SUPPORTING INFORMATION

Design of Potent and Selective Covalent Inhibitors of Bruton's Tyrosine Kinase Targeting an Inactive Conformation

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In Vitro Biology

BTK Biochemical enzyme assay

Biochemical inhibition of BTK was assessed with unphosphorylated full-length human BTK protein. Substrate peptide (FITC-Ahx-TSELKKVVALYDYMPMNAND-NH2) and ATP were added to serial compound dilutions, then the enzymatic reactions were started by adding BTK protein to a final concentration of 3.2 nM. The final ATP concentration was chosen at Km of the enzyme under these conditions (82 μM). After allowing the kinase reactions to proceed for 60 minutes at 30°C these were stopped and analyzed on a Caliper LC3000 workstation by separating phosphorylated and unphosphorylated substrate peptides. Kinase activities were calculated from the amounts of newly formed phospho-peptide.

Biochemical selectivity panel

To profile the compounds against a panel of kinases these methods have been used:

a) Similar methods as described for BTK, using specific substrate and ATP conditions.
b) In brief, recombinant kinases except were incubated within appropriate buffer containing peptide substrate and radiolabelled γ-33P-ATP together with presence or absence of required inhibitor concentration. The reaction was initiated by adding ATP/Mg2+ mix. After incubation for 40 minutes at room temperature (in a few assays this time can be increased to 120 min), the reaction was stopped by adding 0.5% phosphoric acid solution. A portion of reaction mix was spotted onto P30 filtermat to trap peptide and washed 4 times for 4 minutes with phosphoric acid to remove non-specific γ-33P-ATP. The substrate phosphorylation was then measured by scintillation counting, which determined the level of kinase activity inhibition compared to control reactions.

c) Kinases are incubated in assay buffer containing substrate peptide which could be phosphorylated by kinases in the reaction and Mg/ATP (concentration as required). The reaction is initiated by the addition of the Mg/ATP mix. After incubation for 30 minutes at room temperature, the reaction is stopped by the addition of stop solution containing EDTA. Finally, detection buffer is added. The inhibition potency of compounds against these enzymes was assessed using Homogenous Time Resolved Fluorescence approach. The plate is then read in time-resolved fluorescence mode and the homogeneous time-resolved fluorescence (HTRF) signal is determined according to the formula HTRF = 10000 x (Em665nm/Em620nm).

In all enzymatic assays ATP was used at a concentration close to its Km in the same assay format.

Table S1. Biochemical selectivity data for compounds **3**, **4**, **8** and **1**

IC_{50} [µM]	**3**	**4**	**8**	**1**	**1**[a]
BTK	0.013	0.005	0.0012	0.005	0.0015
BMX	1.7	5	0.049	0.001	0.0008
EGFR	>10	>10	>30	0.016	0.005
ERBB2	>10	>10	>30		0.0064
ERBB4	>10	>10		0.007	0.0034
JAK3	>10	>10	>30		0.032
TEC					0.010
ITK					0.0049
TXK					0.002
BLK					0.0001
Addtl. kinases	CDK4D1 2.6, EphB4 2.8, ABL1 4.4, AURKA 7.3, IGF1R 7.3, GSK3B 8.9, RET 8.9, MAPKAPK2 9.6 All others >10 ALK, AXL, AKT1, CAMK2D, CDK2A, CSNK1G3, EPHA4, FGFR1, FGFR2, FGFR3, FGFR4, FLT3, FYN, HCK, INS1R, IRAK1, IRAK4, JAK1, JAK2, KDR, KIT, LCK, LRRK2, LYN, MAP3K8, MAPK14, MAPKAPK5, MERTK, MET, MKNK2, PAK2,	All >10 ABL1, AKT1, ALK, AURKA, AXL, CAMK2D, CDK2A, CDK4D1, CSK, CSNK1G3, EPHA4, EPHB4, FGFR1, FGFR2, FGFR3, FGFR4, FLT3, FYN, GSK3B, HCK, IGF1R, INS1R, IRAK4, JAK1, JAK2, KDR, KIT, LCK, LYN, MAP3K8, MAPK1, MAPK9, MAPK10, MAPK12, MAPK14, MAPKAPK2, MAPKAPK5, MERTK, MET, MKNK1, MKNK2,	All >30 ATM, ABL1, ALK, AMPK, AURKA, AXL, CAMK1, CAMK2D, CDK2A, CDK4D3, CK1, DMPK, EPH4, FGFR2, FGFR3, FGFR4, FLT1, GCN2, GRK1, GSK3B, IGF1R, INSR, IRAK1, JAK1, JAK2, KDR, KIT, LCK, MAPK1, MAPK14, MAPKAPK2, MAPKAPK5, MEKK2, MET, MKK3, MKNK2, NEK11, P70S6K, PDPK1, PIM1,	LSK 0.037, LYN 0.072, CSK 0.079, ABL1 0.22, RET 0.42, SRC 0.54, FGFR3 2.1, AURKA 8.4, EPHB4 9.4 All others >10 ACVR1, AKT1, ALK, CDK2A, CDK4D1, FLT3, INS1R, IRAK1, IRAK4, JAK2, MAPK1, MAPK10, MAPK14, MAPKAPK2, MAPKAPK5, MET, MKNK1, PAK2, PIM2, PKN1, PKN2, PLK1, PRKCA,	

	PDGFRa, PDPK1, PIM2, PKN1, PKN2, PLK1, PRKACA, PRKCA, PRKCQ, RON, ROCK2, RPS6KB1, SRC, SYK, TYK2, WNK1, ZAP70	MST1R, PAK2, PDGFRaV561D, PDPK1, PIM2, PKN1, PKN2, PLK1, PRKACA, PRKCA, PRKCQ, RET, ROCK2, RPS6KB1, SRC, SYK, TYK2, WNK1, YES, ZAP70	PKN2, PRKACA, PRKBA, PRKCA, PRKCQ, RAF, RET, ROCK2, SLK, SYK, TGFBR1, TRB2, TYK2, WEE1, WNK1, ZAP70	PRKCQ, SYK, ZAP70	

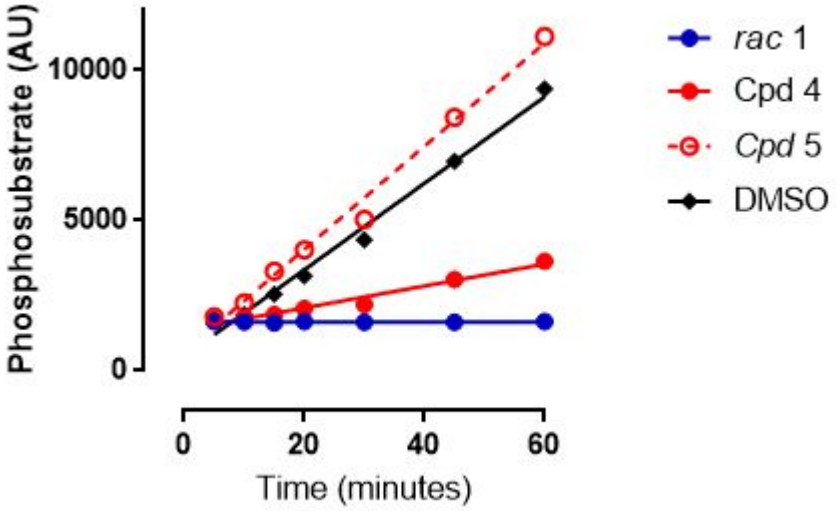
[a]Published biochemical data from Barf et al.[1]

Assessment of irreversible BTK inhibition in dilution experiment

To assess whether the compounds inhibited BTK enzymatic activity irreversible we performed a dilution experiment.[2] Full length human unphosphorylated BTK protein was preincubated with equimolar concentrations of compounds for two hours at a concentration of 5.7 µM at room temperature. Then the compound/enzyme mixture was diluted into kinase assay buffer containing ATP and peptide substrate to reach a final concentration of 10 nM of BTK and compound. The enzyme reaction was then monitored by sampling over one hour and measuring substrate phosphorylation.

Reaction rates showed linear progression for the DMSO solvent control (black squares). The non-covalent compound **5** (red empty circles) showed similar kinase activity as the DMSO control, suggesting reversible inhibition after the dilution step. On the other hand, both *rac*-**1** (blue circles) and compound **4** (red filled circles) showed almost complete absence of enzyme activity after the dilution step, suggesting irreversible inhibition (lines show linear regression fits).

Figure S1



Cellular phospho-Tyr551 BTK assay

Activation of the antigen receptor on B cells (BCR) triggers a cascade of phosphorylation events. This includes the phosphorylation of BTK on its activation loop tyrosine Y551. In human B cells phosphorylation of Tyr551 in BTK can be induced by cross-linking the BCR with anti-IgM and/or

addition of low concentrations of the phosphatase inhibitor H_2O_2. Under these conditions H_2O_2 inhibits the phosphatases that restrict the BCR-dependent activation of LYN.[3–7]

The inhibition of BTK Tyr551 phosphorylation by the compounds was determined after incubation of human B cell lymphoma Ramos cells (ATCC CRL-1596) with compounds for 45 minutes at 37°C. Then the cells were stimulated for 15 minutes with 0.1 % H_2O_2 and then fixed in 1.5 % formaldehyde. After permeabilization with methanol and intracellular staining for phospho-Tyr551 BTK (#53, BD Biosciences) fluorescence was analyzed on a flow cytometer.

To assess time-dependent inhibition of BTK Tyr551 phosphorylation, Ramos cells were activated in bulk with 15 µg/ml polyclonal anti-IgM antibody (SouthernBiotech) for 5 minutes, then compound was added under vortexing at a final concentration of 0.5 µM. At the given timepoints an aliquot of the cell suspension was drawn and added to an appropriate volume of 10-fold concentrated RIPA lysis buffer to stop the reaction. Aliquots of the cell lysates were resolved by SDS-PAGE and immunoblotted for phospho-Tyr551 BTK (#53, BD Biosciences). Chemiluminescence signals were quantified are reported compared to untreated controls samples.

Of note, the increase of Tyr551 phosphorylation by *rac*-**1** is probably due to stabilization of an active conformation of BTK or by inhibition of upstream kinases, e.g. tyrosine-protein kinase Lyn, a known repressor of BCR signaling.[8] In addition, it has been shown that even under conditions of maximal BCR stimulation only a fraction of about 2 % of the total cellular BTK protein pool is phosphorylated at Tyr551.[9]

Human blood B cell inhibition assay

The effects of the inhibitors were assessed in human primary B cells in blood. Blood from healthy volunteers was provided under informed consent and collected through the Novartis Tissue Donor Program in accordance with the Swiss Human Research Act and approval of the responsible ethic committee.

Fresh human heparinized blood diluted to 90 % was preincubated with compound for 1 hour at 37°C. Then stimulated with a polyclonal anti-IgM antibody (30 µg/ml, Southern Biotech) in presence of non-activating levels of recombinant human IL-4 (5 ng/ml, Immunotools). Activation of B cells was measured by flow cytometry 16 hours later after red blood cell lysis and by reading cell surface expression of the activation markers CD69 (FN50 BD Biosciences) on CD19 positive (HIB19 BD Biosciences) B cells.

Cellular FcγR inhibition assay

The effects of the compounds on BTK-dependent signaling from the activating FcγRs was assessed in the human monocytic cell line THP1 (ATCC, TIB-202). The THP1 cell line expresses the two activating FcγRs CD32a (FcγRII2) and CD64 (FcγRI).[10,11] Both signal through the ITAMs that are contained in their intracellular domain (FcγRIIa) or in the associated common γ-chain (FcγRI). In both cases, the proinflammatory signaling is BTK dependent. The activating FcγR signaling leads to production of proinflammatory cytokines and chemokines and IL-8 secretion can be measured.[12]

Briefly, 384 well culture plates were coated with pooled non-specific human IgG fraction (Redimune, CSL Behring). Serial compound dilutions were dispensed into the IgG-coated plates. Then THP1 cells that had been pre-differentiated with vitamin D3 (15 nM, Merck Millipore) for 5 days were added to each well. Twenty-four hours later the IL8 levels in the supernatants was assessed with a homogenous immunoassay for human IL8 (Cisbio Bioassays).

Cellular pEGFR assay

The EGFR is among the kinases that have a conserved cysteine corresponding to Cys481 in BTK and is a clinically relevant off-target for BTK inhibitors. The cellular selectivity against EGFR signaling was assessed after a 5 hour preincubation that reflects clinical compound exposures.

Cells from the EGFR expressing human cancer line A431 (ATCC, CRL-1555) were preincubated with serial dilutions of compounds for 5 hours in 384 well microtiter plates. Then the EGFR was activated by adding its cognate ligand EGF during 10 minutes at room temperature (25 ng/ml, R&D). The EGF-induced phosphorylation of EGFR was assessed by a HTRF-based immunoassay as indicated by the vendor (Cisbio Bioassays). The effect of compounds on the pEGFR levels was measured compared to solvent controls.

Structural Biology

Co-crystal structure of compound **3** with BTK

The region encoding the sequence of the kinase domain of human BTK with boundaries of 385-659 was sub-cloned into a pIEX/Bac-3 derived vector for protein over-expression in *Spodoptera frugiperda* (Sf) cells. The overexpressed construct was designed to contain a hexa-histidine tag followed by an HRV-3C protease cleavage site at the N-terminus of the BTK sequence. Large-scale expression was carried out in Sf9-DE3 cells in a culture volume of 3.9 L Sf900III medium (Gibco) in 5L-bottles. Cells were diluted to a density of $1.8 \cdot 10^6$ ml^{-1} before addition of recombinant baculo viruses, followed by incubation with constant shaking with 90 rpm at 27° C. Cells were harvested at 48 h post infection by centrifugation at 500 x g for 20 min at room temperature. Cell pellets were flash frozen and stored at -80° C.

For protein purification, the cell pellet was thawed in lysis buffer containing 100 mM Tris-HCL pH 7.6, 300 mM NaCl, 10 mM imidazole, 10 % glycerol, 2 mM TCEP, 250 U of benzonase (Sigma Aldrich) and complete EDTA-free protease inhibitor cocktail (Sigma Aldrich, used according to the manufacture's recommendation). Cells were homogenized using a polytron, followed by centrifugation of the crude lysate in a 30-50Ti rotor (Sorvall) at 20000 rpm for 1h at 4° C. The cleared lysate was subjected to immobilized metal affinity chromatography (IMAC) using a pre-packed Nickel Crude FF column (GE healthcare) in IMAC buffer A (100 mM Tris-HCl pH 7.6, 300 mM NaCl, 10 mM imidazole, 10 % glycerol, 2 mM TCEP). The column was washed with 5 column volumes (CV) of buffer A, followed by 5 CV of 10 % IMAC buffer B (100 mM Tris-HCl pH 7.6, 300 mM NaCl, 250 mM imidazole, 10 % glycerol, 2 mM TCEP) and 8 CV of 60 % IMAC buffer B and 5 CV of 100 % IMAC buffer B. Protein fractions eluting at 60% IMAC buffer B were pooled and incubated with HRV-3C protease at 4° C for 10 h. The cleavage products were dialyzed against IEX buffer A (100 mM Tris-HCl pH 7.6, 10 % glycerol, 2 mM TCEP) and further purified using a pre-packed HiLoad MonoQ column. After loading the protein, the column was washed with 5 CV IEX buffer A and eluted from the column by applying a salt gradient from 0 % to 100 % IEX buffer B (100 mM Tris-HCl pH 7.6, 1 M NaCl, 10 % glycerol, 2 mM TCEP) in 30 CV. Eluted fractions were analyzed by SDS PAGE and fractions containing BTK were pooled and subjected to size exclusion chromatography (SEC) in running buffer containing 100 mM Tris-HCL pH 7.6, 100 mM NaCl, 1 mM EDTA, 2 mM TCEP. The correct mass of the protein was confirmed by mass spectroscopy. After SEC, the protein was frozen and stored at -80° C in aliquots.

For crystallization, a frozen aliquot of purified protein was thawed on ice and compound **3** was added with a ten times molar excess. The protein-ligand mixture was concentrated to a final protein

concentration of 7.6 mg/ml. Equal volumes (1 µl) of protein and a reservoir solution (100 mM Tris-HCL pH 8.5, 200 mM sodium formate, 50 mM CaCl2, 25% (w/v) PEGMME 5000) were mixed and the drop was incubated at room temperature in hanging drop VDX plates (Hampton Research) over 500 µl reservoir solution. Single crystals were harvested and flash cooled in liquid nitrogen prior to diffraction data collection.

X-ray diffraction data were collected at beamline X10SA of the Swiss Light Source (Paul Scherrer Insitute, Villigen Switzerland) at 100 K using a MAR225 CCD detector. Diffraction images were processed and intensities were scaled with AutoPROC.[13] The structure of the complex of BTK with ligand **3** was solved by molecular replacement with PHASER using the structure of murine BTK kinase domain as input model (PDB accession number 1K2P).[14,15] At the time of structure determination, 1K2P.pdb were the only BTK coordinates available in the public domain. The initial molecuar replacement solution was rebuilt in COOT and refined with BUSTER.[16,17] Data collection and refinement statistics are summarized in table 1. Coordinates of the refined structure were deposited in the PDB with accession number 6S90.

Table S2. Data collection and refinement statistics. Statistics for the highest-resolution shell are shown in parentheses.

	BTK in complex with compound 3
Wavelength (Å)	0.999900
Resolution range (Å)	34.59 - 1.822 (1.828 - 1.822)
Space group	C 1 2 1
Unit cell	135.827 Å 39.365 Å 105.186 Å 90° 111.18° 90°
Total reflections	167196 (496)
Unique reflections	46201 (317)
Multiplicity	3.6 (1.6)
Completeness (%)	98.4 (68.3)
Mean I/sigma(I)	15.3 (2.3)
Wilson B-factor (Å^2)	13.3
R-merge	0.044 (0.161)
R-meas	0.052 (0.227)
R-pim	0.027 (0.161)
CC1/2	0.998 (0.912)
Resolution range refinement (Å)	49.04 – 1.82 (1.84 – 1.82)

Reflections used in refinement	46198 (924)
Reflections used for R-free	2274 (40)
R-work	0.169 (0.215)
R-free	0.201 (0.298)
Number of non-hydrogen atoms	4712
macromolecules	4157
ligands	88
solvent	467
Protein residues	524
RMS(bond lengths) (Å)	0.010
RMS(angles) (degrees)	0.97
Ramachandran favored (%)	97.88
Ramachandran outliers (%)	0.38
Rotamer outliers (%)	0.68
Clashscore	1.57
Average B-factor (Å^2)	17.01
Number of TLS groups	2

MS experiment of BTK/compound **4** complex

30 µM Btk(385-659) was incubated 10 minutes with 1.2 times excess of cpd **4** (MW 480 Da) and injected into LC/MS (ACQUITY UPLC® Protein BEH C4 2.1 x 100mm Column, 1.7 µm /Xevo G2-S QTof MS, Waters). Mass spectra over a mass range from 700 m/z to 3000 m/z were acquired using positive-ion Electrospray Ionization (ESI). Deconvolution range from 10 – 150 kDa using Maximum Entropy (MaxEnt). In comparison to unmodified Btk at 31715 Da, a single adduct peak with the corresponding mass of 32196 Da was obtained. For the LC, the column temperature was set to 80°C and the following eluents were used. Buffer A: water + 0.05% TFA, buffer B: acetonitrile + 0.04% TFA, gradient: initial 5% B; from 5 % to 60 % B in 8.0 min; from 60 % B to 98 % B in 0.2 % B; 2.1 min 98 % B with a flow rate of 0.5 mL/min. Injection Mode: Partial loop. In brief, the following MS method was chosen: Time: 0 – 9.8 min, mass Range: 700 – 3000 m/z, ionization mode: ES+, data collection in continuum mode with a scan time of 0.1 sec.

Animal studies

All animal studies described here were performed according to Swiss animal welfare guidelines.

Reverse passive Arthus reaction in the skin

Female C57Bl6 mice (Charles River, France), 8–10 weeks of age, were housed under standard conditions with a 12-hour light/dark cycle, and water and food were provided ad libitum.

The back of the mice was shaved 24 hours prior to the intradermal injection to allow any potential irritation of the skin to resolve. Vehicle and compounds were administered orally to groups of 5 mice at 10 ml/kg at the stated doses. The formulation for *rac*-**1** was a suspension of the compound in a solution of 0.5 % methylcellulose in water, and for **4** a solution of the compound in 0.03 M HCl, 20 % (v/v) solutol and 50 mM citrate buffer.

The passive Arthus reaction was triggered two hours after compound dosing by intradermal injection of 50 µl of phosphate-buffered saline (PBS) to the control site or polyclonal rabbit anti-ovalbumin IgG (30 µg per mouse in PBS, Sigma) into the dorsal skin. The intradermal injection was followed immediately by an i.v. injection of 200 µl ovalbumin (20 mg/kg, Fluka) in saline.

Three hours after the injection of the ovalbumin the thickness of the injected control and anti-ovalbumin IgG skin sites was measured using a digital caliper. Then animals were euthanized and tissues were sampled. For each animal the thickness of the anti-ovalbumin IgG injected sites minus the saline control sites were calculated. The percentage inhibition of the treated groups was calculated with reference to the average of vehicle treated animals.

In vivo pharmacokinetic studies in rat

Sprague–Dawley rats, originated from Charles River Wiga Laboratories (Germany), Harlan Laboratories (Netherland) or Iffa Credo (France) and had a body weight of approximately 250–350 g. After their arrival in our animal facilities, they were acclimatized for at least 7days and were housed under controlled environmental conditions (ambient temperature 21 8C, humidity 60%, 12 h light/dark cycle) with ad libitum access to standard food and tap water before dosing and during the entire experimentation period. All animal experimental procedures complied with the Swiss animal welfare regulations and were approved by the Cantonal Veterinarian Office of Basel-City, Switzerland.

Six to four days before drug administration or animal delivery by the supplier, the rats were anesthetized and catheters were surgically implanted into the femoral artery (for blood collection) and femoral vein (for intravenous injection). The catheters were exteriorized at the neck. For analgesic treatment, animals received Temgesic (10 mg/kg s.c.) before surgery and subsequently twice at appropriate times after surgery. Animals were kept individually in standard cages.

Intravenous doses were applied by bolus injection (0.5 mL/kg) of a solution in 1-methyl-2-pyrrolidone and polyethylene glycol 200 (30:70, v/v) at a dose of 1 mg/kg via the femoral vein catheter. Oral administrations were applied via gavage (2.5 mL/kg) of homogenous aqueous suspension in Tween80, carboxymethylcellulose and water (0.5/0.5/99; w/w/w) at a dose of 3 mg/kg. The test substances were administered alone or in combination (cassette) to groups of up to 4 rats. Blood samples (10–50 µL, EDTA coated tubes) were collected from the femoral artery catheter at 0.08 (i.v. only), 0.25, 0.5, 1, 2, 4, 7, and 24 h after dosing.

Blood samples were spiked with a structurally closely related internal standard, then lysed and de-proteinated using 2 volumes of acetonitrile. After centrifugation the supernatant was evaporated to dryness. The remainder was re-dissolved in methanol (60%) and 1% aqueous formic acid (pH 3.0).

The solution was separated on a Synergi Polar-RP HPLC column (particle size: 2.5 µm; column dimensions: 2 × 50 mm) using a linear gradient (0 min, 98% A/2% B; 0.5 min, 98% A/2% B; 5 min, 10% A/90% B; 5.01 min, 0% A/100% B; 5.5 min, 0% A/100% B at a flow rate of 0.35 or 0.40 mL/min. Mobile phase A consisted of 1% formic acid in HPLC grade water. Mobile phase B consisted of 1% formic acid in HPLC grade acetonitrile. The flow from the HPLC system was directly introduced into the ion source of a TSQ Quantum mass spectrometer (ThermoFisher Scientific, Massachusetts, USA) and subjected to electrospray ionization (positive ion mode).

The test substances were detected via a specific daughter ion of its protonated quasi-molecular ion. Quantitation of blood levels of the test substances were based on an 8-level calibration curve (in triplicate) using blank rat blood samples spiked with stock solutions of external and internal standards.

