and then the mixture was heated to 60 °C for 2 h. The reaction mixture was cooled to room temperature and partitioned between EtOAc and saturated aqueous $NaHCO_3$. The aqueous layer was extracted with more EtOAc, and the combined organics were washed with brine, dried over $MgSO_4$, filtered and

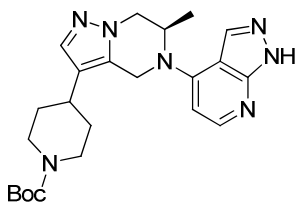
concentrated. The residue was purified on a silica gel column (heptane:EtOAc 1:0 to 3:7) to give the product (91 mg, 0.193 mmol, 76 % yield) as a yellow oil. LCMS (m/z): 471.3 (M+H+).

(R)-5-(6-chloro-1H-pyrrolo[2,3-b]pyridin-4-yl)-6-methyl-3-(1-(methylsulfonyl)piperidin-4-yl)-4,5,6,7-tetrahydropyrazolo[1,5-a]pyrazine (7):



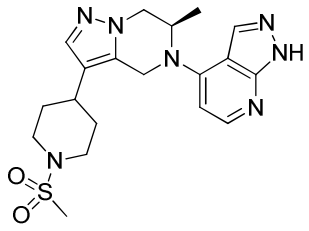
To a stirred solution of (R)-tert-butyl 4-(5-(6-chloro-1H-pyrrolo[2,3-b]pyridin-4-yl)-6-methyl-4,5,6,7-tetrahydropyrazolo[1,5-a]pyrazin-3-yl)piperidine-1-carboxylate (91 mg, 0.193 mmol) in DCM (2.5 mL) at 25 °C was added TFA (0.447 mL, 5.80 mmol) and the mixture was stirred for 1 h. The mixture was concentrated to dryness and then taken up in DCM:MeOH (2:1, 1.5 mL). Si-carbonate resin (0.8 mmol/g, 1200 mg, 0.96 mmol) was added and the mixture was stirred for 30 min and then filtered and concentrated. The so-obtained crude residue was taken up in DCM (2 mL) at 25 °C, and Et_3N (0.081 mL, 0.579 mmol) and Mesyl-Cl (14 µL, 0.174 mmol) were added. The mixture was allowed to stir for 30 min, and then quenched with a few drops of water and concentrated. The residue was purified by prep HPLC. Product fractions were converted to the corresponding free base by treatment with solid Na_2CO_3 followed by extraction into EtOAc. The organic extracts were washed with brine, dried over $MgSO_4$, filtered and concentrated, and then taken up in CH_3CN/H_2O and lyophilized to give the product (43 mg, 0.096 mmol, 50 % yield) as a white solid. HPLC purity >99%. LCMS (m/z): 449.2 (M+H+). 1H NMR (400 MHz, DMSO-*d*6) δ ppm 1.05 - 1.14 (m, 3 H) 1.59 - 1.73 (m, 2 H) 1.90 - 1.99 (m, 2 H) 2.68 - 2.77 (m, 1 H) 2.80 - 2.90 (m, 2 H) 2.92(s, 3 H) 3.65 (br. s., 2 H) 4.13 - 4.20 (m, 1 H) 4.42 - 4.50 (m, 1 H) 4.58 (s, 1 H) 4.70 (s, 1 H) 4.97 - 5.06 (m, 1 H) 6.58 - 6.61 (m, 1 H) 6.65 - 6.70(m, 1 H) 7.31 - 7.37 (m, 1 H) 7.45 (s, 1 H) 11.71 - 11.75 (m, 1 H). ^{13}C NMR (126 MHz, DMSO) δ 150.96, 148.76, 145.28, 136.78, 130.97, 123.26, 120.29, 108.30, 100.88, 100.84, 52.34, 50.34, 46.45, 46.41, 41.04, 34.36, 32.57, 32.26, 30.64, 14.99. HRMS calculated for $C_{20}H_{26}N_6O_2SCl$ 449.1526 Da, measured 449.1527 Da.

(R)-tert-butyl 4-(6-methyl-5-(1H-pyrazolo[3,4-b]pyridin-4-yl)-4,5,6,7-tetrahydropyrazolo[1,5-a]pyrazin-3-yl)piperidine-1-carboxylate:



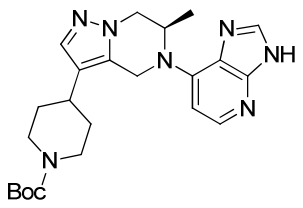
A microwave vial was charged with (R)-tert-butyl 4-(6-methyl-4,5,6,7-tetrahydropyrazolo[1,5-a]pyrazin-3-yl)piperidine-1-carboxylate (67 mg, 0.209 mmol), 4-bromo-1H-pyrazolo[3,4-b]pyridine (45.5 mg, 0.230 mmol), RuPhos palladacycle (22.9 mg, 0.031 mmol), and DavePhos (12.3 mg, 0.031 mmol). The vial was sealed, vacuum flushed three times with Ar, and then taken up in THF (1 mL). LiHMDS (1.0 M in THF, 1.464 mL, 1.464 mmol) was added, and then the mixture was heated to 70 °C for 1 h. The reaction mixture was cooled to room temperature and partitioned between EtOAc and saturated aqueous $NaHCO_3$. The aqueous layer was extracted with more EtOAc, and the combined organics were washed with brine, dried over $MgSO_4$, filtered and concentrated. The residue was purified on a silica gel column (DCM:MeOH 1:0 to 4:1) to give the product (55 mg, 0.126 mmol, 60 % yield) as a yellow oil. LCMS (m/z): 438.3 (M+H+).

(R)-4-(6-methyl-3-(1-(methylsulfonyl)piperidin-4-yl)-6,7-dihydropyrazolo[1,5-a]pyrazin-5(4H)-yl)-1H-pyrazolo[3,4-b]pyridine (17):



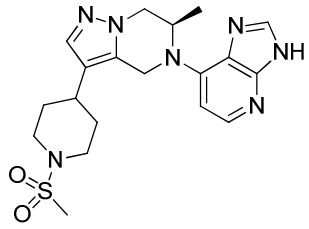
To a stirred solution of (R)-tert-butyl 4-(6-methyl-5-(1H-pyrazolo[3,4-b]pyridin-4-yl)-4,5,6,7-tetrahydropyrazolo[1,5-a]pyrazin-3-yl)piperidine-1-carboxylate (74 mg, 0.103 mmol) in DCM (2 mL) at 25 °C was added TFA (0.391 mL, 5.07 mmol) and the mixture was stirred for 1 h. The mixture was concentrated to dryness and then taken up in MeOH (1.5 mL). Si-carbonate resin (0.8 mmol/g, 455 mg, 0.364 mmol) was added and the mixture was stirred for 30 min and then filtered and concentrated. The so-obtained crude residue was taken up in DCM (1.5 mL) at 25 °C, and Et_3N (0.056 mL, 0.400 mmol) and Mesyl-Cl (12 µL, 0.160 mmol) were added. The mixture was allowed to stir for 30 min, and then quenched with a few drops of water and concentrated. The residue was purified by prep HPLC to provide the product as the corresponding TFA salt (15.6 mg, 0.038 mmol, 28.2 % yield) as a white solid. HPLC purity >99%. LCMS (m/z): 416.2 (M+H+). ^{1}H NMR (of the corresponding free base) (400 MHz, CHLOROFORM-*d*) δ ppm 1.22 - 1.32 (m, 4 H) 1.78 - 1.93 (m, 2 H) 1.98 (br. s., 2 H) 2.55 - 2.69 (m, 1 H) 2.75 - 2.88 (m, 5 H) 3.95 (d, *J*=12.13 Hz, 2 H) 4.35 (s, 1 H) 4.49 (d, *J*=3.91 Hz, 1 H) 4.61 - 4.71 (m, 1 H) 4.89 (d, *J*=15.26 Hz, 2 H) 6.39 (d, *J*=5.87 Hz, 1 H) 7.44 (s, 1 H) 8.13 (s, 1 H) 8.31 (d, *J*=5.87 Hz, 1 H). HRMS calculated for $C_{19}H_{26}N_7O_2S$ 416.1869 Da, measured 416.1874 Da.

(R)-tert-butyl 4-(5-(3H-imidazo[4,5-b]pyridin-7-yl)-6-methyl-4,5,6,7-tetrahydropyrazolo[1,5-a]pyrazin-3-yl)piperidine-1-carboxylate:



A microwave vial was charged with (R)-tert-butyl 4-(6-methyl-4,5,6,7-tetrahydropyrazolo[1,5-a]pyrazin-3-yl)piperidine-1-carboxylate (85 mg, 0.265 mmol), 7-bromo-3H-imidazo[4,5-b]pyridine (73.5 mg, 0.371 mmol), RuPhos palladacycle (29.0 mg, 0.040 mmol), and DavePhos (15.66 mg, 0.040 mmol). The vial was sealed, vacuum flushed three times with Ar, and then taken up in THF (1 mL). LiHMDS (1.0 M in THF, 1.857 mL, 1.857 mmol) was added, and then the mixture was heated to 60 °C for 2 h. The reaction mixture was cooled to room temperature and partitioned between EtOAc and saturated aqueous $NaHCO_3$. The aqueous layer was extracted with more EtOAc, and the combined organics were washed with brine, dried over $MgSO_4$, filtered and concentrated. The residue was purified on a silica gel column (DCM:MeOH 1:0 to 9:1) to give the product (50 mg, 0.114 mmol, 43.1 % yield) as a yellow foam. LCMS (m/z): 438.3 (M+H+).

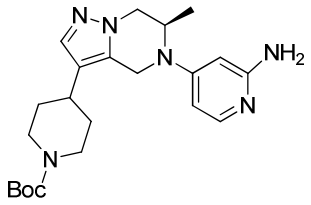
(R)-7-(6-methyl-3-(1-(methylsulfonyl)piperidin-4-yl)-6,7-dihydropyrazolo[1,5-a]pyrazin-5(4H)-yl)-3H-imidazo[4,5-b]pyridine (18):



To a stirred solution of (R)-tert-butyl 4-(5-(3H-imidazo[4,5-b]pyridin-7-yl)-6-methyl-4,5,6,7-tetrahydropyrazolo[1,5-a]pyrazin-3-yl)piperidine-1-carboxylate (45 mg, 0.103 mmol) in DCM (1.5 mL) at 25 °C was added TFA (0.238 mL, 3.09 mmol) and the mixture was stirred for 2 h. The mixture was concentrated to dryness and then taken up in DCM:MeOH (4:1, 1.5 mL). Si-carbonate resin (0.8 mmol/g, 643 mg, 0.515 mmol) was added and the mixture was stirred for 30 min and then filtered and concentrated. The so-obtained crude residue was taken up in DCM (1.5 mL) at 25 °C, and Et_3N (0.043 mL, 0.309 mmol) and Mesyl-Cl (7.22 µL, 0.093 mmol) were added. The mixture was allowed to stir for 30 min, and then quenched with a few drops of water and concentrated. The residue was purified by prep HPLC to provide the product as the corresponding TFA salt (18 mg, 0.043 mmol, 42.1 % yield) as a white solid. HPLC purity >99%. LCMS (m/z): 416.2 (M+H+). ^{1}H NMR (of the corresponding free base) (400 MHz, CHLOROFORM-*d*) δ ppm 1.27 (d, *J*=7.04 Hz, 5 H) 1.79 - 1.94 (m, 2 H) 1.95 - 2.09 (m, 2 H) 2.57 - 2.70 (m, 1 H) 2.77 - 2.90 (m, 5 H) 3.94 (d, *J*=11.74 Hz, 2 H) 4.25 (d, *J*=12.52 Hz, 1 H) 4.45 - 4.59 (m, 2 H) 5.24 (d, *J*=15.65 Hz, 1 H) 6.22 (br. s., 1 H) 6.50 (d, *J*=5.87 Hz, 1 H) 7.41 (s, 1 H) 8.02 (s, 1 H) 8.19 (d, *J*=5.87 Hz, 1 H). ^{13}C NMR (126 MHz, DMSO) δ 148.85, 145.57, 144.97, 138.60, 136.28, 130.54, 123.32, 119.60, 101.87, 51.61, 48.11, 45.98, 45.94, 45.71, 39.93, 33.96,

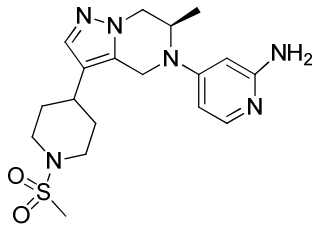
33.90, 31.98, 31.73, 31.60, 30.43, 14.64. HRMS calculated for $C_{19}H_{26}N_7O_2S$ 416.1869 Da, measured 416.1864 Da.

(R)-tert-butyl 4-(5-(2-aminopyridin-4-yl)-6-methyl-4,5,6,7-tetrahydropyrazolo[1,5-a]pyrazin-3-yl)piperidine-1-carboxylate:



A microwave vial was charged with (R)-tert-butyl 4-(6-methyl-4,5,6,7-tetrahydropyrazolo[1,5-a]pyrazin-3-yl)piperidine-1-carboxylate (100 mg, 0.312 mmol), 4-bromopyridin-2-amine (59.4 mg, 0.343 mmol), RuPhos palladacycle (27.3 mg, 0.037 mmol), and DavePhos (14.7 mg, 0.037 mmol). The vial was sealed, vacuum flushed three times with Ar, and then taken up in THF (1 mL). LiHMDS (1.0 M in THF, 2.19 mL, 2.19 mmol) was added, and then the mixture was heated to 70 °C for 1 h. The reaction mixture was cooled to room temperature and partitioned between EtOAc and saturated aqueous $NaHCO_3$. The aqueous layer was extracted with more EtOAc, and the combined organics were washed with brine, dried over $MgSO_4$, filtered and concentrated. The residue was purified on a silica gel column (heptane:EtOAc 9:1 to 3:7) to give the product (59 mg, 0.143 mmol, 46 % yield) as a yellow solid. LCMS (m/z): 413.2 (M+H+).

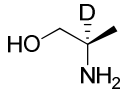
(R)-4-(6-methyl-3-(1-(methylsulfonyl)piperidin-4-yl)-6,7-dihydropyrazolo[1,5-a]pyrazin-5(4H)-yl)pyridin-2-amine (19):



To a stirred solution of (R)-tert-butyl 4-(5-(2-aminopyridin-4-yl)-6-methyl-4,5,6,7-tetrahydropyrazolo[1,5-a]pyrazin-3-yl)piperidine-1-carboxylate (59 mg, 0.143 mmol) in DCM (1 mL) at 25 °C was added TFA (0.220 mL, 2.86 mmol) and the mixture was stirred for 1 h. The reaction mixture was cooled to room temperature and partitioned between EtOAc and saturated aqueous $NaHCO_3$. The aqueous layer was extracted with more EtOAc, and the combined organics were dried over $MgSO_4$, filtered and concentrated. The so-obtained crude residue was taken up in DCM (1 mL) and THF (1 mL) at 25 °C, and Et_3N (0.050 mL, 0.359 mmol) and Mesyl-Cl (16.4 mg, 0.102 mmol) were added. The mixture was allowed to stir for 1 h, and then quenched with a few drops of water and concentrated. The residue was purified by prep HPLC to provide the product as the corresponding TFA salt (15 mg, 0.038 mmol, 38 % yield) as a yellow solid. LCMS (m/z):

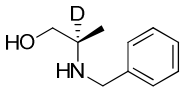
391.1 (M+H+). ^{1}H NMR (500 MHz, DMSO-d_6) δ 0.99 (d, J = 6.6 Hz, 3H), 1.63 (dqd, J = 24.9, 12.5, 4.0 Hz, 2H), 1.89 (d, J = 13.0 Hz, 2H), 2.62 (ddt, J = 11.9, 7.2, 3.7 Hz, 1H), 2.76 (td, J = 12.0, 2.6 Hz, 2H), 2.89 (s, 3H), 3.64 (d, J = 12.0 Hz, 2H), 4.21 (dd, J = 13.1, 1.6 Hz, 1H), 4.30 (dd, J = 13.1, 4.0 Hz, 1H), 4.51 (d, J = 15.9 Hz, 1H), 4.69 (s, 1H), 4.76 (d, J = 15.9 Hz, 1H), 6.12 (d, J = 2.5 Hz, 1H), 6.70 (dd, J = 7.6, 2.6 Hz, 1H), 7.43 (s, 1H), 7.73 (d, J = 6.3 Hz, 1H), 12.53 (s, 1H). ^{13}C NMR (126 MHz, DMSO) δ 155.98, 153.91, 136.50, 135.93, 129.29, 120.25, 100.74, 88.75, 51.22, 48.32, 45.95, 45.93, 40.37, 33.93, 32.00, 31.73, 30.40, 15.37. HRMS calculated for $C_{18}H_{27}N_6O_2S$ 391.1916 Da, measured 391.1916 Da.

D-alaninol-2-d:



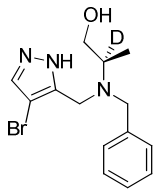
To a stirred 0 °C suspension of LAH (0.682 g, 17.97 mmol) in THF (20 mL) was slowly added D-alanine-2-d (1.031 g, 11.44 mmo), and the mixture was warmed to room temperature and then heated at reflux overnight. The mixture was cooled to room temperature and then quenched by the sequential, dropwise addition of water (0.682 mL), 15% NaOH (4.4 M, 0.682 mL), and water (2.04 mL). The resulting mixture was filtered, washing with THF; the filtrates were dried (Na_2SO_4) and concentrated. The crude residue (829 mg, 10.89 mmol, 95 % yield) was used without further purification.

(R)-2-(benzylamino)propan-1-ol-2-d:



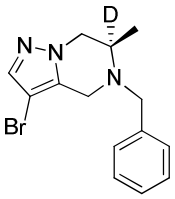
D-alaninol-2-d (829 mg, 10.89 mmol) and benzaldehyde (1.159 ml, 11.44 mmol) were taken up in toluene (25 mL) in a flask outfitted with a Dean-Stark trap and reflux condenser. The mixture was heated at reflux for 4 h and then cooled to 25 °C and concentrated. The resulting residue was taken up in EtOH (25 ml), cooled to 0 °C, and $NaBH_4$ (1030 mg, 27.2 mmol) was added. The pH of the mixture was adjusted to approx. 2 by portionwise addition of 4.0 M HCl in dioxane (13.61 mL, 54.5 mmol), and the mixture was allowed to stir overnight. The mixture was concentrated and partitined between 1.0 M HCl and DCM. The aqueous layer was extracted with more DCM; the organic extracts were disgarded. The aqueous layer was basified with 10 M NaOH and then extracted three times with DCM . The combined organics were dried over $MgSO_4$, filtered and concentrated. The crude product (1.057 g, 6.36 mmol, 58.4 % yield) was used without further purification. LCMS (m/z): 167.0 (M+H+). ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 1.09 (s, 3 H) 3.27 (d, J=10.17 Hz, 1 H) 3.61 (d, J=10.56 Hz, 1 H) 3.72 - 3.78 (m, 1 H) 3.85- 3.91(m, 1 H) 7.33 (d, J=4.30 Hz, 5 H)

(R)-2-(benzyl((4-bromo-1H-pyrazol-5-yl)methyl)amino)propan-1-ol-2-d:



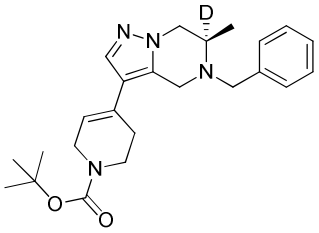
To a stirred mixture of 4-bromo-1H-pyrazole-5-carbaldehyde (637 mg, 3.64 mmol) and (R)-2-(benzylamino)propan-1-ol-2-d (550 mg, 3.31 mmol) in DCE (15 mL) was added $Na(OAc)_3BH$ (1753 mg, 8.27 mmol) and the mixture was stirred at 25 °C for 2.5 h. The mixture was poured onto saturated aqueous NaHCO3 and extracted three times with DCM. The combined organics were dried over MgSO4, filtered and concentrated. The residue was purified on a silica gel column (heptane:EtOAc 1:0 to 0:1) to give the product (854 mg, 2.63 mmol, 79 % yield) as a colorless oil. LCMS (m/z): 325.1/327.1 (M+H+).

