Supporting information for:

Discovery of the TLR7/8 antagonist MHV370 for treatment of systemic autoimmune diseases

AUTHOR NAMES. Phil Alper[1], Claudia Betschart[2], Cédric André[2], Thomas Boulay[2], Dai Cheng[1], Jonathan Deane[1,3], Michael Faller[2], Roland Feifel[2], Ralf Glatthar[2], Dong Han[1], Rene Hemmig[2], Tao Jiang[1], Thomas Knoepfel[2], Jillian Maginnis[1], Daniel Mutnick[1], Wei Pei[1], Giulia Ruzzante[2], Peter Syka[1], Guobao Zhang[1], Yi Zhang[1], Florence Zink[2], Géraldine Zipfel[2], Stuart Hawtin[2], Tobias Junt[2], Pierre-Yves Michellys,[1,4]*

AUTHOR ADDRESS

Novartis Institutes for Biomedical Research, [1], 10675 John Jay Hopkins Drive, San Diego, CA 92121, USA, and [2], Fabrikstrasse 2, Novartis Campus, CH-4056 Basel, Switzerland. [3], Present Address: Kumquat Biosciences, 10770 Wateridge Circle, Ste. 120., San Diego, CA 92121, USA, [4], Present Address: Odyssey Therapeutics, 4242 Campus Point Court, San Diego, CA 92121, USA.

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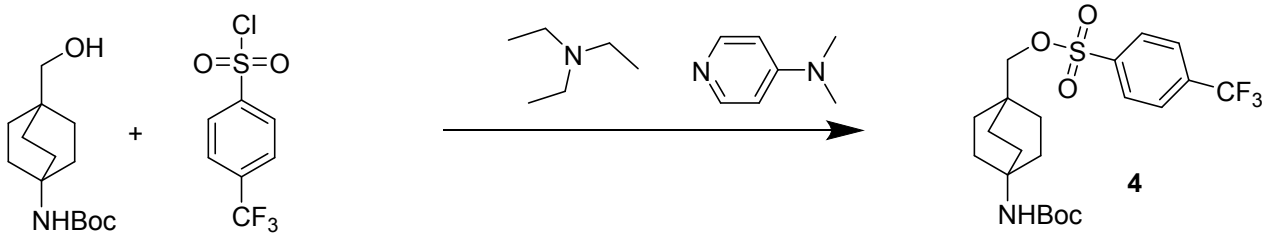
General Synthetic Experimental Details: Unless otherwise stated glassware was air dried prior to use with no special precautions taken for drying. All solvents stated as anhydrous were purchased that way from the manufacturer and used as received. All other purchased reagents were used as received. Unless otherwise stated, all reactions were carried out under a positive pressure of nitrogen. ^{1}H NMR spectral data were recorded on a Bruker 400 MHz instrument and reported as follows: chemical shift on the d scale (using residual protio solvent as the internal standard), multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet), integration and coupling constant in Hertz.

All compounds were >95% pure by HPLC-MS analysis unless otherwise stated.

LC-MS Method: Waters Acquity UPLC/MS system; 2.7um 3.0x50mm Agilent Poroshell 120 C18; 1.25mL/min 50ºC; 5-100%B in 1.8mins, then hold at 100%B Mobile Phase A = H_2O+0.1% formic acid Mobile Phase B = ACN+0.1% formic acid

UV detection 215 nm and 254 nm; Mass detection MS range 100-1000 Da (ESI).

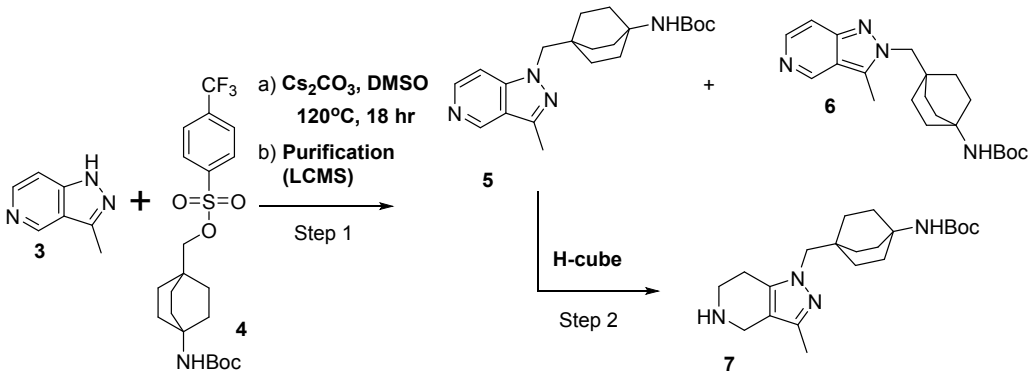
Synthesis of (4-((tert-butoxycarbonyl)amino)bicyclo[2.2.2]octan-1-yl)methyl 4-(trifluoromethyl)benzenesulfonate (**4**)



To the mixture of tert-butyl (4-(hydroxymethyl)bicyclo[2.2.2]octan-1-yl)carbamate (1.021 g, 4 mmol), 4-(trifluoromethyl)benzene-1-sulfonyl chloride (1.566 g, 6.40 mmol) and DCM (Volume: 10 mL) was added triethylamine (1.115 mL, 8.00 mmol) and DMAP (0.049 g, 0.400 mmol) at RT. After addition, the resulting mixture was stirred at RT overnight. LC/MS indicated the reaction was complete: mainly two peaks, product peak with m/z 408 (M+H-56) and intermediate peak (formed from SM2 and DMAP) with m/z 331. Aqueous work up followed by ISCO purification (EtOAc/hexane) to get (4-((tert-butoxycarbonyl)amino)bicyclo[2.2.2]octan-1-yl)methyl 4-(trifluoromethyl)benzenesulfonate as a white solid (1.75g, 94%). ^{1}H NMR (400 MHz, DMSO-d6) δ 8.12 (d, J = 8.4 Hz, 2H), 8.06 (d, J = 8.4 Hz, 2H), 6.39 (s, 1H), 3.74 (s, 2H), 1.75 – 1.57 (m, 6H),

1.42 – 1.26 (m, 15H). LC-MS: m/z calculated for $C_{21}H_{28}F_3NO_5S$ [M+ H^+]: 464; found 408 [M+ H^+ -56]$^+$.

Synthesis of *tert*-butyl (4-((3-methyl-1H-pyrazolo[4,3-c]pyridin-1-yl)methyl)bicyclo[2.2.2]octan-1-yl)carbamate and *tert*-butyl (4-((3-methyl-2H-pyrazolo[4,3-c]pyridin-2-yl)methyl)bicyclo[2.2.2]octan-1-yl)carbamate



Step 1: A suspension of 3-methyl-1H-pyrazolo[4,3-c]pyridine (**3**) (2.26 g, 16.97 mmol), in DMSO (68 ml) was treated with (4-((tert-butoxycarbonyl)amino)bicyclo[2.2.2]octan-1-yl)methyl 4-(trifluoromethyl)benzenesulfonate (**4**) (7.87 g, 16.97 mmol) and cesium carbonate (11.06 g, 33.9 mmol). The mixture was heated to 120 °C for 18 hr to complete, before being cooled to rt and diluted in EtOAc and water. After partitioning, the organic layer was dried over magnesium sulfate and concentrated under reduced pressure. The residue was purified on a 120 g silica gel column using 0-80% EtOAc in hexane and extended to 80% EtOAc in hexane to afford 3.63 g (9.79 mmol, yield: 58%) of *tert*-butyl (4-((3-methyl-1H-pyrazolo[4,3-c]pyridin-1-yl)methyl)bicyclo[2.2.2]octan-1-yl)carbamate (**5**). The gradient was extended 100% EtOAc to elute 903.4 mg (2.44 mmol, yield: 14%) of the by-product tert-butyl (4-((3-methyl-2H-pyrazolo[4,3-c]pyridin-2-yl)methyl)bicyclo[2.2.2]octan-1-yl)carbamate (**6**). Ratio product 1-2 is 4/1.

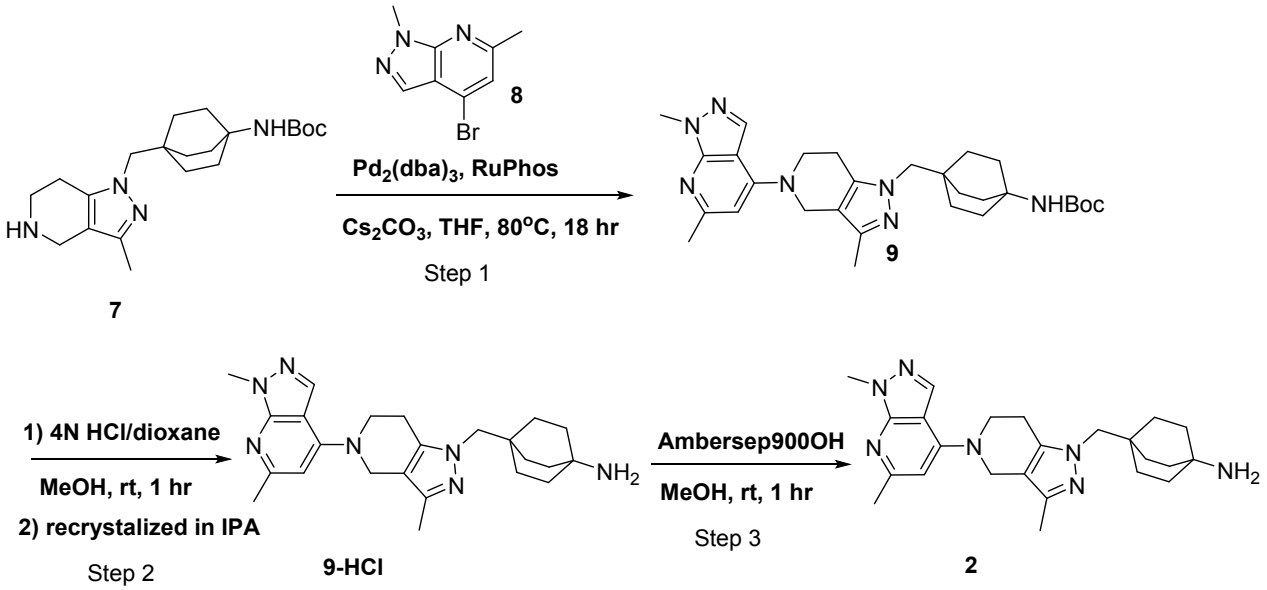
Product 1: LC-MS retention time 1.51 min, m/z calculated for $C_{21}H_{30}N_4O_2$: 370; observed 371 [M+ H]+ 371 [M+ H]+. ^{1}H NMR (400 MHz, Methanol-d_4) δ 9.07 (s, 1H), 8.37 (d, *J* = 6.1 Hz, 1H), 7.60 (d, *J* = 6.2 Hz, 1H), 6.18 (broad s, 1H), 4.17 (s, 2H), 2.71 (s, 3H), 1.87 (dd, *J* = 10.0, 5.9 Hz, 6H), 1.66 (dd, *J* = 10.0, 5.9 Hz, 6H), 1.47 (s, 9H) Product 2: LC-MS: (A) retention time 1.47 min, m/z calculated for $C_{21}H_{30}N_4O_2$ [M+ H]$^+$: 371; found 371.

Step 2: Hydrogenation of Product 1

Tert-butyl (4-((3-methyl-1H-pyrazolo[4,3-c]pyridin-1-yl)methyl)bicyclo[2.2.2]octan-1-yl)carbamate (**5**) (3.6267 g, 9.79 mmol) was hydrogenated using a H-cube system. Once complete, the reaction

solution was concentrated and loaded on a 120 g silica gel column using 0-100% IPA in DCM with 1% ammonia as modifier and then extended to 100% IPA with 1% ammonia as modifier to elute tert-butyl (4-((3-methyl-4,5,6,7-tetrahydro-1H-pyrazolo[4,3-c]pyridin-1-yl)methyl)bicyclo[2.2.2]octan-1-yl)carbamate (**7**): LC-MS: retention time 1.29 min, m/z calculated for $C_{21}H_{34}N_4O_2$ [M+ H]+: 375; found 375. ^{1}H NMR (400 MHz, CD_3OD) δ 3.73 (s, 2H), 3.65 (s, 2H), 3.04 (t, J = 5.8 Hz, 2H), 2.65 (t, J = 5.9 Hz, 2H), 2.12 (s, 3H), 1.87 – 1.74 (m, 6H), 1.61 – 1.47 (m, 6H), 1.39 (s, 9H);

Synthesis of 4-((5-(1,6-dimethyl-1H-pyrazolo[3,4-b]pyridin-4-yl)-3-methyl-4,5,6,7-tetrahydro-1H-pyrazolo[4,3-c]pyridin-1-yl)methyl)bicyclo[2.2.2]octan-1-amine **2**



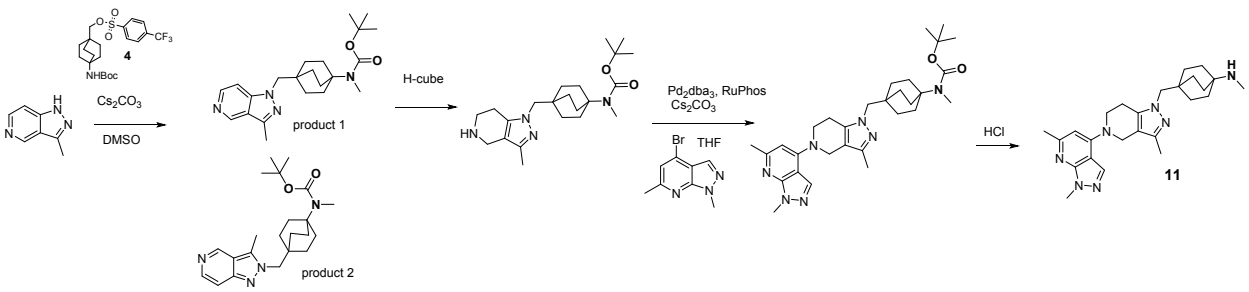
Step 1: To a pressure flask containing tert-butyl (4-((3-methyl-4,5,6,7-tetrahydro-1H-pyrazolo[4,3-c]pyridin-1-yl)methyl)bicyclo[2.2.2]octan-1-yl)carbamate **7** (0.565 g, 42.5 mmol) was added 4-bromo-1,6-dimethyl-1H-pyrazolo[3,4-b]pyridine **8** (0.94g, 2.5 mmol), cesium carbonate (1.63 g, 5.0 mmol),$Pd_2(dba)_3$ (0.057g, 0.062 mmol), RuPhos (0.14 g, 0.3 mmol) and THF (25 mL). The mixture was heated at 80 °C for 18 hrs to complete, then cooled to rt. The mixture was diluted in EtOAc and water. After partition, the aqueous layer was extracted with EtOAc once more. Both organic layers were combined, dried over $MgSO_4$, filtered and concentrated in vacuo. The residue was purified via flash chromatography using 0-100% B/A (A= heptane; B= 25% ethanol in EtOAc) to elute tert-butyl (4-((5-(1,6-dimethyl-1H-pyrazolo[3,4-b]pyridin-4-yl)-3-methyl-4,5,6,7-tetrahydro-1H-pyrazolo[4,3-c]pyridin-1-yl)methyl)bicyclo[2.2.2]octan-1-yl)carbamate **9**: LC-MS:

retention time 1.09 min, m/z calculated for $C_{29}H_{41}N_7O_2$ [M+ H]+: 520; found 520. ^{1}H NMR (400 MHz, CDCl3) δ 7.98 (s, 1H), 6.27 (s, 1H), 4.49 (s, 2H), 4.38 – 4.25 (m, 1H), 4.11 (s, 3H), 3.94 (t, *J* = 5.5 Hz, 2H), 3.70 (s, 2H), 2.87 (t, *J* = 5.6 Hz, 2H), 2.62 (s, 3H), 2.25 (s, 3H), 1.89 – 1.75 (m, 6H), 1.58 (m, 6H), 1.42 (s, 9H).