Table S3. Rat PK parameters[a]

	1[b]	**8[c]**	**9[d]**	**13[e]**	**14[e]**	**15[e]**
Dose [mg·kg^{-1}]	1 i.v. / 3 p.o.	1 i.v.	1 i.v.	1 i.v.	1 i.v.	1 i.v.
CL [mL·min^{-1}·kg^{-1}]	>300	>300	183	>300	176 ± 42	>300
$t_{1/2}$ term. [h]	0.9 ± 1.0	0.2 ± 0.0	0.3	0.3 ± 0.0	2.5 ± 0.1	0.4 ± 0.0
AUC i.v. d.n.[f] [nM·h]	73 ± 8	63 ± 8	178	92 ± 28	162 ± 36	103 ± 32
AUC p.o. d.n.[f] [nM·h]	1.1 ± 0.6					
BAV [%]	2 ± 1					
C_{max} d.n.[f] [nM]	3 ± 2					
T_{max} [h]	0.3 ± 0.0					

[a]Mean values (SD shown); [b]female, n=4; [c]male, cassette dosing, n=4; [d]male, cassette dosing, n=2; [e]male, cassette dosing, n=3; [f]d.n. = dose normalized to 1 mg·kg^{-1}

BTK occupancy PK/PD model

To assess the PK/PD profile of the covalent BTK inhibitors we measured BTK occupancy from tissue samples of animals treated with compounds. Female Wistar Han or OFA rats were dosed orally with compounds formulated as homogenous aqueous suspensions in methylcellulose and water (0.5/99.5; w/w) or Tween80, methylcellulose and water (0.5/0.5/99; w/w/w) at a dose of 10 mg/kg. Blood samples at 0.5, 2 and 5 hours after compound dosing were taken into EDTA tubes under deep anaesthesia for PK analysis. Five hours after dosing the animals were euthanized and the spleen removed to determine BTK target occupancy as described below.

Covalent binding of compounds to BTK was determined in tissues from in vivo studies as a measure for target engagement and pharmacodynamic (PD) activity. BTK occupancy was determined in spleen tissue extracts with immunoassays for free BTK (i.e. not covalently occupied by compound) and total BTK protein using the MSD (Meso Scale Discovery) platform.

For free BTK measurements, a streptavidin-coated MSD assay plate was incubated with the biotinylated covalent BTK probe **S49**, then samples were added to allow binding of the unoccupied free BTK to the plate-bound probe. The binding of the probe **S49** to BTK is mutually exclusive

with compound binding to BTK. Plate-bound BTK was detected with a SULFO TAG-labelled anti-BTK antibody (D3H5, Cell Signaling Technology).

For total BTK measurements, an MSD assay plate was coated with D3H5 anti-BTK to capture total BTK (free BTK and BTK bound to compounds). A SULFO TAG-labelled anti-BTK antibody (#53, BD Biosciences) was then used to detect captured BTK.

The signals from both assays were calibrated against standard curves generated with recombinant BTK protein. The respective free BTK levels for each sample were normalized to the total BTK level in the same sample and these ratios were expressed as percentage of the vehicle control samples

Experimental Part Chemistry

General

Unless otherwise stated, all reagents and solvents were purchased from commercial suppliers and used without further purification. ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker 400 MHz or a Bruker 600 MHz NMR spectrometer. Chemical shifts are reported in parts per million (ppm) relative to an internal solvent reference. Recorded peaks are listed in the order multiplicity (s, singlet; d, doublet; t, triplet; q, quartet; qt, quintet; m, multiplet; br, broad), coupling constants, and number of protons. High resolution mass analyses (HRMS): The analyses were performed by using electrospray ionization in positive ion modus after separation by liquid chromatography (Nexera from Shimadzu). The elemental composition was derived from the mass spectra acquired at the high resolution of about 30'000 on an LTQ Orbitrap XL mass spectrometer (Thermo Scientific). The high mass accuracy below 1 ppm was obtained by using a lock mass. The chromatography was performed at 150 µL/min flow rate with a polar gradient from 5% to 100% acetonitrile in 5 min. 0.04% and 0.05% formic acid were used as the modifier additive in the acetonitrile phase and aqueous phase, respectively, in addition to 3.75 mM ammonium acetate in the aqueous phase. Final compounds were purified to ≥90% purity as assessed by analytical liquid chromatography using the methods below.

LC/MS method 1: column Acquity HSS T3 1.8µm 2.1 x 50 mm at 60 °C; eluent A: water + 0.05% formic acid + 3.75 mM ammonium acetate; eluent B: acetonitrile + 0.04% formic acid; gradient: from 5 to 98% B in 1.4 min; flow 1.0 mL/min. Detection method: UV 220-315 nm – MS mass range ESI +/-: 120 – 1200 m/z.

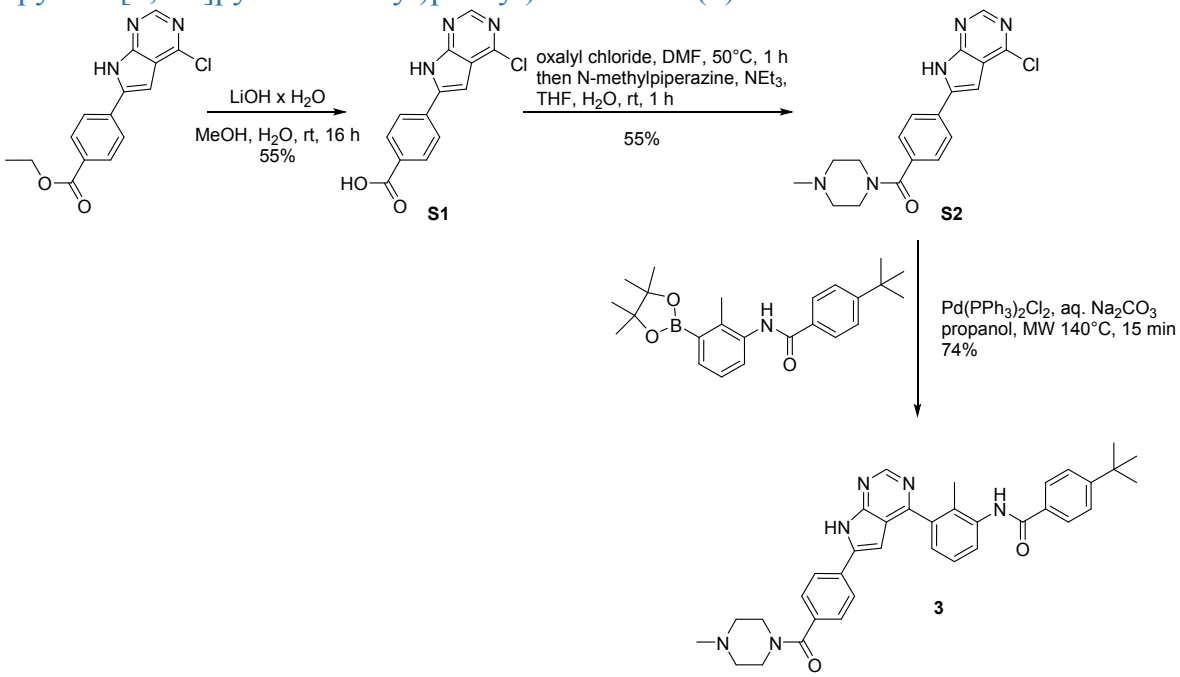
LC/MS method 2: column Ascentis®Express C18 30 x 2.1mm, 2.7µm at 50 °C; eluent A: water + 0.05% formic acid + 3.75 mM ammonium acetate; eluent B: acetonitrile + 0.04% formic acid; gradient: from 10 to 95% B in 2 min; flow 1.2 mL/min. Detection method: UV 220-315 nm – MS mass range ESI +/-: 120 – 1200 m/z.

LC/MS method 3: column SunFire C18 20x4.6mm, 3.5µm, reverse phase at 45 °C; eluent A: water + 0.1% trifluoroacetic acid; eluent B: acetonitrile + 0.1% trifluoroacetic acid; gradient: from 5 to 100% B in 8 min; flow 2.0 mL/min. Detection method: UV – MS.

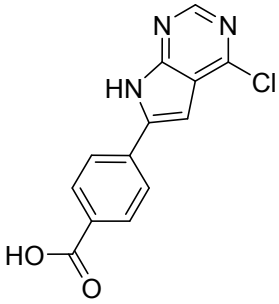
LC/MS method 4: column Ascentis®Express C18 30 x 2.1mm, 2.7µm at 50 °C; eluent A: water + 0.05% formic acid + 3.75 mM ammonium acetate; eluent B: acetonitrile + 0.04% formic acid; gradient: from 2 to 98% B in 1.4 min; flow 1.2 mL/min. Detection method: UV 220-315 nm – MS mass range ESI +/-: 120 – 1200 m/z.

LC/MS method 5: column Acquity UPLC BEH C18, 1.7µm, 2.1x50mm at 80°C; eluent A: water + 4.76 % isopropanol + 0.05 % formic acid + 3.75 mM ammonium acetate, B: isopropanol + 0.05 % formic acide; gradient: concave from 1-98 % B in 1.7 min, flow: 0.6 mL/min. Detection method: UV 220-315 nm – MS mass range ESI +/-: 120 – 1200 m/z.

Synthesis of 4-(tert-Butyl)-N-(2-methyl-3-(6-(4-(4-methylpiperazine-1-carbonyl)phenyl)-7H-pyrrolo[2,3-d]pyrimidin-4-yl)phenyl)benzamide (**3**)

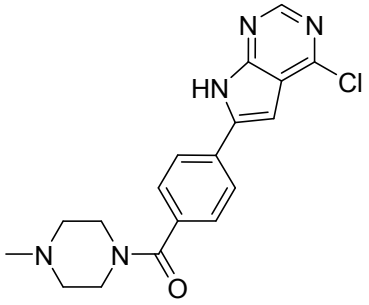


4-(4-Chloro-7H-pyrrolo[2,3-d]pyrimidin-6-yl)benzoic acid (S1)



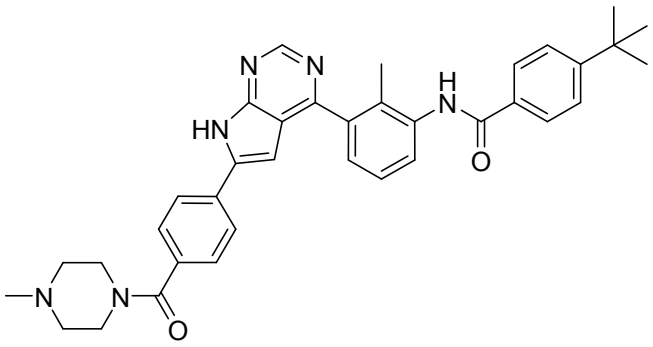
4-(4-Chloro-7H-pyrrolo[2,3-d]pyrimidin-6-yl)benzoic acid ethyl ester (10.0 g, 33.1 mmol) is suspended in MeOH (70 mL), and treated with a solution of LiOH x H_2O (3.48 g, 82.6 mmol) in water (55 mL) at room temperature and stirred for 16 h. The reaction mixture was then acidified with 4 N aqueous HCl solution to pH 2 and the precipitated product is isolated by filtration, washed with cold water and dried *in vacuo* at 60°C to give the title compound (4.96 g, 18.1 mmol, 55%) as a white powder. ^{1}H-NMR (400 MHz, DMSO-d6): δ ppm 13.33-12.98 (m, 2H), 8.65 (s, 1H), 8.24-8.13 (m, 2H), 8.11-8.00 (m, 2H), 7.29 (s, 1H); LC/MS method 5: Rt 1.27 min, calcd for $C_{13}H_9ClN_3O_2$ $[M+H]^+$ m/z 274.0, found 274.1.

(4-(4-Chloro-7H-pyrrolo[2,3-d]pyrimidin-6-yl)phenyl)(4-methylpiperazin-1-yl)methanone (S2)



S1 (500 mg, 1.86 mmol) is suspended in dry THF (20 mL) under an argon atmosphere and oxalyl chloride (0.37 mL, 4.57 mmol) is added at room temperature, followed by 4-5 drops of DMF. The reaction mixture is heated to 50°C for 1 h, then cooled to room temperature and all volatiles are removed *in vacuo* to give a solid yellow residue, which is suspended in THF (30 mL). This suspension is added dropwise to a solution of N-methylpiperazine (0.51 mL, 4.57 mmol) and triethylamine (1.10 mL, 7.66 mmol) in water (1 mL) at 0°C. The reaction is allowed to stir for 1 h at room temperature, then all volatiles are removed *in vacuo* and the residual crude product is taken up in ethyl acetate, washed with water and dried over magnesium sulfate. After filtration and removal of the solvents *in vacuo* the product is crystallized from ethyl acetate/hexane to give the title compound (365 mg, 1.02 mmol, 55%) as a white powder. ^{1}H-NMR (400 MHz, DMSO-d6): δ ppm 13.13 (s_{br}, 1H), 8.63 (s, 1H), 8.17-8.04 (m, 2H), 7.61-7.40 (m, 2H), 7.31-7.12 (m, 1H), 3.79-3.20 (m, 4H), 2.35 (s_{br}, 4H), 2.23 (s, 3H); LC/MS method 5: Rt 0.98 min, calcd for $C_{18}H_{19}ClN_5O$ $[M+H]^+$ m/z 356.1, found 356.2.

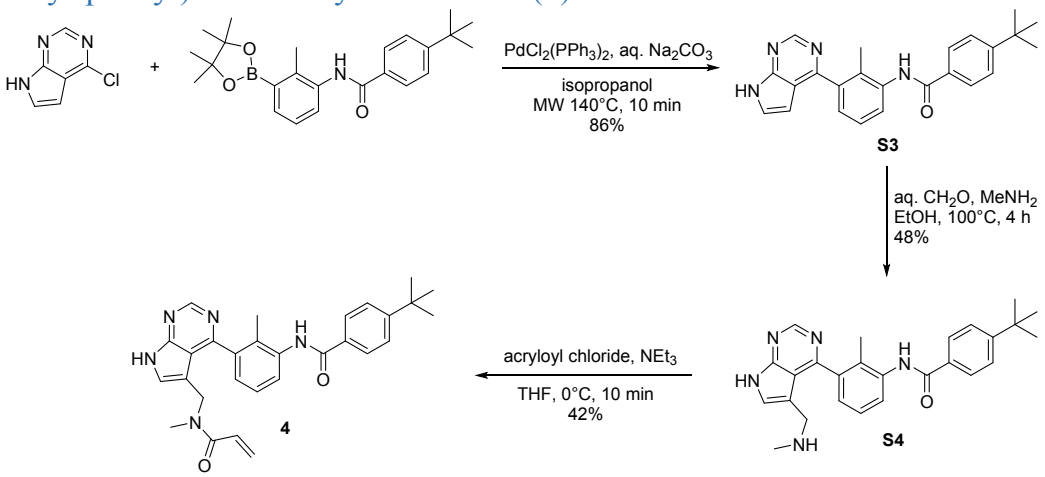
4-(tert-Butyl)-N-(2-methyl-3-(6-(4-(4-methylpiperazine-1-carbonyl)phenyl)-7H-pyrrolo[2,3-d]pyrimidin-4-yl)phenyl)benzamide (3)



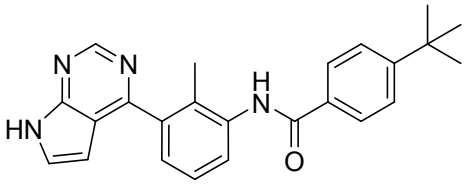
A microwave vial was charged with **S2** (200 mg, 0.562 mmol) and 4-tert-butyl-N-[2-methyl-3-(4,4,5,5-tetramethyl-[1,3,2]dioxaborolan-2-yl)-phenyl]-benzamide[18] (266 mg, 0.676 mmol). *n*-Propanol (6 mL) and 2 M aqueous sodium carbonate solution (2 mL, 4 mmol) were added. The vial was flushed with argon and $PdCl_2(PPh_3)_2$ (40 mg, 0.057 mmol) was added. The sealed tube was heated in a microwave reactor for 15 min at 140°C. The reaction mixture was diluted with saturated aqueous sodium bicarbonate solution and extracted with ethyl acetate. The organic layer was washed with water and brine, dried over sodium sulfate, and concentrated *in vacuo*. The crude product was purified by column chromatography on silica gel with an ethyl acetate/methanol gradient and recrystallized from ethyl acetate/diethylether to provide the title compound (330 mg,

0.562 mmol, 74%) as a yellowish solid. ^{1}H-NMR (400 MHz, DMSO-d6): δ ppm 12.84 (s, 1H), 9.96 (s, 1H), 8.89 (s, 1H), 8.09-8.04 (m, 2H), 8.02-7.95 (m, 2H), 7.61-7.38 (m, 7H), 6.90 (s, 1H), 3.72-3.22 (m, 4H), 2.42-2.28 (m, 4H), 2.22 (m, 6H), 1.35 (s, 9H).; ^{13}C NMR (151 MHz, DMSO-d6) δ ppm 168.39, 165.33, 158.35, 154.45, 153.23, 151.12, 138.62, 138.28, 137.48, 135.86, 132.25, 131.71, 131.65, 127.62, 127.50, 127.41, 125.69, 125.18, 117.95, 97.44, 45.57, 34.65, 30.92, 15.27; LC/MS method 5: Rt 1.37 min, calcd for $C_{36}H_{39}N_6O_2$ $[M+H]^+$ m/z 587.3, found 587.4; HRMS (ESI+) calcd for $C_{36}H_{39}N_6O_2$ $[M+H]^+$ 587.31290, found 587.31293.

Synthesis of N-(3-{5-[(Acryloyl-methyl-amino)-methyl]-7H-pyrrolo[2,3-d]pyrimidin-4-yl}-2-methyl-phenyl)-4-tert-butyl-benzamide (**4**)

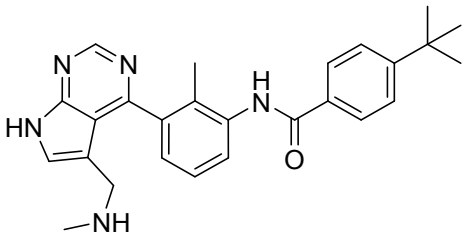


4-*tert*-Butyl-N-[2-methyl-3-(7H-pyrrolo[2,3-d]pyrimidin-4-yl)-phenyl]-benzamide (S3)



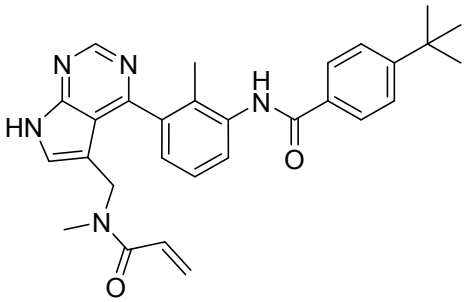
A microwave vial was charged with 4-chloro-7H-pyrrolo[2,3-d]pyrimidine (0.50 g, 3.26 mmol) and 4-tert-butyl-N-[2-methyl-3-(4,4,5,5-tetramethyl-[1,3,2]dioxaborolan-2-yl)-phenyl]-benzamide[18] (1.67 g, 4.23 mmol). Isopropanol (20 mL) and 2 M aqueous sodium carbonate solution (3.26 mL, 6.51 mmol) were added. The vial was flushed with argon and $PdCl_2(PPh_3)_2$ (114 mg, 0.163 mmol) was added. The sealed tube was heated in a microwave reactor for 10 min at 140°C. The reaction mixture was diluted with saturated aqueous sodium bicarbonate solution and extracted three times with ethyl acetate. The combined organic layers were washed with brine, dried over sodium sulfate, and concentrated *in vacuo*. The crude product was purified by column chromatography on silica gel with a cyclohexane / ethyl acetate gradient to provide the title compound (1.07 g, 2.78 mmol, 86%) as a beige solid. ^{1}H-NMR (400 MHz, DMSO-d6): δ ppm 12.25 (s, 1H), 9.98 (s, 1H), 8.88 (s, 1H), 7.99 (m, 2H), 7.64-7.62 (m, 1H), 7.59 (m, 2H), 7.53 (m, 1H), 7.45-7.40 (m, 2H), 6.35 (m, 1H), 2.17 (s, 3H), 1.36 (s, 9H); LC/MS method 1: Rt 1.04 min, calcd for $C_{24}H_{25}N_4O$ $[M+H]^+$ m/z 385.2, found 385.3.

4-*tert*-Butyl-N-[2-methyl-3-(5-methylaminomethyl-7H-pyrrolo[2,3-d]pyrimidin-4-yl)-phenyl]-benzamide (S4)



Formaldehyde solution (37% in water) (6.0 mL, 81.0 mmol) was cooled to -10°C and treated dropwise with a solution of methylamine (33% in ethanol) (5.0 mL, 40.3 mmol). The solution was stirred for 5 min, followed by the addition of **S3** (200 mg, 0.52 mmol). The reaction mixture was heated to 100°C for 4 h. After this time, the solution was allowed to cool to room temperature, diluted with saturated aqueous sodium bicarbonate solution and extracted three times with ethyl acetate. The combined organic layers were washed with brine, dried over sodium sulfate and concentrated in vacuo. The crude product was purified by chromatography on silica gel with a dichloromethane/methanol (containing 1% NH_3) gradient to provide the title compound (107 mg, 0.251 mmol, 48%) as a white solid. ^{1}H-NMR (400 MHz, DMSO-d6): δ ppm 12.06 (s_{br}, 1H), 10.01 (s, 1H), 8.81 (s, 1H), 7.98 (d, *J* = 8.1 Hz, 2H), 7.58 (d, *J* = 8.1 Hz, 2H), 7.51 (d, *J* = 7.9 Hz, 1H), 7.45 (s, 1H), 7.39 (t, *J* = 7.7 Hz, 1H), 7.25 (d, *J* = 7.6 Hz, 1H), 3.27 (s, 2H), 2.04 (s, 3H), 1.96 (s, 3H), 1.36 (s, 9H), NH not visible; LC/MS method 1: Rt 0.78 min, calcd for $C_{26}H_{30}N_5O$ $[M+H]^+$ m/z 428.3, found 428.3.

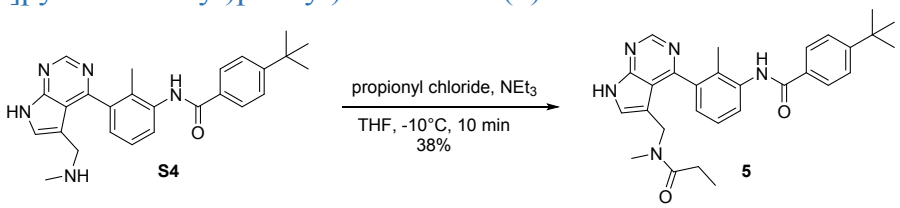
N-(3-{5-[(Acryloyl-methyl-amino)-methyl]-7H-pyrrolo[2,3-d]pyrimidin-4-yl}-2-methyl-phenyl)-4-*tert*-butyl-benzamide (4)

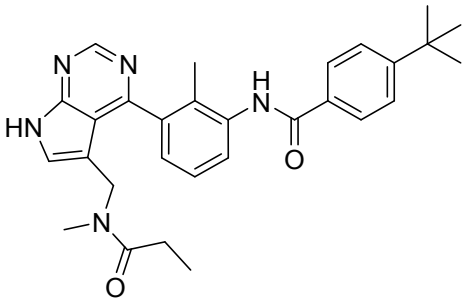


A solution of **S4** (100.0 mg, 0.234 mmol) in tetrahyrofuran (5 mL) was cooled to 0°C and treated with triethyl amine (71.0 mg, 0.702 mmol) followed by fast addition of a solution of acryloyl chloride (42.3 mg, 0.468 mmol) in tetrahyrofuran (1 mL). The reaction mixture was stirred for 10 min, quenched with 2 N NaOH solution and extracted three times with ethyl acetate. The combined organic layers were washed with brine, dried over sodium sulfate, and concentrated *in vacuo*. The crude product was purified by chromatography on silica gel with an ethyl acetate / methanol gradient to provide the title compound as a white solid (47 mg, 0.096 mmol, 42%). ^{1}H NMR (600 MHz, DMSO-d6): δ ppm (rotamers) 12.55, 12.48 (2 s_{br}, 1H), 10.09, 10.06 (2 s, 1H), 8.92, 8.90 (2 s, 1H), 7.94 (d_{br}, *J* = 8.5 Hz, 2H), 7.55 (d, *J* = 8.5 Hz, 2H), 7.51, 7.49 (2 d_{br}, *J* = 7.8 Hz, 1H), 7.41 (t, *J* = 7.4 Hz, 1H), 7.42, 7.34 (2 s_{br}, 1H), 7.29 (d, *J* = 7.6, 1H), 6.71, 6.48 (2 dd, *J* = 16.6, 10.4 Hz, 1H), 6.07 (d_{br}, *J* = 16.8 Hz, 1H), 5.65, 5.56 (2 dd, *J* = 10.5, 2.5 Hz, 1H), 4.18-4.11 (m, 2H), 2.82, 2.81 (2 s, 3H), 1.97, 1.94 (2 s, 3H), 1.32 (s, 9H); ^{13}C NMR (151 MHz, DMSO-d6) δ ppm (rotamers) 165.56, 165.43, 165.32, 158.10, 157.87, 154.56, 152.00, 151.84, 149.97, 137.16, 132.11, 132.03, 131.59, 128.60, 128.08, 127.85, 127.60, 127.38, 127.17, 126.80, 126.62, 125.88, 125.81, 125.22, 115.28, 114.87, 111.36, 111.05, 106.76, 45.27, 42.76, 34.91, 34.69, 33.68, 30.93, 15.08; LC/MS

method 1: Rt 0.96 min, calcd for $C_{29}H_{32}N_5O_2$ $[M+H]^+$ m/z 482.3, found 482.4; HRMS (ESI+) calcd for $C_{29}H_{32}N_5O_2$ $[M+H]^+$ 482.25505, found 482.25519.