(R)-5-benzyl-3-bromo-6-methyl-4,5,6,7-tetrahydropyrazolo[1,5-a]pyrazine-6-d:



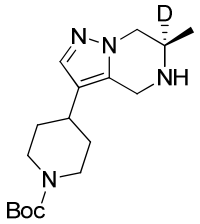
To a solution of (R)-2-(benzyl((4-bromo-1H-pyrazol-5-yl)methyl)amino)propan-1-ol-2-d (854 mg, 2.63 mmol) in DCM (13 mL) was added $SOCl_2$ (0.767 mL, 10.50 mmol) dropwise at RT. The resulting solution was stirred at room temperature for 4 h. The reaction mixture was concentrated and dried under high vacuum. The crude residue was taken up in DMF (10 mL) at RT and NaH (60% in mineral oil, 379 mg, 15.78 mmol) was slowly added. The reaction was stirred for 1 h and then diluted with EtOAc, washed with brine and water and then dried over $MgSO_4$, filtered and concentrated. The residue was purified on a silica gel column (heptane:EtOAc 1:0 to 1:1) to give the product (670 mg, 2.18 mmol, 83 % yield) as a colorless oil. LCMS (m/z): 307.1/309.1 (M+H+).

(R)-tert-butyl 4-(5-benzyl-6-methyl-4,5,6,7-tetrahydropyrazolo[1,5-a]pyrazin-3-yl)-5,6-dihydropyridine-1(2H)-carboxylate-6-d:



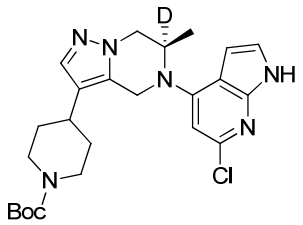
(R)-5-benzyl-3-bromo-6-methyl-4,5,6,7-tetrahydropyrazolo[1,5-a]pyrazine-6-d (670 mg, 2.181 mmol), tert-butyl 4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-5,6-dihydropyridine-1(2H)-carboxylate (944 mg, 3.05 mmol), $PdCl_2(dppf)\cdot CH_2Cl_2$ (178 mg, 0.218 mmol) and 2 M aq. sodium carbonate (3.27 mL, 6.54 mmol) were mixed in DME (17 mL) in a microwave tube. The mixture was purged with argon for 15 min. The tube was sealed and heated to 90 ºC overnight. The reaction mixture was poured onto water and extracted three times with EtOAc. The combined organics were washed with brine, dried over $MgSO_4$, filtered and concentrated. The residue was purified on a silica gel column (heptane:EtOAc 1:0 to 0:1) to give the product (823 mg, 2.010 mmol, 92 % yield) as a yellow foam. LCMS (m/z): 410.3 (M+H+).

(R)-tert-butyl 4-(6-methyl-4,5,6,7-tetrahydropyrazolo[1,5-a]pyrazin-3-yl)piperidine-1-carboxylate-6-d:



(R)-tert-butyl 4-(5-benzyl-6-methyl-4,5,6,7-tetrahydropyrazolo[1,5-a]pyrazin-3-yl)-5,6-dihydropyridine-1(2H)-carboxylate-6-d (814 mg, 1.988 mmol), ammonium formate (627 mg, 9.94 mmol), and 10% Pd/C (423 mg, 0.398 mmol) was taken up in MeOH (17 mL), sealed in a sealed tube, and heated to 70 °C for 1 h. The mixture was filtered through a pad of Celite, washing with EtOAc, and then partitioned between EtOAc and satuated aqueous $NaHCO_3$. The aqeous layer was extracted with more EtOAc, and the combined organics were washed with brine, dried over $MgSO_4$, filtered and concentrated. The crude product (598 mg, 1.860 mmol, 94 % yield) was isolated as a colorless oil which was used without further purification. LCMS (m/z): 322.3 (M+H+).

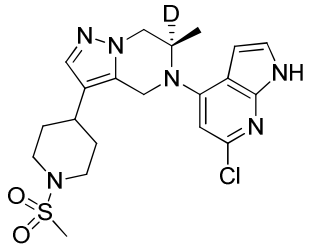
(R)-tert-butyl 4-(5-(6-chloro-1H-pyrrolo[2,3-b]pyridin-4-yl)-6-methyl-4,5,6,7-tetrahydropyrazolo[1,5-a]pyrazin-3-yl)piperidine-1-carboxylate-6-d:



A microwave vial was charged with (R)-tert-butyl 4-(6-methyl-4,5,6,7-tetrahydropyrazolo[1,5-a]pyrazin-3-yl)piperidine-1-carboxylate-6-d (148 mg, 0.460 mmol), 4-bromo-6-chloro-1H-pyrrolo[2,3-b]pyridine (117 mg, 0.506 mmol), RuPhos palladacycle (33.6 mg, 0.046 mmol), and DavePhos (18.12 mg, 0.046 mmol). The vial was sealed, vacuum flushed three times with Ar, and then taken up in THF (2 mL). LiHMDS (1.0 M in THF, 3.22 mL, 3.22 mmol) was added, and then

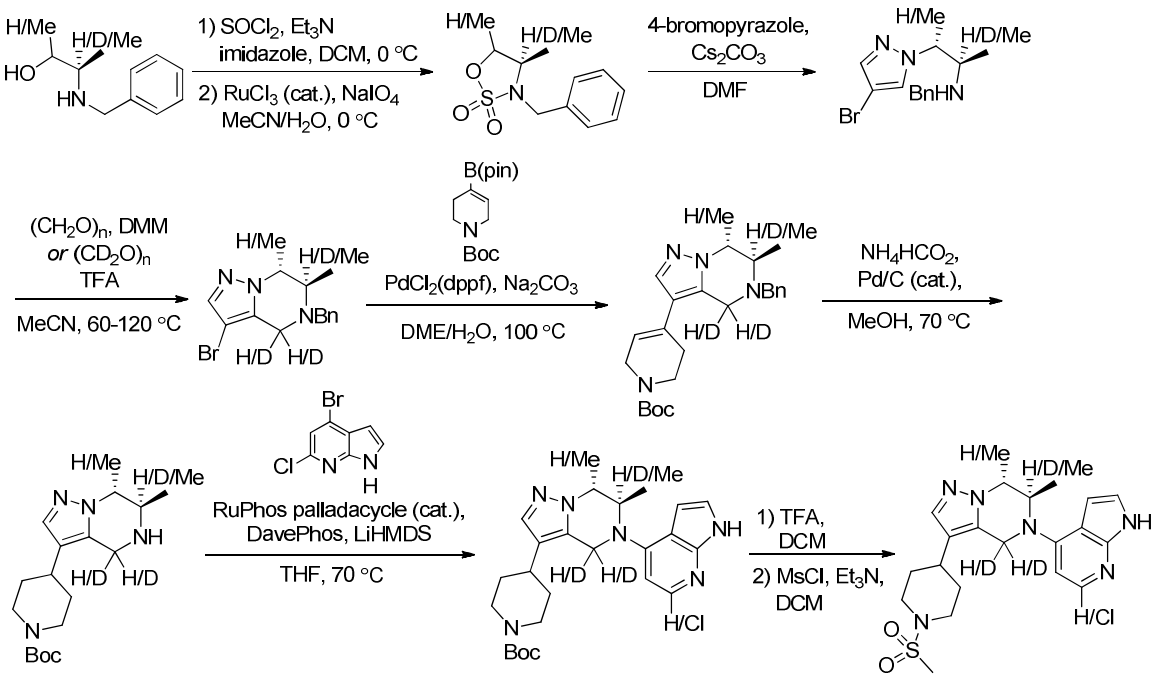
the mixture was heated to 55 °C for 2.5 h. The reaction mixture was cooled to room temperature and partitioned between EtOAc and saturated aqueous $NaHCO_3$. The aqueous layer was extracted with more EtOAc, and the combined organics were washed with brine, dried over $MgSO_4$, filtered and concentrated. The residue was purified on a silica gel column (heptane:EtOAc 1:0 to 0:1) to give the product (154 mg, 0.326 mmol, 70.9 % yield) as a yellow oil. LCMS (m/z): 472.1 (M+H+).

(R)-5-(6-chloro-1H-pyrrolo[2,3-b]pyridin-4-yl)-6-methyl-3-(1-(methylsulfonyl)piperidin-4-yl)-4,5,6,7-tetrahydropyrazolo[1,5-a]pyrazine-6-d (24):



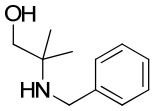
To a stirred solution of (R)-tert-butyl 4-(5-(6-chloro-1H-pyrrolo[2,3-b]pyridin-4-yl)-6-methyl-4,5,6,7-tetrahydropyrazolo[1,5-a]pyrazin-3-yl)piperidine-1-carboxylate-6-d (151 mg, 0.320 mmol) in DCM (4 mL) at 25 °C was added TFA (0.739 mL, 9.60 mmol) and the mixture was stirred for 1 h. The mixture was concentrated to dryness and then taken up in DCM:MeOH (9:1, 1.5 mL). Si-carbonate resin (0.8 mmol/g, 2.0 g, 1.60 mmol) was added and the mixture was stirred for 30 min and then filtered and concentrated. The so-obtained crude residue was taken up in DCM (4 mL) and DMF (0.5 mL) at 25 °C, and Et_3N (0.223 mL, 1.60 mmol) and Mesyl-Cl (22 µL, 0.288 mmol) were added. The mixture was allowed to stir for 30 min, and then quenched with a few drops of water and concentrated. The residue was purified by prep HPLC. Product fractions were converted to the corresponding free base by treatment with solid Na_2CO_3 followed by extraction into EtOAc. The organic extracts were washed with brine, dried over $MgSO_4$, filtered and concentrated, and then taken up in CH_3CN/H_2O and lyophilized to give the product (96 mg, 0.213 mmol, 67 % yield) as a white solid. HPLC purity >99%. LCMS (m/z): 450.3 (M+H+). 1H NMR (400 MHz, DMSO-*d*6) d ppm 1.03 (s, 3 H) 1.53 - 1.68 (m, 2 H) 1.89 (d, *J*=11.74 Hz, 2 H) 2.62 - 2.72 (m, 1 H) 2.79 (t, *J*=11.93 Hz, 2 H) 2.87 (s, 3 H) 3.61 (d, *J*=11.74 Hz, 2 H) 4.10 (d, *J*=12.52 Hz, 1 H) 4.39 (d, *J*=12.91 Hz, 1 H) 4.47 - 4.55 (m, 1 H) 4.62 - 4.72 (m, 1 H) 6.54 (s, 1 H) 6.62 (dd, *J*=3.13, 1.96 Hz, 1 H) 7.28 (d, *J*=2.74 Hz, 1 H) 7.39 (s, 1 H) 11.67 (br. s., 1 H). ^{13}C NMR (126 MHz, DMSO) δ 150.47, 148.29, 144.83, 136.32, 130.51, 122.80, 119.84, 107.81, 100.41, 100.35, 51.81, 45.99, 45.96, 40.55, 33.90, 32.11, 31.80, 30.19, 14.43. HRMS calculated for $C_{20}H_{25}N_6O_2SClD$ 450.1589 Da, measured 450.1602 Da.

E. General method for the synthesis of compounds 20-21 and 25-26



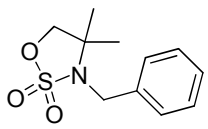
20-21 and **25-26**

2-(benzylamino)-2-methylpropan-1-ol:



To a solution of 2-amino-2-methylpropan-1-ol (1 g, 11.22 mmol) and benzaldehyde (1.310 g, 12.34 mmol) in DCE (15 mL) at RT was added portionwise sodium triacetoxyborohydride (6.66 g, 31.4 mmol), and the mixture was stirred for 3 h. The mixture was diluted with EtOAc and washed with saturated aqueous $NaHCO_3$. The organic layer was dried over $MgSO_4$, filtered, and concentrated. The crude product (1.247 g, 6.96 mmol, 62 % yield) was used without further purification. LCMS (m/z): 180.0 (M+H+).

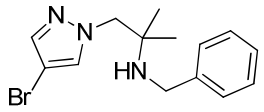
3-benzyl-4,4-dimethyl-1,2,3-oxathiazolidine 2,2-dioxide:



A solution of $SOCl_2$ (0.298 mL, 4.09 mmol) in CH_2Cl_2 (8 mL) was added dropwise to a stirred, 0 °C mixture of 2-(benzylamino)-2-methylpropan-1-ol (666 mg, 3.72 mmol), imidazole (1.265 g, 18.58 mmol), and Et_3N (1.812 mL, 13.00 mmol) in CH_2Cl_2 (25 mL), and the mixture was allowed

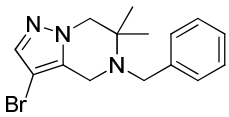
to stir for 2 h. The mixture was quenched with H_2O and extracted twice with CH_2Cl_2. The combined organics were washed with H_2O, dried over $MgSO_4$, filtered and concentrated. The so-obtained residue was carried forward without further purification. To a stirred solution of the crude residue in acetonitrile (20 mL) and water (15 mL) at 0 °C were added $NaIO_4$ (1.31 g, 6.13 mmol), , and $RuCl_3$ (0.079 g, 0.383 mmol) sequentially and the mixture was stirred at 0 °C for 3 h. The two layers were separated and the aqueous layer was extracted three times with EtOAc. The combined organics were washed with saturated aqueous $NaHCO_3$ and brine and then dried over $MgSO_4$, filtered and concentrated. The residue was purified on a silica gel column (heptane:EtOAc 1:0 to 0:1) to give the product (487 mg, 2.02 mmol, 53 % yield) as a colorless oil. LCMS (m/z): 242.1 (M+H+).

N-benzyl-1-(4-bromo-1H-pyrazol-1-yl)-2-methylpropan-2-amine:



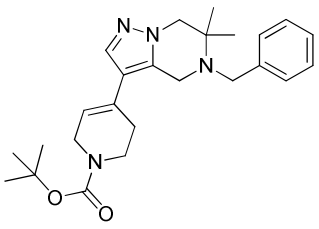
A mixture of 3-benzyl-4,4-dimethyl-1,2,3-oxathiazolidine 2,2-dioxide (487 mg, 2.018 mmol), 4-bromopyrazole (356 mg, 2.422 mmol), and Cs_2CO_3 (1.32 g, 4.04 mmol) in DMF (5 mL) was stirred at 25 °C for 2 h. The mixture was concentrated, taken up in 25 mL of 1:1 DCM:20%aq. H_2SO_4, and stirred vigorously for 2 h. The mixture was then carefully basified with 10 M NaOH, and the layers were separated. The aqeuous layer was extracted twice with DCM, and the combined organics were washed with brine, dried over $MgSO_4$, filtered and concentrated. The residue was purified on a silica gel column (heptane:EtOAc 1:0 to 3:7) to give the product (588 mg, 1.91 mmol, 95 % yield) as a colorless oil. LCMS (m/z): 308.3/310.3 (M+H+).

5-benzyl-3-bromo-6,6-dimethyl-4,5,6,7-tetrahydropyrazolo[1,5-a]pyrazine:



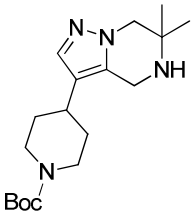
To a stirred solution of N-benzyl-1-(4-bromo-1H-pyrazol-1-yl)-2-methylpropan-2-amine (400 mg, 1.298mmol) in acetonitrile (20 mL) was added dimethoxymethane (0.929 mL, 10.38 mmol) and TFA (0.050 mL, 0.659 mmol) and the mixture was was stirred in a sealed tube at 120 °C for 48 h. The mixture was poured onto saturated aqueous $NaHCO_3$ and extracted three times with EtOAc. The combined organics were washed with brine, dried over $MgSO_4$, filtered, and concentrated. The residue was purified on a silica gel column (heptane:EtOAc 1:0 to 0:1) to provide the product (320 mg, 0.999 mmol, 77 % yield). LCMS (m/z): 319.9/321.8 (M+H+).

Tert-butyl 4-(5-benzyl-6,6-dimethyl-4,5,6,7-tetrahydropyrazolo[1,5-a]pyrazin-3-yl)-5,6-dihydropyridine-1(2H)-carboxylate:



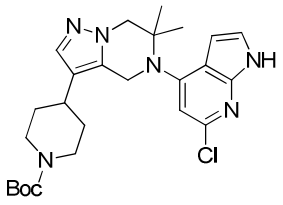
5-benzyl-3-bromo-6,6-dimethyl-4,5,6,7-tetrahydropyrazolo[1,5-a]pyrazine (320 mg, 0.999 mmol), 3,6-dihydro-2H-pyridine-1-N-Boc-4-boronic acid pinacol ester (433 mg, 1.399 mmol), $PdCl_2(dppf)\cdot CH_2Cl_2$ (40.8 mg, 0.050 mmol) and 2 M aq. sodium carbonate (2.0 mL, 4.0 mmol) were mixed in DME (5 mL) in a microwave tube. The mixture was purged with argon for 15 min. The tube was sealed and heated to 90 ºC for 7 h. The reaction mixture was poured onto water and extracted three times with EtOAc. The combined organics were washed with brine, dried over $MgSO_4$, filtered and concentrated. The residue was purified on a silica gel column (heptane:EtOAc 1:0 to 0:1) to give the product (450 mg, 1.065 mmol, 107 % yield). LCMS (m/z): 423.2 (M+H+).