Step 2: To a 500 mL round bottom flask containing tert-butyl (4-((5-(1,6-dimethyl-1H-pyrazolo[3,4-b]pyridin-4-yl)-3-methyl-4,5,6,7-tetrahydro-1H-pyrazolo[4,3-c]pyridin-1-yl)methyl)bicyclo[2.2.2]octan-1-yl)carbamate (1.5 g, 1.45 mmol) was added methanol (6 mL), then 4N HCl in dioxane (7.2 mL, 28.9 mmol). The mixture was stirred at rt for 18 hr then concentrated in vacuo. The residue was treated with isopropanol portionwise at 70°C to get all solid dissolved. The solution was cooled naturally to rt and aged for 18 hrs. Then the solid was filtered and the filtrate was concentrated and the crystallization process repeated. Both batches were combined and dried under vacuum at 40 °C for 18 hrs to afford of 4-((5-(1,6-dimethyl-1H-pyrazolo[3,4-b]pyridin-4-yl)-3-methyl-4,5,6,7-tetrahydro-1H-pyrazolo[4,3-c]pyridin-1-yl)methyl)bicyclo[2.2.2]octan-1-amine **2** as HCl salt: ^{1}H NMR (400 MHz, CD_3OD) δ 8.51 (s, 1H), 6.88 (s, 1H), 5.06 – 4.92 (m, 2H), 4.24 (s, 2H), 4.11 (s, 3H), 3.97 (s, 2H), 3.10 (t, *J* = 5.6 Hz, 2H), 2.69 (s, 3H), 2.38 (s, 3H), 1.88 – 1.59 (m, 12H); m/z calculated for $C_{24}H_{33}N_7$ [M+H$^+$]: 420; observed 420. Ambersep 900OH (17 ml, 0.8 meq/mL, prewashed with 60 mL of MeOH) was added into a solution of 4-((5-(1,6-dimethyl-1H-pyrazolo[3,4-b]pyridin-4-yl)-3-methyl-4,5,6,7-tetrahydro-1H-pyrazolo[4,3-c]pyridin-1-yl)methyl)bicyclo[2.2.2]octan-1-amine as HCl salt **2-HCl** (1.53 g, 2.7 mmol) in MeOH (100 mL). The mixture was stirred at RT for 1 hr then filtered, washed with 50 mL of MeOH and concentrated. The crude product was added (by solid loading) to a 12 g silica gel column and was eluted with 2-9% MeOH (containing small amount of ammonia) in DCM. Collected fractions and concentrated to give the product, 4-((5-(1,6-dimethyl-1H-pyrazolo[3,4-b]pyridin-4-yl)-3-methyl-4,5,6,7-tetrahydro-1H-pyrazolo[4,3-c]pyridin-1-yl)methyl)bicyclo[2.2.2]octan-1-amine, as the free base (**2**): ^{1}H NMR (400 MHz, CD_3OD) δ 8.15 (s, 1H), 6.45 (s, 1H), 4.54 (s, 2H), 4.00 (s, 3H), 3.97 (t, *J* = 5.6 Hz, 2H), 3.73 (s, 2H), 2.90 (t, *J* = 5.6 Hz, 2H), 2.55 (s, 3H), 2.23 (s, 3H), 1.55 (s, 12H); MS calculated for $C_{24}H_{33}N_7$ [M+H$^+$]: 420; observed 420. EA (C, H, N) calcd: 68.70, 7.93, 23.37; found: 68.18, 7.99, 22.94.

Synthesis of final compounds

Synthesis of 4-((5-(1,6-dimethyl-1H-pyrazolo[3,4-b]pyridin-4-yl)-3-methyl-4,5,6,7-tetrahydro-1H-pyrazolo[4,3-c]pyridin-1-yl)methyl)-N-methylbicyclo[2.2.2]octan-1-amine **11**



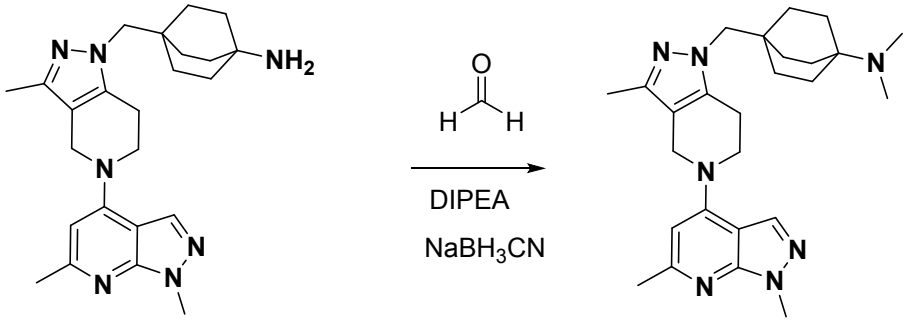
Step 1: A mixture of 133.0 mg (1.0 mmol) of 3-methyl-1H-pyrazolo[4,3-c]pyridine, 525.0 mg (1.1 mmol) of (4-((tert-butoxycarbonyl)(methyl)amino)bicyclo[2.2.2]octan-1-yl)methyl 4-(trifluoromethyl)benzenesulfonate (**4**) and 652.0 mg (2.0 mmol) of cesium carbonate in DMSO was stirred at 80 °C overnight. After completion of the reaction (LC-MS monitoring), the reaction mixture was cooled down to RT and diluted with EtOAc/water. The layers were separated and the organic layer was dried over Na_2SO_4 then concentrated. The residue was added (solid loading) to a 40 g silica gel column and was eluted with 0-100% EtOAc in Hexanes. Collected fractions and concentrated to afford 243.0 mg (0.632 mmol, yield: 63%) of the desired product 1 (coming out first), tert-butyl methyl(4-((3-methyl-1H-pyrazolo[4,3-c]pyridin-1-yl)methyl)bicyclo[2.2.2]octan-1-yl)carbamate, and 67.0 mg (0.174 mmol, yield: 17%) of product 2 (coming out later), tert-butyl methyl(4-((3-methyl-2H-pyrazolo[4,3-c]pyridin-2-yl)methyl)bicyclo[2.2.2]octan-1-yl)carbamate.
Product 1: 1H NMR (400 MHz, Methanol-d4) δ 8.99 (d, J = 1.1 Hz, 1H), 8.29 (d, J = 6.2 Hz, 1H), 7.52 (dd, J = 6.2, 1.2 Hz, 1H), 4.10 – 4.07 (m, 2H), 2.80 (s, 3H), 2.63 (s, 3H), 2.01 – 1.94 (m, 6H), 1.63 – 1.56 (m, 6H), 1.43 (s, 9H). Product 2: 1H NMR (400 MHz, Methanol-d4) δ 9.08 (d, J = 1.3 Hz, 1H), 8.14 (d, J = 6.4 Hz, 1H), 7.48 (dd, J = 6.3, 1.2 Hz, 1H), 4.19 (s, 2H), 2.81 (s, 3H), 2.77 (s, 3H), 2.07 – 1.97 (m, 6H), 1.70 – 1.62 (m, 6H), 1.43 (s, 9H)

Step 2: A mixture of 66.7 mg (0.295 mmol) of 4-bromo-1,6-dimethyl-1H-pyrazolo[3,4-b]pyridine, 115.0 mg (0.295 mmol) of *tert*-butyl (4-((5-(1,6-dimethyl-1H-pyrazolo[3,4-b]pyridin-4-yl)-3-methyl-4,5,6,7-tetrahydro-1H-pyrazolo[4,3-c]pyridin-1-yl)methyl)bicyclo[2.2.2]octan-1-yl)(methyl)carbamate, 6.8 mg (0.008 mmol) of Pd2dba3, 16.5 mg (0.035 mmol) of RuPhos and 192.0 mg (0.59 mmol) of Cs_2CO_3 in THF (2 mL) was purged with argon before heated to 75 °C for 15 hr. After cooling to RT the mixture was diluted in EtOAc and water. The layers were separated and the aqueous layer was extracted with EtOAc. The combined organic layers were dried over Na_2SO_4, filtered and concentrated under reduced pressure. The residue was added to a 12g silica gel column and was eluted with 0-5% methanol in DCM. Collected fractions and concentrated to afford 105.0 mg (0.187 mmol, yield: 63%) of *tert*-butyl (4-((5-(1,6-dimethyl-1H-

pyrazolo[3,4-b]pyridin-4-yl)-3-methyl-4,5,6,7-tetrahydro-1H-pyrazolo[4,3-c]pyridin-1-yl)methyl)bicyclo[2.2.2]octan-1-yl)(methyl)carbamate. ^{1}H NMR (400 MHz, Methanol-d4) δ 8.16 (s, 1H), 6.46 (s, 1H), 4.54 (s, 2H), 4.00 (s, 5H), 3.72 (s, 2H), 2.90 (t, J = 5.6 Hz, 2H), 2.80 (s, 3H), 2.55 (s, 3H), 2.23 (s, 3H), 2.04 – 1.91 (m, 6H), 1.60 – 1.52 (m, 6H), 1.43 (s, 9H).

Step 3: To a solution of 90.0 mg (0.169 mmol) of *tert*-butyl (4-((5-(1,6-dimethyl-1H-pyrazolo[3,4-b]pyridin-4-yl)-3-methyl-4,5,6,7-tetrahydro-1H-pyrazolo[4,3-c]pyridin-1-yl)methyl)bicyclo[2.2.2]octan-1-yl)(methyl)carbamate in dioxane/MeOH (2 mL, v/v 1/1) was added 0.5 mL of HCl (4M solution in dioxane). The resulting mixture was stirred at RT for 2 h. LC-MS showed completion of the reaction. The reaction mixture was concentrated and the residue was dissolved in 2.0 mL of MeOH then Ambersep 900 OH (0.8 meq/mL, 5.0 eq., prewashed with 5.0 mL of MeOH) was added and the mixture was stirred at RT for 1 h, filtered, washed with 10 mL of MeOH, filtrated and concentrated under reduced pressure. The residue was added to a 4 g silica gel column and was eluted with 2-9% MeOH (containing small amount of ammonia) in DCM. Collected fractions and concentrated then lyophilized to afford 41.0 mg (0.09 mmol, yied: 53%) of 4-((5-(1,6-dimethyl-1H-pyrazolo[3,4-b]pyridin-4-yl)-3-methyl-4,5,6,7-tetrahydro-1H-pyrazolo[4,3-c]pyridin-1-yl)methyl)-N-methylbicyclo[2.2.2]octan-1-amine **11**. ESIMS calcd. for $C_{25}H_{35}N_7$ ($M+H^+$), 434, found: 434. 1H NMR (400 MHz, Methanol-d4) δ 8.05 (s, 1H), 6.36 (s, 1H), 4.44 (s, 2H), 3.90 (s, 3H), 3.88 (t, J = 5.8 Hz, 2H), 3.64 (s, 2H), 2.80 (t, J = 5.6 Hz, 2H), 2.44 (s, 3H), 2.17 (s, 3H), 2.13 (s, 3H), 1.49 (s, 12H).

Synthesis of 4-((5-(1,6-dimethyl-1H-pyrazolo[3,4-b]pyridin-4-yl)-3-methyl-4,5,6,7-tetrahydro-1H-pyrazolo[4,3-c]pyridin-1-yl)methyl)-N,N-dimethylbicyclo[2.2.2]octan-1-amine **12**

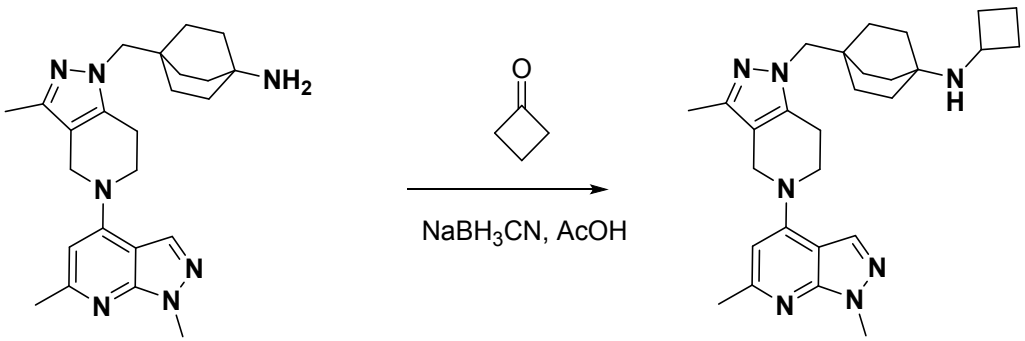


To a solution of 142.0 mg (0.25 mmol) of 4-((5-(1,6-dimethyl-1H-pyrazolo[3,4-b]8yridine-4-yl)-3-methyl-4,5,6,7-tetrahydro-1H-pyrazolo[4,3-c]8yridine-1-yl)methyl)bicyclo[2.2.2]octan-1-amine **2** in THF/MeOH (5/1.5 mL) was added 97.0 mg (0.75 mmol) of DIPEA. The mixture was stirred at

RT for 10 minutes before formaldehyde (0.037 mL, 37wt% in water) was added. The resulting mixture was stirred at RT for 30 minutes before 157.0 mg (2.5 mmol) of $NaBH_3CN$ was added portionwise. The resulting mixture was stirred at RT overnight. The reaction was quenched by adding 2.0 mL of water then extracted with DCM and combined organic layers were dried over Na_2SO_4 then concentrated.

The resulting mixture was added to a 4g silica gel column and was eluted with 0-8% MeOH (containing very small amount of ammonia) in DCM. Collected fractions and concentrated then lyophilized to afford 38.0 mg (0.081 mmol, yield: 32%) of 4-((5-(1,6-dimethyl-1H-pyrazolo[3,4-b]pyridin-4-yl)-3-methyl-4,5,6,7-tetrahydro-1H-pyrazolo[4,3-c]pyridin-1-yl)methyl)-N,N-dimethylbicyclo[2.2.2]octan-1-amine **12**. ESIMS calcd. for $C_{26}H_{37}N_7$ ($M+H^+$), 448, found: 448. 1H NMR (400 MHz, Methanol-d4) δ 8.35 (s, 1H), 6.71 (s, 1H), 4.78 – 4.68 (m, 2H), 4.08 (d, J = 11.4 Hz, 2H), 4.00 (s, 3H), 3.72 (s, 2H), 2.91 (t, J = 5.4 Hz, 2H), 2.65 (s, 6H), 2.57 (s, 3H), 2.15 (s, 3H), 1.81 – 1.72 (m, 6H), 1.64 – 1.55 (m, 6H).

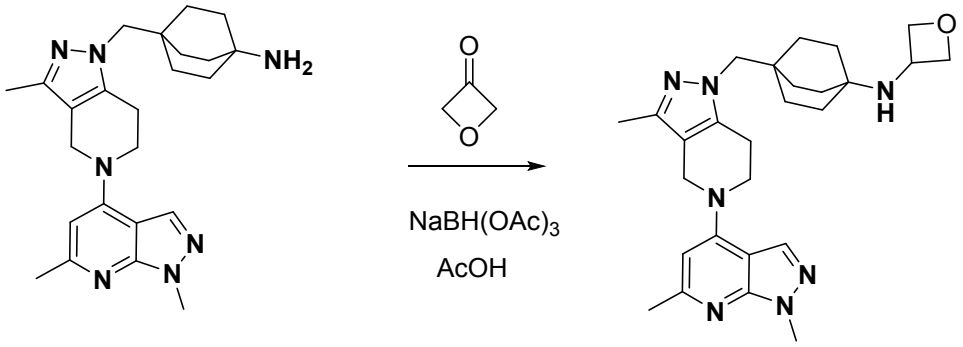
Synthesis of N-cyclobutyl-4-((5-(1,6-dimethyl-1H-pyrazolo[3,4-b]pyridin-4-yl)-3-methyl-4,5,6,7-tetrahydro-1H-pyrazolo[4,3-c]pyridin-1-yl)methyl)bicyclo[2.2.2]octan-1-amine **13**



To a solution of 4-((5-(1,6-dimethyl-1H-pyrazolo[3,4-b]pyridin-4-yl)-3-methyl-4,5,6,7-tetrahydro-1H-pyrazolo[4,3-c]pyridin-1-yl)methyl)bicyclo[2.2.2]octan-1-amine **2** (42.0 mg, 0.1 mmol) in MeOH (2 mL) was added acetic acid (30.0 mg (0.5 mmol) and cyclobutanone (35.8 mg (0.5 mmol). The mixture was stirred at RT for 30 minutes before $NaBH_3CN$ (62.8 mg, 1.0 mmol) was added portionwise. The resulting mixture was stirred at 75 °C overnight. After cooling to RT, the mixture was concentrated and the residue was added (by solid loading) to a 4g silica gel column and was eluted with 0-8% MeOH in DCM. Collected fractions and concentrated then further purified by mass-triggered HPLC to give 32.0 mg (0.064 mmol, yield: 64%) of N-cyclobutyl-4-((5-(1,6-dimethyl-1H-pyrazolo[3,4-b]pyridin-4-yl)-3-methyl-4,5,6,7-tetrahydro-1H-pyrazolo[4,3-

c]pyridin-1-yl)methyl)bicyclo[2.2.2]octan-1-amine **13**, which was neutralized using Ambersep 900 OH (Strong Base Anion Exchanger). ESIMS calcd. for $C_{28}H_{39}N_7$ ($M+H^+$), 474, found: 474. ^1H NMR (400 MHz, Methanol-d4) δ 8.16 (s, 1H), 6.46 (s, 1H), 4.55 (s, 2H), 4.01 (s, 3H), 3.98 (t, J = 5.6 Hz, 2H), 3.72 (s, 2H), 3.32 (m, 1H), 2.90 (t, J = 5.5 Hz, 2H), 2.55 (s, 3H), 2.23 (s, 3H), 2.19 (tt, J = 11.1, 5.3 Hz, 2H), 1.93 – 1.77 (m, 2H), 1.68 (dd, J = 11.1, 5.7 Hz, 2H), 1.63 – 1.51 (m, 12H).