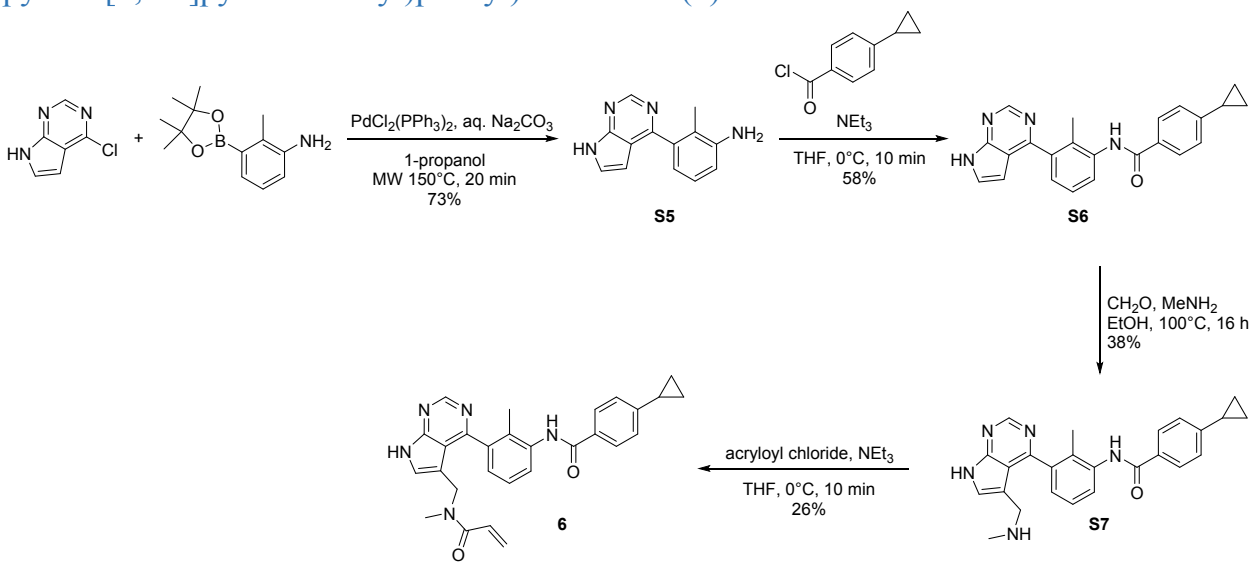
Synthesis of 4-tert-Butyl-N-(2-methyl-3-(5-((N-methylpropionamido)methyl)-7H-pyrrolo[2,3-d]pyrimidin-4-yl)phenyl)benzamide (5)



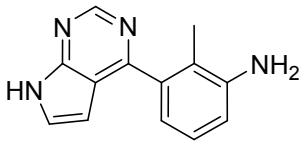


To a solution of **S4** (28.5 mg, 0.067 mmol) in tetrahyrofuran (5 mL) was added triethyl amine (20.2 mg, 0.200 mmol). The mixture was cooled to -10°C before adding a solution of propionyl chloride (12.3 mg, 0.133 mmol) in tetrahyrofuran (1 mL). The reaction mixture was stirred for 10 min at -10°C, quenched with 2N NaOH and ethyl acetate and stirred for 1 h at room temperature. The layers were separated and the aqueous layer was extracted twice with ethyl acetate. The combined organic layers were washed with brine, dried over sodium sulfate, and concentrated *in vacuo*. The crude product was purified by chromatography on silica gel with an ethyl acetate/methanol gradient to provide the title compound as a white solid (12.4 mg, 0.067 mmol, 38%). ^{1}H NMR (400 MHz, DMSO-d6): δ ppm (rotamers) 12.19 (s_{br}, 1H), 10.02 (m, 1H), 8.81 (d, J = 7.8 Hz, 1H), 7.94 (m, 2 H), 7.55 (m, 2H), 7.47 (d, J = 7.8 Hz, 1H), 7.40-7.35 (m, 1H), 7.30-7.20 (m, 2H), 4.05 (m, 2H), 2.74 (s, 3H), 2.31-2.04 (m, 2H), 1.95, 1.91 (2 s, 3H), 1.33 (s, 9H), 0.95, 0.88 (2 t, 3H); LC/MS method 2: Rt 1.61 min, calcd for $C_{29}H_{34}N_5O_2$ $[M+H]^+$ m/z 484.3, found 484.3.

Synthesis of 4-Cyclopropyl-N-(2-methyl-3-(5-((N-methylacrylamido)methyl)-7H-pyrrolo[2,3-d]pyrimidin-4-yl)phenyl)benzamide (6)

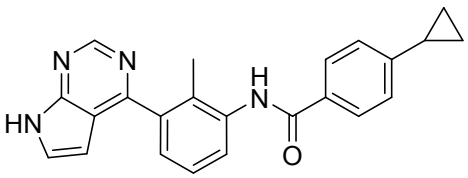


2-Methyl-3-(7H-pyrrolo[2,3-d]pyrimidin-4-yl)aniline (S5)



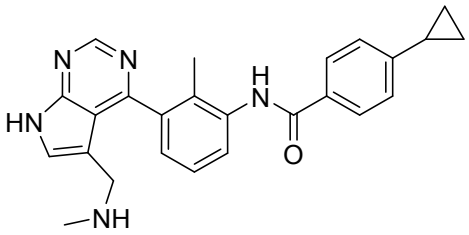
4-Chloro-7H-pyrrolo[2,3-d]pyrimidine (2.0 g, 13.0 mmol) and 2-methyl-3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)aniline[18] (3.95 g, 16.9 mmol) were dissolved in 1-propanol (34 mL), then 2M aqueous sodium carbonate (13.0 mL, 26.0 mmol) was added and the vial was flushed with argon for 20 min. Then bis(triphenylphosphine)palladium(II) chloride (0.091 g, 0.130 mmol) was added, the vial was sealed, and the reaction mixture was heated in the microwave at 150°C for 20 min. The reaction mixture was cooled to room temperature, filtered and extracted twice with ethyl acetate. The combined organic layers were washed with saturated aqueous sodium bicarbonate solution, dried with sodium sulfate and concentrated. The crude product was purified by chromatography on silica gel with an ethyl acetate/methanol gradient to provide the title compound as a brown solid (2.92 mg, 9.53 mmol, 73%). ^{1}H NMR (400 MHz, DMSO-d6): δ ppm 12.11 (s_{br}, 1H), 8.78 (s, 1H), 7.51 (dd, J = 2.3, 3.5 Hz, 1H), 7.01 (t, J = 7.7 Hz, 1H), 6.76 (dd, J = 1.3, 8.0 Hz, 1H), 6.64 (dd, J = 1.3, 7.4 Hz, 1H), 6.27 (dd, J = 1.8, 3.6 Hz, 1H), 4.99 (s, 2H), 1.94 (s, 3H); LC/MS method 1: Rt 0.78 min, calcd for $C_{13}H_{13}N_4$ $[M+H]^+$ m/z 225.1, found 225.1.

4-Cyclopropyl-N-(2-methyl-3-(7H-pyrrolo[2,3-d]pyrimidin-4-yl)phenyl)benzamide (S6)



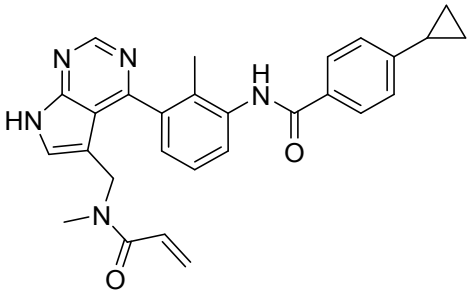
To a suspension of 4-cyclopropylbenzoic acid (1.00 g, 6.17 mmol) in toluene (30 mL) was added thionyl chloride (0.900 ml, 12.3 mmol) and DMF (0.6 mL). The reaction mixture was stirred for 5h at 80°C and then concentrated *in vacuo*. After addition of toluene the mixture was concentrated *in vacuo* and the remaining residue was dissolved in THF (7 mL). The resulting solution was added to a mixture of **S5** (1.40 g, 6.24 mmol) and triethylamine (2.6 mL, 18.7 mmol) in THF (18 mL) at 0°C. The reaction mixture was stirred for 10 min at 0°C, then quenched with 2N NaOH solution and extracted three times with EtOAc. The combined organic layers were washed with brine, dried over sodium sulfate and concentrated *in vacuo*. The crude product was trituated in diethyl ether and filtrated to give the title compound (1.34 g, 3.64 mmol, 58%) as grey solid. ^{1}H NMR (400 MHz, DMSO-d6): δ ppm 12.24 (s_{br}, 1H), 9.94 (s, 1H), 8.85 (s, 1H), 7.92 (d, *J* = 8.1 Hz, 2H), 7.61 (m, 1H), 7.49 (dd, *J* = 2.4, 6.9 Hz, 1H), 7.42-7.34 (m, 2H), 7.23 (d, *J* = 8.3 Hz, 2H), 6.33 (d, *J* = 3.5 Hz, 1H), 2.14 (s, 3H), 2.05-1.99 (m, 1H), 1.08-0.99 (m, 2H), 0.80-0.75 (m, 2H); LC/MS method 1: Rt 1.88 min, calcd for $C_{23}H_{21}N_4O$ $[M+H]^+$ m/z 369.2, found 369.1.

4-Cyclopropyl-N-(2-methyl-3-(5-((methylamino)methyl)-7H-pyrrolo[2,3-d]pyrimidin-4-yl)phenyl)benzamide (S7)



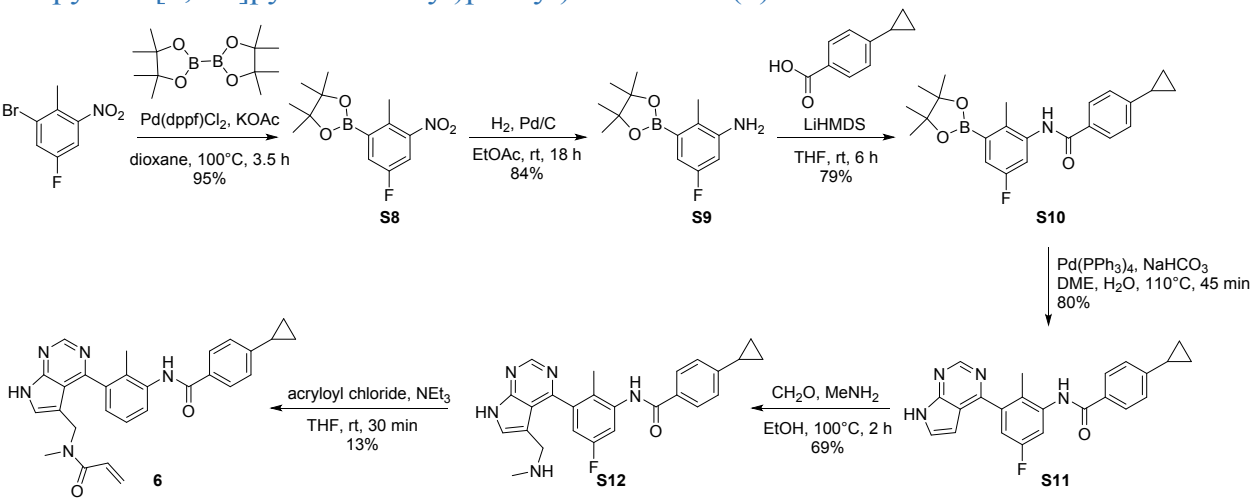
A solution of methylamine in ethanol (9.02 ml, 72.7 mmol) was added slowly to an aqueous formaldehyde solution (37 wt%, 5.42 ml, 72.7 mmol) at 0°C. The reaction mixture was allowed to reach room temperature and was stirred for 20 min. Then a solution of **S6** (1.34 g, 3.64 mmol) in ethanol (7.5 mL) was added and the resulting mixture was stirred at 100°C for 16 h. The reaction was quenched by addition of ethyl acetate and saturated aqueous sodium bicarbonate solution. The layers were separated and the aqueous layer was extracted twice with ethyl acetate. The combined organic layers were washed with brine, dried with sodium sulfate and concentrated *in vacuo*. The crude product was purified by flash chromatography (silica gel, methanol/dichloromethane gradient) to obtain the title compound as a beige solid (572 mg, 1.39 mmol, 38%). ^{1}H NMR (400 MHz, DMSO-d6): δ ppm 12.05 (s_{br}, 1H), 9.96 (s, 1H), 8.77 (s, 1H), 7.92-7.88 (m, 2H), 7.47-7.39 (m, 2H), 7.36-7.29 (m, 1H), 7.22-7.14 (m, 3H), 2.02-1.97 (m, 1H), 3.32 (s_{br}, 1H), 3.25 (s, 2H), 2.01 (s, 3H), 1.92 (s, 3H), 1.04-0.99 (m, 2H), 0.77-0.73 (m, 2H) ; LC/MS method 1: Rt 1.31 min, calcd for $C_{25}H_{26}N_5O$ $[M+H]^+$ m/z 412.2, found 412.3.

4-Cyclopropyl-N-(2-methyl-3-(5-((N-methylacrylamido)methyl)-7H-pyrrolo[2,3-d]pyrimidin-4-yl)phenyl)benzamide (6)

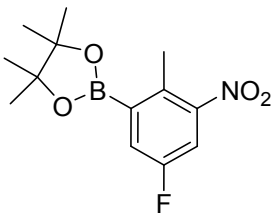


To a solution of **S7** (120 mg, 0.292 mmol) in THF (1.5 mL) at 0°C was added triethylamine (0.061 mL, 0.437 mmol). Then acryloyl chloride (0.035 mL, 0.437 mmol) was added dropwise. The resulting mixture was stirred for 10 min at 0°C. The reaction was allowed to warm to room temperature and quenched with water. The mixture was extracted with ethyl acetate and the organic layer was dried with sodium sulfate, filtered, and concentrated *in vacuo*. The crude product was purified by SFC to afford the title compound (35 mg, 0.075 mmol, 26%) as white solid. ^{1}H NMR (400 MHz, DMSO-d6, 373 K): δ ppm 11.93 (s_{br}, 1H), 9.58 (s, 1H), 8.78 (s, 1H), 7.85 (d, J = 8.0 Hz, 2H), 7.55-7.47 (m, 2H), 7.32 (t, 1H), 7.22-7.16 (m, 3H), 6.53-6.45 (m, 1H), 6.04-5.96 (m, 1H), 5.58-5.50 (m, 1H), 4.11 (m, 2H), 2.77 (s, 3H), 2.02-1.98 (m, 1H), 1.96 (s, 3H), 1.02-0.98 (m, 2H), 0.75-0.71 (m, 2H); LC/MS method 3: Rt 3.17 min, calcd for $C_{28}H_{28}N_5O_2$ $[M+H]^+$ m/z 466.2, found 466.2.

Synthesis of 4-Cyclopropyl-N-(5-fluoro-2-methyl-3-(5-((N-methylacrylamido)methyl)-7H-pyrrolo[2,3-d]pyrimidin-4-yl)phenyl)benzamide (7)



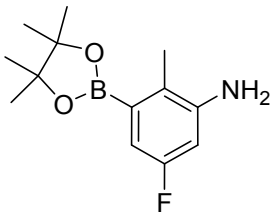
2-(Fluoro-2-methyl-3-nitro-phenyl)-4,4,5,5-tetramethyl-[1,3,2]dioxaborolone (S8)



To a solution of 1-bromo-5-fluoro-2-methyl-3-nitro-benzene (100 g, 427 mmol) in dioxane (4 L) were added bis-(pinacolato)-diboron (163 g, 642 mmol), potassium acetate (147 g, 1498 mmol)

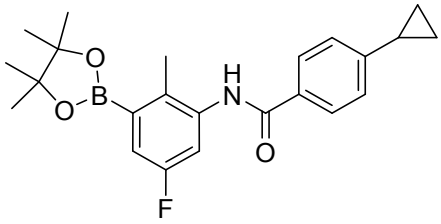
and bis(diphenylphosphino)ferrocenedichloropalladium (II) (18.0 g, 22.0 mmol). The mixture was heated to 100°C for 3.5 hr. After cooling the dark mixture was diluted with ethyl acetate (3 L) and poured into a saturated aqueous sodium bicarbonate solution (3 L). The layers were separated and the aqueous layer was extracted with ethyl acetate (2 L). Both organic layers were washed with brine (3 L), combined, dried over magnesium sulfate, filtered and concentrated *in vacuo*. The dark oil was purified by flash chromatography on silica (heptane/ethyl acetate 9:1) to afford the title compound (114.5 g, 407 mmol, 95%). ^{1}H-NMR (400 MHz, DMSO-d6): δ (ppm) 7.93 (d, J = 8.5 Hz, 1H), 7.62 (d, J = 8.5, 1H), 2.51 (s, 3H), 1.33 (s, 12H); LC/MS method 1: Rt 1.35 min.

5-Fluoro-2-methyl-3-(4,4,5,5-tetramethyl-[1,3,2]dioxaborolan-2-yl)-phenylamine (S9)



S8 (12.4 g, 44.1 mmol) was dissolved in ethyl acetate (300 mL) and palladium on charcoal (10% palladium) (4.0 g) was added. The mixture was hydrogenated at room temperature and normal pressure for 18 hours. The mixture was filtered over Kieselgur and evaporated. The residue was purified by flash chromatography on silica (ethyl acetate) to afford the title compound (9.38 g, 37.3 mmol, 84%) as a beige solid. ^{1}H-NMR (400 MHz, DMSO-d6): δ (ppm) 6.52 (m, 2H), 5.11 (s_{br}, 2NH), 2.19 (s, 3H), 1.29 (s, 12H); LC/MS method 1: Rt 1.11 min, calcd for $C_{13}H_{20}BFNO_2$ $[M+H]^+$ m/z 252.2, found 252.2.

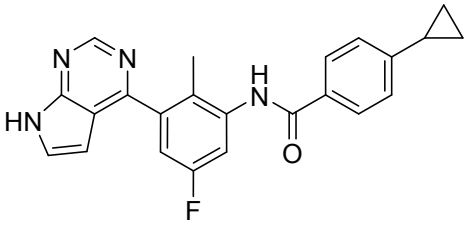
4-Cyclopropyl-N-(5-fluoro-2-methyl-3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl)benzamide (S10)



A solution of the **S9** (15 g, 59.7 mmol) and methyl 4-cyclopropylbenzoate (15.79 g, 90 mmol) in tetrahydrofuran (60 mL) was cooled in an ice-bath. Sodium bis(trimethylsilyl)amide (2 M in tetrahydrofuran, 45 mL, 90 mmol) was added by syringe so that the temperature was kept below 5°C (about 25 minutes). The mixture was then stirred for 10 minutes at this temperature. The cooling bath was removed and the brown solution was stirred for 4 hr. The dark solution was cooled again in the ice-bath and further sodium bis(trimethylsilyl)amide solution (2 M in tetrahydrofuran, 15 mL, 30 mmol) was added. Stirring at room temperature was continued for 1 hr, then another sodium bis(trimethylsilyl)amide solution (2 M in tetrahydrofuran, 5 mL, 10 mmol) was added. After another hour, all aniline starting material was consumed. The reaction mixture was diluted with ethyl acetate (500 mL) and water (250 mL). The organic layer was washed with 10% aqueous ammonium chloride solution (200 mL), 10% aqueous sodium carbonate solution (200 mL) and brine (100 mL). It was then dried over sodium sulfate, filtered and evaporated. A first batch of product was obtained by crystallization from dichloromethane/cyclohexane. A second batch was

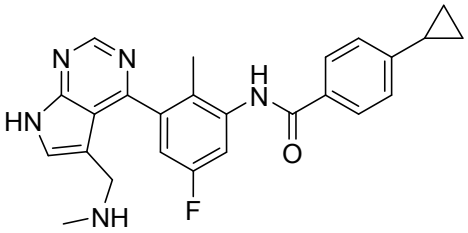
obtained by addition of diethyl ether to the mother liquor and cooling it in an ice-bath. The remaining solution was evaporated and crystallized from ethyl acetate and cyclohexane to give another batch. All batches were combined to yield the title compound (18.6 g, 47.1 mmol, 79%). ^{1}H-NMR (400 MHz, $CDCl_3$): δ (ppm) 8.03 (dd, *J* = 10.6, 2.8 Hz, 1H), 7.77 (d, *J* = 8.1 Hz, 2H), 7.74 (s_{br}, 1H), 7.30 (dd, *J* = 8.6, 2.8 Hz, 1H), 7.17 (d, *J* = 7.9 Hz, 2 H), 2.49 (s, 3H), 1.97 (m, 1H), 1.35 (s, 12H), 1.09-1.04 (m, 2H), 0.80-0.76 (m, 2H); LC/MS method 1: Rt 1.34 min, calcd for $C_{23}H_{28}BFNO_3$ $[M+H]^+$ m/z 396.2, found 396.2.

4-Cyclopropyl-N-(5-fluoro-2-methyl-3-(7H-pyrrolo[2,3-d]pyrimidin-4-yl)phenyl)benzamide (S11)



To a solution of 4-chloro-7H-pyrrolo[2,3-d]pyrimidine (80 mg, 0.521 mmol) and **S10** (227 mg, 0.573 mmol) in dimethoxyethane (10 mL) were added aqueous sodium bicarbonate solution (1.2M, 3 mL) and water (3 mL). The vial was flushed with argon and $Pd(PPh_3)_4$ (18 mg, 0.016 mmol) was added. The mixture was stirred at 110 °C for 45 min. The reaction mixture was diluted with water and ethyl acetate. The organic layer was washed with water, dried with sodium sulfate and concentrated *in vacuo*. The crude product was purified by flash chrmoatography (silica gel, dichloromethane/methanol 98:2) to yield the title compound (178 mg, 0.42 mmol, 80%) as pale yellow solid. ^{1}H-NMR (400 MHz, DMSO-d6): δ (ppm) 12.25 (s_{br}, 1H), 9.94 (s, 1H), 8.83 (s, 1H), 7.88 (d, *J* = 8.0 Hz, 2H), 7.59 (d, *J* = 3.6 Hz, 1H), 7.43 (d, *J* = 9.5 Hz, 1H), 7.21 (d, *J* = 8.0 Hz, 2H), 7.19-7.14 (m, 1H), 6.33 (s, 1H), 2.06 (s, 3H), 2.02-1.96 (m, 1H), 1.08-0.95 (m, 2H), 0.77-0.72 (m, 2H); LC/MS method 1: Rt 0.97 min, calcd for $C_{23}H_{20}FN_4O$ $[M+H]^+$ m/z 387.2, found 387.2.