Tert-butyl 4-(6,6-dimethyl-4,5,6,7-tetrahydropyrazolo[1,5-a]pyrazin-3-yl)piperidine-1-carboxylate:



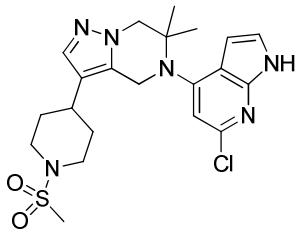
Tert-butyl 4-(5-benzyl-6,6-dimethyl-4,5,6,7-tetrahydropyrazolo[1,5-a]pyrazin-3-yl)-5,6-dihydropyridine-1(2H)-carboxylate (450 mg, 1.065 mmol), ammonium formate (269 mg, 4.26 mmol), and 10% Pd/C (227 mg, 0.213 mmol) were taken up in MeOH (7 mL), sealed in a microwave vial, and heated to 73 °C for 45 min. The mixture was filtered through a pad of Celite, washing with EtOAc, and then partitioned between EtOAc and satuated aqueous $NaHCO_3$. The aqueous layer was extracted with more EtOAc, and the combined organics were washed with brine, dried over $MgSO_4$, filtered and concentrated. The crude product (310 mg, 0.927 mmol, 897 % yield) was used without further purification. LCMS (m/z): 335.2 (M+H+).

Tert-butyl 4-(5-(6-chloro-1H-pyrrolo[2,3-b]pyridin-4-yl)-6,6-dimethyl-4,5,6,7-tetrahydropyrazolo[1,5-a]pyrazin-3-yl)piperidine-1-carboxylate:



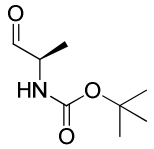
A microwave vial was charged with tert-butyl 4-(6,6-dimethyl-4,5,6,7-tetrahydropyrazolo[1,5-a]pyrazin-3-yl)piperidine-1-carboxylate (77 mg, 0.230 mmol), 4-bromo-6-chloro-1H-pyrrolo[2,3-b]pyridine (58.6 mg, 0.253 mmol), RuPhos palladacycle (26.8 mg, 0.037 mmol), and DavePhos (14.5 mg, 0.037 mmol). The vial was sealed, vacuum flushed three times with Ar, and then taken up in THF (1 mL). LiHMDS (1.0 M in THF) (1.612 mL, 1.612 mmol) was added, and then the mixture was heated to 70 °C for 3 h. The reaction mixture was cooled to RT and partitioned between EtOAc and saturated aqueous $NaHCO_3$. The aqueous layer was extracted with more EtOAc, and the combined organics were washed with brine, dried over $MgSO_4$, filtered and concentrated. The residue was purified on a silica gel column (heptane:EtOAc 1:0 to 3:7) to give the product (41 mg, 0.085 mmol, 37 % yield). LCMS (m/z): 485.2 (M+H+).

5-(6-chloro-1H-pyrrolo[2,3-b]pyridin-4-yl)-6,6-dimethyl-3-(1-(methylsulfonyl)piperidin-4-yl)-4,5,6,7-tetrahydropyrazolo[1,5-a]pyrazine (20):



To a stirred solution of tert-butyl 4-(5-(6-chloro-1H-pyrrolo[2,3-b]pyridin-4-yl)-6,6-dimethyl-4,5,6,7-tetrahydropyrazolo[1,5-a]pyrazin-3-yl)piperidine-1-carboxylate (41 mg, 0.085 mmol) in DCM (2 mL) at 25 °C was added TFA (0.261 mL, 3.38 mmol) and the mixture was stirred for 1 h. The mixture was concentrated to dryness and then taken up in DCM:MeOH (10:1, 6 mL). Si-carbonate resin (0.8 mmol/g, 1.0 g, 0.80 mmol) was added and the mixture was stirred for 20 min and then filtered and concentrated. The so-obtained crude residue was taken up in DCM (2 mL) at 25 °C; and Et_3N (0.038 mL, 0.27 mmol) and methansulfonyl chloride (11.6 mg mL, 0.101 mmol) were added. The mixture was allowed to stir for 30 min, and then quenched with a few drops of water and concentrated. The residue was purified by prep HPLC to provide the product as the corresponding TFA salt (14 mg, 0.030 mmol, 45 % yield). HPLC purity >99%. LCMS (m/z): 463.2 (M+H+). HRMS calculated for $C_{21}H_{28}N_6O_2SCl$ 463.1683 Da, measured 463.1683 Da.

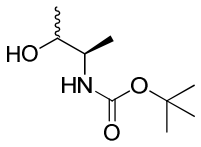
(R)-tert-butyl 1-oxopropan-2-ylcarbamate:



To a stirred solution of N-(tert-Butoxycarbonyl)-L-alanine methyl ester (1.0 g, 4.92 mmol) in DCM (10 mL) at -78 °C was added DIBAL-H (1.0 M in PhMe, 10.33 mL, 10.33 mmol) dropwise over 20 min, and the mixture was allowed to stir at -78 °C for an additional 1.5 h. The reaction was quenched by the slow addition of H_2O and the mixture was allowed to warm to RT. The mixture

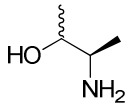
was filtered through a plug of Celite, and the organic phase of the filtrate was separated, washed with brine, dried over $MgSO_4$, filtered and concentrated. The crude product was used without further purification.

(R)-tert-butyl 3-hydroxybutan-2-ylcarbamate:



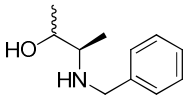
MeLi (1.6 M in Et_2O, 41.1 mL, 65.8 mmol) was slowly added to a -78 °C stirred solution of (R)-tert-butyl 1-oxopropan-2-ylcarbamate (4.56 g, 26.3 mmol) in Et_2O (200 mL) and the mixture was allowed to stir at the same temperature for 1.5 h. The reaction was quenched with saturated aqueous NH_4Cl and extracted three times with EtOAc. The combined organics were washed with brine, dried over MgSO4, filtered and concentrated. The residue was purified on a silica gel column (heptane:EtOAc 1:0 to 1:1) to give the product (2.32 g, 12.26 mmol, 46.6 % yield) as a colorless oil.

(R)-3-aminobutan-2-ol:



To a stirred solution of (R)-tert-butyl 3-hydroxybutan-2-ylcarbamate (2.61 g, 13.79 mmol) in DCM (150 mL) at 25 °C was added TFA (21.25 mL, 276 mmol) and the mixture was stirred for 2.5 h. The mixture was concentrated, azeotroping off excess TFA with PhMe, and then taken up in 10% MeOH:DCM (125 mL). Si-carbonate resin (0.8 mmol/g, 43 g, 34.47 mmol) was added, and the mixture was stirred for 1.5 h and then filtered and concentrated. The so-obtained crude residue was used without further purification

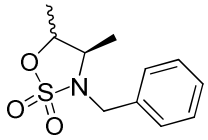
(R)-3-(benzylamino)butan-2-ol:



To a stirred solution of (R)-3-aminobutan-2-ol (1.229 g, 13.79 mmol) and benzaldehyde (1.398 mL, 13.79 mmol) in DCE (125 mL) at 0 °C was slowly added sodium triacetoxyborohydride (5.26 g, 24.82 mmol), maintaing the internal temperature below 25 °C. After the addition was complete, the mixture was allowed to stir overnight. More sodium triacetoxyborohydride (5.26 g, 24.82 mmol) was added, and stirring was continued for another 5 h. The mixture was poured onto 1 M HCl, and the phases were separated. The organic layer was extracted twice with 1 M HCl. The combined aqueous layers were adjusted to pH=13 by slow addition of 10 M NaOH in an ice bath;

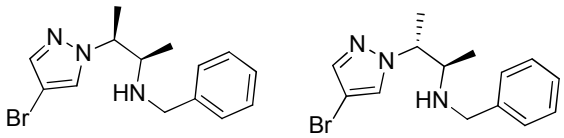
the aqueous layer was then extracted three times with DCM. The combined organics were dried over $MgSO_4$, filtered and concentrated. The crude product was used without further purification. LCMS (m/z): 180.1 (M+H+).

(4R)-3-benzyl-4,5-dimethyl-1,2,3-oxathiazolidine 2,2-dioxide:



A solution of $SOCl_2$ (0.573 mL, 7.85 mmol) in CH_2Cl_2 (25 mL) was added dropwise to a stirred, 0 °C mixture of (R)-3-(dibenzylamino)butan-2-ol (1.28 g, 7.14 mmol), imidazole (1.944 g, 28.6 mmol), and Et_3N (2.190 mL, 15.71 mmol) in CH_2Cl_2 (100 mL), and the mixture was allowed to stir for 1 h. The mixture was quenched with H_2O and extracted twice with CH_2Cl_2. The combined organics were washed with H_2O, dried over $MgSO_4$, filtered and concentrated. The so-obtained residue was carried forward without further purification. To a stirred solution of the crude residue in acetonitrile (70 mL) at 0 °C were added $NaIO_4$ (2.291 g, 10.71 mmol), water (53.8 mL), and $RuCl_3$ (0.015 g, 0.071 mmol) sequentially and the mixture was stirred at 0 °C for 3 h. The two layers were separated and the aqueous layer was extracted three times with EtOAc. The combined organics were washed with saturated aqueous $NaHCO_3$ and brine and then dried over $MgSO_4$, filtered and concentrated. The residue was purified on a silica gel column (heptane:EtOAc 1:0 to 1:1) to give the product (1.12 g, 4.64 mmol, 65.0 % yield) as a colorless oil. LCMS (m/z): 242.2 (M+H+).

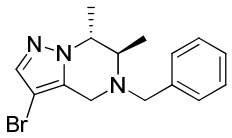
(2R,3R)-N-benzyl-3-(4-bromo-1H-pyrazol-1-yl)butan-2-amine and (2R,3S)-N-benzyl-3-(4-bromo-1H-pyrazol-1-yl)butan-2-amine:



A mixture of (4R)-3-benzyl-4,5-dimethyl-1,2,3-oxathiazolidine 2,2-dioxide (1.12 g, 4.64 mmol), 4-bromopyrazole (1.364 g, 9.28 mmol), and Cs_2CO_3 (3.02 g, 9.28 mmol) in DMF (50 mL) was stirred at 25 °C for 1.5 h. The mixture was concentrated, taken up in 100 mL of 1:1 DCM:20%aq. H_2SO_4, and stirred vigorously overnight. The mixture was then carefully basified with 3 M NaOH, and the layers were separated. The aqeuous layer was extracted twice with DCM, and the combined organics were washed with brine, dried over $MgSO_4$, filtered and concentrated. The residue was purified on a silica gel column (heptane:EtOAc 1:0 to 3:2) to give both the (2R,3S) product (469 mg, 1.522 mmol, 32.8 % yield) and (2R,3R) product (790 mg, 2.56 mmol, 55.2 % yield) as colorless oils. The relative stereochemistry of the two compounds was determined by characterization of subsequent products by 2-D NMR methods. LCMS (m/z): 308.2/310.1 (M+H+). 1H NMR (400 MHz, $CDCl_3$) δ ppm 0.97 (d, *J*=6.65 Hz, 3 H) 1.53 (d, *J*=7.04 Hz, 3 H) 2.92 - 3.00 (m, 1 H) 3.64 - 3.71 (m, 1 H) 3.79 (s, 1 H) 4.25 - 4.34 (m, 1 H) 7.26 - 7.34 (m, 5 H)

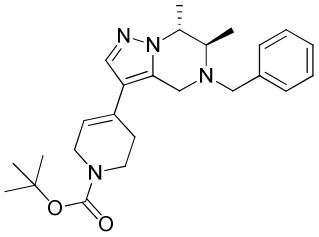
7.45 (s, 1 H) 7.49 (s, 1 H) and 1H NMR (400 MHz, $CDCl_3$) δ ppm 1.07 (d, *J*=6.26 Hz, 3 H) 1.48 (d, *J*=7.04 Hz, 3 H) 2.98 (t, *J*=6.46 Hz, 1 H) 3.56 (d, *J*=13.30 Hz, 1 H) 3.77 (d, *J*=13.30 Hz, 1 H) 4.20 (s, 1 H) 7.19 (d, *J*=7.04 Hz, 2 H) 7.29 (d, *J*=7.43 Hz, 2 H) 7.45 (d, *J*=14.48 Hz, 2 H)

(6R,7R)-5-benzyl-3-bromo-6,7-dimethyl-4,5,6,7-tetrahydropyrazolo[1,5-a]pyrazine:



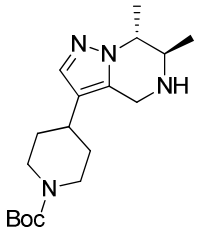
To a stirred solution of (2R,3R)-N-benzyl-3-(4-bromo-1H-pyrazol-1-yl)butan-2-amine (634 mg, 2.057 mmol) in acetonitrile (20 mL) was added paraformaldehyde (247 mg, 8.23 mmol) and TFA (0.032 mL, 0.411 mmol) and the mixture was was stirred at 60 °C for 6 h. The mixture was poured onto saturated aqueous $NaHCO_3$ and extracted three times with EtOAc. The combined organics were washed with brine, dried over $MgSO_4$, filtered, and concentrated. The residue was purified on a silica gel column (heptane:EtOAc 1:0 to 9:1) to provide the product (370 mg, 1.16 mmol, 56 % yield) as a colorless oil. The relative stereochemistry of the product was confirmed by 2-D NMR methods. LCMS (m/z): 320.1/322.2 (M+H+). 1H NMR (400 MHz, $CDCl_3$) □ ppm 1.10 (d, J=6.65 Hz, 3 H) 1.53 (d, J=6.65 Hz, 3 H) 3.03 (dd, J=6.65, 2.74 Hz, 1 H) 3.50 (d, J=16.04 Hz, 1 H) 3.72 - 3.78 (m, 3 H) 4.08 (dd, J=6.26, 2.74 Hz, 1 H) 7.27 - 7.39 (m, 5 H) 7.45 (s, 1 H)

Tert-butyl 4-((6R,7R)-5-benzyl-6,7-dimethyl-4,5,6,7-tetrahydropyrazolo[1,5-a]pyrazin-3-yl)-5,6-dihydropyridine-1(2H)-carboxylate:



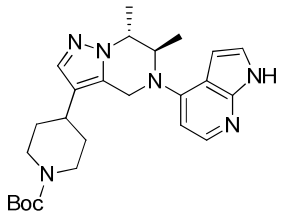
(2R,3R)-N-benzyl-3-(4-bromo-1H-pyrazol-1-yl)-N-methylbutan-2-amine (463 mg, 1.446 mmol), 3,6-dihydro-2H-pyridine-1-N-Boc-4-boronic acid pinacol ester (626 mg, 2.024 mmol), $PdCl_2(dppf)\cdot CH_2Cl_2$ (118 mg, 0.145 mmol) and 2 M aq. sodium carbonate (2.169 mL, 4.34 mmol) were mixed in DME (12 mL) in a microwave tube. The mixture was purged with argon for 15 min. The tube was sealed and heated to 90 ºC for 6 h. The reaction mixture was poured onto water and extracted three times with EtOAc. The combined organics were washed with brine, dried over $MgSO_4$, filtered and concentrated. The residue was purified on a silica gel column (heptane:EtOAc 1:0 to 1:3) to give the product (465 mg, 1.100 mmol, 76 % yield) as a pale yellow oil.. LCMS (m/z): 423.2 (M+H+).

Tert-butyl 4-((6R,7R)-6,7-dimethyl-4,5,6,7-tetrahydropyrazolo[1,5-a]pyrazin-3-yl)piperidine-1-carboxylate :



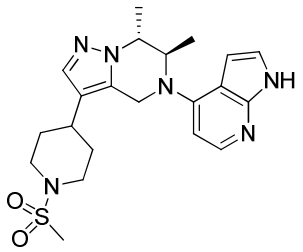
Tert-butyl 4-((6R,7R)-5-benzyl-6,7-dimethyl-4,5,6,7-tetrahydropyrazolo[1,5-a]pyrazin-3-yl)-5,6-dihydropyridine-1(2H)-carboxylate (438 mg, 1.037 mmol), ammonium formate (327 mg, 5.18 mmol), and 10% Pd/C (221 mg, 0.207 mmol) were taken up in MeOH (9 mL), sealed in a microwave vial, and heated to 70 °C for 1.5 h. The mixture was filtered through a pad of Celite, washing with EtOAc, and then partitioned between EtOAc and satuated aqueous $NaHCO_3$. The aqueous layer was extracted with more EtOAc, and the combined organics were washed with brine, dried over $MgSO_4$, filtered and concentrated. The crude product (336 mg, 1.005 mmol, 97 % yield) was isolated as a white solid which was used without further purification. LCMS (m/z): 335.2 (M+H+).

Tert-butyl 4-((6R,7R)-6,7-dimethyl-5-(1H-pyrrolo[2,3-b]pyridin-4-yl)-4,5,6,7-tetrahydropyrazolo[1,5-a]pyrazin-3-yl)piperidine-1-carboxylate:



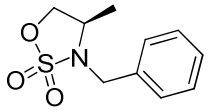
A microwave vial was charged with tert-butyl 4-((6R,7R)-6,7-dimethyl-4,5,6,7-tetrahydropyrazolo[1,5-a]pyrazin-3-yl)piperidine-1-carboxylate (81 mg, 0.242 mmol), 4-bromo-7-azaindole (52.5 mg, 0.266 mmol), RuPhos palladacycle (26.5 mg, 0.036 mmol), and DavePhos (14.30 mg, 0.036 mmol). The vial was sealed, vacuum flushed three times with Ar, and then taken up in THF (1 mL). LiHMDS (1.0 M in THF) (1.695 mL, 1.695 mmol) was added, and then the mixture was heated to 70 °C for 1 h. The reaction mixture was cooled to RT and partitioned between EtOAc and saturated aqueous $NaHCO_3$. The aqueous layer was extracted with more EtOAc, and the combined organics were washed with brine, dried over $MgSO_4$, filtered and concentrated. The residue was purified on a silica gel column (heptane:EtOAc 1:0 to 0:1) to give the product (90 mg, 0.200 mmol, 82 % yield). LCMS (m/z): 451.2 (M+H+).

(6R,7R)-6,7-dimethyl-3-(1-(methylsulfonyl)piperidin-4-yl)-5-(1H-pyrrolo[2,3-b]pyridin-4-yl)-4,5,6,7-tetrahydropyrazolo[1,5-a]pyrazine (21):



To a stirred solution of tert-butyl 4-((6R,7R)-6,7-dimethyl-5-(1H-pyrrolo[2,3-b]pyridin-4-yl)-4,5,6,7-tetrahydropyrazolo[1,5-a]pyrazin-3-yl)piperidine-1-carboxylate (91 mg, 0.202 mmol) in DCM (2.5 mL) at 25 °C was added TFA (0.467 mL, 6.06 mmol) and the mixture was stirred for 1 h. The mixture was concentrated to dryness and then taken up in DCM:MeOH (9:1, 2.5 mL). Si-carbonate resin (0.8 mmol/g, 1.26 g, 1.01 mmol) was added and the mixture was stirred for 30 min and then filtered and concentrated. The so-obtained crude residue was taken up in DCM (2 mL) at 25 °C; and Et_3N (0.084 mL, 0.606 mmol) and methansulfonyl chloride (0.015 mL, 0.192 mmol) were added. The mixture was allowed to stir for 30 min, and then quenched with a few drops of water and concentrated. The residue was purified by prep HPLC to provide the TFA salt (64.8 mg, 0.151 mmol, 74.9 % yield) as a white solid. HPLC purity >99%. LCMS (m/z): 429.3 (M+H+). 1H NMR (400 MHz, DMSO-d6) δ ppm 1.09 (d, J=6.65 Hz, 3 H) 1.37 (d, J=6.65 Hz, 3 H) 1.63 (br. s., 2 H) 1.88 (br. s., 2 H) 2.61 - 2.72 (m, 1 H) 2.73 - 2.82 (m, 2 H) 2.87 (s, 3 H) 3.62 (d, J=10.56 Hz, 2 H) 4.43 (d, J=6.65 Hz, 1 H) 4.87 (d, J=15.65 Hz, 1 H) 5.01 (d, J=6.26 Hz, 1 H) 5.15 (d, J=16.04 Hz, 1 H) 6.91 (d, J=7.43 Hz, 1 H) 6.98 (d, J=1.96 Hz, 1 H) 7.42 (s, 1 H) 7.45 - 7.48 (m, 1 H) 8.10 (d, J=7.43 Hz, 1 H) 12.44 (br. s., 1 H). ^{13}C NMR (126 MHz, DMSO) δ 153.87, 139.57, 137.05, 134.66, 128.61, 124.06, 120.50, 108.71, 104.06, 100.67, 57.71, 55.51, 46.41, 42.45, 34.42, 32.42, 32.32, 30.71, 20.49, 17.16. HRMS calculated for $C_{21}H_{29}N_6O_2S$ 429.2073 Da, measured 429.2076 Da.