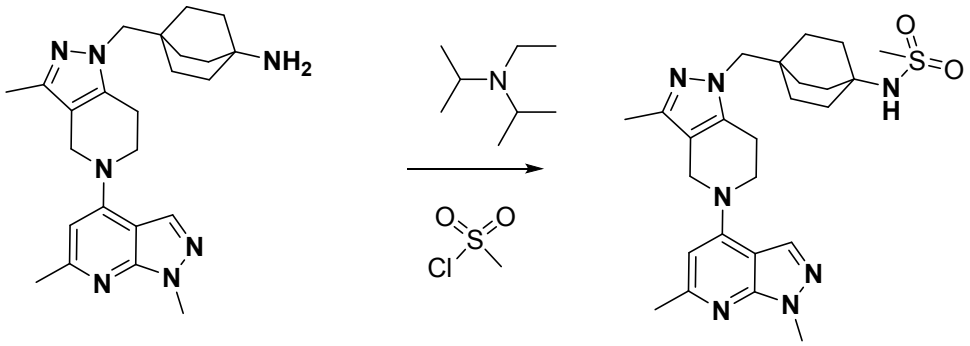
Synthesis of N-(4-((5-(1,6-dimethyl-1H-pyrazolo[3,4-b]pyridin-4-yl)-3-methyl-4,5,6,7-tetrahydro-1H-pyrazolo[4,3-c]pyridin-1-yl)methyl)bicyclo[2.2.2]octan-1-yl)oxetan-3-amine **14**



To a solution of 42.0 mg (0.1 mmol) of 4-((5-(1,6-dimethyl-1H-pyrazolo[3,4-b]pyridin-4-yl)-3-methyl-4,5,6,7-tetrahydro-1H-pyrazolo[4,3-c]pyridin-1-yl)methyl)bicyclo[2.2.2]octan-1-amine **2** in DCE (3 mL) was added AcOH (6.0 mg, 0.1 mmol) and oxetan-3-one (73.5 mg, 1.0 mmol). The mixture was stirred at RT for 30 minutes before sodium triacetoxyhydroborate (65.5 mg, 0.3 mmol) was added. The resulting mixture was stirred at RT overnight. LC-MS showed desired product but the reaction was not complete. More oxetan-3-one (60 uL) and sodium triacetoxyhydroborate (66 mg) was added and the mixture was stirred at RT for additional one day. LC-MS showed completion of the reaction. The mixture was treated with 1N NaOH and extracted with DCM (3X). The organic layers were concentrated and the crude product was added (by solid loading) to a 4g silica gel column and was eluted with 0-50% IPA (containing 0.2 M ammonia) in DCM. Collected fractions and concentrated then lyophilized to afford 35.0 mg (0.07 mmol, yield: 70%) of N-(4-((5-(1,6-dimethyl-1H-pyrazolo[3,4-b]pyridin-4-yl)-3-methyl-4,5,6,7-tetrahydro-1H-pyrazolo[4,3-c]pyridin-1-yl)methyl)bicyclo[2.2.2]octan-1-yl)oxetan-3-amine **14**. ESIMS calcd. for $C_{27}H_{37}N_7O$ ($M+H^+$), 476, found: 476. 1H NMR (400 MHz, Methanol-d4) δ 8.15 (s, 1H), 6.45 (s, 1H), 4.70 (dd, J = 7.3, 6.4 Hz, 2H), 4.54 (s, 2H), 4.44 (t, J = 6.6 Hz, 2H), 4.14 (p, J = 7.2 Hz, 1H), 4.00 (s, 3H),

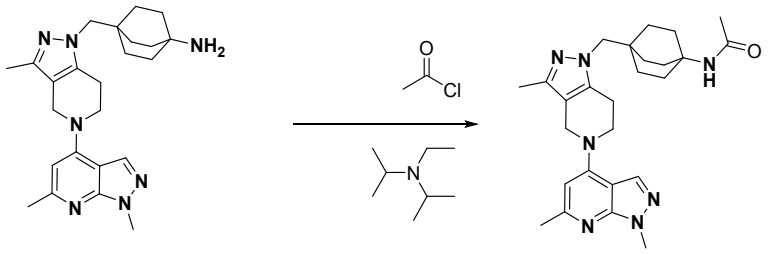
3.97 (t, J = 5.6 Hz, 2H), 3.71 (s, 2H), 2.89 (t, J = 5.6 Hz, 2H), 2.55 (s, 3H), 2.23 (s, 3H), 1.58 – 1.43 (m, 12H). EA calcd for $C_{27}H_{37}N_7O$ (C, H, N): 69.76, 8.33, 21.90; found: 67.14, 8.19, 20.74.

Synthesis of -(4-((5-(1,6-dimethyl-1H-pyrazolo[3,4-b]pyridin-4-yl)-3-methyl-4,5,6,7-tetrahydro-1H-pyrazolo[4,3-c]pyridin-1-yl)methyl)bicyclo[2.2.2]octan-1-yl)methanesulfonamide **15**



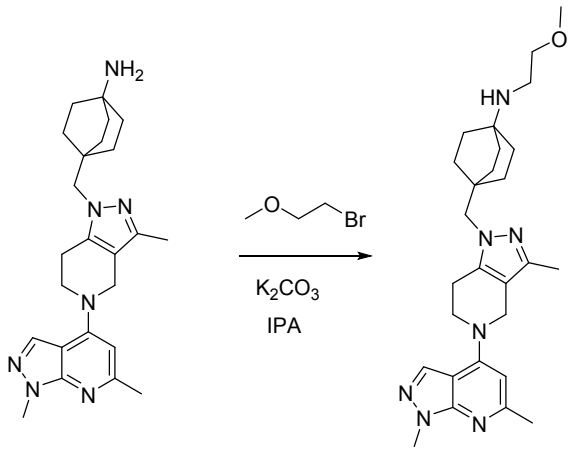
To a mixture of 56.7 mg (0.1 mmol) of 4-((5-(1,6-dimethyl-1H-pyrazolo[3,4-b]pyridin-4-yl)-3-methyl-4,5,6,7-tetrahydro-1H-pyrazolo[4,3-c]pyridin-1-yl)methyl)bicyclo[2.2.2]octan-1-amine **2** and 38.8 mg (0.3 mmol) of DIPEA in DCM (5 mL) was added 14.9 mg (0.13 mmol) of methanesulfonyl chloride at 0 °C. The resulting mixture was slowly warmed up to RT and stirred for 2 hrs until reaction completion. The crude product was added (by solid loading) to a 4g silica gel column and was eluted with 0-7% MeOH (containing very small amount of ammonia) in DCM, and further purified by mass-triggered HPLC. Collected fractions, 1.0 mL of 1N aqueous HCl was added then concentrated and lyophilized to afford 7.0 mg (0.012 mmol, yield: 12%) of N-(4-((5-(1,6-dimethyl-1H-pyrazolo[3,4-b]pyridin-4-yl)-3-methyl-4,5,6,7-tetrahydro-1H-pyrazolo[4,3-c]pyridin-1-yl)methyl)bicyclo[2.2.2]octan-1-yl)methanesulfonamide **15**, as HCl salt. ESIMS calcd. for $C_{25}H_{35}N_7O_2S$ ($M+H^+$), 498, found: 498. ^1H NMR (400 MHz, Methanol-d4) δ 8.53 (s, 1H), 6.89 (s, 1H), 5.06 – 4.94 (m, 2H), 4.25 (s, 2H), 4.12 (s, 3H), 3.98 (s, 2H), 3.18 – 3.06 (m, 2H), 2.94 (s, 3H), 2.70 (s, 3H), 2.43 (s, 3H), 1.96 – 1.85 (m, 6H), 1.67 – 1.57 (m, 6H).

Synthesis of N-(4-((5-(1,6-dimethyl-1H-pyrazolo[3,4-b]pyridin-4-yl)-3-methyl-4,5,6,7-tetrahydro-1H-pyrazolo[4,3-c]pyridin-1-yl)methyl)bicyclo[2.2.2]octan-1-yl)acetamide **16**



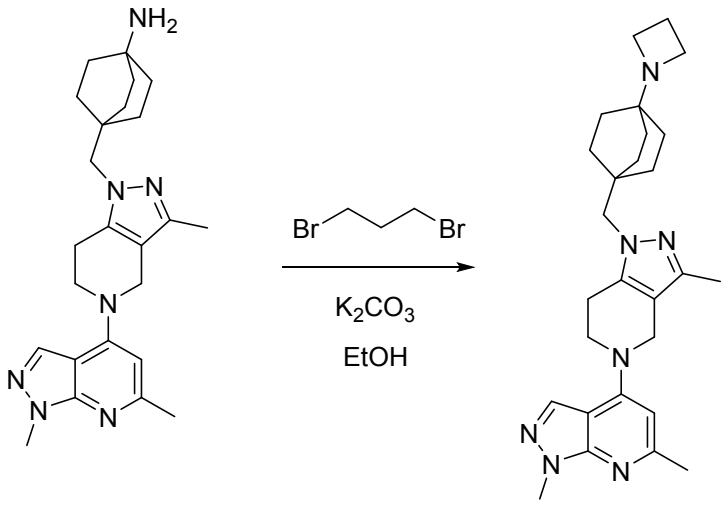
To a mixture of 56.7 mg (0.1 mmol) of 4-((5-(1,6-dimethyl-1H-pyrazolo[3,4-b]pyridin-4-yl)-3-methyl-4,5,6,7-tetrahydro-1H-pyrazolo[4,3-c]pyridin-1-yl)methyl)bicyclo[2.2.2]octan-1-amine **2** and 38.8 mg (0.3 mmol) of DIPEA in DCM was added 10.2 mg (0.13 mmol) of acetyl chloride at 0 °C. The resulting mixture was slowly warmed up to RT and stirred for 2 hrs. The crude product was added (by solid loading) to a 4g silica gel column and was eluted with 0-7% MeOH (containing very small amount of ammonia) in DCM, and further purified by mass-triggered HPLC. Collected fractions, 1.0 mL of 1N aqueous HCl was added then concentrated and lyophilized to afford 30.0 mg (0.057 mmol, yield: 57%) of N-(4-((5-(1,6-dimethyl-1H-pyrazolo[3,4-b]pyridin-4-yl)-3-methyl-4,5,6,7-tetrahydro-1H-pyrazolo[4,3-c]pyridin-1-yl)methyl)bicyclo[2.2.2]octan-1-yl)acetamide, as HCl salt . ESIMS calcd. for $C_{26}H_{35}N_7O$ ($M+H^+$), 462, found: 462. ^1H NMR (400 MHz, Methanol-d4) δ 8.55 (s, 1H), 6.93 (s, 1H), 5.16 – 4.97 (m, 2H), 4.29 (d, J = 7.0 Hz, 2H), 4.13 (s, 3H), 4.10 (s, 2H), 3.20 (t, J = 5.2 Hz, 2H), 2.71 (s, 3H), 2.52 (s, 3H), 1.96 (d, J = 6.0 Hz, 9H), 1.71 – 1.55 (m, 6H).

Synthesis of 4-((5-(1,6-dimethyl-1H-pyrazolo[3,4-b]pyridin-4-yl)-3-methyl-4,5,6,7-tetrahydro-1H-pyrazolo[4,3-c]pyridin-1-yl)methyl)-N-(2-methoxyethyl)bicyclo[2.2.2]octan-1-amine **17**



A mixture of 126.0 mg (0.3 mmol) of 4-((5-(1,6-dimethyl-1H-pyrazolo[3,4-b]pyridin-4-yl)-3-methyl-4,5,6,7-tetrahydro-1H-pyrazolo[4,3-c]pyridin-1-yl)methyl)bicyclo[2.2.2]octan-1-amine **2**, 50.0 mg (0.36 mmol) of 1-bromo-2-methoxyethane and 124.0 mg (0.9 mmol) of K_2CO_3 in 2-propanol (3 mL) was heated at 110 °C for 24 h. After cooling at RT, the solvents were removed under reduced pressure and the residue was added (by solid loading) to a 24 g gold silica gel column and eluted with 0-30% IPA (containing 1% of ammonia) in DCM. Collected fractions and concentrated then lyophilized afforded 44.0 mg (0.09 mmol, yield: 30%) of 4-((5-(1,6-dimethyl-1H-pyrazolo[3,4-b]pyridin-4-yl)-3-methyl-4,5,6,7-tetrahydro-1H-pyrazolo[4,3-c]pyridin-1-yl)methyl)-N-(2-methoxyethyl)bicyclo[2.2.2]octan-1-amine **17**. ESIMS calcd. for $C_{27}H_{39}N_7$ ($M+H^+$), 478, found: 478. 1H NMR (400 MHz, Methanol-d4) δ 8.05 (s, 1H), 6.35 (s, 1H), 4.44 (s, 2H), 3.90 (s, 3H), 3.87 (t, J = 5.6 Hz, 2H), 3.62 (s, 2H), 3.34 (t, J = 5.4 Hz, 2H), 3.21 (s, 3H), 2.80 (t, J = 5.6 Hz, 2H), 2.55 (t, J = 5.4 Hz, 2H), 2.44 (s, 3H), 2.13 (s, 3H), 1.46 (s, 12H).

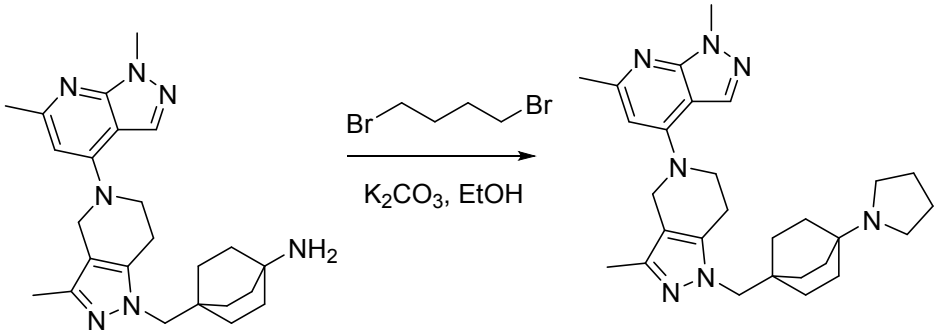
Synthesis of 1-((4-(azetidin-1-yl)bicyclo[2.2.2]octan-1-yl)methyl)-5-(1,6-dimethyl-1H-pyrazolo[3,4-b]pyridin-4-yl)-3-methyl-4,5,6,7-tetrahydro-1H-pyrazolo[4,3-c]pyridine **18**



A mixture of 42.0 mg (0.1 mmol) of 4-((5-(1,6-dimethyl-1H-pyrazolo[3,4-b]pyridin-4-yl)-3-methyl-4,5,6,7-tetrahydro-1H-pyrazolo[4,3-c]pyridin-1-yl)methyl)bicyclo[2.2.2]octan-1-amine **2**, 103 mg (0.5 mmol) of 1,3-dibromopropane and 41.5 mg (0.3 mmol) of K_2CO_3 in EtOH (1 mL) was heated under microwave irradiation at 120 °C for 30 minutes. After cooling to RT the mixture was partitioned between EtOAc and 1N aqueous NaOH. The aqueous layer was extracted with EtOAc. The combined organic layers were dried over Na_2SO_4 and concentrated. The residue was added to a 4 g silica gel column and was eluted with 0-9% MeOH (containing small amount of ammonia)

in DCM. Collected fractions and concentrated then lyophilized to afford 18.0 mg (0.034 mmol, yield: 35%) of 1-((4-(azetidin-1-yl)bicyclo[2.2.2]octan-1-yl)methyl)-5-(1,6-dimethyl-1H-pyrazolo[3,4-b]pyridin-4-yl)-3-methyl-4,5,6,7-tetrahydro-1H-pyrazolo[4,3-c]pyridine **18**. ESIMS calcd. for $C_{27}H_{37}N_7$ ($M+H^+$), 460, found: 460. ^{1}H NMR (400 MHz, Methanol-d4) δ 8.03 (d, J = 0.9 Hz, 1H), 6.33 (s, 1H), 4.42 (s, 2H), 3.89 (s, 3H), 3.85 (q, J = 5.6 Hz, 2H), 3.61 (s, 2H), 3.33 – 3.23 (m, 4H), 2.79 (t, J = 5.6 Hz, 2H), 2.43 (s, 3H), 2.12 (s, 3H), 1.95 (dt, J = 15.3, 7.6 Hz, 2H), 1.49 – 1.36 (m, 12H.