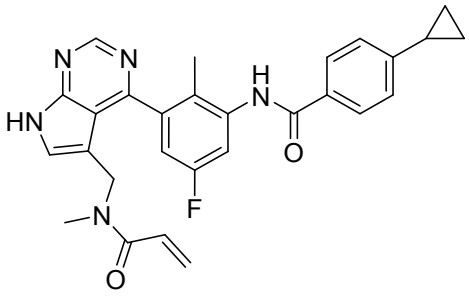
4-Cyclopropyl-N-(5-fluoro-2-methyl-3-(5-((methylamino)methyl)-7H-pyrrolo[2,3-d]pyrimidin-4-yl)phenyl)benzamide (S12)



To a solution of methylamine in ethanol (33 wt%, 19.9 mmol, 2.46 mL) was added an aqueous solution of formaldehyde (37 wt%, 40.1 mmol, 2.99 mL). The mixture was stirred for 5 minutes before adding **S11** (100 mg, 0.259 mmol). The resulting mixture was stirred at 100°C for 2 h, then quenched with water. The mixture was basified by addition of 2N NaOH and extracted three times with ethyl acetate. The combined organic layers were dried with sodium sulfate, filtered and concentrated *in vacuo*. The crude product was purified by flash chromatography (silica gel, ethyl acetate/methanol (containing 10% ammonia) gradient) to yield the title compound (77 mg, 0.179

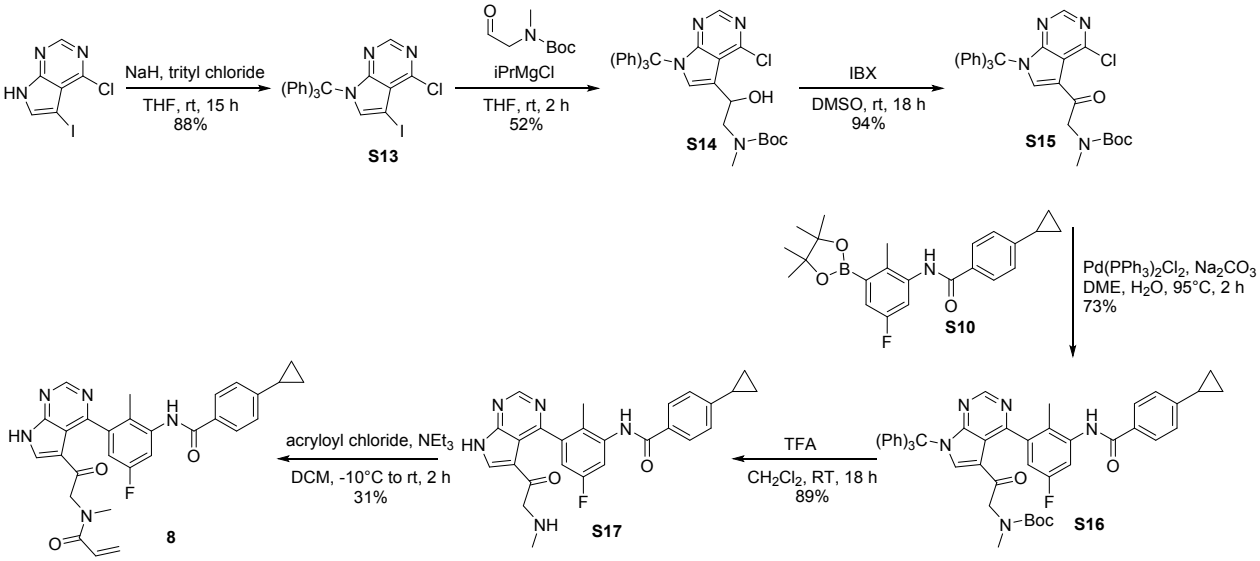
mmol, 69%). LC/MS method 1: Rt 1.43 min, calcd for $C_{25}H_{25}FN_5O$ $[M+H]^+$ m/z 430.2, found 430.3.

4-Cyclopropyl-N-(5-fluoro-2-methyl-3-(5-((N-methylacrylamido)methyl)-7H-pyrrolo[2,3-d]pyrimidin-4-yl)phenyl)benzamide (7)

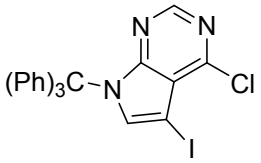


To a solution of **S12** (77 mg, 0.179 mmol) in THF (2 mL) at 0°C was added a solution of acryloyl chloride (16 mg, 0.179 mmol) in THF (1 mL). The resulting mixture was stirred for 30 min at room temperature, then diluted with water and extracted twice with ethyl acetate. The combined organic layers were dried over sodium sulfate, filtered and concentrated *in vacuo*. The crude product was purified by flash chromatography (silica gel, ethyl acetate/methanol gradient) to yield the title compound (11.1 mg, 0.023 mmol, 13%). ^{1}H NMR (400 MHz, DMSO-d6, 373 K): δ ppm 11.99 (s_{br}, 1H), 9.61 (s, 1H), 8.80 (s, 1H), 7.85 (d, *J* = 8.4 Hz, 2H), 7.42 (dd, *J* = 10.2, 2.8 Hz, 1H), 7.27 (s, 1H), 7.19 (d, *J* = 8.3 Hz, 2H), 7.03 (dd, *J* = 8.6, 2.8 Hz, 1H), 6.51 (m, 1H), 6.00 (dd, *J* = 16.7, 2.4 Hz, 1H), 5.53 (dd, *J* = 10.4, 2.4 Hz, 1H), 4.16 (s, 2H), 2.79 (s, 3H), 2.01 (m, 1H), 1.93 (s, 3H), 1.04-0.99 (m, 2H), 0.77-0.73 (m, 2H); LC/MS method 4: Rt 0.93 min, calcd for $C_{28}H_{27}FN_5O_2$ $[M+H]^+$ m/z 484.2, found 484.1.

Synthesis of 4-Cyclopropyl-N-(5-fluoro-2-methyl-3-(5-(2-(N-methylacrylamido)acetyl)-7H-pyrrolo[2,3-d]pyrimidin-4-yl)phenyl)benzamide (**8**)

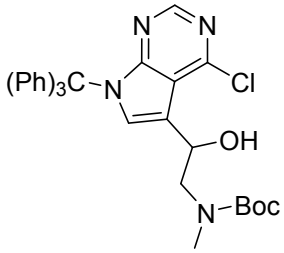


4-Chloro-5-iodo-7-trityl-7H-pyrrolo[2,3-d]pyrimidine (S13)



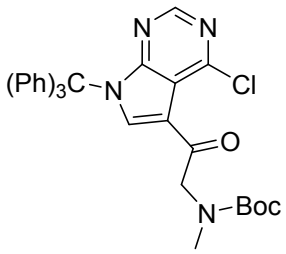
To a suspension of NaH (1.72 g, 42.9 mmol) in THF (150 mL) was added a suspension of 4-chloro-5-iodo-7H-pyrrolo[2,3-d]pyrimidine (10 g, 35.8 mmol) in THF (75 mL) dropwise. The mixture was stirred at room temperature for 15 min. It was then cooled to 0°C and trityl chloride (11.0 g, 39.4 mmol) was added. The reaction mixture was stirred for 15 h while allowing it to reach room temperature. The mixture was carefully poured into saturated aqueous ammonium chloride solution and extracted twice with ethyl acetate. The combined organic layers were washed with water, saturated aqueous sodium bicarbonate solution and brine, dried over magnesium sulfate, filtered and concentrated. The crude product was purified by flash chromatography (silica gel, heptane/dichloromethane gradient) to yield the title compound (17.3 g, 31.5 mmol, 88%) as beige solid. ^{1}H NMR (400 MHz, DMSO-d6): δ ppm 8.29 (s, 1H), 7.47 (s, 1 H), 7.33-7.19 (m, 10H), 7.15-7.09 (m, 5H).; LC/MS method 1: Rt 1.50 min, calcd for $C_{25}H_{18}ClN_3$ $[M+H]^+$ m/z 522.0, found 522.0.

tert-Butyl 2-(4-chloro-7-trityl-7H-pyrrolo[2,3-d]pyrimidin-5-yl)-2-hydroxyethyl(methyl)carbamate (S14)



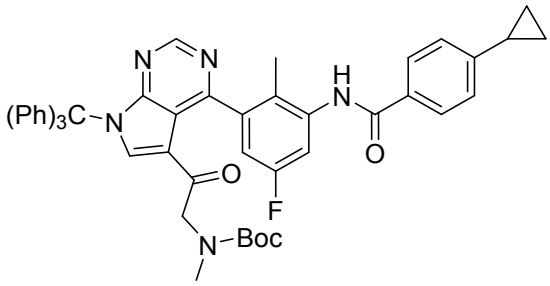
To a solution of **S13** (3.04 g, 5.83 mmol) in THF (40 mL) at -10°C was added isopropylmagnesium chloride (2M in tetrahydrofuran, 4.37 mL, 8.74 mmol) and the resulting mixture was stirred at -10°C for 30 min. Then a solution of *tert*-butyl methyl(2-oxoethyl)carbamate (2.02 g, 6.99 mmol) in tetrahydrofuran (20 mL) was added and the mixture was stirred at room temperature for 2 hr. The mixture was quenched by addition of an aqueous HCl solution (1 M), the layers were separated, and the aqueous layer was extracted with ethyl acetate. The combined organic layers were washed with brine, dried over magnesium sulfate, and concentrated *in vacuo*. The reulting crude mixture was purified by column chromatography on silica gel with an ethyl acetate / cyclohexane gradient to provide the title compound as a white solid (1.92 g, 3.04 mmol, 52%). ^{1}H NMR (400 MHz, DMSO-d6): δ ppm (rotamers) 8.30-8.24 (m, 1H), 7.44-7.29 (m, 10 H), 7.17-7.10 (m, 6H), 5.50-5.42 (m, 1H), 5.33 (s_{br}, 1H), 3.87-3.38 (m, 2H), 2.80-2.74 (m, 3H), 1.31, 1.14 (2 s, 9H); LC/MS method 1: Rt 1.42 min, calcd for $C_{33}H_{34}ClN_4O_3$ $[M+H]^+$ m/z 569.2, found 569.3.

tert-Butyl (2-(4-chloro-7-trityl-7H-pyrrolo[2,3-d]pyrimidin-5-yl)-2-oxoethyl)(methyl)carbamate (S15)



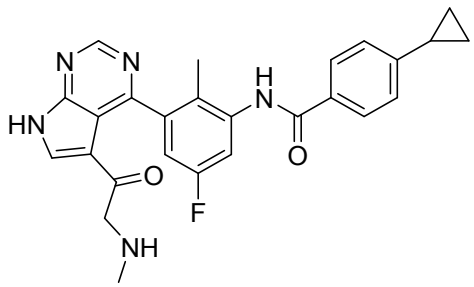
To a solution of **S14** (0.88 g, 1.55 mmol) in dimethyl sulfoxide (10 mL) was added 2-iodoxybenzoic acid (0.476 g, 1.70 mmol) and the resulting mixture was stirred at room temperature overnight. The reaction mixture was quenched with water and filtered. The filter cake was rinsed with ethyl acetate, and the combined filtrates were extracted with ethyl acetate. The organic layer was washed with aqueous sodium hydroxide solution (1 N) and brine, dried over magnesium sulfate and concentrated *in vacuo*. The crude product was purified by column chromatography (silica gel, cyclohexane / ethyl acetate gradient) to afford the title compound (870 mg, 1.46 mmol, 94%) as a white foam. ^{1}H NMR (400 MHz, $CDCl_3$): δ ppm (rotamers) 8.39, 8.36 (2 s, 1H), 8.08, 7.87 (2 s, 1H), 7.35-7.31 (m, 10H), 7.17-7.13 (m, 5H), 4.44, 4.31 (2 s_{br}, 2H), 2.98, 2.96 (2 s, 3H), 1.48, 1.45 (2s, 9H); LC/MS method 1: Rt 1.43 min, calcd for $C_{33}H_{32}ClN_4O_3$ $[M+H]^+$ m/z 567.2, found 567.2.

tert-Butyl (2-(4-(3-(4-cyclopropylbenzamido)-5-fluoro-2-methylphenyl)-7-trityl-7H-pyrrolo[2,3-d]pyrimidin-5-yl)-2-oxoethyl)(methyl)carbamate (S16)



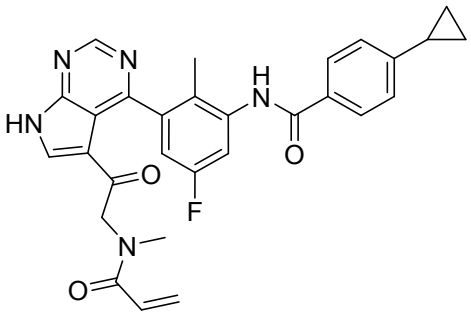
S15 (752 mg, 1.33 mmol) and **S10** (655 mg, 1.33 mmol) were dissolved in a mixture of dimethoxyethane and water (16 mL, 7:1). Then an aqueous solution of sodium carbonate (1 M, 2.65 mL, 2.65 mmol) was added and the mixture was degased for 10 min under argon. Then palladium(II)bis(triphenylphosphine) dichloride (47 mg, 0.067 mmol) was added and the reaction mixture was heated for 2 hr at 95°C. The reaction mixture was quenched with saturated aqueous sodium bicarbonate solution and extracted with ethyl acetate. The combined organic layers were washed with water and brine, dried over sodium sulfate and concentrated *in vacuo*. The crude product was purified by column chromatography (silica gel, cyclohexane/ethyl acetate gradient) to afford the title compound (942 mg, 1.12 mmol, 74%) as a light orange solid. ^{1}H-NMR (400 MHz, DMSO-d6): δ ppm (rotamers) 9.95, 9.78 (2 s, 1H), 8.66 (s, 1H), 8.30, 8.23 (2 s, 1H), 7.90 (m, 2H), 7.43-7.22 (m, 18H), 6.91 (m, 1H), 4.31, 4.25 (2 s, 2H), 2.69, 2.66 (2 s, 3H), 2.03-1.98 (m, 4H), 1.22-1.12 (m, 9H), 1.06-1.03 (m, 2H), 0.79-0.77 (m, 2H); LC/MS method 1: Rt 1.51 min, calcd for $C_{50}H_{47}FN_5O_4$ $[M+H]^+$ m/z 800.4, found 800.4.

4-Cyclopropyl-N-(5-fluoro-2-methyl-3-(5-(2-(methylamino)acetyl)-7H-pyrrolo[2,3-d]pyrimidin-4-yl)phenyl)benzamide (S17)



To a solution of **S16** (936 mg, 1.17 mmol) in dichloromethane (10 mL) was added trifluoroacetic acid (1 mL, 13.0 mmol). After addition of one drop of water the reaction mixture was stirred overnight at room temperature. The reaction mixture was quenched with aqueous sodium carbonate solution (2 M) and extracted with ethyl acetate and tetrahydrofuran. The combined organic layer was washed with brine, dried over magnesium sulfate, filtered and concentrated. The orange solid was dissolved in methanol (2 mL) and the first batch of the title compound was precipitated by addition of diethyl ether. After filtration, the filtrate was concentrated and the remaining crude product was purified by column chromatography (silica gel, dichloromethane/methanol gradient including 2% ammonium hydroxide) to afford a second batch of the title compound. The two batches were combined to obtain the title compound (500 mg, 1.04 mmol, 89%). ^{1}H-NMR (400 MHz, DMSO-d6): δ ppm 9.89 (s, 1H), 8.92 (s, 1H), 8.63 (s, 1H), 7.89 (d, *J* = 8.1 Hz, 2H), 7.39 (dd, *J* = 10.3, 2.8 Hz, 1H), 7.21 (d, *J* = 8.1 Hz, 2H), 6.87 (dd, *J* = 8.9, 2.8 Hz, 1H), 3.82 (s, 2H), 3.32 (s_{br}, 1H), 2.25 (s, 3H), 2.01 (m, 1H), 1.89 (s, 3H), 1.06-0.99 (m, 2H), 0.78-0.71 (m, 2H), one amide NH not visible; LC/MS method 1: Rt 0.68 min, calcd for $C_{26}H_{25}FN_5O_2$ $[M+H]^+$ m/z 458.2, found 458.2.

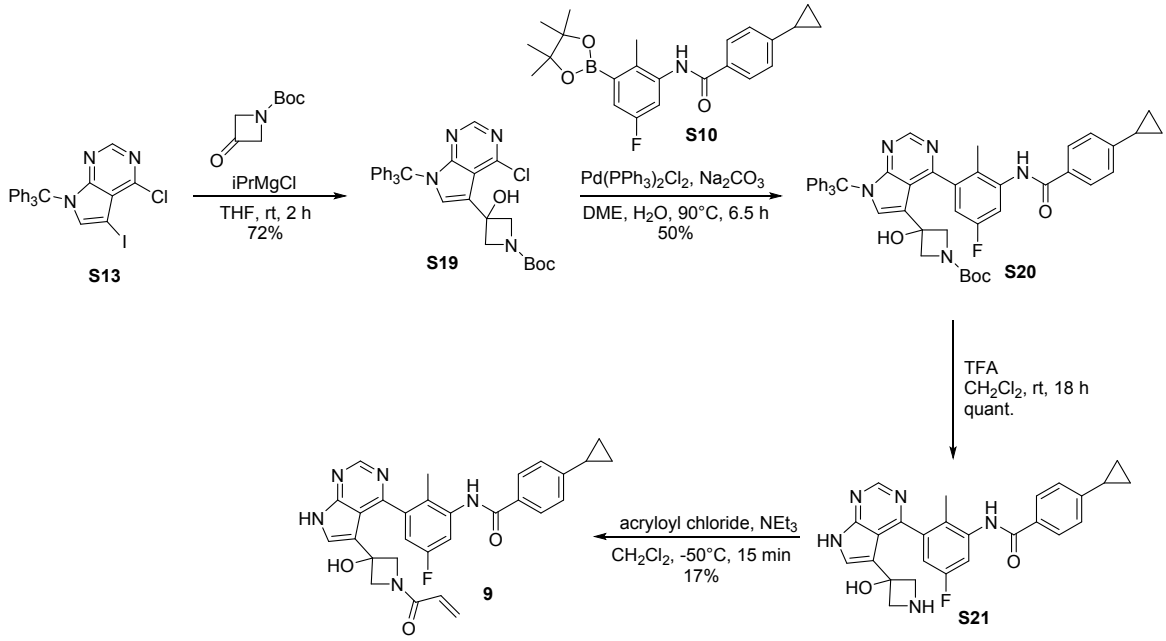
4-Cyclopropyl-N-(5-fluoro-2-methyl-3-(5-(2-(N-methylacrylamido)acetyl)-7H-pyrrolo[2,3-d]pyrimidin-4-yl)phenyl)benzamide (8)



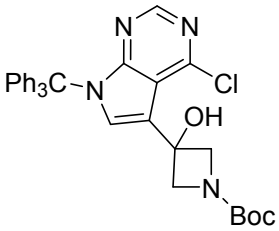
To a solution of 4-cyclopropyl-N-(5-fluoro-2-methyl-3-(5-(2-(methylamino)acetyl)-7H-pyrrolo[2,3-d]pyrimidin-4-yl)phenyl)benzamide (105 mg, 0.230 mmol) in dry dichloromethane (5 mL) and dimethylformamide (1 mL) at -10°C was added triethylamine (0.100 mL, 0.717 mmol) followed by acryloyl chloride (0.022 mL, 0.271 mmol). The reaction mixture was stirred for 20 min at -10°C and then allowed to warm up to room temperature for 1.5 hr. The reaction mixture was quenched with saturated aqueous ammonium chloride solution, extracted with dichloromethane, and the organic layer was dried over magnesium sulfate, filtered and concentrated. The crude product was purified by SFC to afford the title compound (40 mg, 0.072 mmol, 31%). ^{1}H NMR (600 MHz, DMSO-d6): δ ppm (rotamers) 13.11 (s_{br}, 1H), 9.95, 9.77 (2 s, 1H), 8.97, 8.96 (2 s, 1H), 8.72, 8.70 (2 s, 1H), 7.97-7.79 (m, 2H), 7.40, 7.31 (2 dd, *J* = 10.1, 2.8 Hz, 1H), 7.25-7.15 (m, 2H), 6.91, 6.85 (2 dd, *J* = 8.9, 2.8 Hz, 1H), 6.79, 6.44 (2 dd, *J* = 16.6, 10.4

Hz, 1H), 6.08-5.94 (m, 1H), 5.65, 5.51 (2 dd, J = 10.4, 2.5 Hz, 1H), 5.02-4.53 (m, 2H), 2.98, 2.78 (2 s, 3H), 2.07-1.97 (m,1H), 1.95, 1.91 (2 s, 3H), 1.07-0.96 (m, 2H), 0.79-0.71 (m, 2H); ^{13}C NMR (151 MHz, DMSO-d6) δ ppm (rotamers) 188.13, 187.67, 166.40, 165.73, 165.13, 159.73, 159.54, 159.28, 158.15, 153.43, 153.22, 151.85, 148.25, 148.19, 141.70, 141.54, 137.76, 137.47, 135.47, 135.21, 131.18, 128.11, 127.77, 127.65, 126.71, 125.16, 113.98, 113.59, 113.44, 113.40, 113.30, 113.03, 112.83, 112.58, 56.24, 54.84, 36.21, 34.66, 15.19, 14.68, 14.63, 10.22; LC/MS method 1: Rt 0.91 min, calcd for $C_{29}H_{27}FN_5O_3$ $[M+H]^+$ m/z 512.2, found 512.4; HRMS (ESI+) calcd for $C_{29}H_{27}N_5O_3F$ $[M+H]^+$ 512.20924, found 512.20935.

Synthesis of N-(3-(5-(1-Acryloyl-3-hydroxyazetidin-3-yl)-7H-pyrrolo[2,3-d]pyrimidin-4-yl)-5-fluoro-2-methylphenyl)-4-cyclopropylbenzamide (**9**)



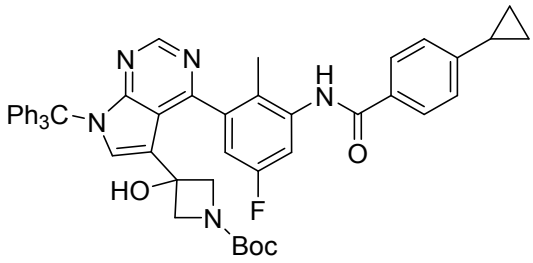
tert-Butyl 3-(4-chloro-7-trityl-7H-pyrrolo[2,3-d]pyrimidin-5-yl)-3-hydroxyazetidine-1-carboxylate (S19)



To a solution of **S13** (2.00 g, 3.83 mmol) in THF (38 mL) at -10°C was added isopropylmagnesium chloride (2.87 mL, 5.75 mmol) dropwise. The pale yellow solution was stirred at -10 °C for 40 min, then a solution of 1-Boc-3-oxoazetidine (0.722 g, 4.22 mmol) in THF (3.0 mL) was added. The reaction mixture was stirred at room temperature for 2.5 h. The mixture was quenched with 1M HCl and diluted with ethyl acetate. The aqueous layer was extracted with ethyl acetate. The organic layers were combined, washed with saturated aqueous sodium bicarbonate solution and brine, dried over magnesium sulfate, filtered and concentrated. The residue was purified by flash chromatography (silica gel, heptane/ethyl acetate gradient) to afford the title compound (1.65 g,

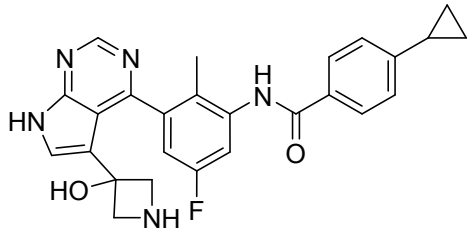
2.76 mmol, 72%) as a white solid. ^{1}H-NMR (400 MHz, DMSO-d6): δ ppm 8.30 (s, 1H), 7.41-7.27 (m, 10H), 7.20-7.08 (m, 6H), 6.24 (s, 1H), 4.24 (m, 2H), 4.04 (m, 2H), 1.38 (s, 9H); LC/MS method 1: Rt 1.33 min, calcd for $C_{33}H_{32}ClIN_4O_3$ $[M+H]^+$ m/z 567.2, found 567.2.