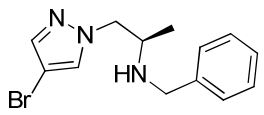
(**4R)-3-benzyl-4-methyl-1,2,3-oxathiazolidine 2,2-dioxide:**



A solution of $SOCl_2$ (0.583 mL, 7.99 mmol) in CH_2Cl_2 (25 mL) was added dropwise to a stirred, 0 °C mixture of (R)-2-(benzylamino)propan-1-ol (1.20 g, 7.26 mmol), imidazole (1.978 g, 29.1 mmol), and Et_3N (2.227 mL, 15.98 mmol) in CH_2Cl_2 (100 mL), and the mixture was allowed to stir for 1 h. The mixture was quenched with H_2O and extracted twice with CH_2Cl_2. The combined organics were washed with H_2O, dried over $MgSO_4$, filtered and concentrated. The so-obtained residue was carried forward without further purification. To a stirred solution of the crude residue in acetonitrile (70 mL) at 0 °C were added $NaIO_4$ (2.329 g, 10.89 mmol), water (53.8 mL), and $RuCl_3$ (0.015 g, 0.073 mmol) sequentially and the mixture was stirred at 0 °C for 3 h. The two layers were separated and the aqueous layer was extracted three times with EtOAc. The combined organics were washed with saturated aqueous $NaHCO_3$ and brine and then dried over $MgSO_4$, filtered and concentrated. The residue was purified on a silica gel column (heptane:EtOAc 1:0 to

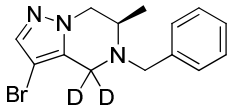
0:1) to give the product (1.45 g, 6.38 mmol, 88 % yield) as a white solid. LCMS (m/z): 228.2 (M+H+).

(R)-N-benzyl-1-(4-bromo-1H-pyrazol-1-yl)propan-2-amine:



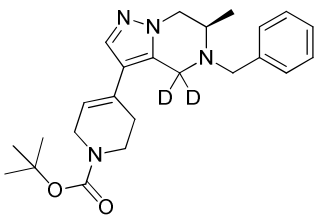
A mixture of (4R)-3-benzyl-4-methyl-1,2,3-oxathiazolidine 2,2-dioxide (1.45 g, 4.64 mmol), 4-bromopyrazole (1.406 g, 9.57 mmol), and Cs_2CO_3 (4.16 g, 12.76 mmol) in DMF (20 mL) was stirred at 25 °C for 2 h. The mixture was concentrated, taken up in 75 mL of 1:1 DCM:20%aq. H_2SO_4, and stirred vigorously overnight. The mixture was then carefully basified with 10 M NaOH, and the layers were separated. The aqeuous layer was extracted twice with DCM, and the combined organics were washed with brine, dried over $MgSO_4$, filtered and concentrated. The residue was purified on a silica gel column (heptane:EtOAc 1:0 to 3:7) to give the product (2.15 g, 7.31 mmol, 115 % yield) as a colorless oil which was contaminated with a small amount of excess 4-bromopyrazole but was nonetheless carried forward without further purification. LCMS (m/z): 294.0/296.0 (M+H+). 1H NMR (400 MHz, CHLOROFORMd) d ppm 1.08 (d, J=6.26 Hz, 3 H) 3.14 (d, J=5.87 Hz, 1 H) 3.71 (s, 1 H) 3.79 (s, 1 H) 3.99 - 4.12 (m, 2 H) 7.24 (s, 3 H) 7.28 - 7.34 (m, 2 H) 7.43 (s, 1 H) 7.47 (s, 1 H).

(R)-5-benzyl-3-bromo-6-methyl-4,5,6,7-tetrahydropyrazolo[1,5-a]pyrazine-4-d_2:



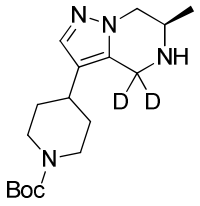
To a stirred solution of (R)-N-benzyl-1-(4-bromo-1H-pyrazol-1-yl)propan-2-amine (520 mg, 1.768 mmol) in acetonitrile (20 mL) was added paraformaldehyde-d_2 (227 mg, 7.07 mmol) and TFA (0.027 mL, 0.354 mmol) and the mixture was was stirred at 60 °C overnight. The mixture was poured onto saturated aqueous $NaHCO_3$ and extracted three times with EtOAc. The combined organics were washed with brine, dried over $MgSO_4$, filtered, and concentrated. The residue was purified on a silica gel column (heptane:EtOAc 1:0 to 7:3) to provide the product (359 mg, 1.17 mmol, 66 % yield) as a colorless oil. LCMS (m/z): 308.1/310.1 (M+H+). 1H NMR (300 MHz, <cdcl3>) d ppm 1.22 (d, J=6.45 Hz, 3 H) 3.18 - 3.38 (m, 1 H) 3.67 (s, 1 H) 3.79 (br. s., 1 H) 3.87 - 3.98 (m, 1 H) 4.21 (d, J=12.31 Hz, 1 H) 7.35 (d, J=1.47 Hz, 5 H) 7.44 (s, 1 H)

Tert-butyl 4-(R)-5-benzyl-6-methyl-4,5,6,7-tetrahydropyrazolo[1,5-a]pyrazin-3-yl)-5,6-dihydropyridine-1(2H)-carboxylate-4-d_2:



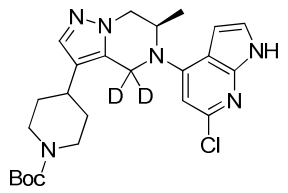
(R)-5-benzyl-3-bromo-6-methyl-4,5,6,7-tetrahydropyrazolo[1,5-a]pyrazine-4-d_2 (419 mg, 1.359 mmol), 3,6-dihydro-2H-pyridine-1-N-Boc-4-boronic acid pinacol ester (588 mg, 1.903 mmol), $PdCl_2(dppf)\cdot CH_2Cl_2$ (111 mg, 0.136 mmol) and 2 M aq. sodium carbonate (2.039 mL, 4.08 mmol) were mixed in DME (10 mL) in a microwave tube. The mixture was purged with argon for 15 min. The tube was sealed and heated to 90 ºC for 6 h. The reaction mixture was poured onto water and extracted three times with EtOAc. The combined organics were washed with brine, dried over $MgSO_4$, filtered and concentrated. The residue was purified on a silica gel column (heptane:EtOAc 1:0 to 0:1) to give the product (483 mg, 1.176 mmol, 87 % yield) as a pale yellow oil.. LCMS (m/z): 411.3 (M+H+).

Tert-butyl 4-(R)-6-methyl-4,5,6,7-tetrahydropyrazolo[1,5-a]pyrazin-3-yl)piperidine-1-carboxylate-4-d_2:



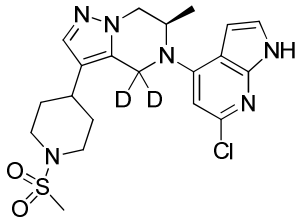
Tert-butyl 4-(R)-5-benzyl-6-methyl-4,5,6,7-tetrahydropyrazolo[1,5-a]pyrazin-3-yl)-5,6-dihydropyridine-1(2H)-carboxylate-4-d_2 (476 mg, 1.159 mmol), ammonium formate (366 mg, 5.80 mmol), and 10% Pd/C (247 mg, 0.232mmol) were taken up in MeOH (10 mL), sealed in a microwave vial, and heated to 70 °C for 1.5 h. The mixture was filtered through a pad of Celite, washing with EtOAc, and then partitioned between EtOAc and satuated aqueous $NaHCO_3$. The aqueous layer was extracted with more EtOAc, and the combined organics were washed with brine, dried over $MgSO_4$, filtered and concentrated. The crude product (355 mg, 1.101 mmol, 95 % yield) was isolated as a white solid which was used without further purification. LCMS (m/z): 323.3 (M+H+).

Tert-butyl 4-((R)-6-methyl-5-(6-chloro-1H-pyrrolo[2,3-b]pyridin-4-yl)-4,5,6,7-tetrahydropyrazolo[1,5-a]pyrazin-3-yl)piperidine-1-carboxylate-4-d_2:



A microwave vial was charged with tert-butyl 4-(R)-6-methyl-4,5,6,7-tetrahydropyrazolo[1,5-a]pyrazin-3-yl)piperidine-1-carboxylate-4-d_2 (98 mg, 0.304 mmol), 4-bromo-6-chloro-1H-pyrollo[2,3-b]pyridine (77 mg, 0.334 mmol), RuPhos palladacycle (33.2 mg, 0.046 mmol), and DavePhos (18 mg, 0.046 mmol). The vial was sealed, vacuum flushed three times with Ar, and then taken up in THF (1 mL). LiHMDS (1.0 M in THF) (2.128 mL, 2.128 mmol) was added, and then the mixture was heated to 55 °C for 2 h. The reaction mixture was cooled to RT and partitioned between EtOAc and saturated aqueous $NaHCO_3$. The aqueous layer was extracted with more EtOAc, and the combined organics were washed with brine, dried over $MgSO_4$, filtered and concentrated. The residue was purified on a silica gel column (heptane:EtOAc 1:0 to 0:1) to give the product (109 mg, 0.230 mmol, 76 % yield) as a yellow foam. LCMS (m/z): 473.2 (M+H+).

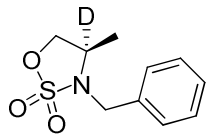
(R)-6-methyl-3-(1-(methylsulfonyl)piperidin-4-yl)-5-(6-chloro-1H-pyrrolo[2,3-b]pyridin-4-yl)-4,5,6,7-tetrahydropyrazolo[1,5-a]pyrazine-4-d_2 (25):



To a stirred solution of tert-butyl 4-((R)-6-methyl-5-(6-chloro-1H-pyrrolo[2,3-b]pyridin-4-yl)-4,5,6,7-tetrahydropyrazolo[1,5-a]pyrazin-3-yl)piperidine-1-carboxylate-4-d_2 (109 mg, 0.230 mmol) in DCM (3 mL) at 25 °C was added TFA (0.533 mL, 6.91 mmol) and the mixture was stirred for 1 h. The mixture was concentrated to dryness and then taken up in DCM:MeOH (9:1, 2.5 mL). Si-carbonate resin (0.8 mmol/g, 1.4 g, 1.12 mmol) was added and the mixture was stirred for 30 min and then filtered and concentrated. The so-obtained crude residue was taken up in DCM (4 mL) at 25 °C; and Et_3N (0.16 mL, 1.15 mmol) and methansulfonyl chloride (0.018 mL, 0.23 mmol) were added. The mixture was allowed to stir for 30 min, and then quenched with a few drops of water and concentrated. The residue was purified by prep HPLC. Product fractions were converted to the corresponding free base by treatment with solid Na_2CO_3 followed by extraction into EtOAc. The organic extracts were washed with brine, dried over $MgSO_4$, filtered and concentrated, and then taken up in CH_3CN/H_2O and lyophilized to give the product (70 mg, 0.155 mmol, 68 % yield) as a white solid. HPLC purity >99%. LCMS (m/z): 451.4 (M+H+). 1H NMR (400 MHz, DMSO-*d6*) δ ppm 1.03 (d, *J*=6.65 Hz, 3 H) 1.60 (ddd, *J*=12.33, 8.41, 3.91 Hz, 2 H) 1.88 (d, *J*=11.35 Hz, 2 H) 2.61 - 2.71 (m, 1 H) 2.78 (t, *J*=11.93 Hz, 2 H) 2.86 (s, 3 H) 3.60

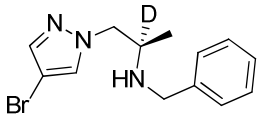
(d, *J*=12.13 Hz, 2 H) 4.09 (d, *J*=12.52 Hz, 1 H) 4.39 (dd, *J*=12.52, 4.30 Hz, 1 H) 4.90 - 5.00 (m, 1 H) 6.53 (s, 1 H) 6.61 (dd, *J*=3.52, 1.96 Hz, 1 H) 7.26 - 7.29 (m, 1 H) 7.38 (s, 1 H) 11.66 (br. s., 1 H). ^{13}C NMR (126 MHz, DMSO) δ 136.31, 122.78, 107.82, 100.41, 100.35, 51.86, 49.83, 45.98, 45.94, 40.10, 39.92, 39.76, 39.59, 39.43, 32.11, 31.80, 14.57. HRMS calculated for $C_{20}H_{24}N_6O_2SClD_2$ 451.1652 Da, measured 451.1660 Da.

(4R)-3-benzyl-4-methyl-1,2,3-oxathiazolidine 2,2-dioxide-4-d:



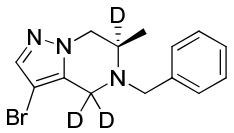
A solution of $SOCl_2$ (0.239 mL, 3.27 mmol) in CH_2Cl_2 (10 mL) was added dropwise to a stirred, 0 °C mixture of (R)-2-(benzylamino)propan-1-ol -2-d(494 mg, 7.26 mmol), imidazole (0.809 g, 11.89 mmol), and Et_3N (0.911 mL, 6.54 mmol) in CH_2Cl_2 (40 mL), and the mixture was allowed to stir for 1 h. The mixture was quenched with H_2O and extracted twice with CH_2Cl_2. The combined organics were washed with H_2O, dried over $MgSO_4$, filtered and concentrated. The so-obtained residue was carried forward without further purification. To a stirred solution of the crude residue in acetonitrile (30 mL) at 0 °C were added $NaIO_4$ (953 mg, 4.46 mmol), water (23 mL), and $RuCl_3$ (6.16 mg, 0.030 mmol) sequentially and the mixture was stirred at 0 °C for 3 h. The two layers were separated and the aqueous layer was extracted three times with EtOAc. The combined organics were washed with saturated aqueous $NaHCO_3$ and brine and then dried over $MgSO_4$, filtered and concentrated. The residue was purified on a silica gel column (heptane:EtOAc 1:0 to 0:1) to give the product (472 mg, 2.068 mmol, 70 % yield) as a white solid. LCMS (m/z): 229.0 (M+H+). 1H NMR (400 MHz, CHLOROFORM-d) d ppm 1.19 (s, 3 H) 4.12 (d, J=8.22 Hz, 1 H) 4.18 - 4.26 (m, 1 H) 4.36 - 4.44 (m, 1 H) 4.56 (d, J=8.22 Hz, 1 H) 7.30 - 7.46 (m, 5 H).

(R)-N-benzyl-1-(4-bromo-1H-pyrazol-1-yl)propan-2-amine-2-d:



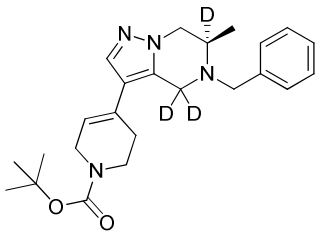
A mixture of (4R)-3-benzyl-4-methyl-1,2,3-oxathiazolidine 2,2-dioxide-4-d (470 mg, 2.059 mmol), 4-bromopyrazole (303 mg, 2.059 mmol), and Cs_2CO_3 (1.34 g, 4.12 mmol) in DMF (6 mL) was stirred at 25 °C for 2 h. The mixture was concentrated, taken up in 25 mL of 1:1 DCM:20%aq. H_2SO_4, and stirred vigorously overnight. The mixture was then carefully basified with 10 M NaOH, and the layers were separated. The aqeuous layer was extracted twice with DCM, and the combined organics were washed with brine, dried over $MgSO_4$, filtered and concentrated. The residue was purified on a silica gel column (heptane:EtOAc 1:0 to 3:7) to give the product (514 mg, 1.74 mmol, 85 % yield) as a colorless oil. LCMS (m/z): 295.1/297.1 (M+H+). 1H NMR (400 MHz, CHLOROFORM-d) d ppm 1.08 (s, 3 H) 3.66 - 3.73 (m, 1 H) 3.78 - 3.84 (m, 1 H) 4.05 (d, J=3.13 Hz, 2 H) 7.21 - 7.26 (m, 3 H) 7.30 (d, J=7.04 Hz, 2 H) 7.45 (d, J=12.91 Hz, 2 H).

(R)-5-benzyl-3-bromo-6-methyl-4,5,6,7-tetrahydropyrazolo[1,5-a]pyrazine-4,6-d_3:



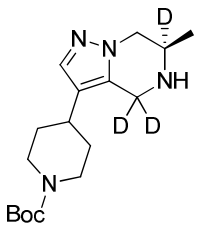
To a stirred solution of (R)-N-benzyl-1-(4-bromo-1H-pyrazol-1-yl)propan-2-amine-2-d (513 mg, 1.738 mmol) in acetonitrile (20 mL) was added paraformaldehyde-d_2 (223 mg, 6.95 mmol) and TFA (0.027 mL, 0.348 mmol) and the mixture was was stirred at 60 °C overnight. The mixture was poured onto saturated aqueous $NaHCO_3$ and extracted three times with EtOAc. The combined organics were washed with brine, dried over $MgSO_4$, filtered, and concentrated. The residue was purified on a silica gel column (heptane:EtOAc 1:0 to 7:3) to provide the product (321 mg, 1.038 mmol, 60 % yield) as a colorless oil. LCMS (m/z): 309.1/311.1 (M+H+). 1H NMR (400 MHz, CHLOROFORM-d) d ppm 1.21 (s, 3 H) 3.62 - 3.72 (m, 1 H) 3.76 - 3.83 (m, 1 H) 3.91 (d, J=12.13 Hz, 1 H) 4.20 (d, J=12.52 Hz, 1 H) 7.27 - 7.39 (m, 5 H) 7.44 (s, 1 H).