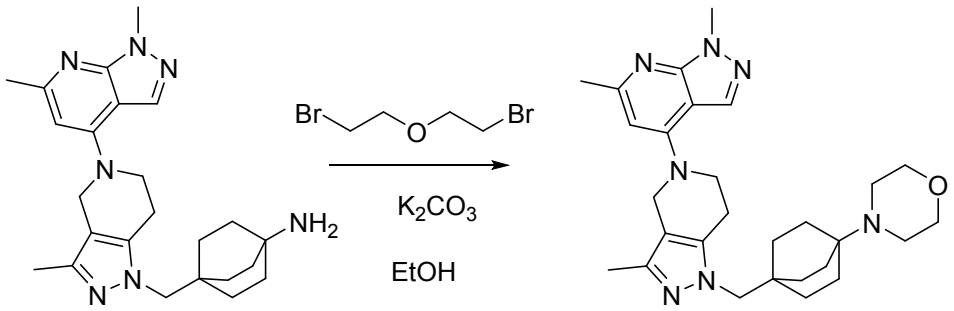
Synthesis of 5-(1,6-dimethyl-1H-pyrazolo[3,4-b]pyridin-4-yl)-3-methyl-1-((4-(pyrrolidin-1-yl)bicyclo[2.2.2]octan-1-yl)methyl)-4,5,6,7-tetrahydro-1H-pyrazolo[4,3-c]pyridine **19**



A slurry of 270 mg (0.644 mmol) of 4-((5-(1,6-dimethyl-1H-pyrazolo[3,4-b]pyridin-4-yl)-3-methyl-4,5,6,7-tetrahydro-1H-pyrazolo[4,3-c]pyridin-1-yl)methyl)bicyclo[2.2.2]octan-1-amine **2** in EtOH (3.2 mL) was treated with K_2CO_3 (178 mg, 1.287 mmol) and 1,4-dibromobutane (417 mg, 1.931 mmol). The mixture was heated to 120 °C for 2 hr by microwave irradiation. The mixture was filtered and washed with methanol and then concentrated under reduced pressure and the residue was loaded on a 12 g silica gel column using 0-50% IPA/DCM with 3% ammonia as modifier. Some fractions were impure, which was further purified by mass-directed HPLC using 1-25% MeCN in water and TFA as modifier. Fractions from HPLC were combined and concentrated under reduced pressure. The residue was suspended in 1N NaOH and extracted with EtOAc (3X). Organic layers were combined, dried over $MgSO_4$ and concentrated in vacuo. Two portions of product were combined to afford 250.0 mg (0.517 mmol, yield: 80%) of 4-((5-(1,6-dimethyl-1H-pyrazolo[3,4-b]pyridin-4-yl)-3-methyl-4,5,6,7-tetrahydro-1H-pyrazolo[4,3-c]pyridin-1-yl)methyl)-N-(4-ethoxybutyl)bicyclo[2.2.2]octan-1-amine **19**. ESIMS calcd. for $C_{28}H_{39}N_7$ ($M+H^+$), 474, found: 474. ^{1}H NMR (400 MHz, Methanol-d_4) δ 8.05 (s, 1H), 6.35 (s, 1H), 4.79 (d, *J* = 1.3 Hz, 9H), 4.44

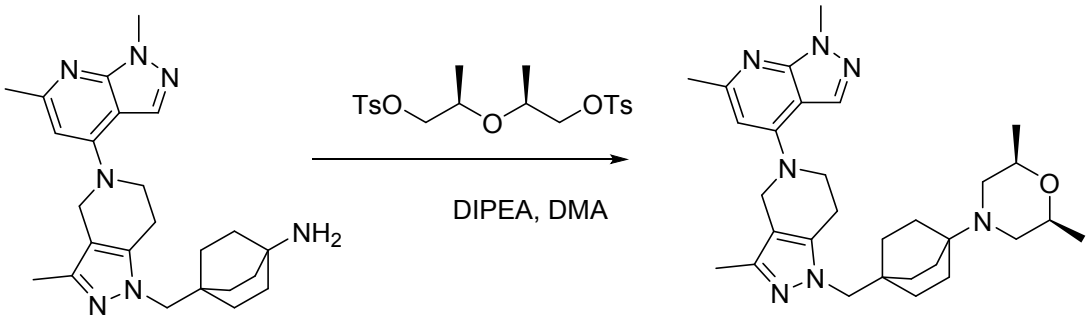
(s, 2H), 3.89 (d, *J* = 9.1 Hz, 5H), 3.63 (s, 2H), 2.80 (t, *J* = 5.6 Hz, 2H), 2.64 (d, *J* = 6.2 Hz, 4H), 2.44 (s, 3H), 2.13 (s, 3H), 1.74 – 1.64 (m, 4H), 1.52 (m, *J* = 43.2, 11.5, 6.0 Hz, 13H).

Synthesis of N-(but-3-en-1-yl)-4-((5-(1,6-dimethyl-1H-pyrazolo[3,4-b]pyridin-4-yl)-3-methyl-4,5,6,7-tetrahydro-1H-pyrazolo[4,3-c]pyridin-1-yl)methyl)-N-(4-ethoxybutyl)bicyclo[2.2.2]octan-1-amine **20**



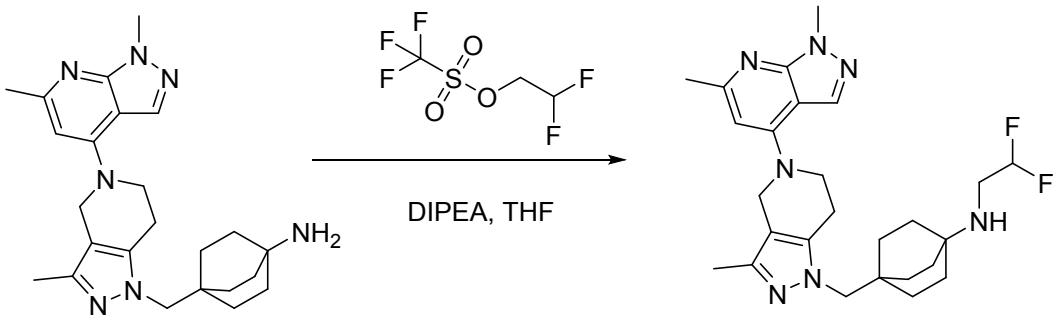
A mixture of 84 mg (0.200 mmol) of 4-((5-(1,6-dimethyl-1H-pyrazolo[3,4-b]pyridin-4-yl)-3-methyl-4,5,6,7-tetrahydro-1H-pyrazolo[4,3-c]pyridin-1-yl)methyl)bicyclo[2.2.2]octan-1-amine **2**, 83 mg (0.601 mmol) of K_2CO_3, 232.0 mg (1.0 mmol) of 1-bromo-2-(2-bromoethoxy)ethane in ethanol (2 mL) was heated to 120 °C for 2 hr by microwave irradiation. After cooling to RT, the mixture was diluted with EtOAc and 1N NaOH. After separation, the aqueous layer was extracted with EtOAc twice, the organic layers were combined, dried over $MgSO_4$ and concentrated under reduced pressure. The residue was loaded on a 4 g silica gel column using 0-30% IPA/DCM with 3% ammonia as modifier to afford 80.4 mg (0.161 mmol, yield: 80%) 4-(4-((5-(1,6-dimethyl-1H-pyrazolo[3,4-b]pyridin-4-yl)-3-methyl-4,5,6,7-tetrahydro-1H-pyrazolo[4,3-c]pyridin-1-yl)methyl)bicyclo[2.2.2]octan-1-yl)morpholine **20**. ESIMS calcd. for $C_{28}H_{39}N_7O$ ($M+H^+$), 490, found: 490. ^{1}H NMR (400 MHz, Methanol-d_4) δ 8.06 (s, 1H), 6.36 (s, 1H), 4.45 (s, 2H), 3.89 (d, *J* = 9.5 Hz, 5H), 3.63 (s, 2H), 3.55 (t, *J* = 4.6 Hz, 4H), 2.81 (t, *J* = 5.6 Hz, 2H), 2.47 (s, 3H), 2.46 (d, *J* = 16.5 Hz, 1H), 2.45 (s, 3H), 2.13 (s, 3H), 1.47 (m, *J* = 15.4, 11.7, 5.7 Hz, 12H).

Synthesis of (2S,6R)-4-(4-((5-(1,6-dimethyl-1H-pyrazolo[3,4-b]pyridin-4-yl)-3-methyl-4,5,6,7-tetrahydro-1H-pyrazolo[4,3-c]pyridin-1-yl)methyl)bicyclo[2.2.2]octan-1-yl)-2,6-dimethylmorpholine **21**



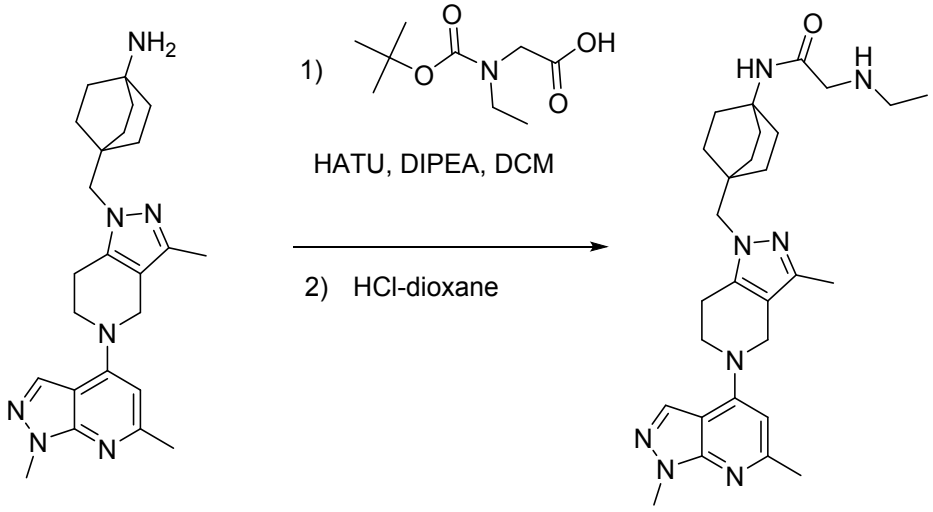
A mixture of 42 mg (0.1 mmol) of 4-((5-(1,6-dimethyl-1H-pyrazolo[3,4-b]16yridine-4-yl)-3-methyl-4,5,6,7-tetrahydro-1H-pyrazolo[4,3-c]16yridine-1-yl)methyl)bicyclo[2.2.2]octan-1-amine **2** was treated with 177 mg (0.400 mmol) of (S)-1-(4-methylbenzenesulfonate)-2-(((R)-1-(4-methylbenzenesulfonate)-2-yl)oxy)propane, 87 µl of DIEA (0.501 mmol) and DMA (1 mL) was heated to 100 °C for 18 hr. The mixture was extracted with water and EtOAc (4X). The organic layers were concentrated and the residue was loaded on a 4 g silica gel column using 0-100% EtOAc in hexane to remove impurity, then 0-10% with extended 10% methanol in DCM with 2.5% NH3 as modifier to afford 19.0 mg (0.036 mmol, yield: 36% of (2S,6R)-4-(4-((5-(1,6-dimethyl-1H-pyrazolo[3,4-b]pyridin-4-yl)-3-methyl-4,5,6,7-tetrahydro-1H-pyrazolo[4,3-c]pyridin-1-yl)methyl)bicyclo[2.2.2]octan-1-yl)-2,6-dimethylmorpholine **21**. ESIMS calcd. for $C_{28}H_{39}N_7O_2$ ($M+H^+$), 518, found: 518. ^{1}H NMR (400 MHz, Methanol-d_4) δ 8.06 (s, 1H), 6.36 (s, 1H), 4.80 (s, 2H), 4.45 (s, 2H), 3.89 (d, *J* = 9.7 Hz, 5H), 3.63 (s, 2H), 3.53 – 3.45 (m, 2H), 2.81 (t, *J* = 5.6 Hz, 2H), 2.71 (d, *J* = 11.3 Hz, 2H), 2.45 (s, 3H), 2.13 (s, 3H), 1.78 (s, 2H), 1.55 – 1.41 (m, 12H), 1.01 (d, *J* = 6.2 Hz, 6H).

N-(2,2-difluoroethyl)-4-((5-(1,6-dimethyl-1H-pyrazolo[3,4-b]pyridin-4-yl)-3-methyl-4,5,6,7-tetrahydro-1H-pyrazolo[4,3-c]pyridin-1-yl)methyl)bicyclo[2.2.2]octan-1-amine **22**



To a mixture of 29.4 mg (0.07 mmol) of 4-((5-(1,6-dimethyl-1H-pyrazolo[3,4-b]pyridin-4-yl)-3-methyl-4,5,6,7-tetrahydro-1H-pyrazolo[4,3-c]pyridin-1-yl)methyl)bicyclo[2.2.2]octan-1-amine **2** and 18.1 mg (0.024 mL, 0.14 mmol) of DIPEA in 5 mL of THF was added 16.5 mg (10.25 mL, 0.077 mmol) of 2,2-difluoroethyl trifluoromethanesulfonate. The resulting mixture was stirred at 85°C for 2 h. LC-MS showed desired product and completion of the reaction. The crude product was added to a 4 g silica gel column and was eluted with 2-9% MeOH (containing small amount of ammonia) in DCM. Collected fractions and concentrated then lyophilized to give the product, N-(2,2-difluoroethyl)-4-((5-(1,6-dimethyl-1H-pyrazolo[3,4-b]pyridin-4-yl)-3-methyl-4,5,6,7-tetrahydro-1H-pyrazolo[4,3-c]pyridin-1-yl)methyl)bicyclo[2.2.2]octan-1-amine **22**. 1H NMR (400 MHz, Methanol-d4) δ 8.05 (s, 1H), 6.36 (s, 1H), 5.79 (tt, J = 55.9, 4.1 Hz, 1H), 4.44 (s, 2H), 3.89 (m, 5H), 3.64 (s, 2H), 2.94 – 2.75 (m, 4H), 2.44 (s, 3H), 2.13 (s, 3H), 1.49 (s, 12H). ESIMS calcd. for $C_{26}H_{35}F_2N_7$ ($M+H^+$), 484, found: 484.