***tert*-Butyl 3-(4-(3-(4-cyclopropylbenzamido)-5-fluoro-2-methylphenyl)-7-trityl-7H-pyrrolo[2,3-d]pyrimidin-5-yl)-3-hydroxyazetidine-1-carboxylate (S20)**



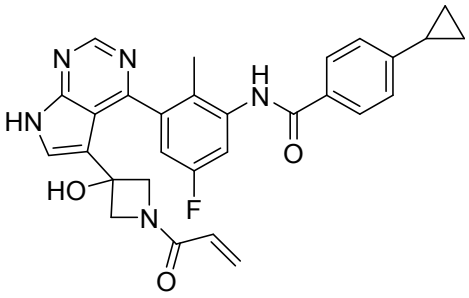
To a solution of **S19** (500 mg, 0.882 mmol) in DME (8.0 mL) was added **S10** (383 mg, 0.970 mmol) followed by saturated aqueous sodium bicarbonate solution (5.48 mL, 6.17 mmol) and water (0.77 mL). The mixture was degased for 10 min, then $Pd(PPh_3)_2Cl_2$ (30.9 mg, 0.044 mmol) was added. The reaction mixture was stirred at 90°C for 4 h. More **S10** (78 mg, 0.197 mmol, 0.22 eq) and $Pd(PPh_3)_2Cl_2$ (10 mg, 0.014 mmol) were added. The reaction mixture was stirred at 90°C for additional 2.5 h. The mixture was diluted with ethyl acetate and washed with saturated aqueous sodium bicarbonate solution. The aqueous layer was extracted with ethyl acetate. The combined organic layers were washed with saturated aqueous sodium bicarbonate solution and brine, dried over magnesium sulfate, filtered and concentrated. The residue was purified by flash chromatography (silica gel, heptane/ethyl acetate gradient) to afford the title compound (354 mg, 0.443 mmol, 50%) a pale yellow solid. ^{1}H-NMR (400 MHz, DMSO-d6): δ ppm 9.89 (s, 1H), 8.51 (s, 1H), 7.88 (d, J = 8.0 Hz, 2H), 7.51-7.02 (m, 20H), 5.75 (s, 1H), 3.98 (m, 1H), 3.68 (m, 1H), 3.46 (m, 1H), 3.19 (d, J = 9.3 Hz, 1H), 2.06-1.96 (m, 1H), 1.90 (s, 3H), 1.29 (s, 9H), 1.08-1.00 (m, 2H), 0.81-0.72 (m, 2H); LC/MS method 1: Rt 1.42 min, calcd for $C_{50}H_{47}FN_5O_4$ $[M+H]^+$ m/z 800.4, found 800.3.

4-Cyclopropyl-N-(5-fluoro-3-(5-(3-hydroxyazetidin-3-yl)-7H-pyrrolo[2,3-d]pyrimidin-4-yl)-2-methylphenyl)benzamide (S21)



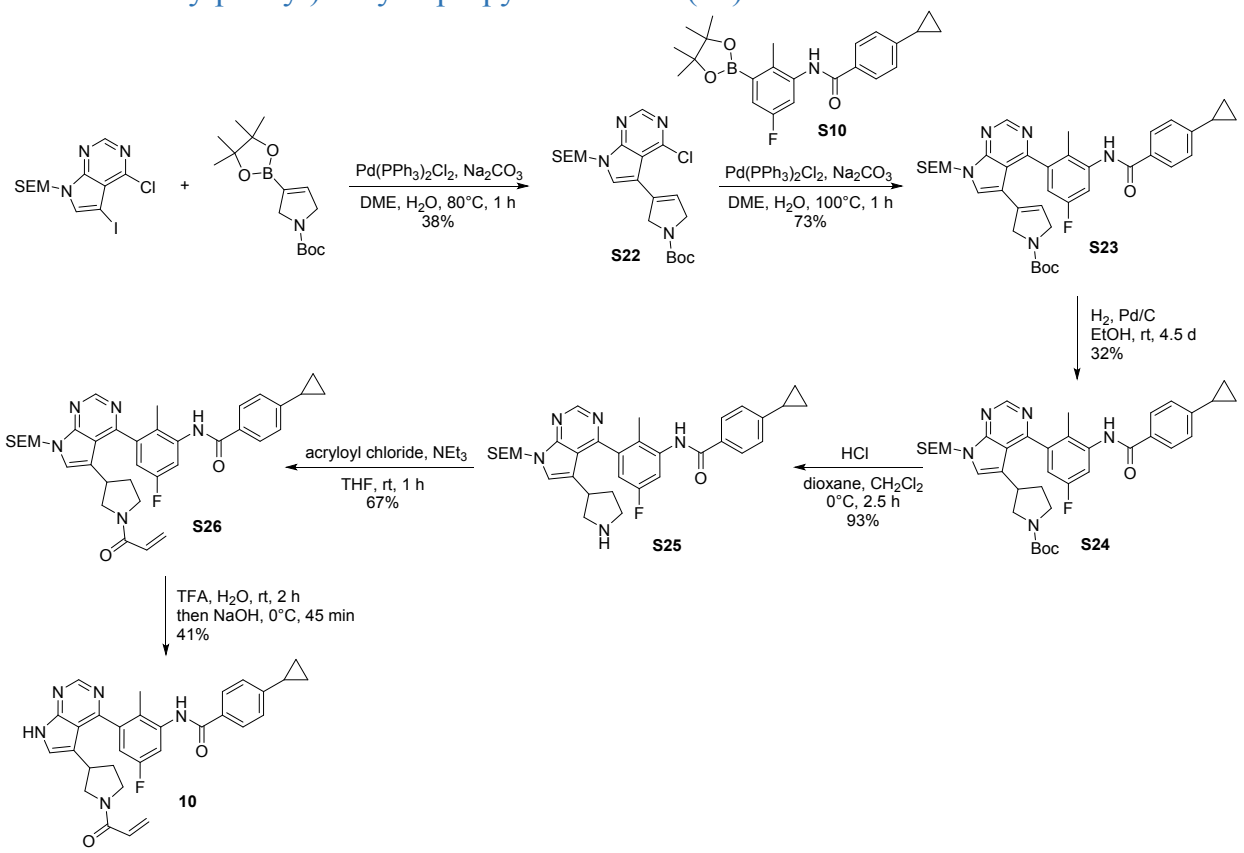
To a solution of **S20** (340 mg, 0.425 mmol) in dichloromethane (10 mL) was carefully added trifluoroacetic acid (0.327 mL, 4.25 mmol) dropwise. The reaction mixture was stirred at room temperature overnight. The reaction mixture was concentrated *in vacuo* to yield the title compound as TFA salt which was used in the next step without purification. LC/MS method 1: Rt 0.67 min, calcd for $C_{26}H_{25}FN_5O_2$ $[M+H]^+$ m/z 458.2, found 458.2.

N-(3-(5-(1-Acryloyl-3-hydroxyazetidin-3-yl)-7H-pyrrolo[2,3-d]pyrimidin-4-yl)-5-fluoro-2-methylphenyl)-4-cyclopropylbenzamide (9)

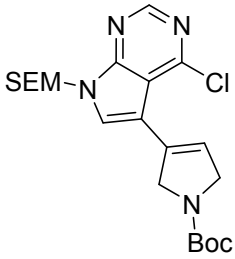


To a solution of **S20** (250 mg, 0.266 mmol) and triethylamine (0.185 mL, 1.329 mmol) in dichloromethane (4 mL) and THF (1 mL) was added at -50°C acryloyl chloride (8.60 µl, 0.106 mmol). The mixture was stirred at this temperature for 15 min. The reaction mixture was quenched at -50°C with saturated aqueous sodium bicarbonate solution and extrated with ethyl acetate. The organic layer was washed with water and brine, dried with magnesium sulfate, filtered and concentrated. The crude product was purified by flash chromatography (silica gel, dichloromethane/methanol containing 2% ammonia gradient) to yield the title compound (25 mg, 0.046 mmol, 17%) as white solid. ^{1}H NMR (400 MHz, MeOD): δ ppm (rotamers) 8.84 (m, 1H), 7.92 (m, 2H), 7.72 (m, 1H), 7.50, 7.35 (2 m, 1H), 7.25 (m, 2H), 7.18, 7.12 (m, 1H), 6.50-6.17 (m, 2H), 5.72 (m, 1H), 4.65, 4.52 (2 m, 1H), 4.27, 4.21 (2 m, 1H), 3.94 (m, 1H), 3.58, 3.52 (2 m, 1H), 2.05-1.96 (m, 4H), 1.11-1.06 (m, 2H), 0.83-0.79 (m, 2H), 2 NH and 1 OH not visible; ^{13}C NMR (151 MHz, DMSO-d6) δ ppm 165.39, 164.53, 159.86, 158.25, 157.46, 153.03, 150.74, 148.24, 140.73, 137.86, 131.01, 127.73, 127.28, 126.11, 125.60, 125.17, 117.09, 114.40, 65.88, 63.56, 61.61, 15.16, 14.41, 10.16; LC/MS method 1: Rt 0.81 min, calcd for $C_{29}H_{27}FN_5O_3$ $[M+H]^+$ m/z 512.2, found 512.2; HRMS (ESI+) calcd for $C_{29}H_{27}FN_5O_3$ $[M+H]^+$ 512.20924, found 512.20917.

Synthesis of N-(3-(5-(1-Acryloylpyrrolidin-3-yl)-7H-pyrrolo[2,3-d]pyrimidin-4-yl)-5-fluoro-2-methylphenyl)-4-cyclopropylbenzamide (10)

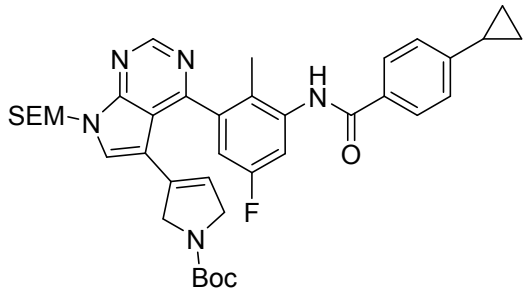


***tert*-Butyl 3-(4-chloro-7-((2-(trimethylsilyl)ethoxy)methyl)-7H-pyrrolo[2,3-d]pyrimidin-5-yl)-2,5-dihydro-1H-pyrrole-1-carboxylate (S22)**



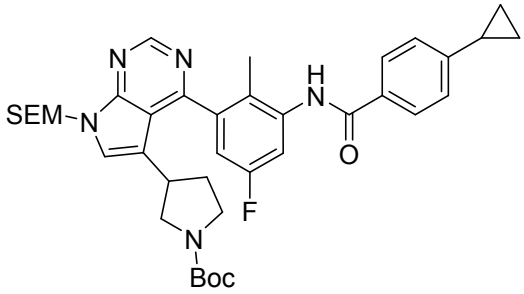
A mixture of 4-chloro-5-iodo-7-((2-(trimethylsilyl)ethoxy)methyl)-7H-pyrrolo[2,3-d]pyrimidine[19] (710 mg, 1.73 mmol), *tert*-butyl 3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-2,5-dihydro-1H-pyrrole-1-carboxylate (1.02 g, 3.47 mmol) and 2M aqueous sodium carbonate solution (4.33 mL, 8.66 mmol) in DME (8.7 mL) was flushed with argon. Then $Pd(PPh_3)_2Cl_2$ (60.8 mg, 0.087 mmol) was added, and the mixture was heated at 100 °C for 1 h. The reaction mixture was cooled to room temperature and concentrated. The crude residue was purified by flash chromatography (silica gel, heptane/ethyl acetate gradient) to yield the title compound (301 mg, 0.667 mmol, 38.5%) as a yellow oil. ^{1}H NMR (400 MHz, DMSO-d6): δ ppm 8.74 (s, 1H), 8.07 (d, J = 4.4 Hz, 1H), 6.32 (m, 1H), 5.67 (s, 2H), 4.52-4.42 (m, 2H), 4.31-4.24 (m, 2H), 3.58 (m, 2H), 1.47 (s, 9H), 0.87 (t, J = 8.0 Hz, 2H), -0.05 (s, 9H). ; LC/MS method 1: Rt 1.46 min, calcd for $C_{21}H_{32}ClN_4O_3Si$ $[M+H]^+$ m/z 451.2, found 451.3.

***tert*-Butyl 3-(4-(3-(4-cyclopropylbenzamido)-5-fluoro-2-methylphenyl)-7-((2-(trimethylsilyl)ethoxy)methyl)-7H-pyrrolo[2,3-d]pyrimidin-5-yl)-2,5-dihydro-1H-pyrrole-1-carboxylate (S23)**



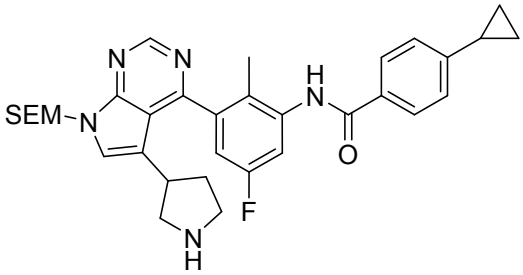
A mixture of **S22** (322 mg, 0.714 mmol), **S10** (310 mg, 0.785 mmol) and 2M aqueous sodium carbonate solution (1.79 mL, 3.57 mmol) in DME (3570 µL) was flushed with argon. Then $Pd(PPh_3)_2Cl_2$ (25.05 mg, 0.036 mmol) was added, and the mixture was heated at 100 °C for 1 h. The reaction mixture was cooled to room temperature and concentrated. The crude residue was purified by flash chromatography (silica gel, heptane/ethyl acetate gradient) to yield the title compound (360 mg, 0.526 mmol, 73%). LC/MS method 1: Rt 1.51 min, calcd for $C_{38}H_{47}FN_5O_4Si$ $[M+H]^+$ m/z 684.3, found 684.5.

***tert*-Butyl 3-(4-(3-(4-cyclopropylbenzamido)-5-fluoro-2-methylphenyl)-7-((2-(trimethylsilyl)ethoxy)methyl)-7H-pyrrolo[2,3-d]pyrimidin-5-yl)pyrrolidine-1-carboxylate (S23)**



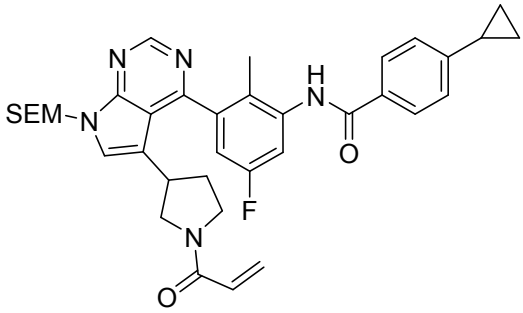
To a solution of **S22** (360 mg, 0.526 mmol) in ethanol (10.5 mL) was added Pd/C (10%, 72 mg) and the resulting mixture was treated with hydrogen (4 bar) for 21 h. Then additional Pd/C (10%, 150 mg) was added and treatment with hydrogen (4 bar) continued for 60 h. The mixture was filtrated and concentrated. The crude product was purified by flash chromatography (silica gel, heptane/ethyl acetate gradient) to yield the title compound (164 mg, 71% purity by LC, 0.170 mmol, 32%) as yellow foam. A byproduct (29% by LC) with M+2 could not be separated. LC/MS method 1: Rt 1.47 min, calcd for $C_{38}H_{49}FN_5O_4Si$ $[M+H]^+$ m/z 686.4, found 686.4.

4-Cyclopropyl-N-(5-fluoro-2-methyl-3-(5-(pyrrolidin-3-yl)-7-((2-(trimethylsilyl)ethoxy)methyl)-7H-pyrrolo[2,3-d]pyrimidin-4-yl)phenyl)benzamide (S24)



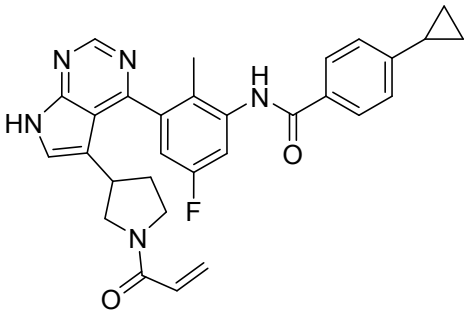
To a solution of **S23** (164 mg, 71% purity by LC, 0.170 mmol) in dichloromethane at 0°C was added HCl in dioxane (4N, 0.29 mL, 1.17 mmol). The mixture was stirred at 0°C for 2.5 h, then diluted with dichloromethane, and washed with saturated aqueous sodium bicarbonate solution and brine. The aqueous layer was extracted with dichloromethane and the combined organic layers were dried over sodium sulfate, filtered and concentrated to yield the crude title compound (137 mg, 68% purity by LC, 159 mmol, 93%) which was taken directly to the next step. The now deprotected byproduct from the previous step (22% by LC) with M+2 could not be separated. LC/MS method 1: Rt 1.03 min, calcd for $C_{33}H_{41}FN_5O_2Si$ $[M+H]^+$ m/z 586.3, found 586.3.

N-(3-(5-(1-Acryloylpyrrolidin-3-yl)-7-((2-(trimethylsilyl)ethoxy)methyl)-7H-pyrrolo[2,3-d]pyrimidin-4-yl)-5-fluoro-2-methylphenyl)-4-cyclopropylbenzamide (S24)



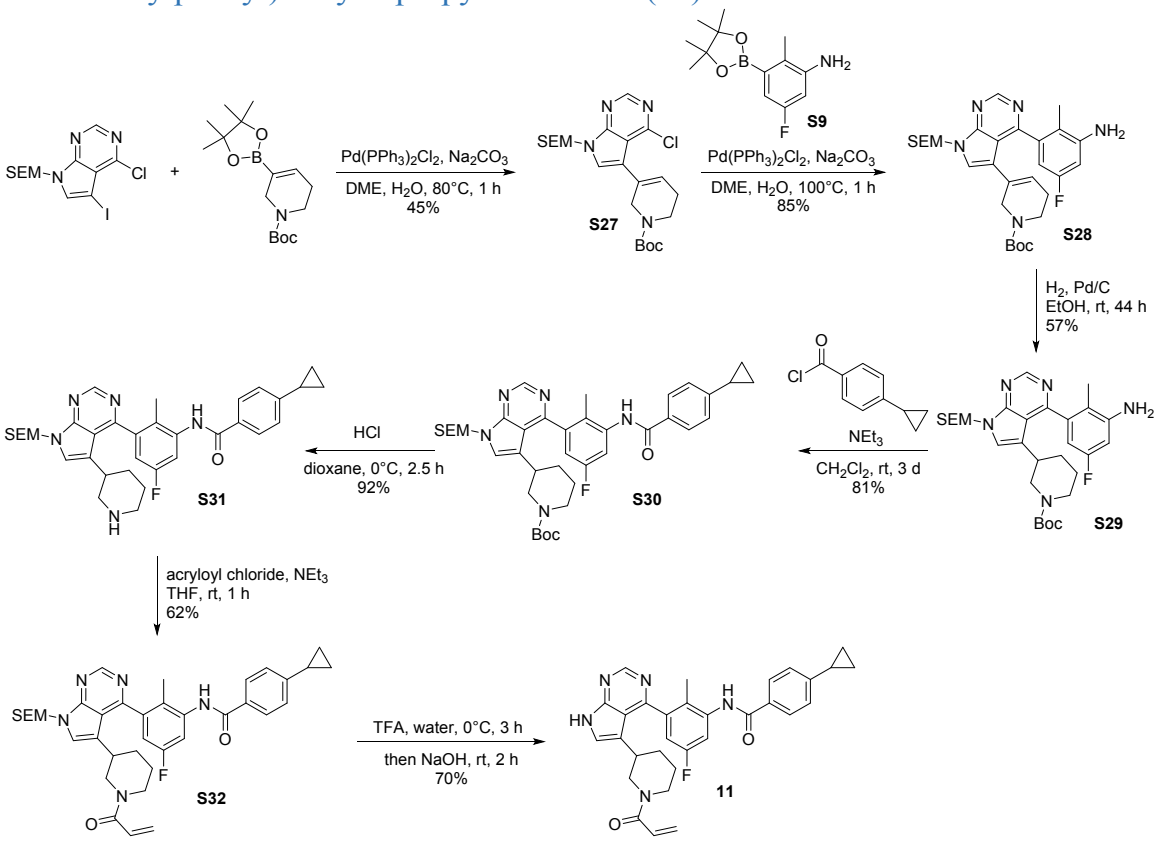
To a solution of **S24** (136 mg, 68% purity, 0.157 mmol) and triethylamine (0.065 mL, 0.464 mmol) in THF (1.2 mL) was added at 0°C acryloyl chloride (20.6 µl, 0.255 mmol). The mixture was stirred at room temperature for 1h. The reaction mixture was diluted with dichloromethane, washed with saturated aqueous sodium bicarbonate solution and brine, and the aqueous layers were extrated with dichloromethane. The combined organic layers were dried with sodium sulfate, filtered and concentrated. The crude product was purified by flash chromatography (silica gel, heptane/ethyl acetate gradient) to yield the title compound (97 mg, 70% purity by LC, 0.106 mmol, 67%) as a white solid. The acrylated byproduct from the previous steps (28% by LC) with M+2 could not be separated. ^{1}H NMR (400 MHz, DMSO-d6) δ 10.08 (s, 1H), 8.92 (s, 1H), 7.91-7.86 (m, 2H), 7.74 (d, *J* = 6.9 Hz, 1H), 7.50-7.34 (m, 2H), 7.25-7.21 (m, 2H), 6.59-6.33 (m, 1H), 6.16-6.02 (m, 1H), 5.70-5.60 (m, 3H), 3.57 (m, 2H), 3.53-2.98 (m, 5H), 2.05-1.99 (m, 1H), 1.92-1.58 (m, 5H), 1.09-1.00 (m, 2H), 0.87-0.72 (m, 4H), -0.11 (s, 9H); LC/MS method 1: Rt 1.27 min, calcd for $C_{36}H_{43}FN_5O_3Si$ $[M+H]^+$ m/z 640.3, found 640.3.

N-(3-(5-(1-Acryloylpyrrolidin-3-yl)-7H-pyrrolo[2,3-d]pyrimidin-4-yl)-5-fluoro-2-methylphenyl)-4-cyclopropylbenzamide (10)

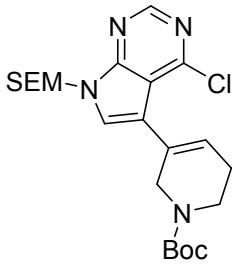


A mixture of **S25** (97 mg, 70% purity by LC, 0.106 mmol) and trifluoroacetic acid (95% in water, 0.68 mL, 8.81 mmol) was stirred at room temperature for 2 h. Then 10 N NaOH (1.76 mL, 17.6 mmol) were added at 0°C and stirring at this temperature was continued for 45 min. The reaction mixture was diluted with ethyl acetate and washed with saturated aqueous sodium bicarbonate solution and brine. The organic layer was dried with sodium sulfate, filtered and concentrated. The crude product was purified by SFC to yield the title compound (22 mg, 0.043 mmol, 41%) as a white solid. ^{1}H NMR (400 MHz, DMSO-d6): δ ppm (rotamers) 12.24 (m, 1H), 10.09, 10.04 (2 s, 1H), 8.85-8.84 (m, 1H), 7.91 (m, 2H), 7.57-7.40 (m, 2 H), 7.27-7.17 (m, 3H), 6.60-6.38 (m, 1H), 6.11 (m, 1H), 5.68-5.56 (m, 1 H), 3.71-2.99 (m, 5H), 2.05 (m, 1H), 1.93-1.68 (m, 5H), 1.10-1.05 (m, 2H), 0.82-0.78 (m, 2H); LC/MS method 1: Rt 0.90 min, calcd for $C_{30}H_{29}FN_5O_2$ $[M+H]^+$ m/z 510.2, found 510.3; HRMS (ESI+) calcd for $C_{30}H_{29}FN_5O_2$ $[M+H]^+$ 510.22998, found 510.23013.

Synthesis of N-(3-(5-(1-Acryloylpiperidin-3-yl)-7H-pyrrolo[2,3-d]pyrimidin-4-yl)-5-fluoro-2-methylphenyl)-4-cyclopropylbenzamide (11)

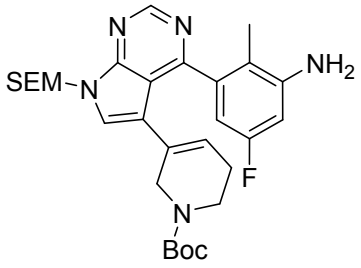


***tert*-Butyl 3-(4-chloro-7-((2-(trimethylsilyl)ethoxy)methyl)-7H-pyrrolo[2,3-d]pyrimidin-5-yl)-5,6-dihydropyridine-1(2H)-carboxylate (S27)**



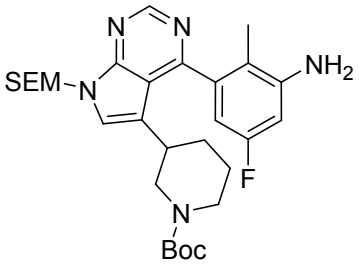
A mixture of 4-chloro-5-iodo-7-((2-(trimethylsilyl)ethoxy)methyl)-7H-pyrrolo[2,3-d]pyrimidine[19] (800 mg, 1.95 mmol), *tert*-butyl 3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-5,6-dihydropyridine-1(2H)-carboxylate (2.01 g, 1.95 mmol) and 2M aqueous sodium carbonate solution (1.04 g, 9.76 mmol) in DME (9.7 mL) was flushed with argon. Then $Pd(PPh_3)_2Cl_2$ (68.5 mg, 0.098 mmol) was added and the mixture was heated at 80 °C for 1 h. The reaction mixture was cooled to room temperature and concentrated. The crude residue was purified by flash chromatography (silica gel, heptane/ethyl acetate gradient) to yield the title compound (408 mg, 0.877 mmol, 45%) as a white foam. ^{1}H NMR (400 MHz, DMSO-d6): δ ppm 8.69 (s, 1H), 7.85 (s_{br}, 1H), 5.97 (s_{br}, 1H), 5.64 (s, 2H), 4.15 (s_{br}, 2H), 3.58-3.48 (m, 4H), 2.27 (m, 2H), 1.43 (s, 9H), 0.83 (t, J = 7.9 Hz, 2H), -0.09 (s, 9H); LC/MS method 1: Rt 1.49 min, calcd for $C_{22}H_{34}ClN_4O_3Si$ $[M+H]^+$ m/z 465.2, found 465.2.