Tert-butyl 4-(R)-5-benzyl-6-methyl-4,5,6,7-tetrahydropyrazolo[1,5-a]pyrazin-3-yl)-5,6-dihydropyridine-1(2H)-carboxylate-4,6-d_3:



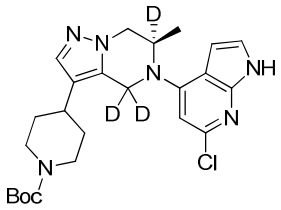
(R)-5-benzyl-3-bromo-6-methyl-4,5,6,7-tetrahydropyrazolo[1,5-a]pyrazine-4,6-d_3 (328 mg, 1.061 mmol), 3,6-dihydro-2H-pyridine-1-N-Boc-4-boronic acid pinacol ester (459 mg, 1.485 mmol), $PdCl_2(dppf)\cdot CH_2Cl_2$ (87 mg, 0.106 mmol) and 2 M aq. sodium carbonate (1.591 mL, 3.18 mmol) were mixed in DME (8 mL) in a microwave tube. The mixture was purged with argon for 15 min. The tube was sealed and heated to 90 ºC overnight. The reaction mixture was poured onto water and extracted three times with EtOAc. The combined organics were washed with brine, dried over $MgSO_4$, filtered and concentrated. The residue was purified on a silica gel column (heptane:EtOAc 1:0 to 0:1) to give the product (381 mg, 0.926 mmol, 87 % yield) as a pale yellow oil.. LCMS (m/z): 412.4 (M+H+).

Tert-butyl 4-(R)-6-methyl-4,5,6,7-tetrahydropyrazolo[1,5-a]pyrazin-3-yl)piperidine-1-carboxylate-4,6-d_3:



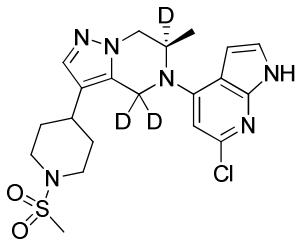
Tert-butyl 4-(R)-5-benzyl-6-methyl-4,5,6,7-tetrahydropyrazolo[1,5-a]pyrazin-3-yl)-5,6-dihydropyridine-1(2H)-carboxylate-4,6-d_3 (371 mg, 0.901 mmol), ammonium formate (284 mg, 4.51 mmol), and 10% Pd/C (192 mg, 0.180 mmol) were taken up in MeOH (8 mL), sealed in a microwave vial, and heated to 70 °C for 1.5 h. The mixture was filtered through a pad of Celite, washing with EtOAc, and then partitioned between EtOAc and satuated aqueous $NaHCO_3$. The aqueous layer was extracted with more EtOAc, and the combined organics were washed with brine, dried over $MgSO_4$, filtered and concentrated. The crude product (286 mg, 0.884 mmol, 98 % yield) was isolated as a white solid which was used without further purification. LCMS (m/z): 324.3 (M+H+).

Tert-butyl 4-((R)-6-methyl-5-(6-chloro-1H-pyrrolo[2,3-b]pyridin-4-yl)-4,5,6,7-tetrahydropyrazolo[1,5-a]pyrazin-3-yl)piperidine-1-carboxylate-4,6-d_3:



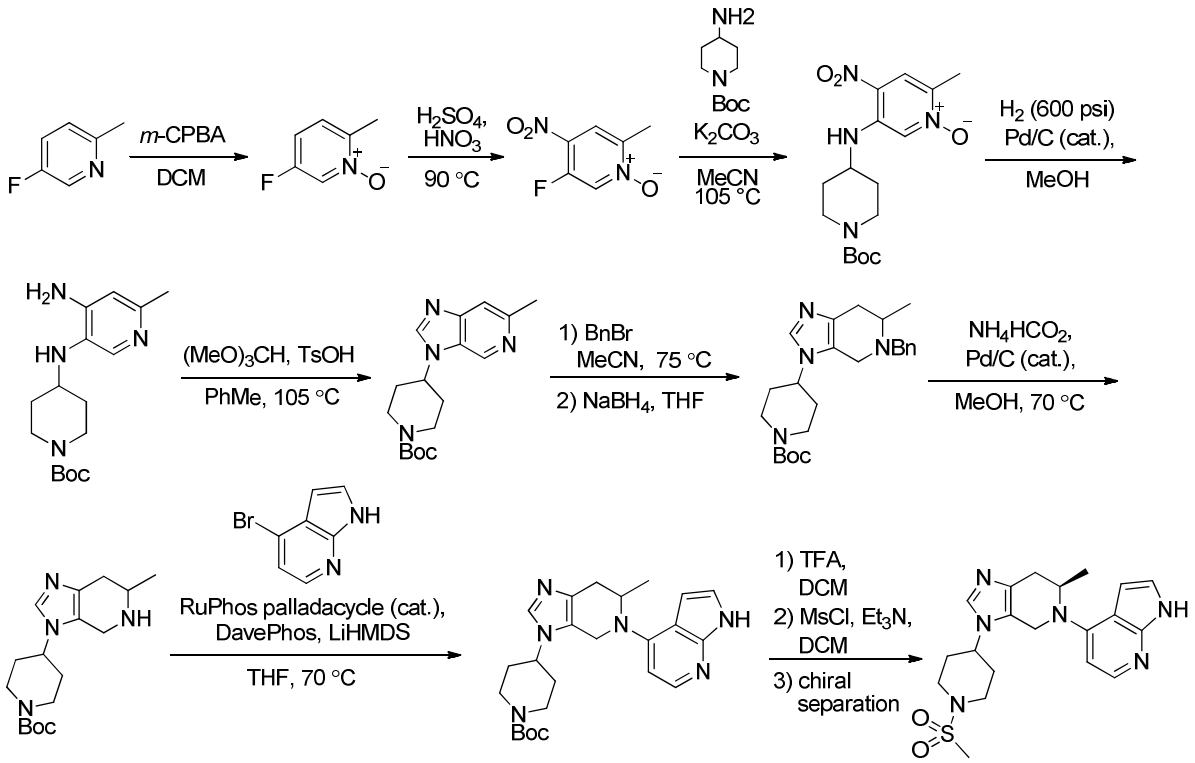
A microwave vial was charged with tert-butyl 4-(R)-6-methyl-4,5,6,7-tetrahydropyrazolo[1,5-a]pyrazin-3-yl)piperidine-1-carboxylate-4,6-d_3 (129 mg, 0.399 mmol), 4-bromo-6-chloro-1H-pyrollo[2,3-b]pyridine (102 mg, 0.439 mmol), RuPhos palladacycle (29.1 mg, 0.040 mmol), and DavePhos (15.7 mg, 0.040 mmol). The vial was sealed, vacuum flushed three times with Ar, and then taken up in THF (1 mL). LiHMDS (1.0 M in THF) (2.79 mL, 2.79 mmol) was added, and then the mixture was heated to 55 °C for 2 h. The reaction mixture was cooled to RT and partitioned between EtOAc and saturated aqueous $NaHCO_3$. The aqueous layer was extracted with more EtOAc, and the combined organics were washed with brine, dried over $MgSO_4$, filtered and concentrated. The residue was purified on a silica gel column (heptane:EtOAc 1:0 to 0:1) to give the product (145 mg, 0.306 mmol, 77 % yield) as a pale yellow oil. LCMS (m/z): 474.3 (M+H+).

(R)-6-methyl-3-(1-(methylsulfonyl)piperidin-4-yl)-5-(6-chloro-1H-pyrrolo[2,3-b]pyridin-4-yl)-4,5,6,7-tetrahydropyrazolo[1,5-a]pyrazine-4,6-d_3 (26):

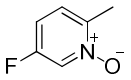


To a stirred solution of tert-butyl 4-((R)-6-methyl-5-(6-chloro-1H-pyrrolo[2,3-b]pyridin-4-yl)-4,5,6,7-tetrahydropyrazolo[1,5-a]pyrazin-3-yl)piperidine-1-carboxylate-4,6-d_3 (144 mg, 0.304 mmol) in DCM (5 mL) at 25 °C was added TFA (0.702 mL, 9.11 mmol) and the mixture was stirred for 1 h. The mixture was concentrated to dryness and then taken up in DCM:MeOH (9:1, 5 mL). Si-carbonate resin (0.8 mmol/g, 1.9 g, 1.52 mmol) was added and the mixture was stirred for 30 min and then filtered and concentrated. The so-obtained crude residue was taken up in DCM (5 mL) at 25 °C; and Et_3N (0.212 mL, 1.52 mmol) and methansulfonyl chloride (0.021 mL, 0.274 mmol) were added. The mixture was allowed to stir for 30 min, and then quenched with a few drops of water and concentrated. The residue was purified by prep HPLC. Product fractions were converted to the corresponding free base by treatment with solid Na_2CO_3 followed by extraction into EtOAc. The organic extracts were washed with brine, dried over $MgSO_4$, filtered and concentrated, and then taken up in CH_3CN/H_2O and lyophilized to give the product (91 mg, 0.199 mmol, 66 % yield) as a white solid. HPLC purity >99%. LCMS (m/z): 452.3 (M+H+). 1H NMR (400 MHz, DMSO-*d*6) δ ppm 1.04 (s, 3 H) 1.53 - 1.69 (m, 2 H) 1.90 (d, *J*=12.13 Hz, 2 H) 2.68 (br. s., 1 H) 2.80 (t, *J*=11.93 Hz, 2 H) 2.88 (s, 3 H) 3.62 (d, *J*=11.74 Hz, 2 H) 4.10 (d, *J*=12.91 Hz, 1 H) 4.39 (d, *J*=12.91 Hz, 1 H) 6.54 (s, 1 H) 6.63 (dd, *J*=3.33, 1.76 Hz, 1 H) 7.27 - 7.32 (m, 1 H) 7.40 (s, 1 H) 11.60 - 11.71 (m, 1 H). ^{13}C NMR (126 MHz, DMSO) δ 150.47, 148.28, 144.81, 136.30, 130.39, 122.75, 119.86, 107.77, 100.39, 100.29, 51.77, 49.51(b), 45.96, 45.92, 40.09, 39.93, 39.76, 39.59, 39.42, 33.91, 32.10, 31.79, 30.18, 14.45.HRMS calculated for $C_{20}H_{23}N_6O_2SClD_3$ 452.1715 Da, measured 452.1714 Da.

F. Synthesis of compound 22

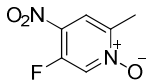


5-fluoro-2-methylpyridine 1-oxide:



To a 0 °C stirred solution of 5-fluoro-2-methylpyridine (2.20 g, 19.80 mmol) in DCM (100 mL) was added m-CPBA (9.76 g, 39.6 mmol) and the mixture was allowed to slowly warm to 25 °C stirred for 48 h. The mixure was quenched with saturated aqueous $Na_2S_2O_3$, stirred vigorously for 15 min, and then poured onto saturated aqueous $NaHCO_3$. The layers were separated, and the aqeous layer was extracted twice more with DCM. The combined organics were dried over $MgSO_4$, filtered and concentrated to the product (2.30 g, 18.09 mmol, 91 % yield) as a crystalline solid which was used without further purification. LCMS (m/z): 128.2 (M+H+). 1H NMR (400 MHz, CHLOROFORM-d) d ppm 2.48 (s, 3 H) 7.00 (br. s., 1 H) 7.22 (s, 1 H) 8.23 (br. s., 1 H)

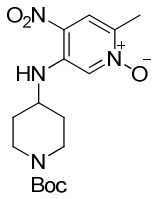
5-fluoro-2-methyl-4-nitropyridine 1-oxide:



Concentrated H_2SO_4 (4 mL) was slowly added to 5-fluoro-2-methylpyridine 1-oxide (1.50 g, 11.80 mmol), maintaning the temperature below 5 °C. A mixture of fuming HNO_3 (2.75 mL, 61.5 mmol)

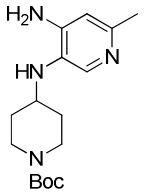
and concentrated H_2SO_4 (4 mL, 75 mmol) was then added dropwise to the mixture, maintaining the same low temperature. After addition was complete, the mixture was heated to 90 °C for 2 h. The mixture was poured slowly onto 100 g of ice and then neutralized with solid NH_4CO_3. The mixture was extracted three times with DCM, and the combined organics were dried over $MgSO_4$, filtered and concentrated to the product (1.82 g, 10.57 mmol, 90 % yield) as a yellow solid which was used without further purification. LCMS (m/z): 173.1 (M+H+). 1H NMR (400 MHz, CHLOROFORM-d) d ppm 2.51 (s, 3 H) 7.98 - 8.08 (m, 1 H) 8.31 (d, J=6.26 Hz, 1 H)

5-(1-(tert-butoxycarbonyl)piperidin-4-ylamino)-2-methyl-4-nitropyridine 1-oxide:



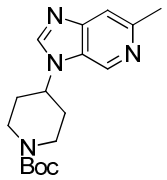
To a stirred solution of 5-fluoro-2-methyl-4-nitropyridine 1-oxide (1.82 g, 10.57 mmol) and 4-amino-1-Boc-piperadine (2.118 g, 10.57 mmol) in acetonitrile (100 mL) was added K_2CO_3 (2.192 g, 15.86 mmol) and the mixture was heated to reflux and stirred overnight. The mixture was cooled and filtered, washing thoroughly with DCM. The resulting solution was concentrated to the product (3.70 g, 10.50 mmol, 99 % yield) of sufficient purity to carry onto the next step without further purification. LCMS (m/z): 353.3 (M+H+). 1H NMR (400 MHz, CHLOROFORM-d) ppm 1.47 (s, 9 H) 1.52 - 1.58 (m, 2 H) 1.99 - 2.10 (m, 2 H) 2.40 (s, 3 H) 2.99 (br. s., 2 H) 3.42 - 3.56 (m, 1 H) 4.04 (br. s., 2 H) 7.64 (d, J=7.43 Hz, 1 H) 8.01 (d, J=6.65 Hz, 2 H)

Tert-butyl 4-(4-amino-6-methylpyridin-3-ylamino)piperidine-1-carboxylate:



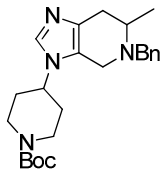
5-(1-(tert-butoxycarbonyl)piperidin-4-ylamino)-2-methyl-4-nitropyridine 1-oxide (3.67 g, 10.41 mmol) and 10% Pd/C (150 mg, 0.141 mmol) were taken up in MeOHl (100 mL) in a steel bomb which was purged three time with H_2 and subsequently pressurized to 250 psi with H_2. The mixture was stirred at 25 °C for 72 hrs. Another addition of 10% Pd/C (50 mg, 0.047 mmol) bomb was further pressurized to 600 psi of H_2 and stirred for 48 hrs more. The mixture was filtered through Celite, washing with MeOH, and concentrated to the product (3.21 g, 10.48 mmol, 101 % yield) as a purple solid which was used without further purification. LCMS (m/z): 307.2 (M+H+). 1H NMR (400 MHz, DMSO-d6) ppm 1.18 (d, J=10.96 Hz, 2 H) 1.30 - 1.42 (m, 9 H) 1.85 (d, J=10.96 Hz, 2 H) 2.12 (s, 3 H) 2.75 - 2.93 (m, 2 H) 3.13 (d, J=2.74 Hz, 1 H) 3.84 (d, J=12.52 Hz, 3 H) 3.97 - 4.02 (m, 1 H) 5.29 - 5.35 (m, 1 H) 6.27 (s, 1 H) 7.48 (s, 1 H)

Tert-butyl 4-(6-methyl-3H-imidazo[4,5-c]pyridin-3-yl)piperidine-1-carboxylate:



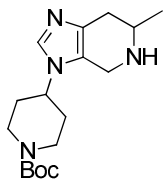
To a stirred mixture of tert-butyl 4-(4-amino-6-methylpyridin-3-ylamino)piperidine-1-carboxylate (3.07 g, 10.02 mmol) and toluene (6 mL) was added trimethyl orthoformate (6.65 mL, 60.1 mmol) and TsOH (0.191 g, 1.002 mmol) and the mixture was heated to 105 °C and stirred for 30 hr. The mixture was cooled to room temperature and partitioned between EtOAc and water. The aqueous layer was extracted twice with more EtOAc and the combined organics were washed with brine, dried over $MgSO_4$ and concentrated to the product (2.67 g, 8.44 mmol, 84 % yield) as a yellow solid which was pure enough to use without further purification. . LCMS (m/z): 317.2 (M+H+). 1H NMR (400 MHz, CHLOROFORM-d) d ppm 1.50 (s, 9 H) 1.96 - 2.11 (m, 2 H) 2.16 - 2.26 (m, 2 H) 2.68 (s, 3 H) 2.90 - 3.03 (m, 2 H) 4.33 - 4.48 (m, 2 H) 7.53 - 7.56 (m, 1 H) 8.02 (s, 1 H) 8.74 - 8.78 (m, 1 H)

Tert-butyl 4-(5-benzyl-6-methyl-4,5-dihydro-3H-imidazo[4,5-c]pyridin-3-yl)piperidine-1-carboxylate:



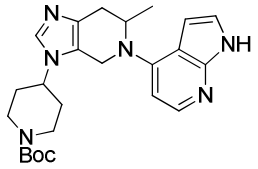
A mixture of tert-butyl 4-(6-methyl-3H-imidazo[4,5-c]pyridin-3-yl)piperidine-1-carboxylate (575 mg, 1.817 mmol) and BnBr (0.238 mL, 2.00 mmol) in acetonitrile (20 mL) was heated to 75 °C and stirred for 4 h. The mixture was cooled to room temperature, concentrated and taken back up in THF (20.00 mL). $NaBH_4$ (275 mg, 7.27 mmol) was slowly added, and the mixture was allowed to stir for 1 h. The mixture was partitioned between saturated aqueous NH_4Cl and EtOAc. The aqueous layer was extracted twice more with EtOAc, and the combined organics were washed with brine , dried over $MgSO_4$, filtered and concentrated. The residue was purified on a silica gel column (heptane:EtOAc 1:0 to 0:1 followed by DCM:MeOH 1:0 to 1:9) to give the product (69 mg, 0.168 mmol, 9 % yield) as a yellow oil. LCMS (m/z): 411.3 (M+H+). 1H NMR (400 MHz, CHLOROFORM-d) d ppm 1.18 (d, J=6.65 Hz, 3 H) 1.41 - 1.51 (m, 9 H) 1.75 (dt, J=12.03, 5.92 Hz, 2 H) 1.94 (d, J=12.52 Hz, 2 H) 2.45 (dd, J=15.85, 4.89 Hz, 1 H) 2.75 (t, J=11.93 Hz, 2 H) 2.86 (dd, J=15.65, 5.09 Hz, 1 H) 3.20 - 3.30 (m, 1 H) 3.56 (d, J=5.48 Hz, 2 H) 3.62 - 3.78 (m, 3 H) 4.23 (br. s., 2 H) 7.23 - 7.39 (m, 5 H) 7.41 (s, 1 H).