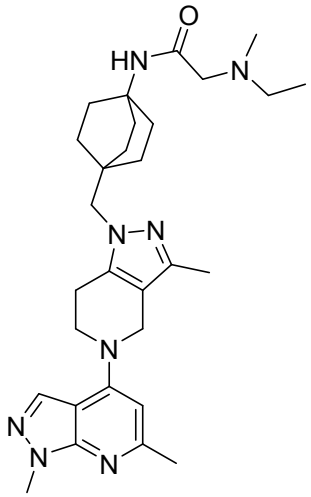
Synthesis of N-(4-((5-(1,6-dimethyl-1H-pyrazolo[3,4-b]pyridin-4-yl)-3-methyl-4,5,6,7-tetrahydro-1H-pyrazolo[4,3-c]pyridin-1-yl)methyl)bicyclo[2.2.2]octan-1-yl)-2-(ethylamino)acetamide **23**



A mixture of 126.0 mg (0.3 mmol) of 4-((5-(1,6-dimethyl-1H-pyrazolo[3,4-b]pyridin-4-yl)-3-methyl-4,5,6,7-tetrahydro-1H-pyrazolo[4,3-c]pyridin-1-yl)methyl)bicyclo[2.2.2]octan-1-amine **2**, 73.2 mg (0.36 mmol) of 2-((tert-butoxycarbonyl)(ethyl)amino)acetic acid and 78 mg (0.6 mmol) of DIPEA in DCM (3mL) was added 137.0 mg (0.36 mmol) of HATU and was stirred at RT for 30 minutes. After completion of the reaction (LC-MS monitoring), the mixture was concentrated and purified by ISCO (0-40% IPA in DCM as eluent, IPA contained 0.02M of ammonia). The fractions were concentrated and the residue was dissolved in MeOH/1,4-dioxane (1.5 mL, 1:2 v/v). 4M HCl in

1,4-dioxane (2.0 mL) was added and the mixture was stirred at RT for 1 h, then concentrated. The residue was neutralized using basic resin then lyophilized to give 136.0 mg (0.264 mmol, yield: 88%) the desired product, N-(4-((5-(1,6-dimethyl-1H-pyrazolo[3,4-b]pyridin-4-yl)-3-methyl-4,5,6,7-tetrahydro-1H-pyrazolo[4,3-c]pyridin-1-yl)methyl)bicyclo[2.2.2]octan-1-yl)-2-(ethylamino)acetamide **23**. ESIMS calcd. for $C_{28}H_{40}N_8O$ ($M+H^+$), 505, found: 505. ^1H NMR (400 MHz, Methanol-d4) δ 8.16 (s, 1H), 6.46 (s, 1H), 4.54 (s, 2H), 4.00 (s, 3H), 3.98 (t, J = 5.6 Hz, 2H), 3.73 (s, 2H), 3.13 (s, 2H), 2.91 (t, J = 5.6 Hz, 2H), 2.59 (q, J = 7.2 Hz, 2H), 2.55 (s, 3H), 2.24 (s, 3H), 1.92 – 1.85 (m, 6H), 1.62 – 1.53 (m, 6H), 1.10 (t, J = 7.2 Hz, 3H). EA for $C_{28}H_{40}N_8O$ (C, H, N) calcd: 66.64, 7.99, 22.20; found: 65.48, 8.04, 21.82.

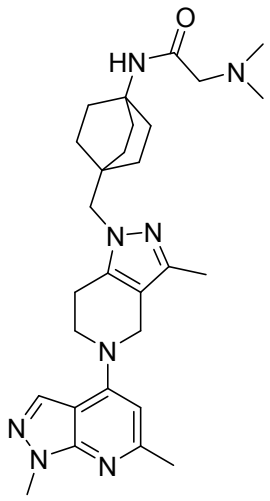
Synthesis of N-(4-((5-(1,6-dimethyl-1H-pyrazolo[3,4-b]pyridin-4-yl)-3-methyl-4,5,6,7-tetrahydro-1H-pyrazolo[4,3-c]pyridin-1-yl)methyl)bicyclo[2.2.2]octan-1-yl)-2-(ethyl(methyl)amino)acetamide **24**



Same preparation as **23** using 126.0 mg (0.3 mmol) of 4-((5-(1,6-dimethyl-1H-pyrazolo[3,4-b]pyridin-4-yl)-3-methyl-4,5,6,7-tetrahydro-1H-pyrazolo[4,3-c]pyridin-1-yl)methyl)bicyclo[2.2.2]octan-1-amine **2**, 42.2 mg (0.36 mmol) of 2-(ethyl(methyl)amino)acetic acid, 78.0 mg (0.6 mmol) of DIPEA and 137.0 mg (0.36 mmol) of HATU in DCM (3mL). 127.0 mg (0.24 mmol, yield: 80%) of N-(4-((5-(1,6-dimethyl-1H-pyrazolo[3,4-b]pyridin-4-yl)-3-methyl-4,5,6,7-tetrahydro-1H-pyrazolo[4,3-c]pyridin-1-yl)methyl)bicyclo[2.2.2]octan-1-yl)-2-(ethyl(methyl)amino)acetamide **24** was isolated. ESIMS calcd. for $C_{29}H_{48}N_8O$ (M+H+), 519, found: 519. 1H NMR (400 MHz, Methanol-d4) δ 8.16 (s, 1H), 6.46 (s, 1H), 4.55 (s, 2H), 4.00 (s, 3H), 3.98 (t, J = 5.5 Hz, 2H), 3.73 (s, 2H), 2.91 (t, J = 5.6 Hz, 2H), 2.88 (s, 2H), 2.55 (s, 3H), 2.47

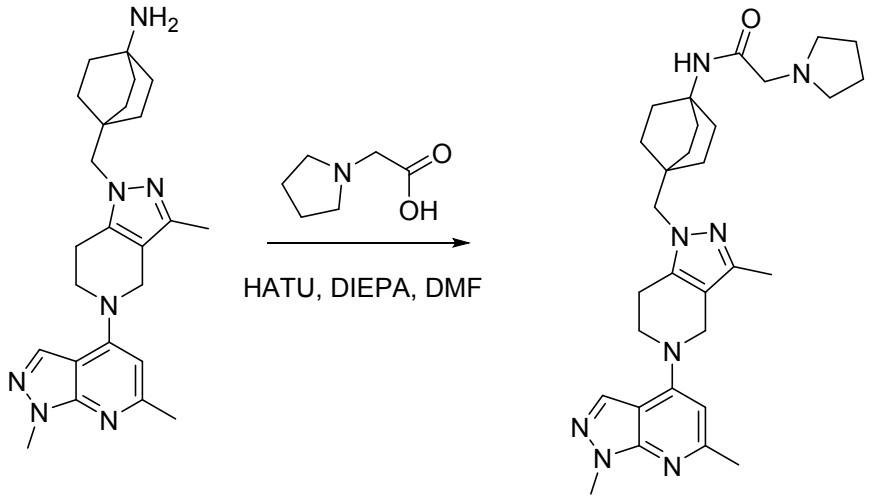
(q, J = 7.2 Hz, 2H), 2.26 (s, 3H), 2.24 (s, 3H), 1.93 – 1.85 (m, 6H), 1.63 – 1.55 (m, 6H), 1.05 (t, J = 7.2 Hz, 3H).

Synthesis of N-(4-((5-(1,6-dimethyl-1H-pyrazolo[3,4-b]pyridin-4-yl)-3-methyl-4,5,6,7-tetrahydro-1H-pyrazolo[4,3-c]pyridin-1-yl)methyl)bicyclo[2.2.2]octan-1-yl)-2-(dimethylamino)acetamide **25**



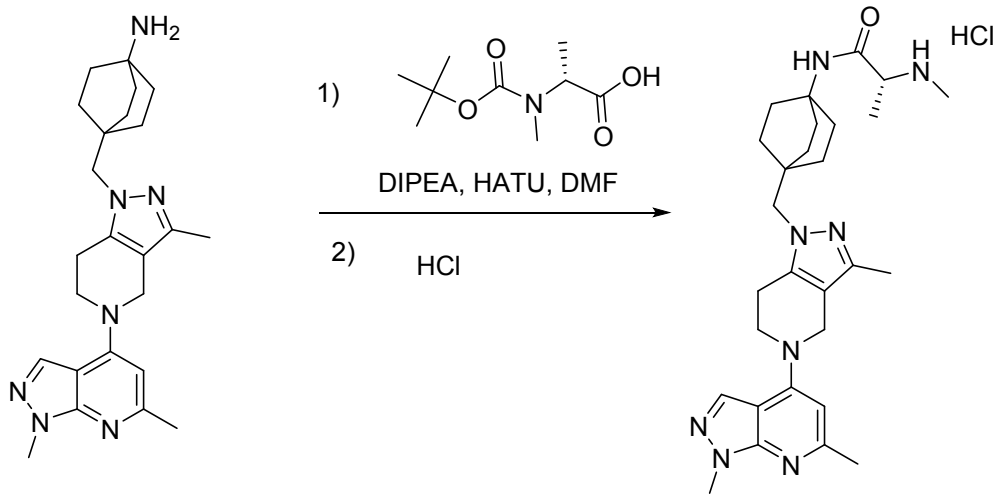
Same preparation as **23** using 42.0 mg (0.1 mmol) of 4-((5-(1,6-dimethyl-1H-pyrazolo[3,4-b]pyridin-4-yl)-3-methyl-4,5,6,7-tetrahydro-1H-pyrazolo[4,3-c]pyridin-1-yl)methyl)bicyclo[2.2.2]octan-1-amine **2**, 12.4 mg (0.12 mmol) of 2-(dimethylamino)acetic acid and 25.8 mg (0.2 mmol) of DIPEA in DCM (3mL) and 45.6 mg (0.12 mmol) of HATU. 30.0 mg (0.056 mmol, yield: 57%) of N-(4-((5-(1,6-dimethyl-1H-pyrazolo[3,4-b]pyridin-4-yl)-3-methyl-4,5,6,7-tetrahydro-1H-pyrazolo[4,3-c]pyridin-1-yl)methyl)bicyclo[2.2.2]octan-1-yl)-2-(dimethylamino)acetamide **25** was isolated. ESIMS calcd. for $C_{28}H_{40}N_8O$ (M+H+), 505, found: 505. 1H NMR (400 MHz, Methanol-d4) δ 8.16 (s, 1H), 6.46 (s, 1H), 4.55 (s, 2H), 4.00 (s, 3H), 3.98 (t, J = 5.7 Hz, 2H), 3.73 (s, 2H), 2.91 (t, J = 5.6 Hz, 2H), 2.88 (s, 2H), 2.55 (s, 3H), 2.29 (s, 6H), 2.24 (s, 3H), 1.93 – 1.85 (m, 6H), 1.63 – 1.54 (m, 6H). EA for $C_{28}H_{40}N_8O$ (C, H, N) calcd: 66.64, 7.99, 22.20; found: 67.14, 8.19, 20.73.

Synthesis of N-(4-((5-(1,6-dimethyl-1H-pyrazolo[3,4-b]pyridin-4-yl)-3-methyl-4,5,6,7-tetrahydro-1H-pyrazolo[4,3-c]pyridin-1-yl)methyl)bicyclo[2.2.2]octan-1-yl)-2-(pyrrolidin-1-yl)acetamide **26**



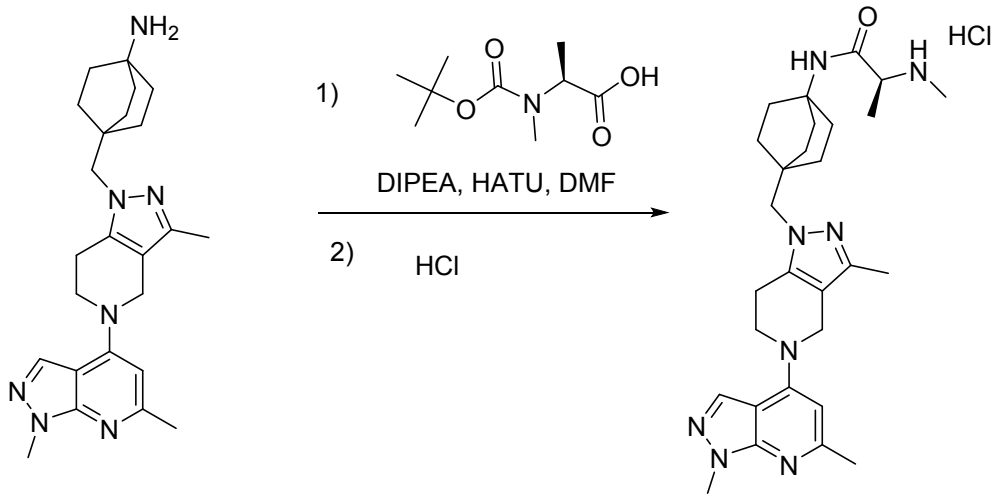
To a solution of 2-(pyrrolidin-1-yl)acetic acid (19.9 mg, 0.12 mmol) in DMF (2 mL) was added HATU (45.6 mg, 0.12 mmol) and DIPEA (25.8 mg, 0.12 mmol). The mixture was stirred at RT for 5 minutes before **2** (42.0 mg, 0.1 mmol) was added. The resulting mixture was then stirred at RT for 2 h. LC-MS showed completion of the reaction. The mixture was concentrated, and the crude product was added to a 4 g silica gel column and was eluted with 0-30% IPA (containing 1% of ammonia) in DCM. Collected fractions and concentrated then lyophilized to give the desired product, N-(4-((5-(1,6-dimethyl-1H-pyrazolo[3,4-b]pyridin-4-yl)-3-methyl-4,5,6,7-tetrahydro-1H-pyrazolo[4,3-c]pyridin-1-yl)methyl)bicyclo[2.2.2]octan-1-yl)-2-(pyrrolidin-1-yl)acetamide **26** (49.0 mg, 0.09 mmol, yield: 90%). ESIMS calcd. for $C_{30}H_{42}N_8O$ (M+H+), 531, found: 531. ^{1}H NMR (600 MHz, Methanol-d4) δ 8.05 (s, 1H), 6.36 (s, 1H), 4.44 (s, 2H), 3.90 (s, 3H), 3.90 – 3.86 (m, 2H), 3.63 (s, 2H), 3.03 (s, 2H), 2.83 – 2.77 (m, 2H), 2.62 – 2.53 (m, 4H), 2.45 (s, 3H), 2.14 (s, 3H), 1.81 – 1.76 (m, 6H), 1.75 – 1.71 (m, 4H), 1.51 – 1.46 (m, 6H).

Synthesis of (R)-N-(4-((5-(1,6-dimethyl-1H-pyrazolo[3,4-b]pyridin-4-yl)-3-methyl-4,5,6,7-tetrahydro-1H-pyrazolo[4,3-c]pyridin-1-yl)methyl)bicyclo[2.2.2]octan-1-yl)-2-(methylamino)propanamide **27**



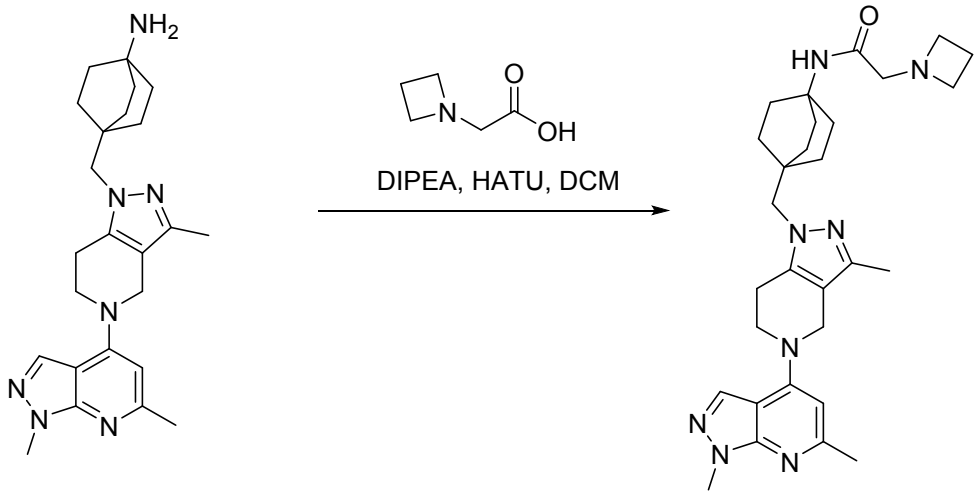
In a 5 mL vial, a mixture of 4-((5-(1,6-dimethyl-1H-pyrazolo[3,4-b]pyridin-4-yl)-3-methyl-4,5,6,7-tetrahydro-1H-pyrazolo[4,3-c]pyridin-1-yl)methyl)bicyclo[2.2.2]octan-1-amine **2** (42 mg, 0.1 mmol), (*R*)-2-((tert-butoxycarbonyl)(methyl)amino)propanoic acid (22.4 mg, 0.12 mmol), DIPEA (25.8 mg, 0.2 mmol) in DMF (1 mL) was added HATU (45.6 mg, 0.12 mmol). The mixture was stirred at RT for 30 minutes. LC-MS confirmed completion of the reaction. Purified the intermediate by mass-triggered HPLC (15-35% ACN in H_2O over 3.5 minutes). Collected fractions were concentrated and the residue was dissolved in MeOH/1,4-dioxane (1.5 mL, 1:2 v/v). 4M HCl in 1,4-dioxane (1.0 mL) was added and the mixture was stirred at RT for 1 h. The mixture was then concentrated and lyophilized to give the desired product, (*R*)-N-(4-((5-(1,6-dimethyl-1H-pyrazolo[3,4-b]pyridin-4-yl)-3-methyl-4,5,6,7-tetrahydro-1H-pyrazolo[4,3-c]pyridin-1-yl)methyl)bicyclo[2.2.2]octan-1-yl)-2-(methylamino)propenamide **27**, as HCl salt. ESIMS calcd. for $C_{28}H_{40}N_8O$ ($M+H^+$), 505, found: 505. ^{1}H NMR (400 MHz, Methanol-d4) δ 8.52 (s, 1H), 6.89 (s, 1H), 5.06 – 4.92 (m, 2H), 4.25 (s, 2H), 4.12 (s, 3H), 3.98 (s, 2H), 3.69 (q, J = 6.2, 5.6Hz, 1H), 3.20 – 3.06 (m, 2H), 2.70 (s, 3H), 2.62 (s, 3H), 2.42 (s, 3H), 2.00 – 1.88 (m, 6H), 1.69 – 1.57 (m, 6H), 1.44 (d, J = 6.9 Hz, 3H).