***tert*-Butyl 3-(4-(3-amino-5-fluoro-2-methylphenyl)-7-((2-(trimethylsilyl)ethoxy)methyl)-7H-pyrrolo[2,3-d]pyrimidin-5-yl)-5,6-dihydropyridine-1(2H)-carboxylate (S28)**



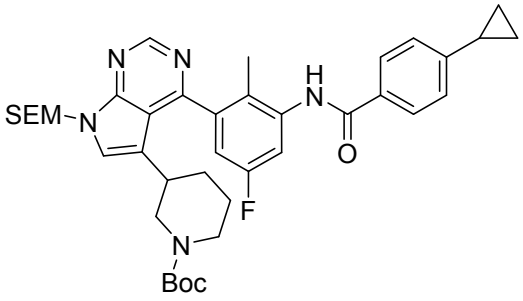
A mixture of **S27** (295 mg, 0.634 mmol), **S9** (175 mg, 0.698 mmol) and 2M aqueous sodium carbonate solution (1.59 mL, 3.17 mmol) in DME (3.2 mL) was flushed with argon. Then $Pd(PPh_3)_2Cl_2$ (22.2 mg, 0.032 mmol) was added and the mixture was heated at 100°C for 1 h. The reaction mixture was cooled to room temperature and concentrated. The crude residue was purified by flash chromatography (silica gel, heptane/ethyl acetate gradient) to yield the title compound (299 mg, 0.540 mmol, 85%) as a yellow foam. ^{1}H NMR (400 MHz, DMSO-d6): δ ppm 8.90 (s, 1H), 7.79 (s, 1H), 6.50 (dd, J = 11.4, 2.7 Hz, 1H), 6.24 (dd, J = 9.2, 2.7 Hz, 1H), 5.67 (s, 2H), 5.33 (s, 2H), 3.69 (s_{br}, 2H), 3.58 (m, 3H), 3.22 (s_{br}, 1H), 1.84 (s_{br}, 2H), 1.66 (s, 3H), 1.40 (s, 9H), 1.08 (s, 1H), 0.82 (m, 2H), -0.10 (s, 9H); LC/MS method 1: Rt 1.38 min, calcd for $C_{29}H_{41}FN_5O_3Si$ $[M+H]^+$ m/z 554.3, found 554.3.

***tert*-Butyl 3-(4-(3-amino-5-fluoro-2-methylphenyl)-7-((2-(trimethylsilyl)ethoxy)methyl)-7H-pyrrolo[2,3-d]pyrimidin-5-yl)piperidine-1-carboxylate (S29)**



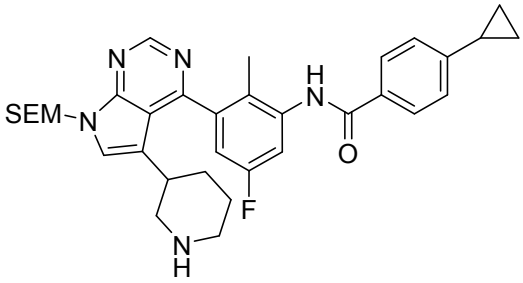
To a solution of **S28** (295 mg, 0.533 mmol) in ethanol (15 mL) was added Pd/C (10%, 45 mg) and the resulting mixture was treated with hydrogen for 44 h. The mixture was filtered and concentrated. The crude product (269 mg, 63% purity my LC, 0.305 mmol, 57%) was used without purification in the next step. LC/MS method 1: Rt 1.37 min, calcd for $C_{29}H_{43}FN_5O_3Si$ $[M+H]^+$ m/z 556.3, found 556.3.

***tert*-Butyl 3-(4-(3-(4-cyclopropylbenzamido)-5-fluoro-2-methylphenyl)-7-((2-(trimethylsilyl)ethoxy)methyl)-7H-pyrrolo[2,3-d]pyrimidin-5-yl)piperidine-1-carboxylate (S30)**



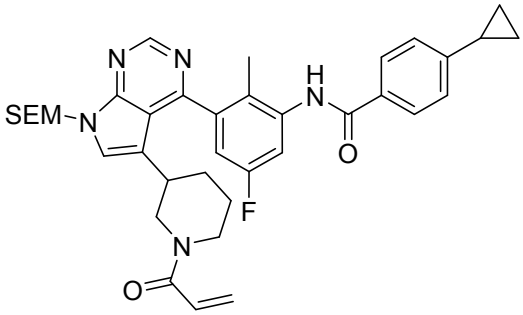
A solution of 4-cyclopropylbenzoic acid (157 mg, 0.969 mmol), thionylchloride (1.41 mL, 19.4 mmol) and DMF (3.8 µL, 0.048 mmol) in toluene (0.88 mL) was stirred under an argon atmosphere for 2 h at 80°C. The mixture was concentrated *in vacuo* and the residue was dissolved in dichloromethane (0.6 mL). The resulting solution was added to a mixture of **S29** (269 mg, 63% purity my LC, 0.305 mmol) and triethylamine (0.20 mL, 1.45 mmol) in dichloromethane (3.6 mL) at 0°C. The mixture was stirred at room temperature for 3 d. The reaction mixture was diluted with ethyl acetate and washed twice with saturated aqueous sodium bicarbonate solution and brine. The aqueous layers were backextracted with ethyl acetate and the combined organic layers were dried over sodium sulfate, filtered and evaporated to dryness. The crude residue was purified by flash chromatography (silica gel, heptane/ethyl acetate gradient) to yield the title compound (184 mg, 0.247 mmol, 81%) as a yellow oil. ^{1}H NMR (400 MHz, DMSO-d6): δ ppm (rotamers) 10.03 (s_{br}, 1H), 8.90 (s, 1H), 7.88 (m, 2H), 7.65 (s, 1H), 7.40 (m, 1H), 7.23 (d, J = 8.1 Hz, 2H), 7.16-7.03 (m, 1H), 5.70-5.59 (m, 2H), 3.83-3.67 (m, 2H), 3.57 (m, 2H), 2.80-2.60 (m, 2H), 2.29-2.13 (m, 1H), 2.02 (m, 1H), 1.93, 1.84 (2 s, 3H), 1.73-1.29 (m, 4H), 1.35, 1.25 (2 s, 9H), 1.07-1.02 (m, 2H), 0.89-0.75 (m, 4H), -0.12 (s, 9H); LC/MS method 1: Rt 1.55 min, calcd for $C_{39}H_{51}FN_5O_4Si$ $[M+H]^+$ m/z 700.4, found 700.3.

4-Cyclopropyl-N-(5-fluoro-2-methyl-3-(5-(piperidin-3-yl)-7-((2-(trimethylsilyl)ethoxy)methyl)-7H-pyrrolo[2,3-d]pyrimidin-4-yl)phenyl)benzamide (S31)



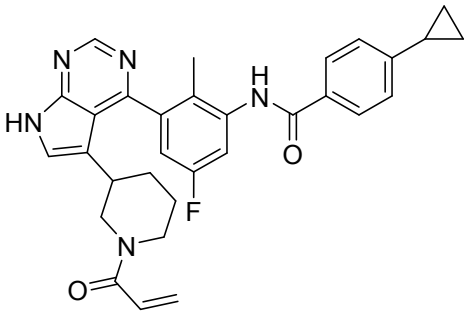
To **S30** (182 mg, 0.260 mmol) 4N HCl in dioxane (650 µL, 2.60 mmol) was added slowly at 0°C. The reaction mixture was stirred for 3.5 h at 0°C. The reaction mixture was quenched with saturated aqueous sodium bicarbonate solution at 0°C, diluted with dichloromethane and extracted. The aqueous layer was extracted with dichloromethane and the combined organic layers were washed with brine, dried over sodium sulfate, filtered and evaporated to dryness. The crude product (160 mg, 0.240 mmol, 92%) was used without purification in the next step. ^{1}H NMR (400 MHz, DMSO-d6): δ ppm (rotamers) 10.14, 10.08 (2 s, 1H), 8.88 (s, 1H), 7.95-7.85 (m, 2H), 7.60, 7.58 (2 s, 1H), 7.49-7.38 (m, 1H), 7.30-7.06 (m, 3H), 5.69-5.55 (m, 2H), 3.59-3.52 (m, 4H), 2.84 (m, 2H), 2.47-2.20 (m, 2H), 2.03 (m, 1H), 1.87, 1.86 (2 s, 3H), 1.64 (m, 1H), 1.48 (m, 1H), 1.30-1.15 (m, 2H), 1.09-0.99 (m, 2H), 0.86-0.71 (m, 4H), -0.10 (s, 9H); LC/MS method 1: Rt 1.05 min, calcd for $C_{34}H_{43}FN_5O_2Si$ $[M+H]^+$ m/z 600.3, found 600.3.

N-(3-(5-(1-Acryloylpiperidin-3-yl)-7-((2-(trimethylsilyl)ethoxy)methyl)-7H-pyrrolo[2,3-d]pyrimidin-4-yl)-5-fluoro-2-methylphenyl)-4-cyclopropylbenzamide (S32)



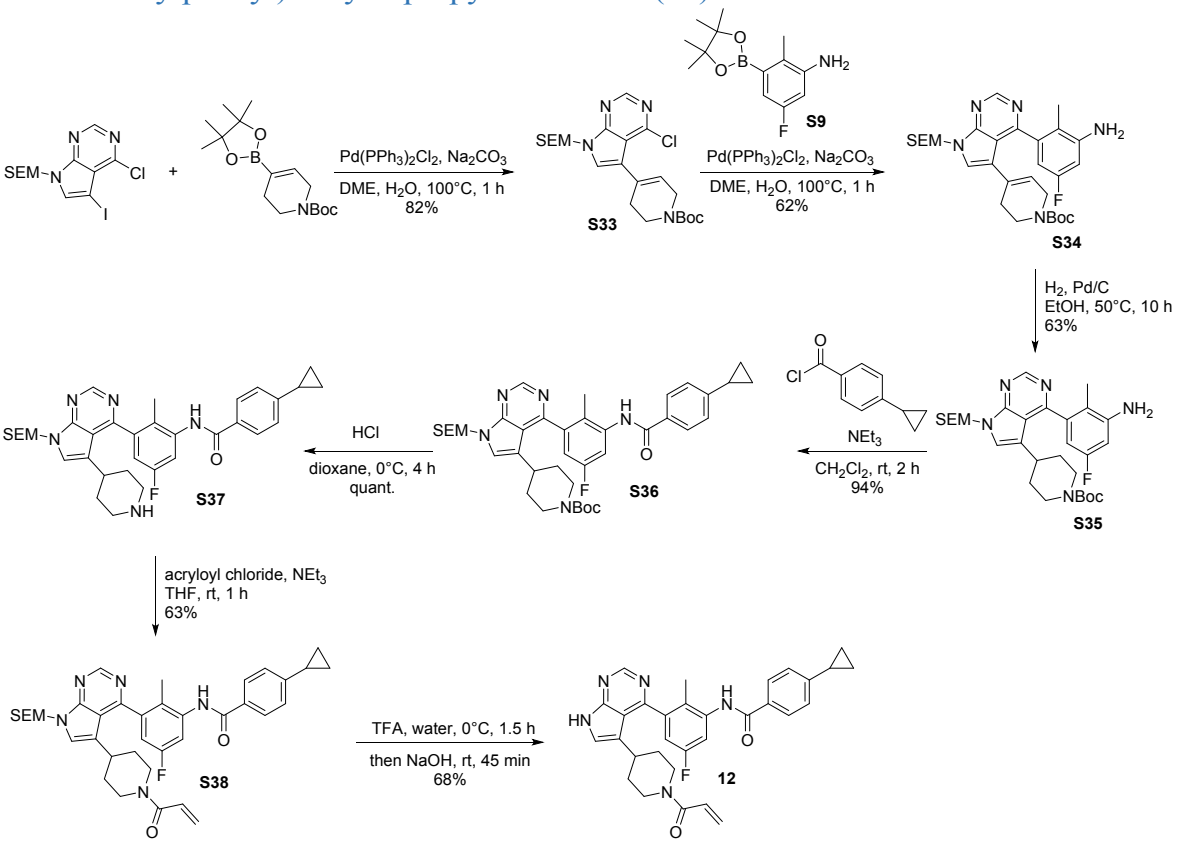
To a solution of **S31** (80 mg, 0.120 mmol) and triethylamine (0.033 mL, 0.240 mmol) in THF (0.6 mL) was added at 0°C acryloyl chloride (10.7 µl, 0.132 mmol). The mixture was stirred at room temperature for 1 h. The reaction mixture was diluted with ethyl acetate, washed with saturated aqueous sodium bicarbonate solution and brine, and the aqueous layers were extrated with ethyl acetate. The combined organic layers were dried with sodium sulfate, filtered and concentrated. The crude product was purified by flash chromatography (silica gel, heptane/ethyl acetate gradient) to yield the title compound (49 mg, 0.075 mmol, 62%) as a pale yellow oil. LC/MS method 1: Rt 1.38 min, calcd for $C_{37}H_{45}FN_5O_3Si$ $[M+H]^+$ m/z 654.3, found 654.3.

N-(3-(5-(1-Acryloylpiperidin-3-yl)-7H-pyrrolo[2,3-d]pyrimidin-4-yl)-5-fluoro-2-methylphenyl)-4-cyclopropylbenzamide (11)

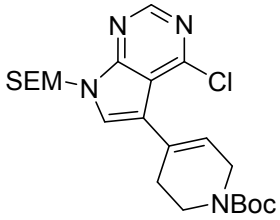


Trifluoroacetic acid (95% in water, 346 µl, 4.50 mmol) was added dropwise to **S32** (49 mg, 0.075 mmol) at 0°C, and the reaction mixture was stirred for 3 h at this temperature. The solvents were evaporated and the remaining residue was dissolved in THF (450 µl), then NaOH (10N, 450 µl, 4.50 mmol) was slowly added at 0°C. The yellow solution was stirred for 2 h at room temperature. The reaction mixture was evaporated to dryness, diluted with ethyl acetate and washed once with 2M aqueous sodium carbonate solution and brine. The aqueous layers were backextracted with ethyl acetate, and the combined organic layers were dried over sodium sulfate, filtered and evaporated to dryness. The crude product was purified by SFC to yield the title compound (27 mg, 0.052 mmol, 69%) as yellow crystals. ^{1}H NMR (400 MHz, DMSO-d6): δ ppm (rotamers) 12.23 (m, 1H), 10.07, 10.00 (2 s, 1H), 8.83 (s, 1H), 7.97-7.89 (m, 2H), 7.56-7.07 (m, 5H), 6.78-6.41 (m, 1H), 6.10-5.86 (m, 1H), 5.63-5.45 (m, 1H), 4.38-4.22 (m, 1H), 3.93-3.78 (m, 1H), 3.21-2.58 (m, 2H), 2.08-1.20 (m, 9H), 1.09-1.02 (m 2H), 0.83-0.77 (m, 2H); LC/MS method 1: Rt 0.98 min, calcd for $C_{31}H_{31}FN_5O_2$ $[M+H]^+$ m/z 524.2, found 524.2; HRMS (ESI+) calcd for $C_{31}H_{31}FN_5O_2$ $[M+H]^+$ 524.24563, found 524.24579.

Synthesis of N-(3-(5-(1-Acryloylpiperidin-4-yl)-7H-pyrrolo[2,3-d]pyrimidin-4-yl)-5-fluoro-2-methylphenyl)-4-cyclopropylbenzamide (12)

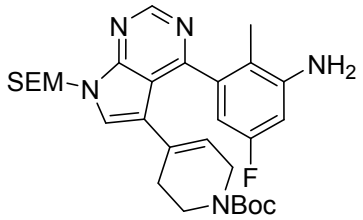


***tert*-Butyl 4-(4-chloro-7-((2-(trimethylsilyl)ethoxy)methyl)-7H-pyrrolo[2,3-d]pyrimidin-5-yl)-5,6-dihydropyridine-1(2H)-carboxylate (S33)**



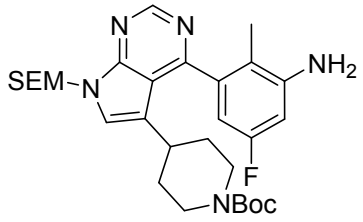
A mixture of 4-chloro-5-iodo-7-((2-(trimethylsilyl)ethoxy)methyl)-7H-pyrrolo[2,3-d]pyrimidine[19] (3.75 g, 9.15 mmol), *tert*-butyl 4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-5,6-dihydropyridine-1(2H)-carboxylate (2.83 g, 9.15 mmol) and 2M aqueous sodium carbonate solution (22.9 mL, 45.8 mmol) in DME (46 mL) was flushed with argon. Then $Pd(PPh_3)_2Cl_2$ (321 mg, 0.458 mmol) was added and the mixture was heated at 100°C for 1 h. The reaction mixture was cooled to room temperature and concentrated. The crude residue was purified by flash chromatography (silica gel, heptane/ethyl acetate gradient) to yield the title compound (3.47 g, 7.46 mmol, 82%) as a yellow oil. ^{1}H NMR (400 MHz, DMSO-d6): δ ppm 8.68 (s, 1H), 7.80 (s, 1H), 5.86 (s_{br}, 1H), 5.63 (s, 2H), 4.06-3.96 (m, 2H), 3.62-3.48 (m, 4H), 2.47 (d, *J* = 3.9 Hz, 2H), 1.45 (s, 9H), 0.88-0.81 (m, 2H), -0.08 (s, 9H); LC/MS method 1: Rt 1.48 min, calcd for $C_{22}H_{34}ClN_4O_3Si$ $[M+H]^+$ m/z 465.2, found 465.3.

***tert*-butyl 4-(4-(3-amino-5-fluoro-2-methylphenyl)-7-((2-(trimethylsilyl)ethoxy)methyl)-7H-pyrrolo[2,3-d]pyrimidin-5-yl)-5,6-dihydropyridine-1(2H)-carboxylate (S34)**



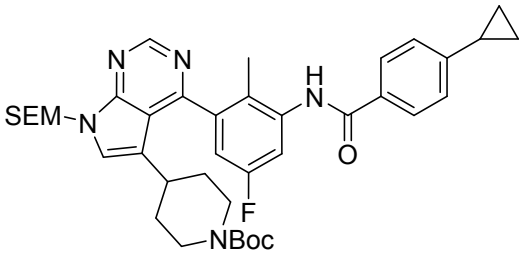
A mixture of **S33** (1.00 g, 2.15 mmol), **S9** (0.648 g, 2.58 mmol) and 2M aqueous sodium carbonate solution (5.38 mL, 10.75 mmol) in DME (10.8 mL) was flushed with argon. Then $Pd(PPh_3)_2Cl_2$ (75 mg, 0.108 mmol) was added and the mixture was heated at 100°C for 1 h. The reaction mixture was cooled to room temperature and concentrated. The crude residue was purified by flash chromatography (silica gel, heptane/ethyl acetate gradient) to yield the title compound (744 mg, 1.34 mmol, 62%) as a yellow foam. ^{1}H NMR (400 MHz, DMSO-d6): δ ppm 8.90 (s, 1H), 7.71 (s, 1H), 6.55 (dd, *J* = 2.7, 11.4 Hz, 1H), 6.24 (dd, *J* = 2.7, 9.1 Hz, 1H), 5.67 (s, 2H), 5.36 (s, 2H), 5.14 (s, 1H), 3.66 (m, 2H), 3.60 (t, *J* = 7.9 Hz, 2H), 3.20 (m, 2H), 2.01 (m, 2H), 1.71 (s, 3H), 1.44 (s, 9H), 0.85 (t, *J* = 7.9 Hz, 2H), -0.07 (s, 9H); LC/MS method 1: Rt 1.38 min, calcd for $C_{29}H_{41}FN_5O_3Si$ $[M+H]^+$ m/z 554.3, found 554.4.

***tert*-butyl 4-(4-(3-amino-5-fluoro-2-methylphenyl)-7-((2-(trimethylsilyl)ethoxy)methyl)-7H-pyrrolo[2,3-d]pyrimidin-5-yl)piperidine-1-carboxylate (S35)**



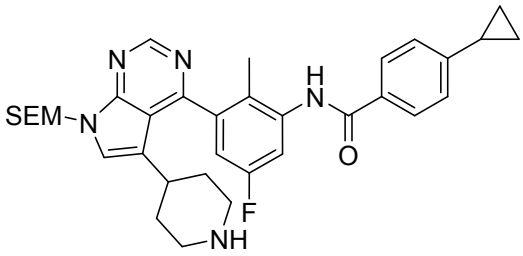
A solution of **S34** (742 mg, 1.34 mmol) in ethanol (27 mL) was hydrogenated using the H-Cube® (Pd/C 10%, 50°C, 70 bar H_2) for 10 h. The mixture was filtered and concentrated. The crude product was purified by flash chromatography (silica gel, heptane/ethyl acetate gradient) to yield the title compound (472 mg, 0.849 mmol, 63%) as a white foam. 1H NMR (400 MHz, DMSO-d6) δ (ppm) 8.85 (s, 1H), 7.55 (s, 1H), 6.59 (dd, *J* = 2.7, 11.4 Hz, 1H), 6.28 (dd, *J* = 2.7, 9.0 Hz, 1H), 5.68-5.55 (m, 2H), 5.41 (s, 2H), 3.91 (m, 2H), 3.56 (t, *J* = 7.8 Hz, 2H), 2.34-2.20 (m, 3H), 1.69 (s, 3H), 1.56-1.19 (m, 4H), 1.38 (s, 9H), 0.81 (dt, *J* = 2.4, 7.7 Hz, 2H), -0.11 (s, 9H); LC/MS method 1: Rt 1.36 min, calcd for $C_{29}H_{43}FN_5O_3Si$ $[M+H]^+$ m/z 556.3, found 556.3.

***tert*-butyl 4-(4-(3-(4-cyclopropylbenzamido)-5-fluoro-2-methylphenyl)-7-((2-(trimethylsilyl)ethoxy)methyl)-7H-pyrrolo[2,3-d]pyrimidin-5-yl)piperidine-1-carboxylate (S36)**



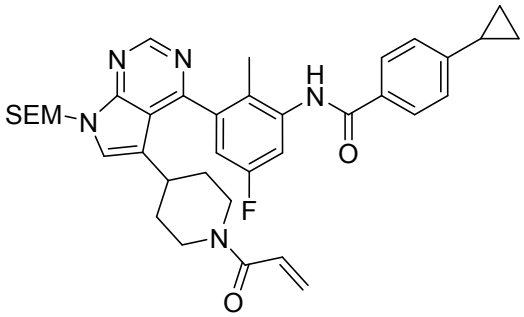
A solution of 4-cyclopropylbenzoic acid (220 mg, 1.36 mmol), thionylchloride (1.97 mL, 27.1 mmol) and one drop of DMF in toluene (1.2 mL) was stirred under an argon atmosphere for 2 h at 80°C. The mixture was concentrated *in vacuo* and the residue was dissolved in dichloromethane (6.7 mL). To this solution, **S35** (375 mg, 0.675 mmol) and triethylamine (0.28 mL, 2.02 mmol) were added. The resulting mixture was stirred at room temperature for 2 h, then it was concentrated *in vacuo*. The crude residue was purified by flash chromatography (silica gel, heptane/ethyl acetate gradient) to yield the title compound (444 mg, 0.634 mmol, 94%) as a white foam. ^{1}H NMR (400 MHz, DMSO-d6): δ ppm 10.11 (s, 1H), 8.90 (s, 1H), 7.96-7.82 (m, 2H), 7.59 (s, 1H), 7.41 (dd, *J* = 2.8, 10.0 Hz, 1H), 7.27-7.21 (m, 2H), 7.18 (dd, *J* = 2.7, 8.8 Hz, 1H), 5.63 (m, 2H), 3.98-3.77 (m, 2H), 3.57 (t, *J* = 7.8 Hz, 2H), 2.34 (m, 2H), 2.10-1.91 (m, 1H), 1.84 (s, 3H), 1.58-1.09 (m, 5H), 1.38 (s, 9H), 1.05 (m, 2H), 0.92-0.71 (m, 4H), -0.11 (s, 9H); LC/MS method 1: Rt 1.51 min, calcd for $C_{39}H_{51}FN_5O_4Si$ $[M+H]^+$ m/z 700.4, found 700.3.