Tert-butyl 4-(6-methyl-4,5,6,7-tetrahydro-3H-imidazo[4,5-c]pyridin-3-yl)piperidine-1-carboxylate:



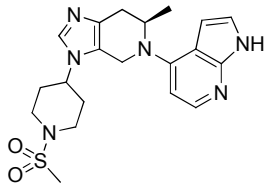
Tert-butyl 4-(5-benzyl-6-methyl-4,5-dihydro-3H-imidazo[4,5-c]pyridin-3-yl)piperidine-1-carboxylate (69 mg, 0.168 mmol), ammonium formate (53.0 mg, 0.840 mmol), and 10% Pd/C (35.8 mg, 0.034 mmol) was taken up in MeOH (2 mL), sealed in a microwave vial, and heated to 70 °C for 1 h. The mixture was filtered through a pad of Celite, washing with EtOAc, and then partitioned between EtOAc and satuated aqueous $NaHCO_3$. The aqueous layer was extracted with more EtOAc, and the combined organics were washed with brine, dried over $MgSO_4$, filtered and concentrated. The crude product (46 mg, 0.144 mmol, 85 % yield) was isolated as a colorless oil which was used without further purification. LCMS (m/z): 321.2 (M+H+).

Tert-butyl 4-(6-methyl-5-(1H-pyrrolo[2,3-b]pyridin-4-yl)-4,5,6,7-tetrahydro-3H-imidazo[4,5-c]pyridin-3-yl)piperidine-1-carboxylate:



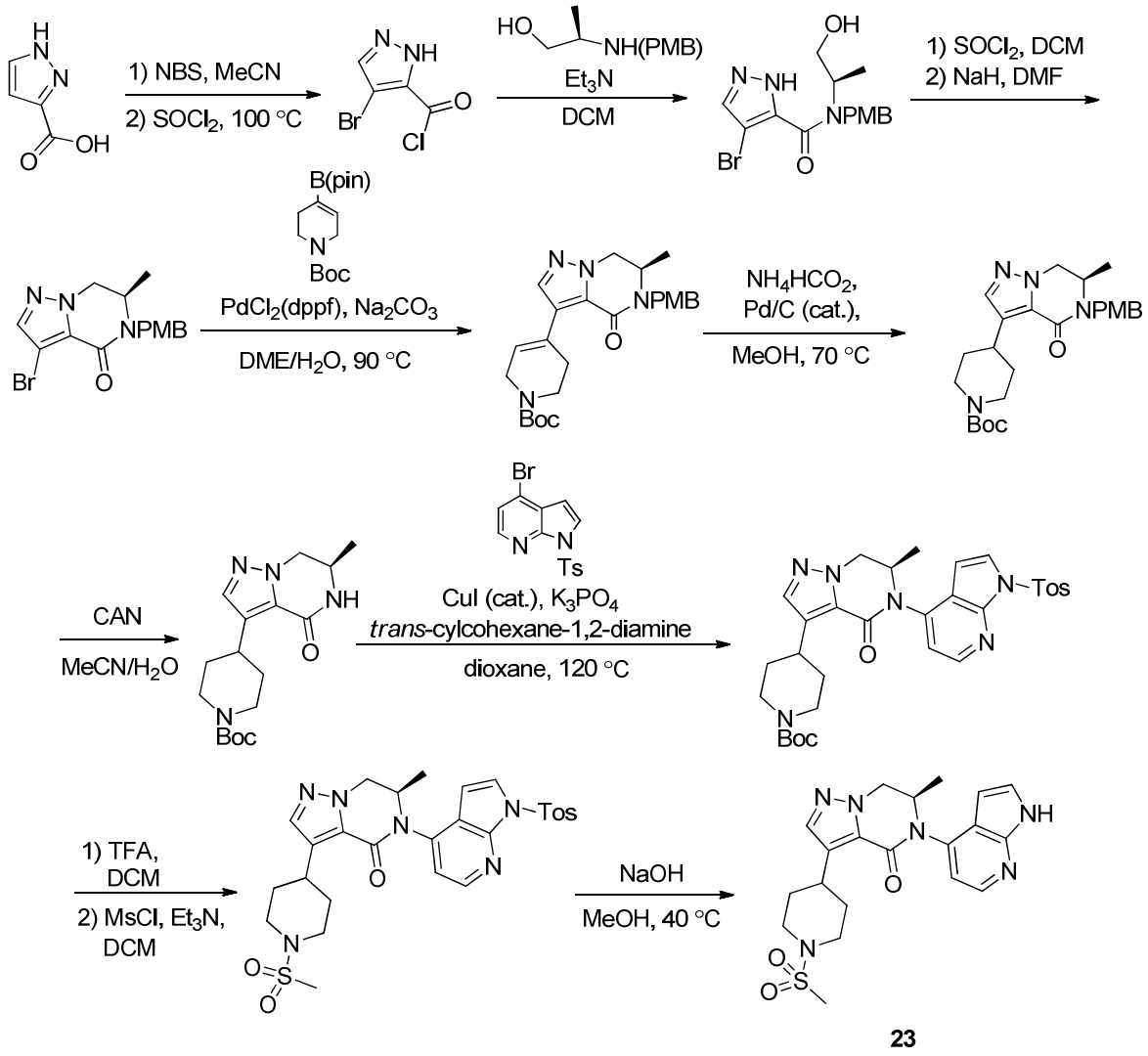
A microwave vial was charged with tert-butyl 4-(6-methyl-4,5,6,7-tetrahydro-3H-imidazo[4,5-c]pyridin-3-yl)piperidine-1-carboxylate (46 mg, 0.144 mmol), 4-bromo-7-azaindole (31.1 mg, 0.158 mmol), RuPhos palladacycle (15.69 mg, 0.022 mmol), and DavePhos [213697-53-1] (8.47 mg, 0.022 mmol). The vial was sealed, vacuumed and flushed three times with Ar, and then taken up in THF (1 mL). LiHMDS (1.0 M in THF) (1.005 mL, 1.005 mmol) was added, and then the mixture was heated to 70 °C for 1 h. The reaction mixture was cooled to RT and partitioned between EtOAc and saturated aqueous $NaHCO_3$. The aqueous layer was extracted with more EtOAc, and the combined organics were washed with brine, dried over $MgSO_4$, filtered and concentrated. The residue was purified on a silica gel column (DCM:MeOH 1:0 to 9:1) to give the product (60 mg, 0.137 mmol, 96 % yield) as a yellow oil. LCMS (m/z): 437.3 (M+H+).

(R)-6-methyl-3-(1-(methylsulfonyl)piperidin-4-yl)-5-(1H-pyrrolo[2,3-b]pyridin-4-yl)-4,5,6,7-tetrahydro-3H-imidazo[4,5-c]pyridine:

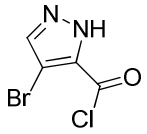


To a stirred solution of tert-butyl 4-(6-methyl-5-(1H-pyrrolo[2,3-b]pyridin-4-yl)-4,5,6,7-tetrahydro-3H-imidazo[4,5-c]pyridin-3-yl)piperidine-1-carboxylate (60 mg, 0.137 mmol) in DCM (2 mL) at 25 °C was added TFA (0.318 mL, 4.12 mmol) and the mixture was stirred for 1 h. The mixture was concentrated to dryness and then taken up in DCM:MeOH (4:1, 2.5 mL). Si-carbonate resin (0.8 mmol/g, 0.85 g, 0.68 mmol) was added and the mixture was stirred for 30 min and then filtered and concentrated. The so-obtained crude residue was taken up in DCM (3 mL) at 25 °C; and Et_3N (0.095 mL, 0.685 mmol) and methansulfonyl chloride (10.68 µL, 0.137 mmol) were added. The mixture was allowed to stir for 30 min, and then quenched with a few drops of water and concentrated. The residue was purified by prep HPLC. Product fractions were converted to the corresponding free base by treatment with solid Na_2CO_3 followed by extraction into EtOAc. The organic extracts were washed with brine, dried over $MgSO_4$, filtered and concentrated, and then taken up in CH_3CN/H_2O and lyophilized to give the racemic product (13.5 mg, 0.033 mmol, 23.77 % yield) as a white solid. The enantiomers were separated using an AD-H column, eluting with 3:2 heptane:EtOH. Both enantiomers were isolated and tested in the ATR biochemical assay; the later-eluting enantiomer was assigned to be the (R)-stereoisomer (4.4 mg, 10.61 µmol, 7.75 % yield) due to it's 100-fold better potency, drawing analogy to similar matched pairs in the THPP series. HPLC purity >99%. LCMS (m/z): 415.2 (M+H+). 1H NMR (400 MHz, DMSO-d6) δ ppm 0.99 (d, J=6.65 Hz, 3 H) 1.84 - 2.03 (m, 2 H) 2.06 (d, J=2.35 Hz, 2 H) 2.44 (br. s., 1 H) 2.84 - 2.96 (m, 5 H) 3.04 - 3.13 (m, 1 H) 3.68 (d, J=11.74 Hz, 2 H) 4.14 (br. s., 1 H) 4.36 (d, J=13.69 Hz, 2 H) 4.80 (t, J=6.26 Hz, 1 H) 6.46 - 6.49 (m, 1 H) 6.51 (d, J=5.48 Hz, 1 H) 7.21 - 7.25 (m, 1 H) 7.81 (br. s., 1 H) 7.95 (d, J=5.48 Hz, 1 H) 11.41 (br. s., 1 H).

G. Synthesis of compound 23

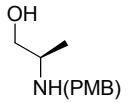


4-bromo-1H-pyrazole-5-carbonyl chloride:



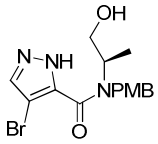
To a stirred solution of 1H-pyrazole-3-carboxylic acid (1260 mg, 11.24 mmol) in acetonitrile (50 mL) was added NBS (2001 mg, 11.24 mmol) at RT, and the reaction mixture was stirred overnight. The resulting mixture was diluted with EtOAc, washed with brine, dried over $MgSO_4$, filtered, and concentrated. A mixture of the so-obtained crude product and thionyl chloride (1.5 mL, 20.55 mmol) was sealed and stirred at 100 °C for 3 h and then cooled to RT. The mixture was concentrated and dried under high vacumm. The crude product (603 mg, 2.88 mmol, 100 % yield) was used without further purification.

(R)-2-(4-methoxybenzylamino)propan-1-ol :



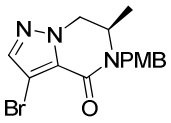
To a solution of (D)-alaninol (9.59 g, 128 mmol) in THF (15 mL) at RT was added dropwise 1-(chloromethyl)-4-methoxybenzene (2 g, 12.77 mmol) and the mixture was stirred for 48 h. The solution was diluted with EtOAc, washed five times with water, dried over $MgSO_4$, filtered, and concentrated. The crude product (1.8 g, 9.22 mmol, 72.2 % yield) was used without further purification. LCMS (m/z): 196.1 (M+H+).

(R)-4-bromo-N-(1-hydroxypropan-2-yl)-N-(4-methoxybenzyl)-1H-pyrazole-5-carboxamide:



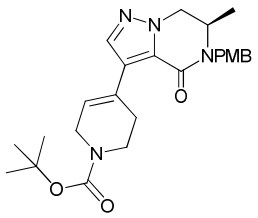
To a solution of (R)-2-(4-methoxybenzylamino)propan-1-ol (503 mg, 2.58 mmol) and Et_3N (2.396 mL, 17.19 mmol) in DCM (7 mL) at RT was added dropwise a solution of 4-bromo-1H-pyrazole-5-carbonyl chloride (450 mg, 2.149 mmol) in of DCM (5 mL), and the mixture was stirred for 2 h. The reaction mixture was concentrated, and the residue was purified on a silica gel column (heptane:EtOAc 4:1 to 0:1) to provide the product (514 mg, 1.397 mmol, 65 % yield). LCMS (m/z): 368.1/370.1 (M+H+).

(R)-3-bromo-5-(4-methoxybenzyl)-6-methyl-6,7-dihydropyrazolo[1,5-a]pyrazin-4(5H)-one:



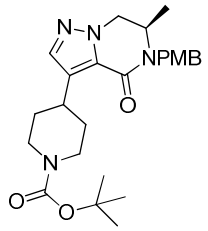
To a mixture of (R)-4-bromo-N-(1-hydroxypropan-2-yl)-N-(4-methoxybenzyl)-1H-pyrazole-5-carboxamide (500 mg, 1.358 mmol) in DCM (10 mL) ar RT was added $SOCl_2$ (0.991 ml, 13.58 mmol) dropwise, and the mixture was stirred for 3 h. The reaction mixture was concentrated and dried under high vacuum. The so-obtained crude product was taken up in DMF (10 mL) ar RT and NaH (60% in mineral oil, 750 mg, 31.3 mmol) was added. The mixture was stirred for 3 h and then diluted with EtOAc and poured onto brine. The organic layer was separated, washed with water and brine, dried over $MgSO_4$, filtered and concentrated. The residue was purified on a silica gel column (heptane:EtOAc 1:0 to 1:1). Isolated the product (210 mg, 0.600 mmol, 48.0 % yield). LCMS (m/z): 350.0/351.9 (M+H+).

(R)-tert-butyl 4-(5-(4-methoxybenzyl)-6-methyl-4-oxo-4,5,6,7-tetrahydropyrazolo[1,5-a]pyrazin-3-yl)-5,6-dihydropyridine-1(2H)-carboxylate:



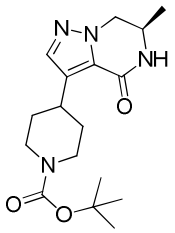
(R)-3-bromo-5-(4-methoxybenzyl)-6-methyl-6,7-dihydropyrazolo[1,5-a]pyrazin-4(5H)-one (115 mg, 0.328 mmol), tert-butyl 4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-5,6-dihydropyridine-1(2H)-carboxylate (132 mg, 0.427 mmol), $PdCl_2(dppf)\cdot CH_2Cl_2$ (26.8 mg, 0.033 mmol) and 2 M aq. sodium carbonate (0.492 mL, 0.985 mmol) were mixed in DME (3 mL) in a microwave tube. The mixture was purged with argon for 15 min. The tube was sealed and heated to 90 ºC for 5 h. The reaction mixture was poured onto water and extracted three times with EtOAc. The combined organics were washed with brine, dried over $MgSO_4$, filtered and concentrated. The residue was purified on a silica gel column (heptane:EtOAc 1:0 to 2:3) to give the product (86 mg, 0.190 mmol, 58 % yield) as a colorless oil. LCMS (m/z): 453.3 (M+H+).

(R)-tert-butyl 4-(5-(4-methoxybenzyl)-6-methyl-4-oxo-4,5,6,7-tetrahydropyrazolo[1,5-a]pyrazin-3-yl)piperidine-1-carboxylate:



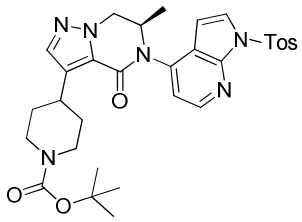
(R)-tert-butyl 4-(5-(4-methoxybenzyl)-6-methyl-4-oxo-4,5,6,7-tetrahydropyrazolo[1,5-a]pyrazin-3-yl)-5,6-dihydropyridine-1(2H)-carboxylate (150 mg, 0.331 mmol), ammonium formate (84 mg, 1.326 mmol), and 10% Pd/C (70.5 mg, 0.663 mmol) were taken up in MeOH (2 mL), sealed in a microwave vial, and heated to 72 °C for 1 h. The mixture was filtered through a pad of Celite, washing with EtOAc, and then partitioned between EtOAc and satuated aqueous $NaHCO_3$. The aqeous layer was extracted with more EtOAc, and the combined organics were washed with brine, dried over $MgSO_4$, filtered and concentrated. The crude product (140 mg, 0.308 mmol, 93 % yield) was used without further purification. LCMS (m/z): 455.4 (M+H+).

(R)-tert-butyl 4-(6-methyl-4-oxo-4,5,6,7-tetrahydropyrazolo[1,5-a]pyrazin-3-yl)piperidine-1-carboxylate:



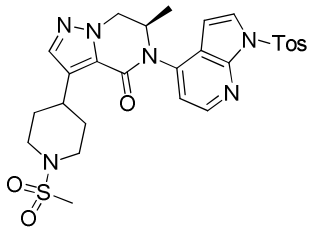
To a mixture of (R)-tert-butyl 4-(5-(4-methoxybenzyl)-6-methyl-4-oxo-4,5,6,7-tetrahydropyrazolo[1,5-a]pyrazin-3-yl)piperidine-1-carboxylate (140 mg, 0.308 mmol) in CH_3CN (7 ml) and H_2O (2.5 ml) at RT was added CAN (675 mg, 1.232 mmol), and the mixture was stirred for 40 min. The reaction was partitioned between water and EtOAc; the organic layer was washed with saturated aqueous $NaHCO_3$ and brine, and then dried over $MgSO_4$, filtered, and concentrated. The crude product was used without further purification. LCMS (m/z): 335.2 (M+H+).

(R)-tert-butyl 4-(6-methyl-4-oxo-5-(1-tosyl-1H-pyrrolo[2,3-b]pyridin-4-yl)-4,5,6,7-tetrahydropyrazolo[1,5-a]pyrazin-3-yl)piperidine-1-carboxylate:



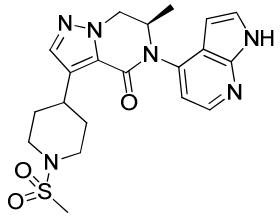
An oven-dried microwave vial was charged with (R)-tert-butyl 4-(6-methyl-4-oxo-4,5,6,7-tetrahydropyrazolo[1,5-a]pyrazin-3-yl)piperidine-1-carboxylate (90 mg, 0.269 mmol), 4-bromo-1-tosyl-1H-pyrrolo[2,3-b]pyridine (142 mg, 0.404 mmol), CuI (20.50 mg, 0.108 mmol) and K_3PO_4 (229 mg, 1.077 mmol). The flask was purged and back-filled with argon. 1,4-dioxane (3 ml) and trans-cyclohexane-1,2-diamine (21.51 mg, 0.188) were added, and the suspension was heated to 120 °C overnight. After cooling to RT, the mixture was diluted with H_2O and EtOAc. The layers were separated, and the aqueous layer was extracted twice with EtOAc. The combined organic layers were dried over Na_2SO_4, filtered, and concentrated. The residue was purified on a silica gel column (heptane:EtOAc 1:0 to 1:3) to give the product (110 mg, 0.182 mmol, 67.6 % yield). LCMS (m/z): 605.4 (M+H+).

(R)-6-methyl-3-(1-(methylsulfonyl)piperidin-4-yl)-5-(1-tosyl-1H-pyrrolo[2,3-b]pyridin-4-yl)-6,7-dihydropyrazolo[1,5-a]pyrazin-4(5H)-one:



To a stirred solution of (R)-tert-butyl 4-(6-methyl-4-oxo-5-(1-tosyl-1H-pyrrolo[2,3-b]pyridin-4-yl)-4,5,6,7-tetrahydropyrazolo[1,5-a]pyrazin-3-yl)piperidine-1-carboxylate (110 mg, 0.182 mmol) in DCM (2 mL) at 25 °C was added TFA (400 µL, 5.19 mmol) and the mixture was stirred for 1 h. The mixture was concentrated to dryness and then taken up in DCM:MeOH (10:1, 10 mL). Si-carbonate resin (0.8 mmol/g, 800 mg, 0.640 mmol) was added and the mixture was stirred for 30 min and then filtered and concentrated. The so-obtained crude residue was taken up in DCM (5 mL) at 25 °C and Et_3N (0.110 ml, 0.793 mmol) and methanesulfonyl chloride (32.7 mg, 0.285 mmol) were added. The mixture was allowed to stir for 1 h, and then concentrated. The residue was purified on a silica gel column (heptane:EtOAc 1:0 to 1:1) to give the product (50 mg, 0.086 mmol, 54.1 % yield). LCMS (m/z): 583.2 (M+H+).