Synthesis of (*S*)-N-(4-((5-(1,6-dimethyl-1H-pyrazolo[3,4-b]pyridin-4-yl)-3-methyl-4,5,6,7-tetrahydro-1H-pyrazolo[4,3-c]pyridin-1-yl)methyl)bicyclo[2.2.2]octan-1-yl)-2-(methylamino)propanamide **28**



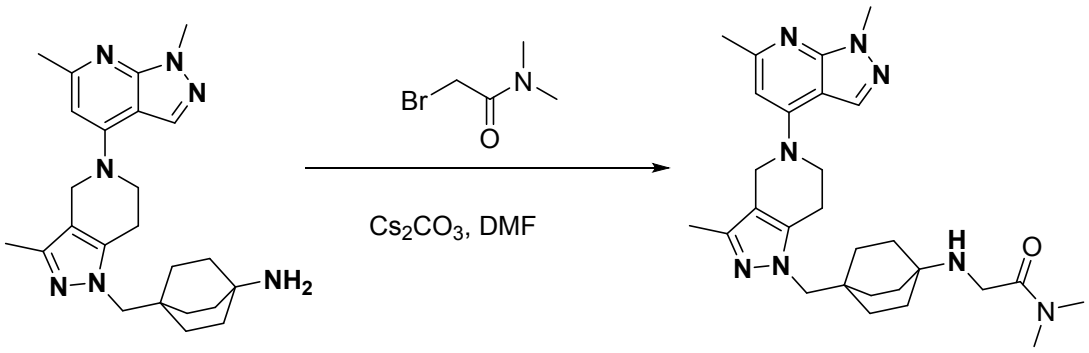
In a 5 mL vial, a mixture of 4-((5-(1,6-dimethyl-1H-pyrazolo[3,4-b]pyridin-4-yl)-3-methyl-4,5,6,7-tetrahydro-1H-pyrazolo[4,3-c]pyridin-1-yl)methyl)bicyclo[2.2.2]octan-1-amine **2** (42 mg, 0.1 mmol), (*S*)-2-((tert-butoxycarbonyl)(methyl)amino)propanoic acid (22.4 mg, 0.12 mmol), DIPEA (25.8 mg, 0.2 mmol) in DMF (1 mL) was added HATU (45.6 mg, 0.12 mmol). The mixture was stirred at RT for 30 minutes. LC-MS confirmed completion of the reaction. Purified the intermediate by mass-triggered HPLC (15-35% ACN in H_2O over 3.5 minutes). Collected fractions were concentrated and the residue was dissolved in MeOH/1,4-dioxane (1.5 mL, 1:2 v/v). 4M HCl in 1,4-dioxane (1.0 mL) was added and the mixture was stirred at RT for 1 h. The mixture was then concentrated and lyophilized to give the desired product, (*S*)-N-(4-((5-(1,6-dimethyl-1H-pyrazolo[3,4-b]pyridin-4-yl)-3-methyl-4,5,6,7-tetrahydro-1H-pyrazolo[4,3-c]pyridin-1-yl)methyl)bicyclo[2.2.2]octan-1-yl)-2-(methylamino)propenamide **28**, as HCl salt. ESIMS calcd. for $C_{28}H_{40}N_8O$ (M+H+), 505, found: 505. ^{1}H NMR (400 MHz, Methanol-d4) δ 8.53 (s, 1H), 6.90 (s, 1H), 5.10 – 4.92 (m, 2H), 4.26 (s, 2H), 4.12 (s, 3H), 4.02 (s, 2H), 3.73 – 3.66 (m, 1H), 3.19 – 3.09 (m, 2H), 2.70 (s, 3H), 2.62 (s, 3H), 2.45 (s, 3H), 2.00 – 1.90 (m, 6H), 1.70 – 1.57 (m, 6H), 1.44 (d, J = 6.9 Hz, 3H).

Synthesis of 2-(azetidin-1-yl)-N-(4-((5-(1,6-dimethyl-1H-pyrazolo[3,4-b]pyridin-4-yl)-3-methyl-4,5,6,7-tetrahydro-1H-pyrazolo[4,3-c]pyridin-1-yl)methyl)bicyclo[2.2.2]octan-1-yl)acetamide **29**



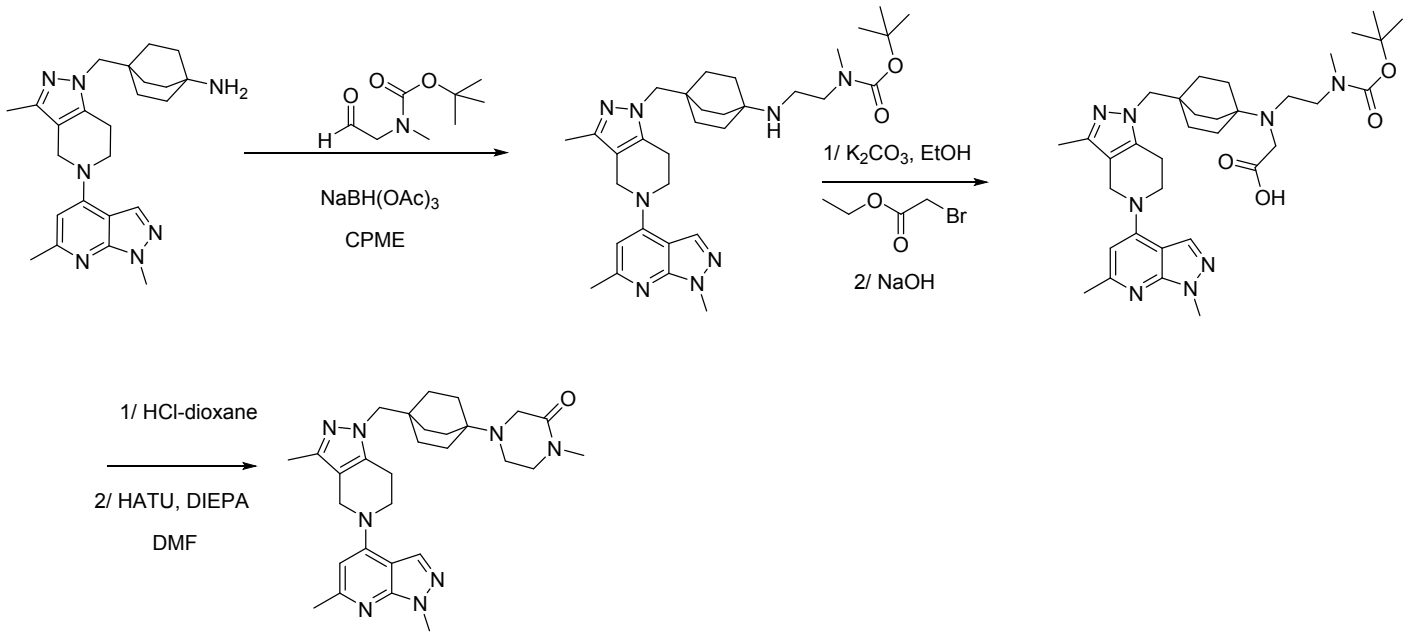
To a mixture of 18.2 mg (0.12 mmol) of 2-(azetidin-1-yl)acetic acid and 42.0 mg (0.1 mmol) of 4-((5-(1,6-dimethyl-1H-pyrazolo[3,4-b]pyridin-4-yl)-3-methyl-4,5,6,7-tetrahydro-1H-pyrazolo[4,3-c]pyridin-1-yl)methyl)bicyclo[2.2.2]octan-1-amine **2** in 5 mL of in DCM was added HATU (45.6 mg, 0.12 mmol) and DIPEA (38.8 mg, 0.052 mL, 0.3 mmol). The resulting mixture was then stirred at RT for 1 h. LC-MS showed completion of the reaction. The mixture was concentrated and the crude product was added to a 4 g silica gel column and was eluted with 0-50% IPA (containing 0.02 M of ammonia) in DCM. Collected fractions and concentrated then further purified by mass-triggered HPLC (10-30% ACN in H_2O over 3.5 minutes). 1N aqueous HCl was added and lyophilized to give the desired product, 2-(azetidin-1-yl)-N-(4-((5-(1,6-dimethyl-1H-pyrazolo[3,4-b]pyridin-4-yl)-3-methyl-4,5,6,7-tetrahydro-1H-pyrazolo[4,3-c]pyridin-1-yl)methyl)bicyclo[2.2.2]octan-1-yl)acetamide **29**, as HCl salt. ^{1}H NMR (400 MHz, Methanol-d4) δ 8.52 (s, 1H), 6.89 (s, 1H), 4.95 (d, J = 13.8 Hz, 2H), 4.26 (tdd, J = 9.2, 5.1, 1.8 Hz, 4H), 4.12 (s, 3H), 4.11 – 4.02 (m, 2H), 3.94 (d, J = 7.7 Hz, 4H), 3.20 – 3.06 (m, 2H), 2.70 (s, 3H), 2.59 (dp, J = 11.9, 9.2 Hz, 1H), 2.41 (s, 4H), 2.21 – 2.10 (m, 0H), 1.91 (dd, J = 10.9, 5.1 Hz, 6H), 1.67 – 1.56 (m, 6H). ESIMS calcd. for $C_{29}H_{40}N_8O$ (M+H+), 517; found 517.

Synthesis of 2-((4-((5-(1,6-dimethyl-1H-pyrazolo[3,4-b]pyridin-4-yl)-3-methyl-4,5,6,7-tetrahydro-1H-pyrazolo[4,3-c]pyridin-1-yl)methyl)bicyclo[2.2.2]octan-1-yl)amino)-N,N-dimethylacetamide **30**



A mixture of 42.0 mg (0.1 mmol) of 4-((5-(1,6-dimethyl-1H-pyrazolo[3,4-b]pyridin-4-yl)-3-methyl-4,5,6,7-tetrahydro-1H-pyrazolo[4,3-c]pyridin-1-yl)methyl)bicyclo[2.2.2]octan-1-amine **2**, 18.3 mg (0.11 mmol) of 2-bromo-N,N-dimethylacetamide and 65.2 mg (0.2 mmol) of Cs_2CO_3 in DMF (1 mL) was stirred at RT overnight. The crude product was directly added to a 4 g silica gel column and was eluted with 0-30% IPA (containing 1% of ammonia) in DCM. The collected fractions were concentrated then lyophilized to give 26.0 mg (0.049 mmol, yield: 49%) of the desired product, 2-((4-((5-(1,6-dimethyl-1H-pyrazolo[3,4-b]pyridin-4-yl)-3-methyl-4,5,6,7-tetrahydro-1H-pyrazolo[4,3-c]pyridin-1-yl)methyl)bicyclo[2.2.2]octan-1-yl)amino)-N,N-dimethylacetamide **30**. ESIMS calcd. for $C_{28}H_{40}N_8O$ ($M+H^+$), 505, found: 505. ^1H NMR (400 MHz, Methanol-d4) δ 8.17 (d, J = 1.8 Hz, 1H), 6.47 (s, 1H), 4.56 (s, 2H), 4.02 (s, 3H), 4.00 (t, J = 5.7 Hz, 2H), 3.76 (m, 2H), 3.56 (m, 2H), 3.03 – 2.95 (m, 6H), 2.92 (t, J = 5.6 Hz, 2H), 2.56 (s, 3H), 2.25 (s, 3H), 1.73 – 1.56 (m, 12H).

Synthesis of 4-(4-((5-(1,6-dimethyl-1H-pyrazolo[3,4-b]pyridin-4-yl)-3-methyl-4,5,6,7-tetrahydro-1H-pyrazolo[4,3-c]pyridin-1-yl)methyl)bicyclo[2.2.2]octan-1-yl)-1-methylpiperazin-2-one **31**



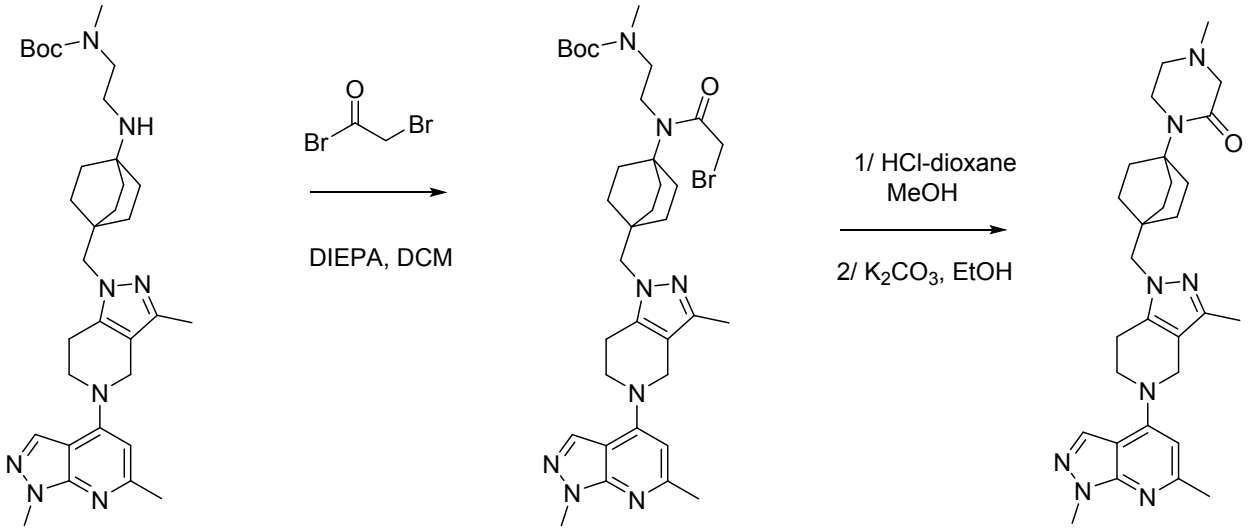
Step 1: To a solution of 4-((5-(1,6-dimethyl-1H-pyrazolo[3,4-b]pyridin-4-yl)-3-methyl-4,5,6,7-tetrahydro-1H-pyrazolo[4,3-c]pyridin-1-yl)methyl)bicyclo[2.2.2]octan-1-amine **2** (50 mg, 0.10 mmol) in CPME (Volume: 5 mL) was added *tert*-butyl methyl(2-oxoethyl)carbamate (52.8 mg, 0.305 mmol). The mixture was stirred at 25°C for 30 minutes before sodium triacetoxyborohydride (64.6 mg, 0.305 mmol) was added. Then the mixture was stirred at 25 °C for 3 hr. The mixture was concentrated and purified on a 4 g silica gel column using 0-40% IPA in DCM with 2% NH_3 as modifier to afford 32.0 mg (0.055 mmol, yield: 55%) of tert-butyl (2-((4-((5-(1,6-dimethyl-1H-pyrazolo[3,4-b]pyridin-4-yl)-3-methyl-4,5,6,7-tetrahydro-1H-pyrazolo[4,3-c]pyridin-1-yl)methyl)bicyclo[2.2.2]octan-1-yl)amino)ethyl)(methyl)carbamate. ESIMS calcd. for $C_{32}H_{48}N_8O_2$ ($M+H^+$), 577, found: 577.