4-Cyclopropyl-N-(5-fluoro-2-methyl-3-(5-(piperidin-4-yl)-7-((2-(trimethylsilyl)ethoxy)methyl)-7H-pyrrolo[2,3-d]pyrimidin-4-yl)phenyl)benzamide (S37)



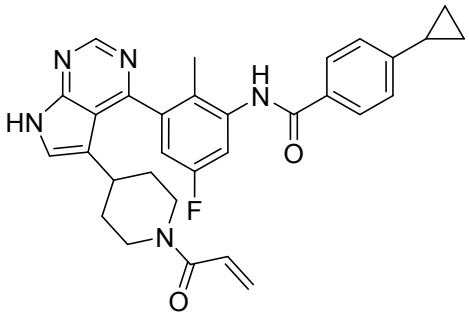
To a solution of **S36** (442 mg, 0.631 mmol) in dichloromethane (0.6 mL) was added 4N HCl in dioxane (790 µL, 3.16 mmol) slowly at 0°C. The reaction mixture was stirred for 4 h at 0°C. The reaction mixture was diluted with dichloromethane and washed with saturated aqueous sodium bicarbonate solution and brine. The aqueous layers were extracted with dichloromethane. The combined organic layers were dried over sodium sulfate, filtered and evaporated to dryness. The crude product (379 mg, 0.632 mmol, quant.) was used without purification in the next step. ^{1}H NMR (400 MHz, DMSO-d6): δ ppm (rotamers) 10.05 (s, 1H), 8.88 (s, 1H), 7.95-7.85 (m, 2H), 7.52 (s, 1H), 7.44 (dd, *J* = 2.9, 10.1 Hz, 1H), 7.27-7.19 (m, 2H), 7.13 (dd, *J* = 2.8, 8.7 Hz, 1H), 5.64 (m, 2H), 3.60-3.54 (m, 2H), 2.82 (m, 2H), 2.34-1.95 (m, 4H), 1.85 (s, 3H), 1.52-1.12 (m, 5H), 1.09-0.99 (m, 2H), 0.89-0.71 (m, 4H), -0.11 (s, 9H).; LC/MS method 1: Rt 1.03 min, calcd for $C_{34}H_{43}FN_5O_2Si$ $[M+H]^+$ m/z 600.3, found 600.4.

N-(3-(5-(1-Acryloylpiperidin-4-yl)-7-((2-(trimethylsilyl)ethoxy)methyl)-7H-pyrrolo[2,3-d]pyrimidin-4-yl)-5-fluoro-2-methylphenyl)-4-cyclopropylbenzamide (S38)



To a solution of **S37** (120 mg, 0.200 mmol) and triethylamine (0.056 mL, 0.400 mmol) in THF (1 mL) was added at 0°C acryloyl chloride (17.8 µl, 0.220 mmol). The mixture was stirred at room temperature for 1 h. The reaction mixture was diluted with dichloromethane, washed with saturated aqueous sodium bicarbonate solution and brine, and the aqueous layers were extrated with dichloromethane. The combined organic layers were dried with sodium sulfate, filtered and concentrated. The crude product was purified by flash chromatography (silica gel, heptane/ethyl acetate gradient) to yield the title compound (83 mg, 0.127 mmol, 63%) as a white solid. ^{1}H NMR (400 MHz, DMSO-d6): δ ppm 10.13 (s, 1H), 8.90 (s, 1H), 7.95-7.80 (m, 2H), 7.59 (s, 1H), 7.40 (dd, *J* = 2.8, 9.9 Hz, 1H), 7.30-7.14 (m, 3H), 6.77 (dd, *J* = 10.6, 16.7 Hz, 1H), 6.05 (d, *J* = 16.8 Hz, 1H), 5.69-5.56 (m, 3H), 4.49-4.29 (m, 1H), 4.09-3.85 (m, 1H), 3.56 (t, *J* = 7.9 Hz, 2H), 3.03-2.12 (m, 2H), 2.02 (m, 1H), 1.85 (s, 3H), 1.70-1.09 (m, 5H), 1.09-1.01 (m, 2H), 0.90-0.72 (m, 4H), -0.12 (s, 9H).; LC/MS method 1: Rt 1.31 min, calcd for $C_{37}H_{45}FN_5O_3Si$ $[M+H]^+$ m/z 654.3, found 654.3.

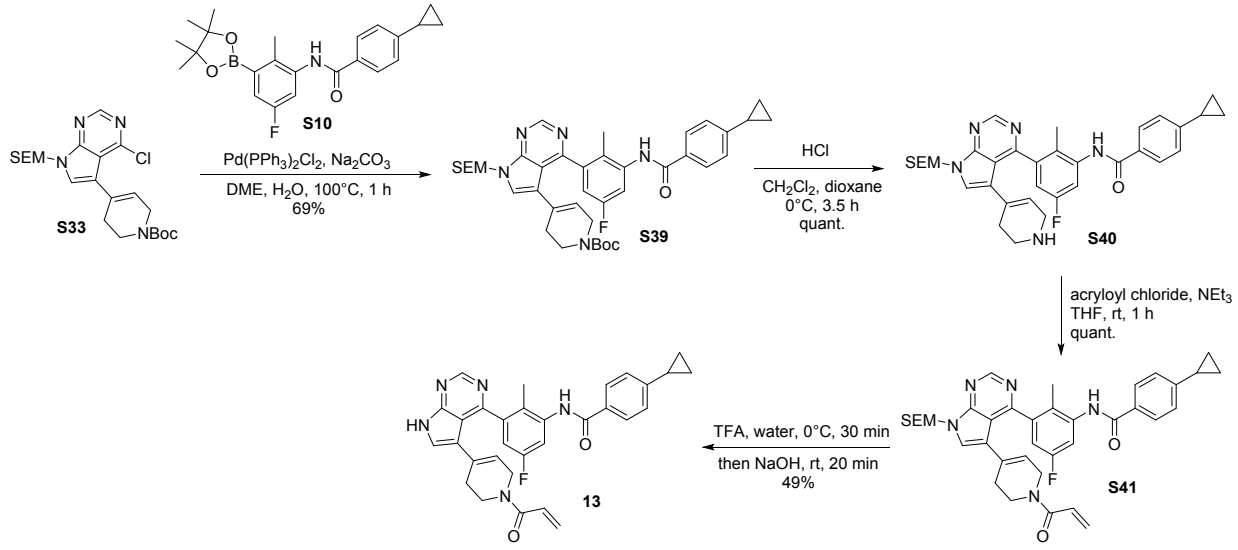
N-(3-(5-(1-Acryloylpiperidin-4-yl)-7H-pyrrolo[2,3-d]pyrimidin-4-yl)-5-fluoro-2-methylphenyl)-4-cyclopropylbenzamide (12)



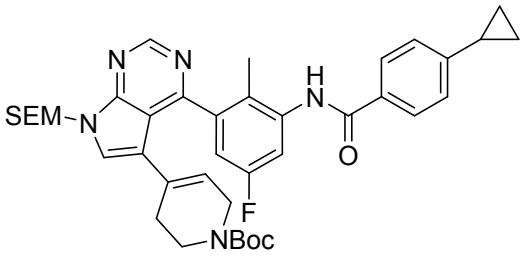
Trifluoroacetic acid (95% in water, 587 µl, 7.62 mmol) was added dropwise to **S38** (83 mg, 0.127 mmol) at 0°C, and the reaction mixture was stirred for 1.5 h at this temperature. Then NaOH (10N, 1.52 mL, 15.2 mmol) was slowly added at 0°C and resulting yellow suspension was stirred for 45 min at room temperature. The reaction mixture was diluted with ethyl acetate and washed with saturated aqueous sodium bicarbonate solution and brine. The aqueous layers were backextracted with ethyl acetate, and the combined organic layers were dried over sodium sulfate, filtered and evaporated to dryness. The crude product was purified by SFC to yield the title compound (45 mg, 0.086 mmol, 68%) as a white solid. ^{1}H NMR (400 MHz, DMSO-d6): δ ppm (rotamers) 11.05 (s_{br}, 1H), 8.80 (s, 1H), 7.93 (m, 2H), 7.43-7.40 (m, 2 H), 7.26 (m, 2H), 7.16 (m, 1H), 6.79 (dd, J = 16.7, 10.5 Hz, 1H), 6.08 (m, 1H), 5.65 (m, 1 H), 4.48-4.33 (m, 1H), 4.05-3.93 (m, 1H), 2.33 (m 1H), 2.96-2.62 (m, 1H), 2.45 (m, 1H), 2.05 (m, 1H), 1.89 (s, 3H), 1.64-1.42 (m, 3H), 1.24-1.15 (m, 1H), 1.09-1.05 (m, 2H), 0.82-0..78 (m, 2H), one NH not visible; LC/MS method 1: Rt 0.93 min, calcd

for $C_{31}H_{31}FN_5O_2$ $[M+H]^+$ m/z 524.2, found 524.3; HRMS (ESI+) calcd for $C_{31}H_{31}FN_5O_2$ $[M+H]^+$ 524.24563, found 524.24607.

Synthesis of N-(3-(5-(1-Acryloyl-1,2,3,6-tetrahydropyridin-4-yl)-7H-pyrrolo[2,3-d]pyrimidin-4-yl)-5-fluoro-2-methylphenyl)-4-cyclopropylbenzamide (13)

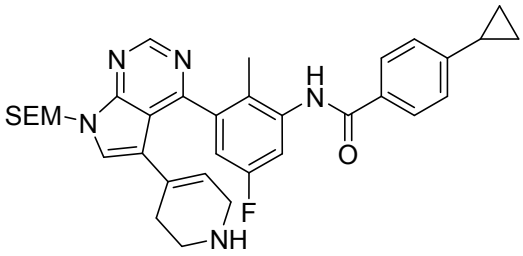


***tert*-Butyl 4-(4-(3-(4-cyclopropylbenzamido)-5-fluoro-2-methylphenyl)-7-((2-(trimethylsilyl)ethoxy)methyl)-7H-pyrrolo[2,3-d]pyrimidin-5-yl)-5,6-dihydropyridine-1(2H)-carboxylate (S39)**



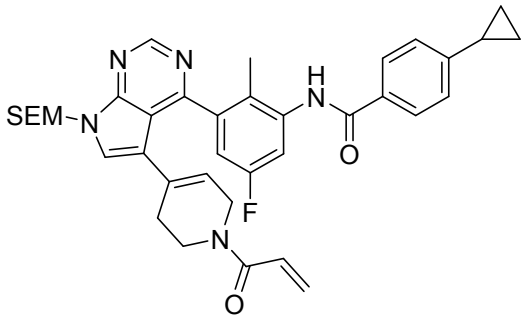
A mixture of **S33** (0.66 g, 1.42 mmol), **S10** (0.673 g, 1.70 mmol) and 2M aqueous sodium carbonate solution (3.55 mL, 7.10 mmol) in DME (7.1 mL) was flushed with argon. Then $Pd(PPh_3)_2Cl_2$ (49.8 mg, 0.071 mmol) was added and the mixture was heated at 100°C for 1 h. The reaction mixture was cooled to room temperature and concentrated. The crude residue was purified by flash chromatography (silica gel, heptane/ethyl acetate gradient) to yield the title compound (682 mg, 0.977 mmol, 69%) as a yellow solid. ^{1}H NMR (400 MHz, DMSO-d6): δ ppm 9.94 (s_{br}, 1H), 8.94 (s, 1H), 7.90 (d, J = 8.0 Hz, 2H), 7.76 (s, 1H), 7.41 (dd, J = 2.8, 10.1 Hz, 1H), 7.24 (d, J = 8.1 Hz, 2H), 7.00 (m, 1H), 5.68 (s, 2H), 5.13 (m, 1H), 3.68-3.55 (m, 4H), 3.26-2.95 (m, 2H), 2.10-1.97 (m, 3H), 1.94 (s, 3H), 1.40 (s, 9H), 1.10-1.00 (m, 2H), 0.85 (t_{br}, J = 7.9 Hz, 2H), 0.81-0.74 (m, 2H), -0.08 (s, 9H); LC/MS method 1: Rt 1.54 min, calcd for $C_{39}H_{49}FN_5O_4Si$ $[M+H]^+$ m/z 698.4, found 698.5.

4-Cyclopropyl-N-(5-fluoro-2-methyl-3-(5-(1,2,3,6-tetrahydropyridin-4-yl)-7-((2-(trimethylsilyl)ethoxy)methyl)-7H-pyrrolo[2,3-d]pyrimidin-4-yl)phenyl)benzamide (S40)



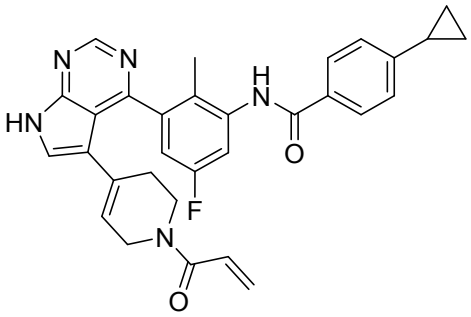
To a solution of **S39** (682 mg, 0.977 mmol) in dichloromethane (1 mL) was added 4N HCl in dioxane (1.2 mL, 4.89 mmol) slowly at 0°C. The reaction mixture was stirred for 3.5 h at 0°C. The reaction mixture was diluted with dichloromethane and washed with saturated aqueous sodium bicarbonate solution and brine. The aqueous layers were extracted with dichloromethane. The combined organic layers were dried over sodium sulfate, filtered and evaporated to dryness. The crude product (636 mg, 0.977 mmol, quant.) was used without purification in the next step. ^{1}H NMR (400 MHz, DMSO-d6): δ ppm 9.98 (s_{br}, 1H), 8.93 (s, 1H), 7.90 (d, *J* = 8.4 Hz, 3H), 7.73 (s, 1H), 7.41 (dd, *J* = 2.8, 10.0 Hz, 1H), 7.28-7.21 (m, 2H), 7.05 (dd, *J* = 2.8, 8.8 Hz, 1H), 5.68 (s, 2H), 5.16 (s, 1H), 3.66-3.54 (m, 1H), 3.09-2.95 (m, 2H), 2.71-2.62 (m, 2H), 2.08-1.94 (m, 4H), 1.89 (s, 3H), 1.09-1.00 (m, 2H), 0.89-0.70 (m, 4H), -0.09 (s, 9H); LC/MS method 1: Rt 1.12 min, calcd for $C_{34}H_{41}FN_5O_2Si$ $[M+H]^+$ m/z 598.3, found 598.3.

N-(3-(5-(1-Acryloyl-1,2,3,6-tetrahydropyridin-4-yl)-7-((2-(trimethylsilyl)ethoxy)methyl)-7H-pyrrolo[2,3-d]pyrimidin-4-yl)-5-fluoro-2-methylphenyl)-4-cyclopropylbenzamide (S41)



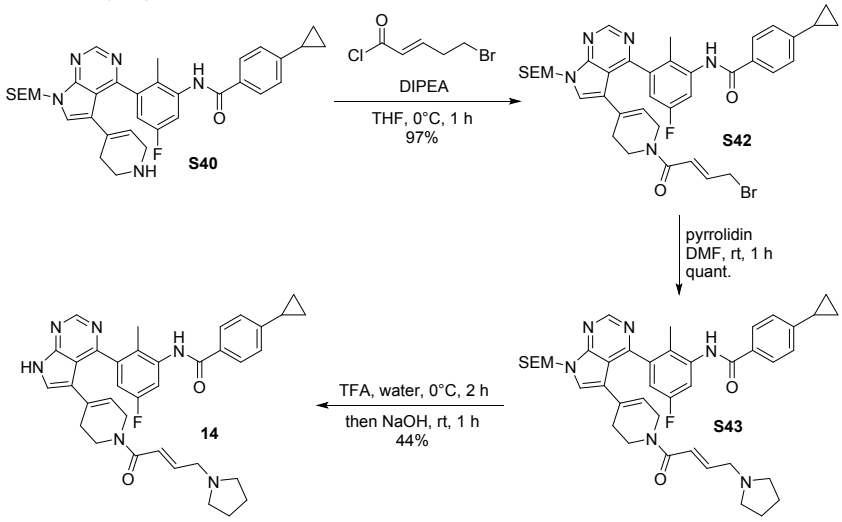
To a solution of **S40** (216 mg, 0.325 mmol) and triethylamine (0.090 mL, 0.650 mmol) in THF (1.6 mL) was added at 0°C acryloyl chloride (28.9 µl, 0.358 mmol). The mixture was stirred at room temperature for 1 h. The reaction mixture was diluted with dichloromethane, washed with saturated aqueous sodium bicarbonate solution and brine, and the aqueous layers were extrated with dichloromethane. The combined organic layers were dried with sodium sulfate, filtered and concentrated. The crude product (212 mg, 0.325 mmol, quant.) was used in the next step without purification. LC/MS method 1: Rt 1.35 min, calcd for $C_{37}H_{43}FN_5O_3Si$ $[M+H]^+$ m/z 652.3, found 652.3.

N-(3-(5-(1-Acryloyl-1,2,3,6-tetrahydropyridin-4-yl)-7H-pyrrolo[2,3-d]pyrimidin-4-yl)-5-fluoro-2-methylphenyl)-4-cyclopropylbenzamide (13)

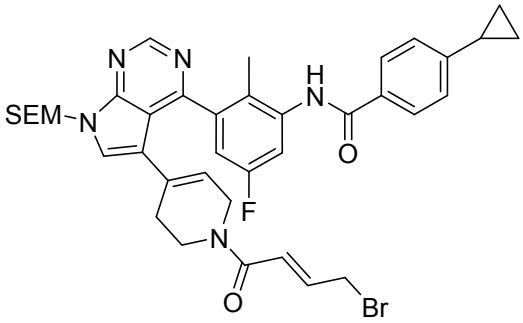


Trifluoroacetic acid (95% in water, 1.5 mL, 19.5 mmol) was added dropwise to **S41** (212 mg, 0.325 mmol) at 0°C, and the reaction mixture was stirred for 30 min at this temperature. Then NaOH (10N, 3.9 mL, 39.0 mmol) was slowly added at 0°C and the resulting yellow suspension was stirred for 20 min at room temperature. The reaction mixture was diluted with ethyl acetate and washed with saturated aqueous sodium bicarbonate solution and brine. The aqueous layers were backextracted with ethyl acetate, and the combined organic layers were dried over sodium sulfate, filtered and evaporated to dryness. The crude product was purified by SFC to yield the title compound (83 mg, 0.159 mmol, 49%) as a white foam. ^{1}H NMR (400 MHz, DMSO-d6): δ ppm (rotamers) 12.33 (s_{br}, 1H), 9.94, 9.86 (2 s, 1H), 8.85 (s, 1H), 7.90 (m, 2H), 7.59 (d, J = 10.0 Hz, 1 H), 7.35 (d, J = 9.9 Hz, 1H), 7.23 (d, J = 7.9 Hz, 2H), 7.01 (m, 1H), 6.74 (m, 1H), 6.10 (dd, J = 16.7, 2.4 Hz, 1H), 5.67 (m, 1 H), 5.15-5.04 (m, 1H), 3.88-3.41 (m, 4H), 2.15-1.99 (m, 3H), 1.95-1.89 (m, 3H), 1.07-1.02 (m, 2H), 0.79-0.75 (m, 2H); ^{13}C NMR (151 MHz, DMSO-d6) δ ppm (rotamers) 165.50, 165.40, 164.42, 164.01, 160.49, 160.38, 158.08, 157.98, 157.62, 157.53, 152.04, 152.00, 150.66, 148.29, 140.70, 140.61, 138.35, 138.25, 131.08, 131.05, 128.52, 128.39, 127.82, 127.20, 127.12, 125.15, 123.03, 122.38, 116.28, 114.08, 113.85, 113.62, 44.34, 42.00, 41.93, 38.03, 30.49, 29.29, 15.19, 14.29, 10.23; LC/MS method 1: Rt 0.96 min, calcd for $C_{31}H_{29}FN_5O_2$ $[M+H]^+$ m/z 522.2, found 522.4; HRMS (ESI+) calcd for $C_{31}H_{29}FN_5O_2$ $[M+H]^+$ 522.22998, found 522.22998.

Synthesis of (*E*)-4-Cyclopropyl-N-(5-fluoro-2-methyl-3-(5-(1-(4-(pyrrolidin-1-yl)but-2-enoyl)-1,2,3,6-tetrahydropyridin-4-yl)-7H-pyrrolo[2,3-d]pyrimidin-4-yl)phenyl)benzamide (14)

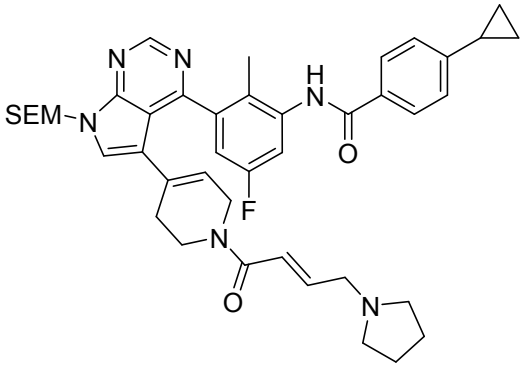


(*E*)-N-(3-(5-(1-(4-bromobut-2-enoyl)-1,2,3,6-tetrahydropyridin-4-yl)-7-((2-(trimethylsilyl)ethoxy)methyl)-7H-pyrrolo[2,3-d]pyrimidin-4-yl)-5-fluoro-2-methylphenyl)-4-cyclopropylbenzamide (S42)



To a solution of **S40** (398 mg, 0.666 mmol) in THF (2.6 mL) was added a solution of (*E*)-4-bromobut-2-enoyl chloride[20] (183 mg, 0.999 mmol) in THF (1.1 mL) at 0°C. Then a solution of N,N-diisopropylethylamine (0.171 mL, 0.999 mmol) was added and the resulting mixture was stirred at 0°C for 1 h. The reaction mixture was diluted with ethyl acetate, washed with water and brine, and the aqueous layers were extrated with ethyl acetate. The combined organic layers were dried with sodium sulfate, filtered and concentrated. The crude product (483 mg, 0.649 mmol, 97%) was used in the next step without purification. ^{1}H NMR (400 MHz, DMSO-d6): δ ppm (rotamers) 10.00, 9.90 (2 s, 1H), 8.95 (s, 1H), 7.91 (d, *J* = 8.3 Hz, 2H), 7.81, 7.77 (2 s, 1H), 7.37 (dd, *J* = 2.9, 9.9 Hz, 1H), 7.25 (d, *J* = 8.1 Hz, 2H), 7.09-6.95 (m, 1H), 6.87-6.60 (m, 2H), 5.68 (s_{br}, 2H), 5.23, 5.06 (2 s_{br}, 1H), 4.43-4.21 (m, 2H), 3.98-3.73 (m, 2H), 3.60 (t, *J* = 7.8 Hz, 2H), 2.20-1.98 (m, 3H), 1.96, 1.89 (2 s, 3H), 1.32-1.15 (m, 2H), 1.08-1.01 (m, 2H), 0.89-0.73 (m, 4H), -0.09 (s, 9H); LC/MS method 1: Rt 1.39 min, calcd for $C_{38}H_{44}BrFN_5O_3Si$ $[M+H]^+$ m/z 744.2, found 744.3.

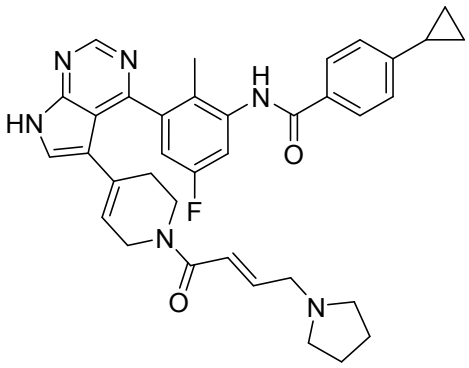
(*E*)-4-Cyclopropyl-N-(5-fluoro-2-methyl-3-(5-(1-(4-(pyrrolidin-1-yl)but-2-enoyl)-1,2,3,6-tetrahydropyridin-4-yl)-7-((2-(trimethylsilyl)ethoxy)methyl)-7H-pyrrolo[2,3-d]pyrimidin-4-yl)phenyl)benzamide (S43)



A solution of **S42** (190 mg, 0.255 mmol) and pyrrolidin (46.4 µL, 0.561 mmol) in DMF (1.3 mL) was stirred at room temperature for 1 h. The mixture was concentrated *in vacuo*. The crude product (188 mg, 0.255 mmol, quant.) was used in the next step without purification. ^{1}H NMR (400 MHz, DMSO-d6): δ ppm (rotamers) 9.98, 9.88 (2 s, 1H), 8.94 (s, 1H), 8.73-8.43 (m, 1H), 7.93-7.83 (m,

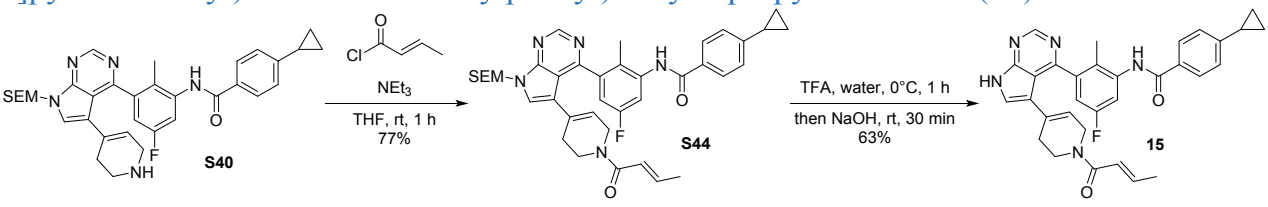
2H), 7.82-7.72 (m, 1H), 7.34 (dd, *J* = 2.8, 9.9 Hz, 1H), 7.23 (d, *J* = 8.4 Hz, 2H), 7.13-6.92 (m, 1H), 6.70-6.46 (m, 1H), 5.67 (s, 2H), 5.21, 5.06 (2 m, 1H), 3.96-3.66 (m, 2H), 3.59 (t, *J* = 7.9 Hz, 2H), 3.49-3.19 (m, 4H), 3.15-3.03 (m, 4H), 2.18-1.63 (m, 10H), 1.07-0.99 (m, 2H), 0.90-0.67 (m, 4H), -0.10 (s, 9H); LC/MS method 1: Rt 1.09 min, calcd for $C_{42}H_{52}FN_6O_3Si$ $[M+H]^+$ m/z 735.4, found 735.5.

(*E*)-4-Cyclopropyl-N-(5-fluoro-2-methyl-3-(5-(1-(4-(pyrrolidin-1-yl)but-2-enoyl)-1,2,3,6-tetrahydropyridin-4-yl)-7H-pyrrolo[2,3-d]pyrimidin-4-yl)phenyl)benzamide (14)

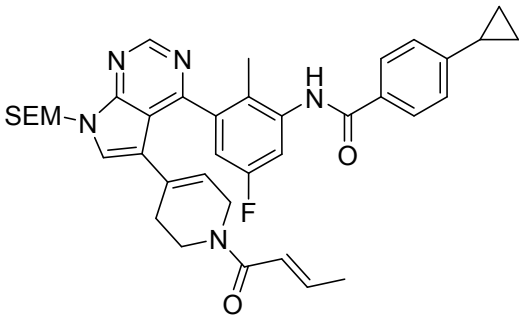


Trifluoroacetic acid (95% in water, 1.18 mL, 15.4 mmol) was added dropwise to **S43** (188 mg, 0.256 mmol) at 0°C, and the reaction mixture was stirred for 2 h at this temperature. Then NaOH (10N, 3.07 mL, 30.7 mmol) was slowly added at 0°C and resulting yellow suspension was stirred for 1 h at room temperature. The reaction mixture was diluted with ethyl acetate and washed with saturated aqueous sodium bicarbonate solution and brine. The aqueous layers were backextracted with ethyl acetate, and the combined organic layers were dried over sodium sulfate, filtered and evaporated to dryness. The crude product was purified by SFC to yield the title compound (69 mg, 0.114 mmol, 44%) as a yellow foam. ^{1}H NMR (400 MHz, DMSO-d6): δ ppm 9.93 (s_{br}, 1H), 8.81 (s, 1H), 7.94 (m, 2H), 7.61 (m, 1 H), 7.35 (dd, *J* = 9.8, 2.8 Hz, 1H), 7.26 (d, *J* = 8.1 Hz, 2H), 7.06-6.96 (m, 1H), 6.70-6.48 (m, 2H), 5.09-4.98 (m, 1H), 3.89-3.31 (m, 8H), 3.20 (d, *J* = 6.1 Hz, 2H), 2.16 (m, 2H), 2.05 (m, 1H), 1.99-1.89 (m, 3H), 1.70 (m, 4H), 1.09-1.05 (m, 2H), 0.81-0.78 (m, 2H), one NH not visible; LC/MS method 1: Rt 0.76 min, calcd for $C_{36}H_{38}FN_6O_2$ $[M+H]^+$ m/z 605.3, found 605.3; HRMS (ESI+) calcd for $C_{36}H_{38}FN_6O_2$ $[M+H]^+$ 605.30348, found 605.30365.

Synthesis of (*E*)-N-(3-(5-(1-But-2-enoyl-1,2,3,6-tetrahydropyridin-4-yl)-7H-pyrrolo[2,3-d]pyrimidin-4-yl)-5-fluoro-2-methylphenyl)-4-cyclopropylbenzamide (15)

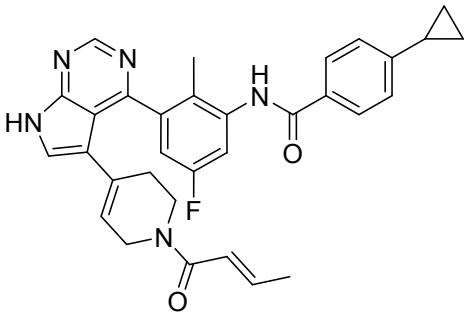


(*E*)-N-(3-(5-(1-but-2-enoyl-1,2,3,6-tetrahydropyridin-4-yl)-7-((2-(trimethylsilyl)ethoxy)methyl)-7H-pyrrolo[2,3-d]pyrimidin-4-yl)-5-fluoro-2-methylphenyl)-4-cyclopropylbenzamide (S44)



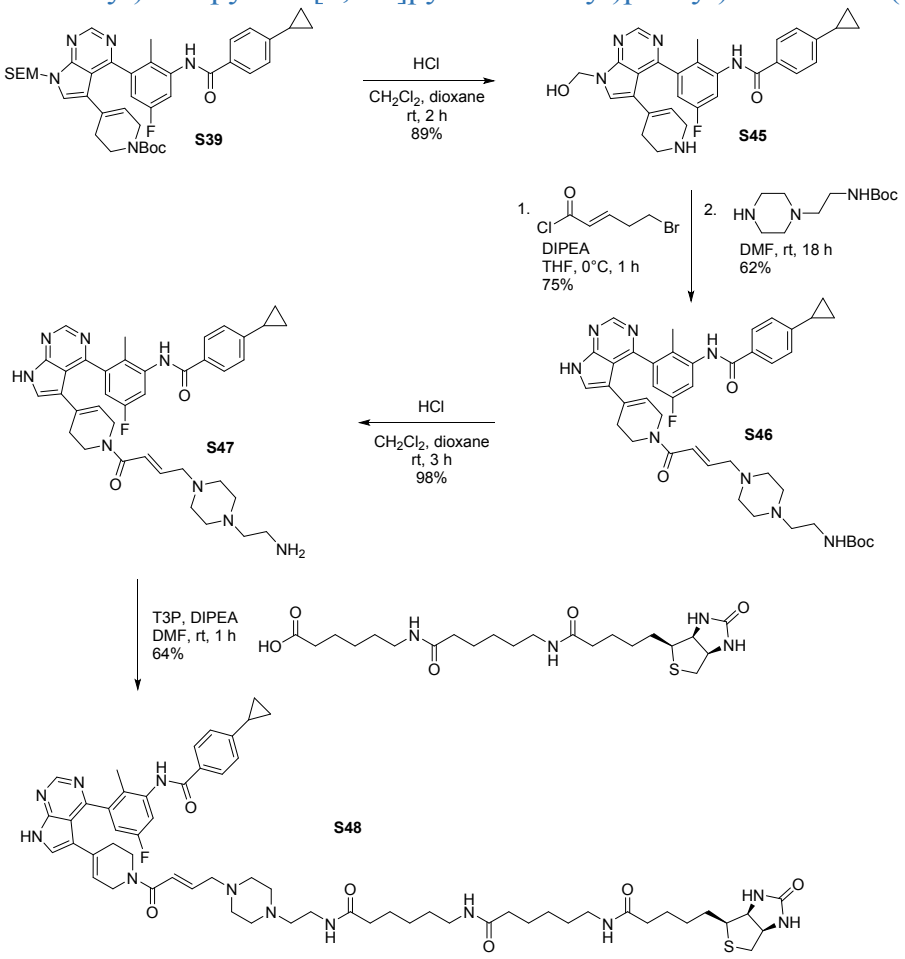
To a solution of **S40** (135 mg, 0.226 mmol) and triethylamine (0.063 mL, 0.452 mmol) in THF (1.1 mL) was added at 0°C (*E*)-but-2-enoyl chloride (26.4 µl, 0.248 mmol). The mixture was stirred at room temperature for 1 h. The reaction mixture was diluted with dichloromethane, washed with saturated aqueous sodium bicarbonate solution and brine, and the aqueous layers were extrated with dichloromethane. The combined organic layers were dried with sodium sulfate, filtered and concentrated. The crude product (140 mg, 83% purity by LC, 0.174 mmol, 77%) was used in the next step without purification. LC/MS method 1: Rt 1.39 min, calcd for $C_{38}H_{45}FN_5O_3Si$ $[M+H]^+$ m/z 666.3, found 666.5.

(*E*)-N-(3-(5-(1-But-2-enoyl-1,2,3,6-tetrahydropyridin-4-yl)-7H-pyrrolo[2,3-d]pyrimidin-4-yl)-5-fluoro-2-methylphenyl)-4-cyclopropylbenzamide (15)

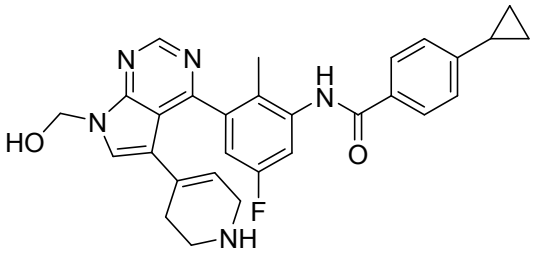


Trifluoroacetic acid (95% in water, 0.97 mL, 12.6 mmol) was added dropwise to **S44** (140 mg, 83% purity by LC, 0.174 mmol) at 0°C, and the reaction mixture was stirred for 2 h at this temperature. Then NaOH (10N, 2.52 mL, 25.2 mmol) was slowly added at 0°C and resulting yellow suspension was stirred for 30 min at room temperature. The reaction mixture was diluted with ethyl acetate and washed with saturated aqueous sodium bicarbonate solution and brine. The aqueous layers were backextracted with ethyl acetate, and the combined organic layers were dried over sodium sulfate, filtered and evaporated to dryness. The crude product was purified by SFC to yield the title compound (59 mg, 0.110 mmol, 63%) as a white solid. ^{1}H NMR (400 MHz, DMSO-d6): δ ppm (rotamers) 12.35 (s_{br}, 1H), 9.97, 9.87 (2 s, 1H), 8.88 (s, 1H), 7.93 (d, *J* = 7.8 Hz, 2H), 7.61 (m, 1 H), 7.37 (d, *J* = 9.8 Hz, 1H), 7.26 (d, *J* = 7.8 Hz, 2H), 7.01 (m, 1H), 6.68 (m, 1H), 6.47 (m, 1H), 5.67 (m, 1 H), 5.16-5.06 (m, 1H), 3.92-3.39 (m, 3H), 2.15-1.91 (m, 6H), 1.86-1.84 (m, 3H), 1.10-1.05 (m, 2H), 0.82-0.78 (m, 2H); LC/MS method 1: Rt 1.00 min, calcd for $C_{32}H_{31}FN_5O_2$ $[M+H]^+$ m/z 536.3, found 536.4; HRMS (ESI+) calcd for $C_{32}H_{31}FN_5O_2$ $[M+H]^+$ 536.24563, found 536.24561.

Synthesis of 4-Cyclopropyl-N-(5-fluoro-2-methyl-3-(5-(1-((*E*)-4-(4-(2-(6-(6-(5-((3aS,4S,6aR)-2-oxohexahydro-1H-thieno[3,4-d]imidazol-4-yl)pentanamido)hexanamido)hexanamido)ethyl)piperazin-1-yl)but-2-enoyl)-1,2,3,6-tetrahydropyridin-4-yl)-7H-pyrrolo[2,3-d]pyrimidin-4-yl)phenyl)benzamide (S49)



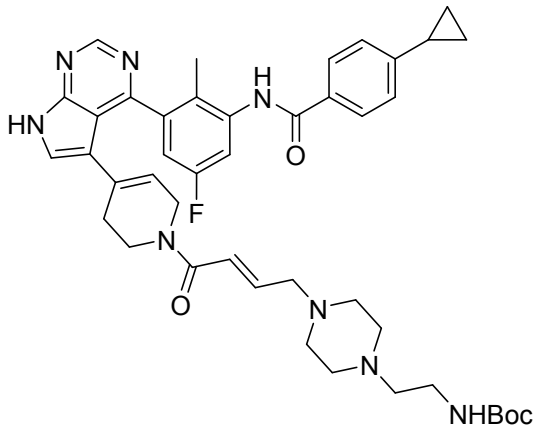
4-Cyclopropyl-N-(5-fluoro-3-(7-(hydroxymethyl)-5-(1,2,3,6-tetrahydropyridin-4-yl)-7H-pyrrolo[2,3-d]pyrimidin-4-yl)-2-methylphenyl)benzamide (S45)



To a solution of **S39** (4.77 g, 6.49 mmol) in dichloromethane (70 mL) was added HCl (4M in dioxan, 16.23 ml, 64.9 mmol). The resulting mixture was stirred for 2 h at room temperature. The mixture was diluted with dichloromethane and it was was carefully adjusted to pH9 by addition of saturated aqueous sodium bicarbonate solution. The precipitate was filtered off, washed with dichloromethane, and dried to yield the title compound (3.20 g, 5.79 mmol, 89%) as beige solid. ^{1}H NMR (400 MHz, DMSO-d6): δ ppm 10.19 (s_{br}, 1H), 8.95 (s, 1H), 8.77 (s_{br}, 1H), 7.96-7.87 (m,

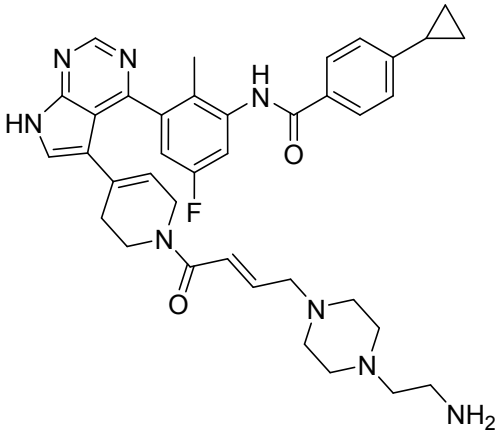
2H), 7.78 (s, 1H), 7.33 (dd, *J* = 2.8, 9.7 Hz, 1H), 7.24 (d, *J* = 8.1 Hz, 2H), 7.18 (dd, *J* = 2.8, 8.7 Hz, 1H), 6.79 (t, *J* = 7.3 Hz, 1H), 5.73-5.62 (m, 2H), 5.18-5.00 (m, 1H), 3.55-3.33 (m, 2H), 3.20-2.96 (m, 2H), 2.43-2.21 (m, 2H), 2.07-1.96 (m, 1H), 1.77 (s, 3H), 1.09-1.00 (m, 2H), 0.81-0.71 (m, 2H); LC/MS method 1: Rt 0.74 min, calcd for $C_{29}H_{29}FN_5O_2$ $[M+H]^+$ m/z 498.2, found 498.3.

(*E*)-*tert*-Butyl (2-(4-(4-(4-(4-(3-(4-cyclopropylbenzamido)-5-fluoro-2-methylphenyl)-7H-pyrrolo[2,3-d]pyrimidin-5-yl)-5,6-dihydropyridin-1(2H)-yl)-4-oxobut-2-en-1-yl)piperazin-1-yl)ethyl)carbamate (S46)



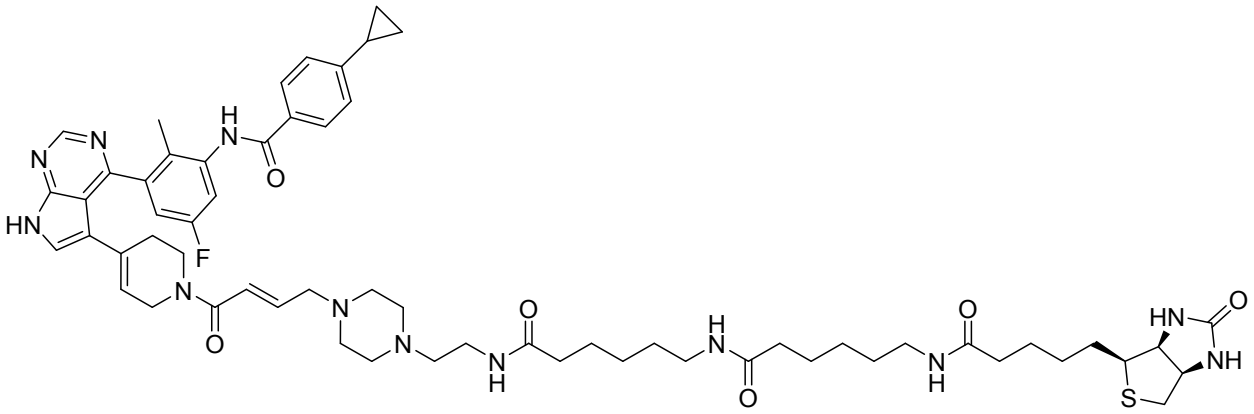
To a suspension of **S45** (3.10 g, 5.61 mmol) in THF (60 mL) at -20°C was added a solution of (*E*)-4-bromobut-2-enoyl chloride[20] (1.14 g, 5.61 mmol). The resulting mixture was stirred for 1 h at -20°C. The mixture was diluted with ethyl acetate and washed with saturated aqueous sodium bicarbonate solution and brine. The organic layer was dried over sodium sulfate, filtered and concentrated *in vacuo*. The residue was dissolved in DMF (45 mL) and 1-(2-Boc-aminoethyl)piperazine (2.13 g, 9.28 mmol) was added. The resulting mixture was stirred at room temperature for 18 h. The mixture was diluted with ethyl acetate and washed with saturated aqueous sodium bicarbonate solution and brine. The organic layer was dried over sodium sulfate, filtered and concentrated *in vacuo*. The crude product was purified by flash chromatography (silica gel, dichloromethane/methanol gradient) to yield the title compound (2.09 g, 2.60 mmol, 46%) as a beige solid. ^{1}H NMR (400 MHz, DMSO-d6): δ ppm (rotamers) 12.35 (s_{br}, 1H), 9.97, 9.85 (2 s, 1H), 8.85 (s, 1H), 7.94-7.85 (m, 2H), 7.60 (m, 1H), 7.33 (m, 1H), 7.23 (d, *J* = 8.0 Hz, 2H), 7.07-6.90 (m, 1H), 6.66-6.43 (m, 3H), 5.13, 4.99 (2 m, 1H), 3.95-3.66 (m, 2H), 3.17 (m, 2H), 3.09-2.93 (m, 4H), 2.45-2.22 (m, 10H), 2.13 (m, 2H), 2.07-1.98 (m, 1H), 1.96, 1.87 (2 s, 3H), 1.37 (s, 9H) 1.11-0.98 (m, 2H), 0.84-0.72 (m, 2H); LC/MS method 1: Rt 0.86 min, calcd for $C_{43}H_{52}FN_8O_4$ $[M+H]^+$ m/z 763.4, found 763.6.

(*E*)-N-(3-(5-(1-(4-(4-(2-Aminoethyl)piperazin-1-yl)but-2-enoyl)-1,2,3,6-tetrahydropyridin-4-yl)-7H-pyrrolo[2,3-d]pyrimidin-4-yl)-5-fluoro-2-methylphenyl)-4-cyclopropylbenzamide (S47)



To a solution of **S46** (1.00 g, 1.25 mmol) in dichloromethane (20 mL) was added HCl (4M in dioxan, 3.11 ml, 12.45 mmol). The resulting mixture was stirred for 3 h at room temperature. The mixture was concentrated *in vacuo* to yield the title compound (1.05 g, 1.22 mmol, 98%) as HCl salt, which was used in the next step without purification. ^{1}H NMR (400 MHz, DMSO-d6): δ ppm (rotamers) 12.89 (s, 1H), 9.98, 9.85 (2 s, 1H), 8.83 (s, 1H), 8.13-7.84 (m, 3H), 7.74-7.65 (m, 2H), 7.64-7.53 (m, 1H), 7.23 (m, 1H), 7.07-6.87 (m, 3H), 6.82-6.61 (m, 1H), 6.52-6.36 (m, 1H), 4.99, 4.80 (2 m, 1H), 3.80-3.45 (m, 4H), 3.29-2.73 (m, 13H), 2.02-1.78 (m, 3H), 1.74, 1.70 (2 s, 3H), 0.86-0.72 (m, 2H), 0.64-0.49 (m, 2H); LC/MS method 1: Rt 0.65 min, calcd for $C_{38}H_{44}FN_8O_2$ $[M+H]^+$ m/z 663.4, found 663.5.

4-Cyclopropyl-N-(5-fluoro-2-methyl-3-(5-(1-((E)-4-(4-(2-(6-(6-(5-((3aS,4S,6aR)-2-oxohexahydro-1H-thieno[3,4-d]imidazol-4-yl)pentanamido)hexanamido)hexanamido)ethyl)piperazin-1-yl)but-2-enoyl)-1,2,3,6-tetrahydropyridin-4-yl)-7H-pyrrolo[2,3-d]pyrimidin-4-yl)phenyl)benzamide (S48)



N-Biotinylcaproylaminocaproic acid (16.2 mg, 0.034 mmol) was dissolved in DMF (3 mL), then propylphosphonic anhydride solution (T3P) (50% in DMF, 0.019 ml, 0.031 mmol) and diisopropylethylamine (0.025 ml, 0.143 mmol) were added. The resulting suspension was stirred for 30 min at room temperature before adding **S47** (25 mg, 0.029 mmol). The reaction mixture was stirred for 30 min at room temperature. The mixture was diluted with ethyl acetate and washed with saturated aqueous sodium bicarbonate solution and brine. The organic layer was dried over

sodium sulfate, filtered and concentrated *in vacuo*. The crude product was purified by flash chromatography (silica gel, dichloromethane/methanol gradient) to yield the title compound (21.6 mg, 0.018 mmol, 64%) as a white solid. ^{1}H NMR (600 MHz, DMSO-d6): δ ppm (rotamers) 12.38 (s_{br}, 1H), 10.0-9.90 (m, 1H), 8.88 (s, 1H), 7.94 (m, 2H), 7.78-7.73 (m, 3H), 7.62 (m, 1H), 7.37 (m, 1H), 7.26 (d_{br}, J = 8.1 Hz, 2H), 7.01 (m, 1H), 6.65-6.37 (m, 4H), 5.15-5.05 (m, 1H), 4.34 (m, 1H), 4.16 (m, 1H), 3.91-3.40 (m, 13H), 3.21-3.09 (m, 4H), 3.05-3.01 (m, 3H), 2.85 (dd, J = 12.4, 5.1 Hz, 1H), 2.61 (d, 1H), 2.50-2.32 (m, 5H), 2.20-1.97 (m, 9H), 1.91 (s_{br}, 1H), 1.65 (m, 1H), 1.57-1.21 (m, 16H), 1.07 (m, 2H), 0.95 (m, 1H), 0.80 (m, 2H); LC/MS method 1: Rt 0.77 min, calcd for $C_{60}H_{80}FN_{12}O_6S$ $[M+H]^+$ m/z 1115.6, found 1115.9; HRMS (ESI+) calcd for $C_{60}H_{80}FN_{12}O_6S$ $[M+H]^+$ 1115.60230, found 1115.60151.

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