(R)-6-methyl-3-(1-(methylsulfonyl)piperidin-4-yl)-5-(1H-pyrrolo[2,3-b]pyridin-4-yl)-6,7-dihydropyrazolo[1,5-a]pyrazin-4(5H)-one (23)



To a solution of (R)-6-methyl-3-(1-(methylsulfonyl)piperidin-4-yl)-5-(1-tosyl-1H-pyrrolo[2,3-b]pyridin-4-yl)-6,7-dihydropyrazolo[1,5-a]pyrazin-4(5H)-one (50 mg, 0.086 mmol) in MeOH (3 mL) at RT was added 10 M aq. NaOH (0.3 mL, 3.00 mmol), and the mixture was heated to 40 ºC for 2 h. The mixture was poured into a mixture of EtOAc and saturated aqueous $NaHCO_3$. The organic layer was separated, and the aqueous layer was extracted two times with EtOAc. The combined organics were, dried over $MgSO_4$, filtered and concentrated. The residue was purified by prep HPLC to provide the product as the corresponding TFA salt (15 mg, 0.035 mmol, 40.8 % yield). HPLC purity >99%. LCMS (m/z): 429.2 (M+H+). ^{1}H NMR (500 MHz, DMSO-d_6) δ 1.16 (d, J = 6.6 Hz, 3H), 1.50 – 1.82 (m, 2H), 1.96 (dd, J = 13.2, 7.7 Hz, 2H), 2.76 (tdd, J = 12.0, 7.0, 2.6 Hz, 2H), 2.86 (s, 3H), 3.14 (ddd, J = 12.0, 8.4, 3.6 Hz, 1H), 3.55 – 3.74 (m, 2H), 4.39 (dd, J = 13.2, 3.6 Hz, 1H), 4.53 (dt, J = 6.7, 4.0 Hz, 1H), 4.84 (dd, J = 13.2, 4.4 Hz, 1H), 6.45 (dd, J = 3.5, 1.8 Hz, 1H), 7.18 (d, J = 5.3 Hz, 1H), 7.55 (dd, J = 3.5, 2.4 Hz, 1H), 7.64 (s, 1H), 8.32 (d, J = 5.3 Hz, 1H), 12.00 (s, 1H). ^{13}C NMR (126 MHz, DMSO) δ 156.38, 148.94, 141.84, 140.53, 137.07, 129.01, 128.00, 126.91, 117.67, 114.31, 98.96, 54.29, 51.58, 45.91, 33.90, 31.60, 31.46, 30.97, 17.74. HRMS calculated for $C_{20}H_{25}N_6O_3S$ 429.1709 Da, measured 429.1719 Da.

II. Supporting Information: Protein expression and purification

H. ATR-Atrip complex expression and purification

Full length native human ATR coding sequence with a N-terminal FLAG tag (MAHNHRHKHADYKDDDDKA) was cloned into pcDNA4/TO vector (Invitrogen) and full length human ATRIP with a N-myc tag was cloned into pTriEx-3 vector (Novagen) under CMV promoter control. These two plasmids were used at 1:1 ratio for transient co-transfection into mammalian 293 FreeStyle (FS) cells (Invitrogen #K9000-01) according to manufacture recommendation and cells were harvested 72 hours post transfection.
Cells from 200 mL mammalian 293FS culture overexpressed with ATR-Atrip were mixed to homogeneous in five mL ice cold lysis buffer (20 mM Tris-HCl, pH 7.5 at RT, 137 mM NaCl, 10% glycerol, 1 mM DTT, 1% (v/v) Tween-20, 0.1% (v/v) NP-40, phosphatase inhibitor cocktail tablets (Roche), complete protease inhibitor cocktail tablets (Roche) and benzonase (EMD)). Lysate was incubated on a rotating platform for 30 minutes at 4°C. This lysate was then clarified by centrifugation and the supernatant was mixed with equilibrated 400 µL of anti-FLAG M2 affinity gel (Sigma-Aldrich catalog #A2220). Clear lysate and anti-FLAG M2 affinity gel were then rotated on a rotating platform for two hours at 4°C to ensure overexpressed FLAG-tagged ATR bound to anti-FLAG M2 affinity gel. Supernatant was removed by centrifugation after binding for 2 hours, and anti-FLAG M2 affinity gel was washed three times, 10 CV each time, with wash buffer (20 mM Tris-HCl, pH 7.5 at RT, 137 mM NaCl and 10% glycerol). Bound proteins, containing ATR-Atrip complex, were eluted with elution buffer (20 mM Tris-HCl, pH 7.5 at RT, 137 mM NaCl and 10% glycerol, 200 ug/ml of 3x FLAG peptide (Sigma-Aldrich catalog #F4799) and 1 mM DTT). Elution fractions were collected as 2 CV for four fractions. All fractions were analyzed by SDS-PAGE (4-12% Tris-Glycine gel, with HiMark Pre-Stained HMW Protein Standard (Invitrogen catalog #LC5699)) with Coomassie stain. ATR-Atrip containing fractions were pooled and protein concentration was measured by Bradford Protein Assay using BSA as standard. Activity of this purified ATR-Atrip complex was measured in biochemical assay.

I. TopBP1 expression and purification

TopBP1 protein used in the ATR biochemical assay is a full length human TopBP1 construct with N-terminal 6His tag and TEV protease cleavage site followed a FLAG tag before the first Ser in TopBP1 gene. This was cloned into pDEST8 insect expression vector (Invitrogen) and protein was expressed from baculovirus infected Sf9 insect cells. Cells were harvested 48 hours post infection by low speed centrifugation. Cell pellet from 2 L insect cell expression was thawed and re-suspended in 100ml of chillled base buffer (50mM Tris HCl @pH7.5 RT, 300mM NaCl, 5% glycerol, 1mM TCEP) that also contained 10mM imidazole, 1% Tween 20, 1X Complete protease inhibitor cocktail (Roche), benzonase (EMD), 50 µg/ml E-64 and 50 µg/ml leupeptin. Cells were lysed by douncing 10 strokes on ice using a hand homogenizer. The lysate is then cleared by centrifugation at 40,000 x g for 40 minutes at 4°C. The clear lysate is uploaded to a pre-equilibrated 5ml prepack Ni-NTA column (GE Healthcare) in base buffer containing 10mM imidazole (50mM Tris HCl @pH7.5 RT, 300mM NaCl, 5% glycerol, 1mM TCEP, 10mM imidazole). The column was washed to baseline with IMAC buffer and then step elute with base buffer containing 100mM imidazole. Fractions containing TopBP1 were pooled based on SDS-PAGE gel and overnight TEV protease cleavage was set up at 4°C in a 10kDa MW cutoff dialysis cassette (Pierce) against base buffer. Uncleaved protein and TEV protease (N-terminal His6 tagged TEV produced in house)

were removed by flowing through the same 5ml prepack Ni-NTA column that was re-equilibrated in base buffer containing 10mM imidazole. Flowthrough sample was collected and concentrated using Centricon 50 and then injected onto size exclusion column superdex 200 16/60 (GE Healthcare) that was pre-equilibrated with the same base buffer. TopBP1 protein elutes as a monomer so fractions that elute at ~60 ml were pooled, concentrated, stored as small aliquots at -80°C after flash frozen in liquid N_2. Protein concentration was determined using Coomassie Plus Protein Assay Reagent (Pierce) with BSA as standard. The purified protein was >90% pure as shown via SDS-PAGE, identification confirmed by both western blot analysis using TopBP1 polyclonal antibody (Santa Cruz Bio) and by LC-MS/MS analysis following Trypsin digestion.

J. ATM expression and purification

The ATM cDNA was obtained from Origen (Origene SC300001). The ATM gene was PCR amplified using two sets of primers: KAC1993 + KAC1998 to generate a 5' gene fragment and KAC1997 + KAC1996 to generate a 3' gene fragment. The PCR product from KAC1993+1998 was digested with SalI and SpeI the PCR product from KAC1997+1996 was digested with SpeI and XhoI. Both PCR fragments were ligated into pENTR1A previously digested with SalI and XhoI. The resulting product was designated Orf 5780 (3111-1); full-length ATM Y54C with an amino-terminal Flag tag in a gateway Entry vector. The Y54C mutation was determined to be in the original Origen clone.

In order to repair the Y54C mutation a 2201bp synthetic fragment was synthesized by Genewiz (ATM_nFlag_OptHs). The synthetic fragment was designed to repair the mutation and to use mammalian optimized codons. The synthetic fragment was digested with SalI and BmgBI. The synthetic fragment was combined with two Orf5780 fragments; one fragment digested NcoI and SalI and the other fragment digested BmgBI and NcoI. A three-part ligation was performed that resulted in the generation of Orf 6533 (3241-6); nFlag-ATM_Opt wt in pENTR1A. Orf 6533 was recombined with several destination vectors to generate the various final expression vectors. One important protein expression construct was LR6557 pCMVII: nFlag-ATM_Opt wt.

ATM protein was produced from LR6557 in HEK293FreeStyle™ cells using a 293Fectin™ protocol. The manufactures recommended protocol was employed with the following modification. The standard 293Fectin™ protocol for 1 liter production requires 1mg of plasmid DNA and 2mL of 293Fectin™. Due to the large size of the ATM gene the protocol was modified to use 3xDNA and 2x293Fectin™. So, for a 1 liter production, 3 mg of plasmid DNA and 4 mL of 293Fectin™ reagent were used. The HEK293FreeStyle™ transfected cells were harvested 72 hours post-transfection.

Cells from 200 mL mammalian HEK293FreeStyle™ culture overexpressing ATM were mixed to homogeneous in five mL ice cold lysis buffer (20 mM Tris-HCl, pH 7.5 at RT, 137 mM NaCl, 10% glycerol, 1 mM DTT, 1% (v/v) Tween-20, 0.1% (v/v) NP-40, phosphatase inhibitor cocktail tablets (Roche), complete protease inhibitor cocktail tablets (Roche) and benzonase (EMD). Lysate was incubated on a rotating platform for 30 minutes at 4°C. This lysate was then clarified by centrifugation and the supernatant was mixed with equilibrated 400 µL of anti-FLAG M2 affinity gel (Sigma-Aldrich catalog #A2220). Clear lysate and anti-FLAG M2 affinity gel were then rotated on a rotating platform for two hours at 4°C to ensure overexpressed FLAG-tagged ATM bound to anti-FLAG M2 affinity gel. Supernatant was removed by centrifugation after binding for 2 hours, and anti-FLAG M2 affinity gel was washed three times, 10 CV each time, with wash buffer (20 mM Tris-HCl, pH 7.5 at RT, 137 mM NaCl and 10% glycerol). Bound proteins, containing ATM, were eluted with elution buffer (20 mM Tris-HCl, pH 7.5 at RT, 137 mM NaCl and 10% glycerol, 200 ug/ml of 3x FLAG peptide (Sigma-Aldrich catalog #F4799) and 1 mM DTT). Elution fractions were collected as 2 CV for four fractions. All fractions were analyzed by SDS-PAGE (4-12% Tris-Glycine gel, with HiMark Pre-Stained HMW Protein Standard (Invitrogen

catalog #LC5699) with Coomassie stain. ATM containing fractions were pooled and protein concentration was measured by Bradford Protein Assay using BSA as standard. Activity of this purified ATM was measured in biochemical assay.

K. CHK1 kinase dead construct expression and purification

CHK1 protein substrate is a kinase dead construct of full length Chk1-D148A with both a N-terminal 6His followed by TEV protease cleavage site and a C-terminal Avi tag cloned into pDEST8 insect expression vector (Invitrogen). Protein was co-expressed with biotin ligase BirA as baculovirus infected Sf9 insect cells supplemented with 50 μM Biotin. Cells were harvested 48 hours post infection by low speed centrifugation.

Cell pellet from 5 L insect cell expression was thawed and re-suspended in 250ml of chillled lysis buffer (50mM Tris HCl @pH7.5 RT, 300mM NaCl, 5% glycerol, 10mM imidazole, 1mM TCEP, 1% Tween 20, 1X Complete protease inhibitor cocktail (Roche), benzonase, 50 μg/ml E-64 and 50 μg/ml leupeptin). Cells were lysed by douncing 10 strokes on ice using a hand homogenizer. The lysate is then cleared by centrifugation at 40,000 x g for 40 minutes at 4°C. The clear lysate is uploaded to a pre-equilibrated 5ml prepack Ni-NTA column (GE Healthcare) in lysis buffer. The column was washed to baseline with buffer containing 25mM imidazole and then step elute with buffer containing 200mM imidazole. Fractions containing Chk1 were pooled based on SDS-PAGE gel, concentrated to >2 mg/ml using Centricon 30 and then injected onto size exclusion column superdex 75 26/60 (GE Healthcare) that was pre-equilibrated with 50mM Tris pH7.5 RT, 300mM NaCl, 5% glycerol, 1mM TCEP. His6-CHK1-D148A-Avi protein elutes as a monomer so fractions that elute at ~158 ml were pooled, concentrated, stored as small aliquots at -80°C after flash frozen in liquid N_2. Protein concentration was determined using Coomassie Plus Protein Assay Reagent (Pierce) with BSA as standard. The purified protein was >95% pure as shown via SDS-PAGE, confirmed by western blot analysis using specific CHK1 monoclonal antibody (Sigma Aldrich C9358) and characterized by mass spectroscopy analysis to be 100% biotinylated.

III. Supporting Information: Biological assays

L. In vitro assay of ATR inhibition

The biochemical ATR assays are carried out in 50mM Hepes, pH7.5, 625uM MgCl2, 0.05% Bovine Serum Albumin, 0.02% Tween-20, 1mM Dithiolthreitol, 2.5% Dimethyl Sulfoxide. Stop and detection steps are combined using 50mM Tris-HCl, pH7.0, 120mM EDTA, 0.01% Tween-20. In the reaction, the final concentration of ATR-ATRIP is 62.5pM; TopBP-1 is 1nM; Chk1 D148A Avi-tagged protein substrate is 2.5nM; ATP is 5uM. The ATP substrate (Adenosine-5'-triphosphate) is purchased from Roche Diagnostics. Detection format is AlphaScreen (PerkinElmer). Phospho-Chk1(Ser345) substrate antibody is purchased from Cell Signaling Technology. The final dilution of antibody is 1:1000. The Alpha Screen Protein A detection kit containing donor and acceptor beads is purchased from PerkinElmer Life Sciences. The final concentration of both donor and acceptor beads is 10ug/ml.
General protocol is as follows: 2X ATR.ATRIP and 2X TopBP1 are pre-incubated for 30 minutes. 5ul of 2X ATR.ATRIP.TopBP1 is added to 0.25ul of test compound in dimethyl sulfoxide and allowed to incubate for 60 minutes. 2X Chk1 D148A Avi-tagged protein substrate and 2X ATP are mixed. 5ul of the Chk1 D148A Avi-tagged protein substrate /ATP mix is added to start the

reaction. The reaction is allowed to proceed for 3hrs. 10uL of Antibody / Alpha Screen beads/Stop-detection buffer is added. Care is taken to keep Alpha Screen beads in the dark at all times. Plates are incubated at room temperature overnight, in the dark, to allow for detection development before being read. The assay is run is a 384-well format using white polypropylene Greiner plates.

M. In vitro assay of ATM inhibition

The biochemical ATM assays are carried out in 50mM Hepes, pH7.5, 10mM MgCl2, 5mM MnCl2, 50mM NaCl, 0.05% Bovine Serum Albumin, 0.01% Tween-20, 1mM Dithiolthreitol, and 2.5% Dimethyl Sulfoxide. Stop and detection steps are combined using 50mM Tris-HCl, pH7.0, 120mM EDTA, 0.01% Tween-20. In the reaction, the final concentration of ATM wild type full length protein is 250pM; substrate peptide p53-Q10-K17 (biotin-GGGGSQEPPLSQKTFSD-NH2) is 200nM ; ATP is 5uM. The ATP substrate (Adenosine-5'-triphosphate) is purchased from Roche Diagnostics. Detection format is AlphaScreen (PerkinElmer). Peptide substrate is made by Tufts University. The detection antibody, phospho-p53 (Ser15), was purchased from Cell Signaling Technology Cat #9284.The final dilution of antibody is 1:500. The Alpha Screen Protein A detection kit containing donor and acceptor beads is purchased from PerkinElmer Life Sciences Cat. #6760617R. The final concentration of both donor and acceptor beads is 20ug/ml. General protocol is as follows: 5ul of 2X ATM is added to 0.25ul of test compound in dimethyl sulfoxide and allowed to incubate for 30 minutes. 2X peptide substrate and 2X ATP are mixed. 5ul of the peptide substrate /ATP mix is added to start the reaction. The reaction is allowed to proceed for 210 min. 10uL of Antibody / Alpha Screen beads/Stop-detection buffer is added. Care is taken to keep Alpha Screen beads in the dark at all times. Plates are incubated at room temperature overnight, in the dark, to allow for detection development before being read. The assay is run is a 384-well format flat bottom small volume white polypropylene Greiner (Cat.#784075) plates.

N. In vitro assay of DNA-PK inhibition

The biochemical DNA-PK assays are carried out in 50 mM HEPES pH 7.5, 10mM MgCl2, 50mM KCl, 10% glycerol, 1mM DTT, 0.01% Tween-20, 0.05% BSA. Incubate DNA-PK (Life Technologies Cat# PV5865, MW 600 Kd) with calf thymus DNA (CT-DNA, Invitrogen Cat# PR9141A) in 384-well plates (Greiner Bio-One Cat# 784075) at room temperature for 90 minutes. Add 5 µL of DNA-PK (final concentration 0.1 nM) /CT-DNA (final concentration 0.63 µg/mL) mixture into each well containing compounds in 0.5 µL of DMSO. Centrifuge plates at 1000 rpm for one minute and incubate at room temperature for another 30 minutes. Add 5 µL of ATP (final concentration 20 µM) and CK1D peptide substrate (final concentration 0.2 µM, GGGGMEEPQSDPSVEPPLSQETFSDLWKLLPE) into each well. Centrifuge plates at 1000 rpm for one minute and incubate at room temperature for another 60 minutes. Stop reaction by adding 10 µL of AlphaScreen detection mixture (20 µg/mL AlphaScreen Protein A Beads, PerkinElmer AlphaScreen Protein A Detection Kit Cat# 6760617R, and 40 ng/ mL Phospho-p53 (Ser15) Antibody, Cell Signaling Cat#9284 phospho-p53 (Ser15) antibody, in 95 mM Tris pH 7.5, 25mM EDTA, 0.01% Tween-20). Seal and keep plates in dark overnight. Read the plates on Envision Multilabel Reader (PerkinElmer 2101 Multilabel Reader, Ex 630 nm Em 520 nm).