Step 2: A mixture of 40 mg (0.069 mmol) of *tert*-butyl (2-((4-((5-(1,6-dimethyl-1H-pyrazolo[3,4-b]pyridin-4-yl)-3-methyl-4,5,6,7-tetrahydro-1H-pyrazolo[4,3-c]pyridin-1-yl)methyl)bicyclo[2.2.2]octan-1-yl)amino)ethyl)(methyl)carbamate, 35.5 mg (0.21 mmol) of ethyl 2-bromoacetate and 10.5 mg (0.076 mmol) of K_2CO_3 in EtOH (1 mL) was heated under microwave irradiation at 150 °C for 1 h. After completion of the reaction (LC-MS monitoring), the mixture was cooled at RT. 1N aqueous NaOH (0.35 mL) was added and the mixture was stirred at RT for 3 h. The mixture was diluted with 2 mL of water and the organic solvents removed under reduced pressure. The aqueous layer was carefully acidified with 1N aqueous HCl to ~pH 3, then purified by mass-triggered HPLC to give 27.0 mg (0.043 mmol, yield: 61%) of 2-((2-((tert-

butoxycarbonyl)(methyl)amino)ethyl)(4-((5-(1,6-dimethyl-1H-pyrazolo[3,4-b]pyridin-4-yl)-3-methyl-4,5,6,7-tetrahydro-1H-pyrazolo[4,3-c]pyridin-1-yl)methyl)bicyclo[2.2.2]octan-1-yl)amino)acetic acid. ESIMS calcd. for $C_{34}H_{50}N_8O_4$ ($M+H^+$), 635, found: 635.

Step 3: To a mixture of 27.0 mg (0.043 mmol) of 2-((2-((*tert*-butoxycarbonyl)(methyl)amino)ethyl)(4-((5-(1,6-dimethyl-1H-pyrazolo[3,4-b]pyridin-4-yl)-3-methyl-4,5,6,7-tetrahydro-1H-pyrazolo[4,3-c]pyridin-1-yl)methyl)bicyclo[2.2.2]octan-1-yl)amino)acetic acid in 1 mL of 1,4-dioxane was added 0.3 mL of MeOH to get a clear solution. 4 M HCl in dioxane was added dropwise and the resulting mixture was stirred at RT for 1 h (LC-MS monitoring). The mixture was concentrated, and the residue was added 2.0 mL of DMF and DIPEA (38 uL, 5 eq.) then added dropwise into a solution of HATU (21 mg, 1.3 eq.) in 3.0 mL of DMF at 0 °C. The resulting mixture was stirred at 0 °C for 30 minutes until completion (LC-MS monitoring). The crude product was purified by mass-triggered HPLC (10-20% ACN in H_2O over 3.5 minutes) then neutralized using basic resin to give 6.0 mg (0.0015 mmol, yield: 24%) of the desired product, 4-(4-((5-(1,6-dimethyl-1H-pyrazolo[3,4-b]pyridin-4-yl)-3-methyl-4,5,6,7-tetrahydro-1H-pyrazolo[4,3-c]pyridin-1-yl)methyl)bicyclo[2.2.2]octan-1-yl)-1-methylpiperazin-2-one **31**. ESIMS calcd. for $C_{29}H_{40}N_8O$ ($M+H^+$), 517, found: 517. ^{1}H NMR (400 MHz, Methanol-d4) δ 8.05 (s, 1H), 6.35 (s, 1H), 4.44 (s, 2H), 3.90 (s, 3H), 3.87 (t, J = 5.5 Hz, 2H), 3.62 (s, 2H), 3.17 (dd, J = 6.3, 4.5 Hz, 2H), 3.08 (s, 2H), 2.80 (m, 5H), 2.66 (dd, J = 6.3, 4.5 Hz, 2H), 2.44 (s, 3H), 2.13 (s, 3H), 1.48 (tq, J = 9.4, 6.4, 4.7 Hz, 12H).

Synthesis of 1-(4-((5-(1,6-dimethyl-1H-pyrazolo[3,4-b]pyridin-4-yl)-3-methyl-4,5,6,7-tetrahydro-1H-pyrazolo[4,3-c]pyridin-1-yl)methyl)bicyclo[2.2.2]octan-1-yl)-4-methylpiperazin-2-one **32**

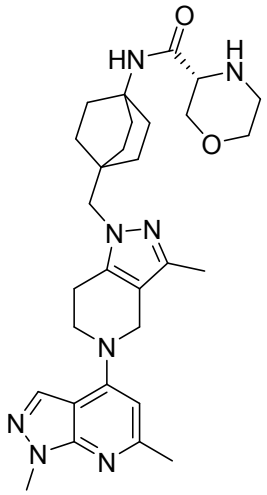


Step 1: A mixture of 40.0 mg (0.069 mmol) of tert-butyl (2-((4-((5-(1,6-dimethyl-1H-pyrazolo[3,4-b]pyridin-4-yl)-3-methyl-4,5,6,7-tetrahydro-1H-pyrazolo[4,3-c]pyridin-1-yl)methyl)bicyclo[2.2.2]octan-1-yl)amino)ethyl)(methyl)carbamate (see step 1 for **31**) and 17.9 mg (0.14 mmol) of DIPEA in 1.0 mL of DCM was added dropwise into a solution of 228.6 mg (0.14 mmol) of 2-bromoacetyl bromide in DCM (1.0 mL) at 0 °C. The resulting mixture was slowly warmed up to RT and stirred for 2 h. LC-MS showed desired product, but the reaction was not complete. More 2-bromoacetyl bromide (2.6 eq.) was added and the mixture was stirred at RT for additional 2 h. Upon completion (LC-MS monitoring), the reaction was quenched by adding 0.1 mL of water and concentrated. The crude product was added to a 4 g silica gel column and was eluted with 0-30% IPA (containing 0.02 M ammonia) in DCM. Collected fractions and concentrated to give the desired product, tert-butyl (2-(2-bromo-N-(4-((5-(1,6-dimethyl-1H-pyrazolo[3,4-b]pyridin-4-yl)-3-methyl-4,5,6,7-tetrahydro-1H-pyrazolo[4,3-c]pyridin-1-yl)methyl)bicyclo[2.2.2]octan-1-yl)acetamido)ethyl)(methyl)carbamate, which contained some DIPEA based on LC-MS and used directly for the next step without further purification. ESIMS calcd. for $C_{34}H_{49}BrN_8O_3$ (M+H+), 698, found: 698..

Step 2: To a mixture of 55.0 mg (0.063 mmol) of *tert*-butyl (2-(2-bromo-N-(4-((5-(1,6-dimethyl-1H-pyrazolo[3,4-b]pyridin-4-yl)-3-methyl-4,5,6,7-tetrahydro-1H-pyrazolo[4,3-c]pyridin-1-yl)methyl)bicyclo[2.2.2]octan-1-yl)acetamido)ethyl)(methyl)carbamate in 1 mL of 1,4-dioxane was added 0.2 mL of MeOH to get a clear solution. 4 M HCl in dioxane was added dropwise and the resulting mixture was stirred at RT for 30 minutes. After completion of the reaction (LC-MS monitoring), the mixture was concentrated and 5.0 mL of EtOH followed by 26.1 mg (0.19 mmol) of K_2CO_3 was added. The resulting mixture was heated at 120 °C for 40 minutes under microwave

irradiation. After removal of the solvents under reduced pressure, the residue was purified by mass-triggered HPLC (10-20% ACN in H_2O over 3.5 minutes) then 1N aqueous HCl was added and lyophilized to give 3.8 mg (0.006 mmol, yield: 10%) of 1-(4-((5-(1,6-dimethyl-1H-pyrazolo[3,4-b]pyridin-4-yl)-3-methyl-4,5,6,7-tetrahydro-1H-pyrazolo[4,3-c]pyridin-1-yl)methyl)bicyclo[2.2.2]octan-1-yl)-4-methylpiperazin-2-one **32**, as HCl salt. ESIMS calcd. for $C_{29}H_{40}N_8O$ ($M+H^+$), 517, found: 517. ^1H NMR (400 MHz, Methanol-d4) δ 8.53 (s, 1H), 6.90 (s, 1H), 4.99 (s, 2H), 4.27 (s, 2H), 4.12 (s, 3H), 4.02 (s, 2H), 3.92 (d, J = 12.1 Hz, 1H), 3.75 (dd, J = 35.3, 18.9 Hz, 5H), 3.20 – 3.11 (m, 2H), 2.94 (s, 3H), 2.71 (s, 3H), 2.46 (s, 3H), 2.16 (d, J = 7.8 Hz, 6H), 1.67 (d, J = 6.7 Hz, 6H).

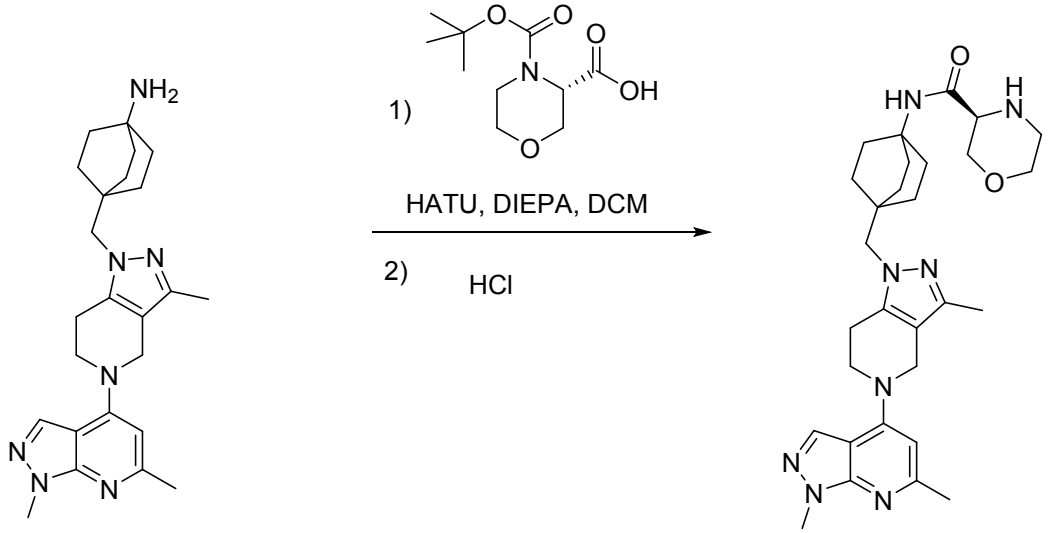
Synthesis of (*R*)-N-(4-((5-(1,6-dimethyl-1H-pyrazolo[3,4-b]pyridin-4-yl)-3-methyl-4,5,6,7-tetrahydro-1H-pyrazolo[4,3-c]pyridin-1-yl)methyl)bicyclo[2.2.2]octan-1-yl)morpholine-3-carboxamide **33**



Same preparation as **23** using 42.0 mg (0.1 mmol) of 4-((5-(1,6-dimethyl-1H-pyrazolo[3,4-b]pyridin-4-yl)-3-methyl-4,5,6,7-tetrahydro-1H-pyrazolo[4,3-c]pyridin-1-yl)methyl)bicyclo[2.2.2]octan-1-amine **2**, 23.1 mg (0.1 mmol) of (R)-4-(tert-butoxycarbonyl)morpholine-3-carboxylic acid, 25.8 mg (0.2 mmol) of DIPEA and 45.6 mg (0.12 mmol) of HATU in DCM (3mL). 58.0 mg (0.091 mmol, yield: 91%) of (*R*)-N-(4-((5-(1,6-dimethyl-1H-pyrazolo[3,4-b]pyridin-4-yl)-3-methyl-4,5,6,7-tetrahydro-1H-pyrazolo[4,3-c]pyridin-1-yl)methyl)bicyclo[2.2.2]octan-1-yl)morpholine-3-carboxamide **33** was isolated. ESIMS calcd. for $C_{29}H_{48}N_8O_2$ ($M+H^+$), 533, found: 533. ^1H NMR (400 MHz, Methanol-d4) δ 8.51 (s, 1H), 7.92 (s, 1H), 6.88 (s, 1H), 5.03 – 4.92 (m, 1H), 4.32 – 4.18 (m, 2H), 4.20 – 4.09 (m, 4H), 4.03 – 3.89 (m,

4H), 3.78 – 3.54 (m, 4H), 3.22 (ddd, J = 13.0, 11.2, 3.8 Hz, 1H), 3.15 – 3.05 (m, 2H), 2.69 (s, 3H), 2.39 (s, 3H), 1.98 – 1.85 (m, 6H), 1.62 (dd, J = 10.3, 5.9 Hz, 6H).

Synthesis of (S)-N-(4-((5-(1,6-dimethyl-1H-pyrazolo[3,4-b]pyridin-4-yl)-3-methyl-4,5,6,7-tetrahydro-1H-pyrazolo[4,3-c]pyridin-1-yl)methyl)bicyclo[2.2.2]octan-1-yl)morpholine-3-carboxamide **34**

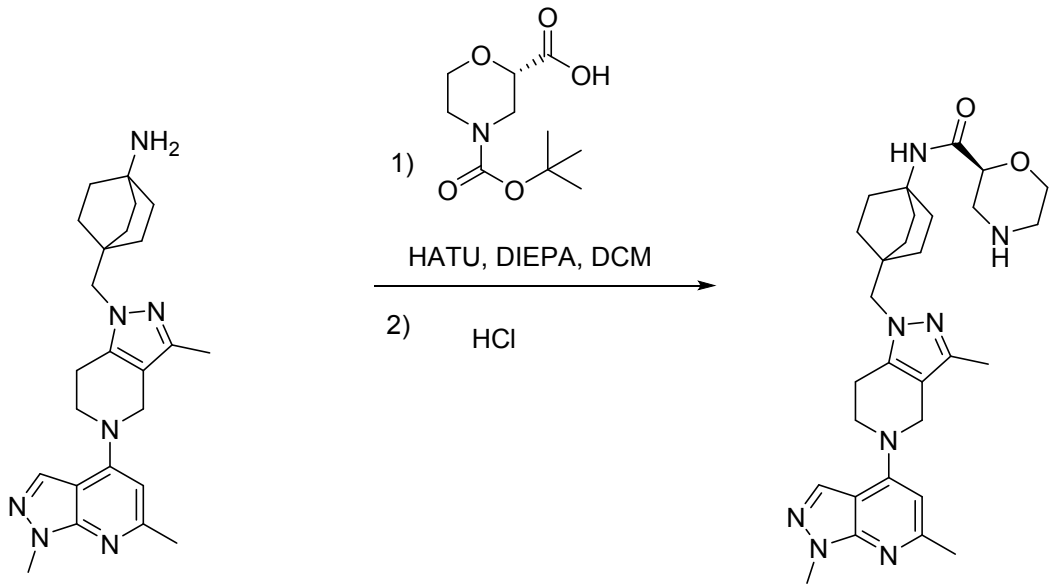


Step 1: Same prep as **23** using 5.0 g (10.73 mmol) of 4-((5-(1,6-dimethyl-1H-pyrazolo[3,4-b]pyridin-4-yl)-3-methyl-4,5,6,7-tetrahydro-1H-pyrazolo[4,3-c]pyridin-1-yl)methyl)bicyclo[2.2.2]octan-1-amine **2**, 2.53 g (10.94 mmol) of (*S*)-4-(tert-butoxycarbonyl)morpholine-3-carboxylic acid , 2.079 g (11.15 mmol) of DIPEA and 4.24 g (11.15 mmol) of HATU in DCM (100 mL). The crude (S)-tert-butyl 3-((4-((5-(1,6-dimethyl-1H-pyrazolo[3,4-b]pyridin-4-yl)-3-methyl-4,5,6,7-tetrahydro-1H-pyrazolo[4,3-c]pyridin-1-yl)methyl)bicyclo[2.2.2]octan-1-yl)carbamoyl)morpholine-4-carboxylate was directly used into step 2.

Step 2: Crude (S)-tert-butyl 3-((4-((5-(1,6-dimethyl-1H-pyrazolo[3,4-b]pyridin-4-yl)-3-methyl-4,5,6,7-tetrahydro-1H-pyrazolo[4,3-c]pyridin-1-yl)methyl)bicyclo[2.2.2]octan-1-yl)carbamoyl)morpholine-4-carboxylate was dissolved in 4N HCl in dioxane (53.6 ml, 215 mmol) and methanol (6 mL). The mixture was stirred at RT overnight then concentrated. The residue was re-dissolved in EtOAc and washed with 1N NaOH. The organic layer was dried over $MgSO_4$, filtered and concentrated under reduced pressure. The residue was loaded on a 40 g silica gel column using 0-100% IPA in DCM with 3% NH_3 as modifier to elute the product. One pure fraction

was selected, concentrated and then suspended in ethyl ether. The solid was filtered and wash twice with ethyl ether. The solid was dried under high vacuum at 90 °C for 2 days to afford (S)-N-(4-((5-(1,6-dimethyl-1H-pyrazolo[3,4-b]pyridin-4-yl)-3-methyl-4,5,6,7-tetrahydro-1H-pyrazolo[4,3-c]pyridin-1-yl)methyl)bicyclo[2.2.2]octan-1-yl)morpholine-3-carboxamide **34** (5.77 g, 10.51 mmol, yield: 98%) . ESIMS calcd. for $C_{29}H_{40}N_8O_2$ ($M+H^+$), 533, found: 533. ^1H NMR (400 MHz, Methanol-d4) δ 8.52 (s, 1H), 7.92 (s, 1H), 6.88 (s, 1H), 5.06 – 4.91 (m, 2H), 4.25 (s, 2H), 4.19 – 4.09 (m, 4H), 4.03 – 3.89 (m, 4H), 3.78 – 3.56 (m, 4H), 3.22 (ddd, J = 12.9, 11.2, 3.7 Hz, 1H), 3.17 – 3.05 (m, 2H), 2.70 (s, 3H), 2.41 (s, 3H), 1.93 (dd, J = 8.9, 5.0 Hz, 6H), 1.72 – 1.54 (m, 6H).

Synthesis of (*2*)-N-(4-((5-(1,6-dimethyl-1H-pyrazolo[3,4-b]pyridin-4-yl)-3-methyl-4,5,6,7-tetrahydro-1H-pyrazolo[4,3-c]pyridin-1-yl)methyl)bicyclo[2.2.2]octan-1-yl)morpholine-2-carboxamide **35**



Step 1: To a mixture of 100 mg (0.24 mmol) of 4-((5-(1,6-dimethyl-1H-pyrazolo[3,4-b]pyridin-4-yl)-3-methyl-4,5,6,7-tetrahydro-1H-pyrazolo[4,3-c]pyridin-1-yl)methyl)bicyclo[2.2.2]octan-1-amine **2**, 60 mg (0.26 mmol) of (*S*)-4-(*tert*-butoxycarbonyl)morpholine-2-carboxylic acid , 40 mg (0.31 mmol) of DIPEA and 100 mg (0.26 mmol) of HATU in DCM (10 mL). The crude (S)-tert-butyl 3-((4-((5-(1,6-dimethyl-1H-pyrazolo[3,4-b]pyridin-4-yl)-3-methyl-4,5,6,7-tetrahydro-1H-pyrazolo[4,3-c]pyridin-1-yl)methyl)bicyclo[2.2.2]octan-1-yl)carbamoyl)morpholine-4-carboxylate was directly used into step 2.

Step 2: Crude (S)-tert-butyl 2-((4-((5-(1,6-dimethyl-1H-pyrazolo[3,4-b]pyridin-4-yl)-3-methyl-4,5,6,7-tetrahydro-1H-pyrazolo[4,3-c]pyridin-1-yl)methyl)bicyclo[2.2.2]octan-1-yl)carbamoyl)morpholine-4-carboxylate was dissolved in 4N HCl in dioxane (0.5 ml, 2 mmol) and methanol (1 mL). The mixture was stirred at RT for overnight then concentrated. The residue was concentrated, re-dissolved in EtOAc and washed with 1N NaOH. The organic layer was dried over $MgSO_4$, filtered and concentrated under reduced pressure. The residue was loaded on a 12 g silica gel column using 0-100% IPA in DCM with 3% NH_3 as modifier to elute the product. One pure fraction was selected, concentrated and then suspended in ethyl ether. The solid was filtered and wash twice with ethyl ether. The solid was dried under high vacuum at 90 °C for 2 days to afford (S)-N-(4-((5-(1,6-dimethyl-1H-pyrazolo[3,4-b]pyridin-4-yl)-3-methyl-4,5,6,7-tetrahydro-1H-pyrazolo[4,3-c]pyridin-1-yl)methyl)bicyclo[2.2.2]octan-1-yl)morpholine-2-carboxamide **35** (5.77 g, 10.51 mmol, yield: 98%) . ESIMS calcd. for $C_{29}H_{40}N_8O_2$ ($M+H^+$), 533, found: 533. ^1H NMR (400 MHz, Methanol-d4) δ 8.19 (s, 1H), 6.49 (s, 1H), 4.55 (s, 2H), 3.99 (s, 3H), 3.54-3.98 (m, 6H), 3.12 (m, 1H), 2.71-2.89 (m, 4H), 2.67 (s, 3H),2.54 (m, 4H), 2.31 (s, 3H), 1.94 (m, 6H), 1.74 – 1.55 (m, 6H).

In vitro kinase assays for selectivity profiling

Biochemical kinase assays against a panel of 46 kinases were performed at Novartis (Switzerland) using microfluidic mobility shift technology as described (Drueckes P. Protein Kinase Selectivity Profiling Using Microfluid Mobility Shift Assays. Methods Mol Biol. 2016;1439:143-57. doi: 10.1007/978-1-4939-3673-1_9. PMID: 27316993). All assays were performed in 384 well microtiter plates and liquid handling and incubation steps using an Innovadyne Nanodrop Express equipped with a robotic arm (Thermo CatX, Caliper Twister II) and an incubator (Liconic STX40, Thermo Cytomat 2C450). In brief, each kinase reaction was started by stepwise addition of 4.5 µl per well of peptide/ATP-solution (50 mM HEPES, pH 7.5, 1 mM DTT, 0.02% Tween20, 0.02% BSA, 10mM beta-glycerophosphate, and 10 µM sodium orthovanadate, Mg/Mn chloride, ATP, and peptide) and 4.5 µl per well of enzyme solution (50mM HEPES, pH 7.5, 1mM DTT, 0.02% Tween20, 0.02% BSA, 10mM beta-glycerophosphate, and 10 µM sodium orthovanadate, Mg/Mn chloride, and enzyme). Concentrations for Mg/Mn and enzyme were adjusted to the assay specific requirements. Peptides were used at 2 µM or at apparent K_M (if K_M<2 µM). ATP concentrations were adjusted to the apparent K_M of the specific enzyme. Following incubation at 60 min at 30 °C, assays were terminated with the addition of buffer (100 mM HEPES pH 7.5, 5% DMSO, 0.1% Caliper coating reagent, 10 mM ETDA, and 0.015% Brij35) and transferred to the Caliper LC3000 workstation (PerkinElmer) for reading. Phosphorylated and unphosphorylated peptides were separated using the Caliper microfluidic mobility shift technology. Kinase activities were calculated from the amounts of formed phospho-peptide. IC_{50} values were determined from percent inhibition values by non-linear regression analysis.

Animals

All animal experiments were approved by the Veterinary Office Basel Stadt and performed under strict adherence to the Swiss law or animal protection and AAALAC requirements. C57BL/6 and 129/Sv mice were obtained from Charles River (Saint-Germain-sur-l'Arbresle, France) or Janvier (Le Genest St. Isle, France) and housed under specific pathogen free conditions.

Mouse splenocyte assays for TLR7 and TLR9

Single cell splenocyte suspensions were prepared from 129Sv mouse spleens by dissection into PBS w/o Ca^{2+}/Mg^{2+} (Life Technologies) and homogenization through a 40 µm cell strainer (BD Falcon). Splenocytes were washed in PBS, red blood cells lysed using RBC Lysis Buffer (Amimed

3-13F00-H) and resuspended in Assay Medium (RPMI1640, 10% FBS, 1x Pen/Strep, non-essential amino acids, Sodium Pyruvate, 0.05 mM β-mercaptoethanol; ThermoFisher Scientific). Cells were seeded into 96-well plates and incubated with increasing concentrations of compound or vehicle control (0.5 % v/v DMSO final per well) for 1h at 37 °C and 5 % CO_2. For TLR7 stimulation, 0.2 µg/mL ssRNA40 (Microsynth, Switzerland) was pre-complexed with N-[1-(2,3 dioleoyloxy) propyl]-N,N,N tri-methyl ammonium methylsulfate (DOTAP) (2.5 µg/mL; Roche life sciences). For TLR9, 0.4 µM of ODN1585 (Microsynth). After 20 h at 37 °C in a humidified incubator, IFNα levels from supernatants were quantified by mouse IFNα Platinum ELISA (EBioSciences, San Diego, CA, USA), using a Spectramax reader M5 (Molecular Devices). Cell viability was assessed by a CellTiter-Glo assay (Promega) according to manufacturer's instructions.

Mouse splenocyte assays for TLR4

Single cell splenocyte suspensions were prepared from C57BL/6 mice and stimulated with the TLR4 agonist LPS011:B4 (Sigma) at 3 µg/ml as indicated above. After 20 h, IL-6 levels in supernatants were quantified by mouse IL-6 DuoSet ELISA (RnD Systems), using a Spectramax reader M5. Cell viability was assessed by a CellTiter-Glo assay.

PBMC assays for TLR4, TLR7, TLR8 and TLR9

PBMC isolation: Fresh human blood, collected in S-Monovette Heparin tubes (Starstedt) were obtained from healthy individuals with patient informed consent from Scripps Normal Donor Service San Diego or Santémed Gesundheitszentrum AG Basel Switzerland. PBMCs were prepared as described previously (Knoepfel 2020, ref 14 in main paper)

TLR7 and TLR9 Assay: Isolated PBMCs were seeded in 384-well plates as outlined in Knoepfel 2020 (ref 14 in main paper) and stimulated. For TLR7 stimulation, cells were activated with 10 µg/ml ssRNA40 (Microsynth, Switzerland or Trilink, San Diego) pre-complexed with 25 µg/ml DOTAP (Roche Life Sciences). For TLR9 stimulation, cells were activated with 0.3 µM of ODN2216 (Invivogen). After 20 h at 37 °C in a humidified incubator, IFNα was quantified from supernatants using the human interferon AlphaLISA kit (PerkinElmer) and an Enspire or EnVision multiplate reader (PerkinElmer) according to manufacturer's protocols. Cell viability was assessed by CellTiter-Glo (Promega).

TLR4 and TLR8 Assay: Isolated PBMCs were seeded in 384-well plates as outlined in Knoepfel 2020 (ref 14 in main paper) and stimulated. For TLR4 stimulation, cells were activated with 5

ng/ml LPS011:B4 (Sigma), and for TLR8 stimulation, cells were activated with 1 µg/ml ssRNA40 pre-complexed with 25 µg/ml DOTAP. After 20 h at 37 °C in a humidified incubator, IL-6 was quantified from supernatants by HTRF (CisBio) and a RUBYstar or PHERAstar (BMG Labtech) fluorescent plate reader according to manufacturer's protocols. Cell viability was assessed by a CellTiter-Glo (Promega).

Human and mouse whole blood assays

Fresh human blood was collected into citrate S-Monovette 9NC tubes (Sarstedt) and blood from 129/Sv mice (for TLR7 or TLR9 assays) or C57BL/6 mice (for TLR4 assays) was collected into Eppendorf tubes containing 4% sodium citrate solution (Sigma Aldrich). Blood was diluted 1:1 with RPMI1640 and incubated with increasing concentrations of MHV370 or vehicle control (0.25% v/v DMSO final per well) in RPMI1640 for 30 min at 37°C and 5% CO_2 (1h pre-incubation for TLR4 assays). Human blood was stimulated for TLR7-mediated IFNα and for TLR8-mediated IL-6 with ssRNA40 (1 µg/ml, precomplexed with 15 µg/ml DOTAP). For TLR9-driven IFNα, human blood was stimulated with ODN2216 (0.2 µM, precomplexed with 15 µg/ml DOTAP). For TLR4-driven IL-6, human blood was stimulated with LPS011:B4 (5 ng/ml, Sigma).

Mouse blood was stimulated for TLR7 responses with ssRNA40 (1 µg/ml precomplexed with 15 µg/ml DOTAP), for TLR9 responses with ODN1585 (0.2 µM precomplexed with 25 µg/ml DOTAP for IFNα), and for TLR4 responses with LPS011:B4 (1 µg/ml). All blood assays were carried out in a final assay volume of 100 µl/well. After incubation for 20 h at 37°C in a humidified incubator, cytokines were quantified from supernatants as reported previously (Knoepfel 2020, ref 14 in main paper). Cell viability was assessed by ATPlite Luminescence assay (PerkinElmer) according to manufacturer's protocols.

Mouse acute cytokine release assay

MHV370 in MC/Tween (0.5 % methylcellulose/ 0.5 % Tween-80) or vehicle alone was administered p.o. to 129/Sv mice (n=5 per group). At 1 h, mice were injected i.v. with 20 µg R0006 (Microsynth) or 20 µg CpG1585, each pre-complexed with 140 µg DOTAP in Hanks Balanced Salt solution (HBSS). At 3 h, blood was withdrawn into EDTA Microvettes (Sarstedt) and plasma TNF, IL-6 and and IFNα quantified using a mouse IFNα Platinum ELISA, a DuoSet IL-6 or Duoset

TNF ELISA (all R&D Systems). MHV370 serum concentrations were quantified by liquid chromatography coupled to mass spectrometry (LC-MS/MS).

Data analysis

Cytokine concentrations from cell-based and *in vivo* experiments were determined following extrapolation to standard curves using appropriate reference cytokine. Data were analysed using Excel XL fit 5.0 (Microsoft) with XL*fit* add-in (IDBS; version 5.2.0) GraphPad Prism (San Diego, CA, USA), or proprietary internal data analysis software. Individual IC_{50} values were determined by nonlinear regression after fitting of curves to the experimental data with each point performed in biological triplicate.

Structure determination

Protein expression and purification: The extracellular domain of TLR8 (residues 27-827) was expressed by Express^{2}ion and purified in-house according to published literature. In brief, TLR8 was expressed in a *Drosophila* S2 expression system. The protein was purified by IgG Sepharose affinity chromatography, subsequent saccharide trimming using EndoHF and Superdex 200 gel filtration chromatography followed by HiTrap Q anion exchange chromatography.

Crystallization: Purified protein buffered in 50 mM Tris pH 8.0; 175 mM NaCl at a concentration of 7.4 mg mL^{-1} and containing 2 mM NVP-MHV370-NX-10, was used to setup a sparse-matrix screen. 0.2 µL protein solution was mixed with 0.2 µL well solution and equilibrated against 80 µL reservoir using SWISSCI MRC 2 Well crystallization plates. Crystallization plates were incubated at 4°C. Crystals were found under several conditions after 5 to 10 days, while crystals suitable for X-ray diffraction experiments were obtained in condition: 25% PEG 3350, 200 mM Ammonium Acetate, 100 mM Bis-Tris pH5.5 reaching 100 µm length on day 14. Crystals were cryoprotected with 10% glycerol, and then vitrified by plunging them into liquid nitrogen.

Data collection and structure determination: Data sets were collected at 1.0 Å wavelength with a PILATUS 6M detector at the Swiss Light Source beamline X10SA (Villigen, Switzerland). Data were collected by Expose GmbH. Diffraction images were processed and scaled using autoPROC. Structures were solved by molecular replacement (Phaser). The initial model was subjected to iterative cycles of manual rebuilding and subsequent structure refinement in Coot and autoBuster, respectively. The ligand structure was built into unbiased Fo-Fc difference electron density calculated by autoBuster. Final structure refinement statistics are summarized in

Supplementary Table 1. Refined coordinates were deposited to the PDB with entry number 8PFI

Wavelength (Å)	1
Resolution range (Å)	66.04-2.79 (2.89 - 2.79)
Space group	C121
a, b, c (Å)	164.6 86.9 153.5
α, β, γ (°)	90 120.6 90
Total reflections	162743 (16776)
Unique reflections	46433 (4593)
R-merge	0.058 (0.576)
R-meas	0.069 (0.675)
I / σ(I)	16.07 (2.31)
Completeness (%)	98.92 (98.84)
Multiplicity	3.5 (3.7)
Wilson B-factor (Å^2)	64.7
CC1/2	1 (0.791)
CC*	1 (0.940)
Reflections used in refinement	46411 (4593)
Reflections used for R-free	2372 (245)
R-work	0.228 (0.273)
R-free	0.263 (0.334)
CC(work)	0.916 (0.802)
CC(free)	0.886 (0.721)
Number of non-hydrogen atoms	12701
macromolecules	11630
ligands	568
solvent	503
Protein residues	1467
R.m.s. deviation bond length (Å)	0.009
R.m.s. deviation angles (°)	1.34
Ramachandran statistics	
Favored (%)	93.43
Allowed (%)	6.09
Outliers (%)	0.48
Rotamer outliers (%)	2.57
Clashscore	3.78
Average B-factor (Å^2)	76.70
Macromolecules (Å^2)	77.30
Ligands (Å^2)	77.35
Solvent (Å^2)	62.05

Supplementary Table 1: Final structural refinement statistics for the cocrystal structure of TLR8 and compound **34** (MHV370).