O. Cellular CHK1 target modulation assay

HeLa S3 cells were grown in AIM-V AlbuMAX (Gibco) cell culture medium as a suspension culture in spinner flasks. HeLa S3 cells (15,000) were treated with 1 uM Gemzar (Eli Lilly) + test compound for 4 hrs. at 37 ^{o}C., 5% CO_2. Samples were lysed and processed using the AlphaScreen SureFire CHK1 (p-Ser345) Assay Kit as recommended by the manufacturer (Perkin Elmer).

P. Celluar mTOR inhibition assay

Subconfluent TSC1 -/- MEF cells were harvested by trypsinization. The cells were added in growth medium (4,000 cells) into wells of a 384-well plate and cultured overnight at 37 ^{o}C., 5% CO_2. Test compounds were added to the cells for 1 hr at 37 ^{o}C., 5% CO_2. Samples were lysed, frozen for 15 min. at -80 ^{o}C., and thawed by shaking at room temperature. Samples were further processed using the AlphaScreen SureFire p70 S6K (p-Thr389) Assay Kit as recommended by the manufacturer (Perkin Elmer).

Q. Time dependent CYP3A4 inactivation assay

Test compound (50 μM with serial dilutions) is incubated with human liver microsome (0.5 mg/mL) in phosphate buffer (100 mM) with NADPH (1 mM) for 0, 5, 15, and 30 minutes. The incubation mixture is then diluted 20 times and incubated with CYP3A4 substrate midazolam (20 μM) to determine residual CYP3A4 enzyme activity. The enzyme activity vs. incubation time is plotted to obtain kinetic parameters for TDI.

R. GSH trapping assay

Compound (10 μM) was incubated in human liver microsomes (1 mg/mL) fortified with KH2PO4 (0.1 M; pH: 7.4) buffer, MgCl2 (3 mM) and GSH and stable label GSH (4 mM; 50:50). The incubate mixture was pre-warmed for 5 minutes at 37 oC and the reaction was started with adding NADPH (2 mM). The reaction was stopped at 60 minutes by adding 1 fold cold acetonitrile. The incubate/acetonitrile mixture was centrifuged at 4000 RPM for 10 minutes and the supernatants were transferred and concentrated under gentle stream of N2 gas before LC/MS/MS analysis.

S. KCN trapping assay

Compound (20 μM) was incubated in rat liver microsomes (1 mg/mL) fortified with KH2PO4 (0.1 M; pH: 7.4) buffer, MgCl2 (5 mM) and KCN and stable label K13C15N (1 mM; 70:30). The incubate mixture was pre-warmed for 3 minutes at 37 oC and the reaction was started with adding NADPH (2 mM). A 300 μL aliquots from incubate were taken at 0, 30 and 60 minutes and added to 300 μL of acetonitrile. The incubate/acetonitrile mixture was centrifuged at 10000 RPM for 5 minutes and the supernatants were transferred and concentrated under gentle stream of N2 gas before LC/MS/MS analysis.

IV. Supporting Information: Physico-chemical property measurements

T. Miniaturized shake flask solubility assay

Test compounds are used as 10 mM DMSO stock solutions. 20 µl of 10mM DMSO stock solution is transferred into a 96 deep well plate labeled as buffer plate and 5 µl is transferred to another plate labeled as compound standard plate. The buffer plate is placed in a Multi-Tainer MT-4 container (FTS Systems) and lyophilized overnight to remove DMSO. 100 µl of PBS (pH 7.0) is added to the dried compound in the buffer plate and 95 µl of DMSO is added to the standard plate. The buffer plate is sonicated in a water bath for 10 min. The two plates are then placed onto a VWR orbital shaker to equilibrate for 24 hours at room temperature. The buffer plate is centrifuged at 4000 rpm for 30 min. 10 µl aliquots of supernatant from the buffer plate are transferred to a sample plate and dilute 5 fold. Both compound standard and sample are injected into the UPLC/UV/CLND/MS to generate multi detector qualitative and quantitive analytical data. Data is processed with Xcalibur , CLND equimolar response used for measuring compound concentration of DMSO solution and UV270 nm or MS relative ratio for solubility determination.

U. Direct logD assay

10 µl of 10 mM DMSO stock solutions of test compounds are placed in each well of a 96 well plate. 10 µl of 2 mM Halodipine (logD = 3.00) is added to each well as an internal standard and mixed. The DMSO is removed using a lyophilizer (overnight). 250 µl of water (PBS pH = 7.4)-saturated octanol is added to each well. 250 µl of octanol-saturated water (PBS pH = 7.4) is added to each well and plate is vortexed overnight. 10 µl of the octanol phase is removed, diluted 1:100 with DMSO and 1 ul is injected into a LC/UV/qTOF (RP column). 100 µl of the water phase is removed from well and 10 µl is injected into the LC/UV/qTOF (RP column). Data is processed with ProfileLynx. Mass chromatograms are integrated, ratio'ed, corrected for dilution and injection volumes and, finally, corrected using area ratio of internal standard.

V. Supporting Information: Generation of crystal structures

V. Expression, purification, and crystallization of PI3Kα mutants

A PI3KCA insect cell expression construct was generated. This construct consists of vector pFastBacDual containing a p85 fragment and full-length p110alpha linked to individual promoters. The p85 fragment encodes p85 307-593aa (nSH2-iSH) and contains an amino-terminal epitope tag (Glu-tag). The p85 gene was cloned adjacent to the p10 promoter. The p110alpha gene is comprised of full-length p110 containing a carboxyl-terminal 6xHis purification tag. In addition, the p110alpha gene contains two substitutions, M232K and L233K, that were engineered for improve crystal packing. The p110alpha gene was cloned adjacent to the Poly-Hedron promoter. To this base construct, further substitutions were engineered using a site-direct mutagenesis methodology.

The various PI3Kalpha constructs were used to generate baculovirus using the Bac-to-Bac method. The baculovirus was amplified in SF21 cells and later used to infect Tn5 insect cells for protein production. The protein production was carried out at various volumes, 1 liter shake flask to 5 liter wave-bag , depending on the expected expression level.

PI3Ka mutant (I800M/F930V) construct was expressed in insect system using Tn5 cells. Cell pellet was re-suspended in 20 mM Tris buffer (pH 7.5 at room temperature), containing 200 mM NaCl, 1 mM MgCl2, 1% Betaine, 1% Ethlene Glycol and 5 mM β-Mercaptoethanol. Cells were lysed by douncing and lysate was cleared by centrifugation. Cleared lysate was then loaded onto Ni

Sepharose 6 Fast Flow resin (GE Healthcare) using FPLC AKTA explorer (GE HealthCare). PI3K mutant protein eluted by gradient elution using Tris buffer containing 5 mM Imidazole to 250 mM Imidazole. Fractions contained PI3Ka mutant protein were pooled and salt in the buffer was diluted to 50 mM NaCl with 50 mM Hepes buffer pH7.5, containing 1% Betaine, 1% Ethlene Glycol, 0.02% CHAPS and 5 mM DTT. This diluted protein solution then became the load for cation exchange chromatography using HiTrap SP-HP column (GE Healthcare). PI3K mutant protein eluted from HiTrap SP-HP column by gradient elution using Hepes buffer containing NaCl from 50 mM to 1 M. Fractions contained PI3K mutant protein were combined and polished on size exclusion column Superdex 200 26/60 (GE Healthcare). Purified protein was in 20 mM Tris (pH 7.2 at room temperature) containing 200 mM NaCl, 1% Betaine, 1% Ethlene Glycol, 0.02% CHAPS and 5 mM DTT. Protein was concentrated to about 8 mg/mL for crystallography. Protein yield of this PI3Ka mutant after three column purification was 2-3 mg/L culture.

Test compound was dissolved in DMSO and added to the protein solution containing PI3Kα mutant protein for a final concentration of 1μM. This solution was incubated on ice for 1 hour and then passed through a 0.2μm filter. Crystals were grown by the hanging-drop vapor diffusion method at 30 °C by mixing 5μL protein-inhibitor solution with 3μL well solution composed of 120 mM potassium thiocyanate and 12% w/v polyethylene glycol (PEG) 3350. The drops were streak seeded and then sealed with a screw cap. Crystals were transferred to a new drop containing the well solution supplemented with 20% ethylene glycol and then flash cooled in liquid nitrogen. Diffraction data was collected at beamline 5.0.2 of the Advanced Light Source.

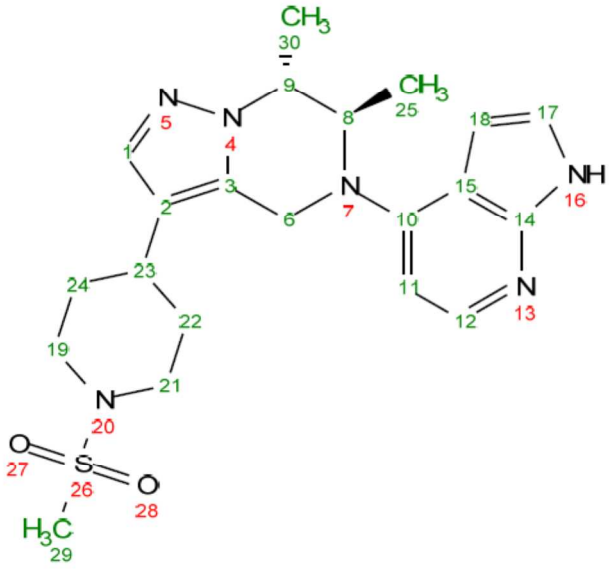
W. Data collection and refinement statistics (molecular replacement)

	mutPI3Kα – compound 4
Data collection	
Space group	$P2_12_12_1$
Cell dimensions	
a, *b*, *c* (Å)	104.48, 106.53, 133.51
α, β, γ (°)	90, 90, 90
Resolution (Å)	2.389Å
R_{merge}	0.068(0.974)
$I / \sigma I$	19.4(2.1)
Completeness (%)	100(100)
Redundancy	6.7(6.7)
Refinement	
Resolution (Å)	2.60
No. reflections	46489
R_{work} / R_{free}	0.1797/0.2358
No. atoms	
Protein	10070
Ligand/ion	321
Water	290
B-factors	
From Wilson plot	69.53
Mean B value	65.10
R.m.s. deviations	
Bond lengths (Å)	0.009
Bond angles (°)	1.03

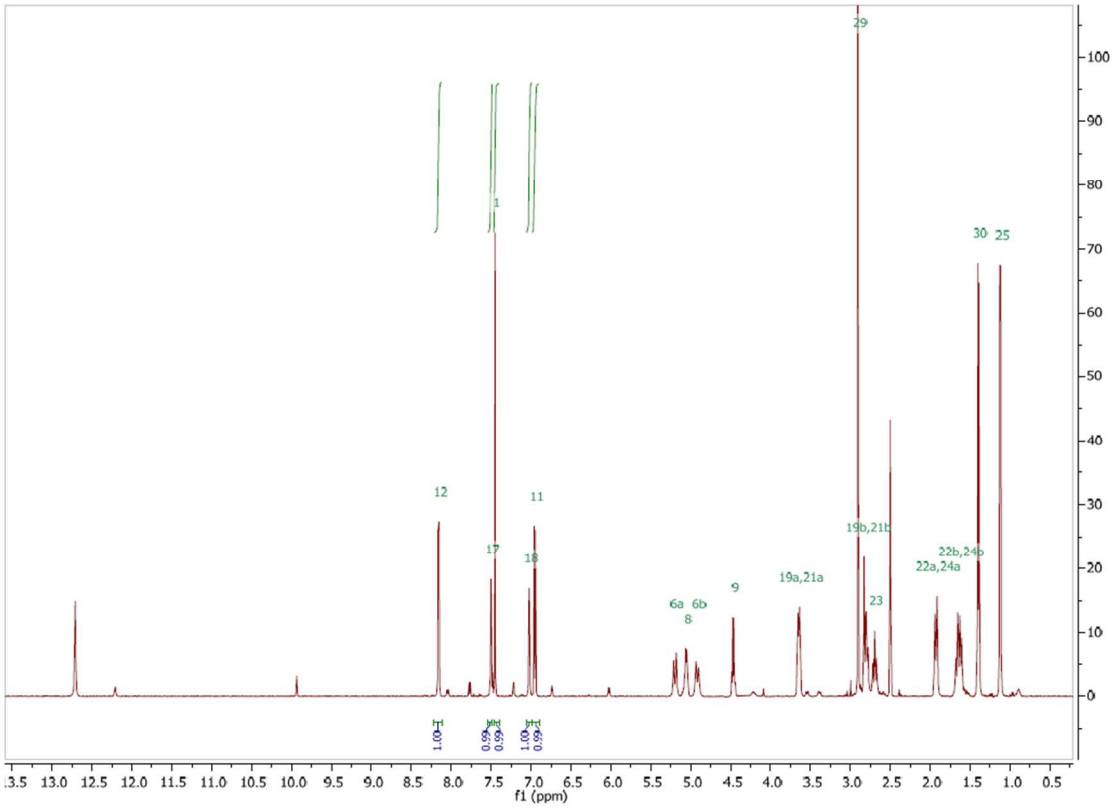
*Values in parentheses are for highest-resolution shell.

VI. Supporting Information: Analytics

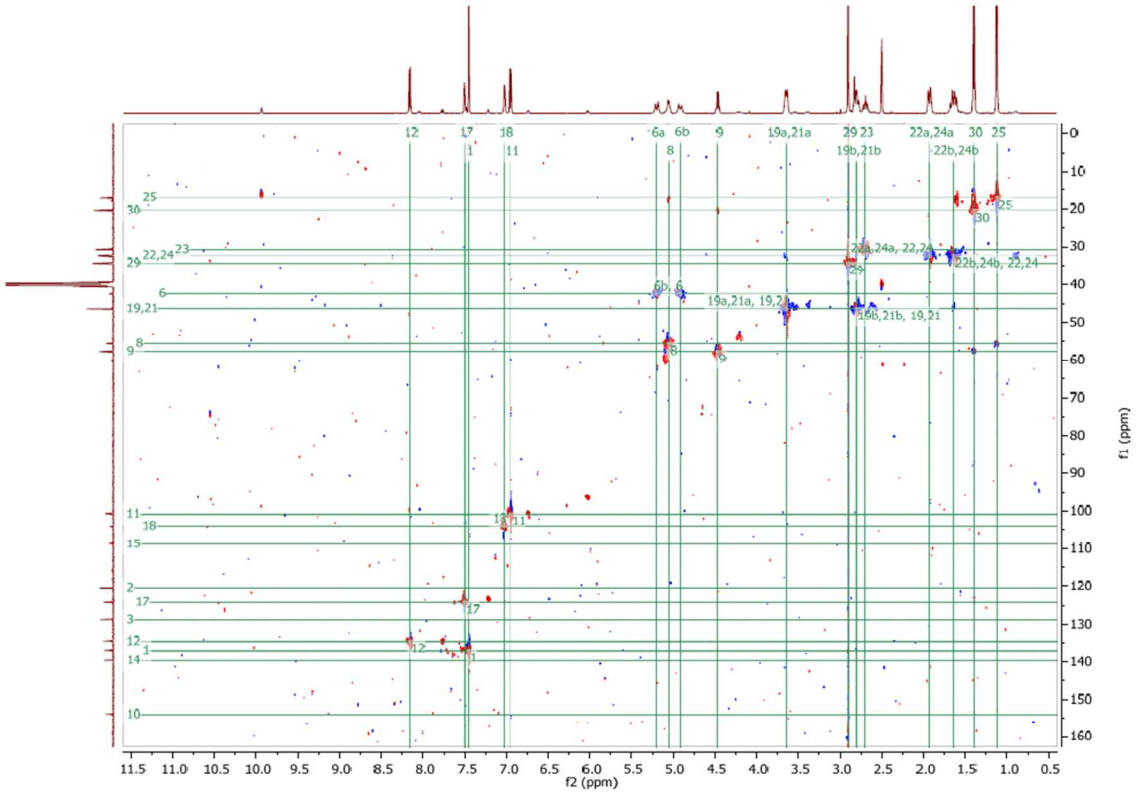
X. 2-D NMR studies confirming the relative configuration of compound 21



Proton NMR assignment:



HSQC:



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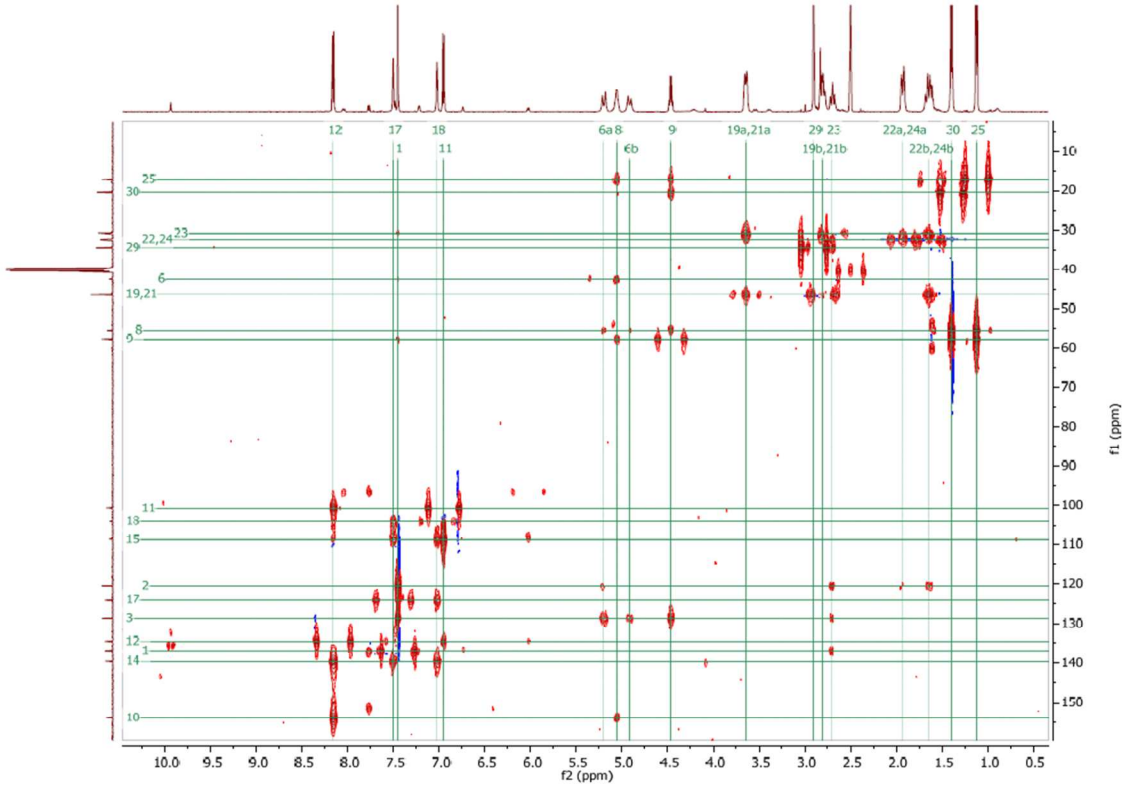

^{13}C NMR assignment: