Structure-Based Design of Selective, Covalent G Protein-Coupled Receptor Kinase 5 Inhibitors

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General Chemistry: All reagents from commercial sources were used without further purification unless otherwise noted. ^{1}H-NMR spectra were taken in DMSO-d6, MeOD or $CDCl_3$ at room temperature on: Varian MR 400 MHz; Varian Vnmrs 500 MHz; and Varian Vnmrs 700 MHz instruments. The reported chemical shifts for the ^{1}HNMR spectra were recorded in parts per million (ppm) on the δ scale from an internal tetramethylsilane (TMS) standard (0.0 ppm). Small molecule mass spectrometry data was measured using a Waters Corporation Micromass LCT or Agilent6230 Q-TOF instrument. HPLC was used to determine purity of compounds on an Agilent 1100 series with an Agilent Zorbax Eclipse Plus-C18 column. A gradient of 10-90% acetonitrile/water over 6 min followed by 90% acetonitrile/water for 7 min was used with detection at 254 nm.

Intact Protein MS and Tandem MS/MS: Intact protein MS was acquired with a Phenomenex C4 column paired with an Agilent 6545 Q-TOF LC/MS. For intact MS and Tandem MS, all samples were prepared with 20 μM GRK in assay buffer (see below), 1 mM compound, and incubated at 4 ˚C for 3 hr before being quenched with 1.0 μL of formic acid. In Tandem MS/MS, we chose Glu-C as the restricting enzyme to avoid small fragments with mass-to-charge ratios below the limit of detection of our instrument.[1] All samples were digested with Glu-C sequencing enzyme, procured from Sigma Aldrich (Roche Life Sciences subsidiary) and used without further purification. MS/MS experiments were run on a nano-LC (Dionex RSLC-nano) with an Orbitrap Fusion Tribrid ETD mass spectrometer. This work was conducted by the Proteomics Resource Facility at the University of Michigan.

Inhibition Assays: For compounds **4**-**9**, IC_{50} values for human GRK5, bovine GRK2, and bovine GRK1 were determined using a radiometric assay as follows. 50 nM GRK was incubated with 500 nM porcine brain tubulin (Cytoskeleton) and 0 - 333 μM inhibitor in 20 mM HEPES pH 7.0, 2 mM $MgCl_2$, 0.025% dodecylmaltoside (DDM), 3% DMSO for 20-30 min for GRK1 and 2 and 0-1 hr for GRK5, as denoted in **Table 1**, on ice, prior to initiation with the addition of 5 μM ATP supplemented with radioactive [γ-^{32}P]-ATP (PerkinElmer Life Sciences). Reactions were quenched at 5 min by addition of 6 μL of 4X SDS gel loading dye to the 6 μL reactions. 8 μL samples were separated on a 4-15% Criterion TGX precast gel (Bio-Rad). Experiments were performed with an n = 3 in duplicate.

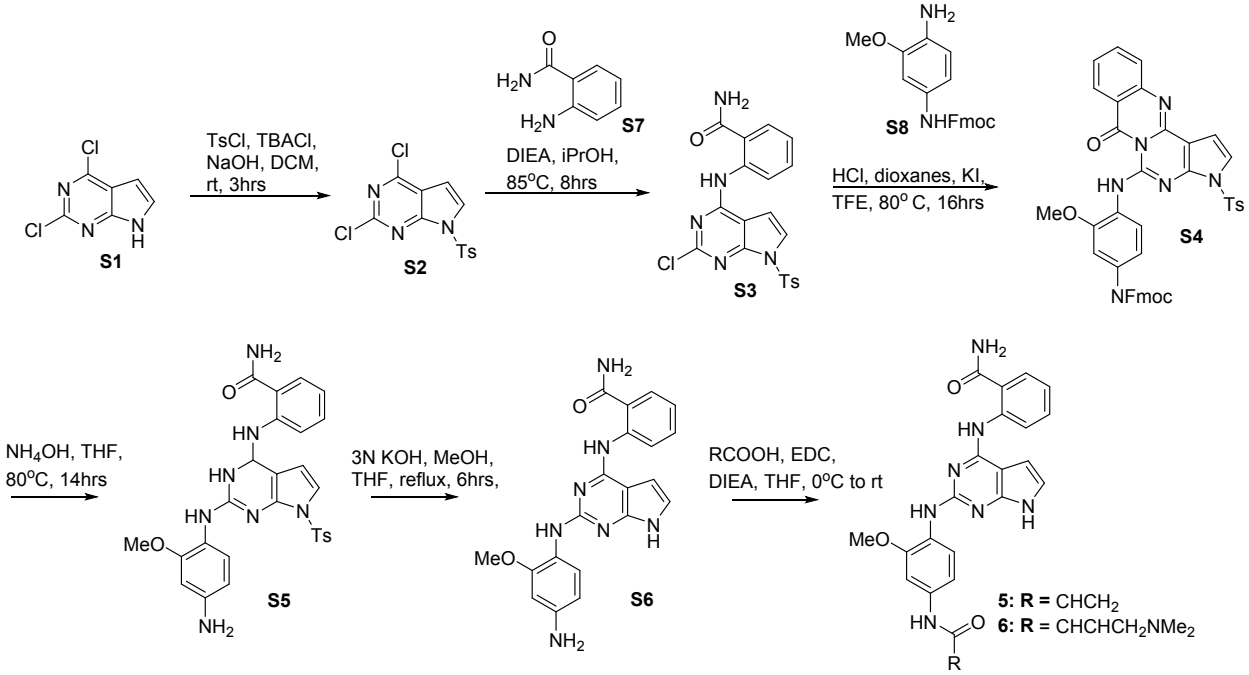
For the remaining compounds **16a**-**16j**, IC_{50} values for human GRK5, bovine GRK2, bovine GRK6, and bovine GRK5-C474S were similarly determined with the following differences. Inhibitors were assessed over the range of 1 nM–1 mM in 20 mM HEPES pH 7.5, 10 mM NaCl, 10 mM $MgCl_2$, 2 mM DTT after a 4 hr incubation at room temperature prior to reaction initiation with ATP. Reactions were quenched at 8 min by transferring 10 μL reaction into 5 μL 4X SDS gel loading dye. 10 μL samples were separated on a 4-15% Criterion TGX precast gel. These conditions were also used to assess compound **5** for GRK5 (4 hr), GRK2, and GRK5-C474S but with the addition of 3% DMSO (final) to the incubation buffer. Experiments were performed with an n = 3 except for assays involving **5** against GRK5, GRK6, and GRK5-C474S with 4 hr incubations, which were performed 4 times.

For compounds **5** and **16d**, IC_{50} values were also determined when opsin was used as the substrate. 50 nM of GRK5 were preincubated with 0-750 μM inhibitor in buffer containing 20 mM HEPES pH 7.0, 2 mM $MgCl_2$, 0.025% dodecylmaltoside (DDM), 3% DMSO for 0, 30, 60 or 240 min. Rhodopsin in the rod outer segment was exposed to the light for approximately 3 min right before phosphorylation. 5 μM ATP supplemented with radioactive [γ-^{32}P]-ATP (PerkinElmer Life Sciences) was added to initiate the reaction. Reactions were quenched at 5 min by addition of 10 μL of 4X SDS gel loading dye to the 20 μL reactions. 12 μL samples were separated on a 4-15% Criterion TGX precast gel (Bio-Rad). Experiments were performed with an n = 2 for compound 5 an n=3 for compound 16d.

Gels were dried, exposed to a storage phosphor screen overnight, and scanned using a Typhoon scanner. Bands corresponding to phosphorylated tubulin were quantified using ImageQuant, plotted as a function of log[inhibitor], and fit to the three-parameter log(inhibitor) vs. response model in GraphPad Prism 7.03 to determine the IC_{50}., and mean and standard deviation values were calculated using the column statistics function of GraphPad Prism 7.03.

Standard control compounds are run during each assay to assess consistency across time, experimenters, and subtle changes in assay conditions that are sometimes required to keep some of the compounds soluble and disperse (such as through addition of DDM or 3% DMSO). Paroxetine, GSK180736A, and CCG215022 were used as controls for compounds **4**-**9**, and CCG215022 for compounds **16a**-**16j**.

Synthetic Protocols:



Scheme S1: Synthesis of non-homologated *para*-substituted analogues **5** and **6**.

2,4-dichloro-7-tosyl-7H-pyrrolo[2,3-d]pyrimidine (**S2**): To a 100 mL round bottom flask was added 2,4-dichloro-7H-pyrrolo[2,3-d]pyrimidine **S1** (1.0 g, 5.32 mmol), 4 – methylbenzene – 1 – sulfonyl

chloride (1.12 g, 5.85 mmol), tetra – butyl ammonium chloride (0.07 g, 0.27 mmol), and dichloromethane (20 mL). Then 6N sodium hydroxide (2.66 mL, 15.96 mmol) was added dropwise. The slurry was stirred at room temperature vigorously for 1.5 hours going from cloudy to clear. The reaction was then diluted with water and the layers were separated. The organic layer was washed with NaCl (1x), and then dried with $MgSO_4$. The $MgSO_4$ was filtered off and the filtrate was then concentrated. The resulting off white solid was purified using 100% dichloromethane to give the title compound as a white solid (1.64 g, 4.79 mmol, 89% yield). 1H NMR (500 MHz, DMSO-d6) δ 8.13 (d, J = 4.0 Hz, 1H), 8.07 – 8.01 (m, 2H), 7.51 (d, J = 8.2 Hz, 2H), 6.99 (d, J = 4.1 Hz, 1H), 2.39 (s, 4H). HPLC (gradient A): retention time = 8.207 min; purity = 98%.

2-((2-chloro-7-tosyl-7H-pyrrolo[2,3-d]pyrimidin-4-yl)amino)benzamide (**S3**): To a 100 mL round bottom flask was added 2,4-dichloro-7-tosyl-7H-pyrrolo[2,3-d]pyrimidine **S2** (0.93 g, 2.72 mmol) and 2 – aminobenzamide **S7** (1.48 g, 10.88 mmol). Isopropanol (16 mL) and di-isopropylethylamine (2.38 mL, 13.6 mmol) were then added and the reaction was heated to reflux at 85 ˚C. All solids went into solution upon heating. After refluxing overnight, the reaction was cooled down and the resulting white precipitate was filtered off and washed with additional isopropanol to give the title compound (1.10 g, 2.49 mmol, 92% yield). 1H NMR (400 MHz, DMSO-d6) δ 12.34 (s, 1H), 8.50 – 8.43 (m, 1H), 8.32 (s, 1H), 8.00 (d, J = 8.2 Hz, 2H), 7.85 (d, J = 7.9 Hz, 1H), 7.81 (s, 1H), 7.77 (d, J = 3.9 Hz, 1H), 7.59 (t, J = 7.9 Hz, 1H), 7.49 (d, J = 8.1 Hz, 2H), 7.19 (t, J = 7.6 Hz, 1H), 6.70 (d, J = 4.0 Hz, 1H), 2.38 (s, 3H). HPLC (gradient A): retention time = 7.890 min; purity = 99%.

5-((4-amino-2-methoxyphenyl)amino)-3-tosylpyrrolo[2',3':4,5]pyrimido[6,1-b]quinazolin-7(3H)-one (**S4**): To a 100 mL pressure vessel was added 2-((2-chloro-7-tosyl-7H-pyrrolo[2,3-d]pyrimidin-4-yl)amino)benzamide **S3** (0.221 g, 0.500 mmol), (9H-fluoren-9-yl)methyl (4-amino-3-methoxyphenyl)carbamate **S8** (0.189 g, 0.525 mmol), 4M HCl in dioxanes (0.50 mL, 2.0 mmol), potassium iodide (0.01 g, 0.06 mmol), and trifluoroethanol (10 mL). The sealed reaction was then heated overnight at 90 ˚C. The following day the reaction was cooled to room temperature and then a light yellow/orange solid was filtered off and taken as is to the next step.

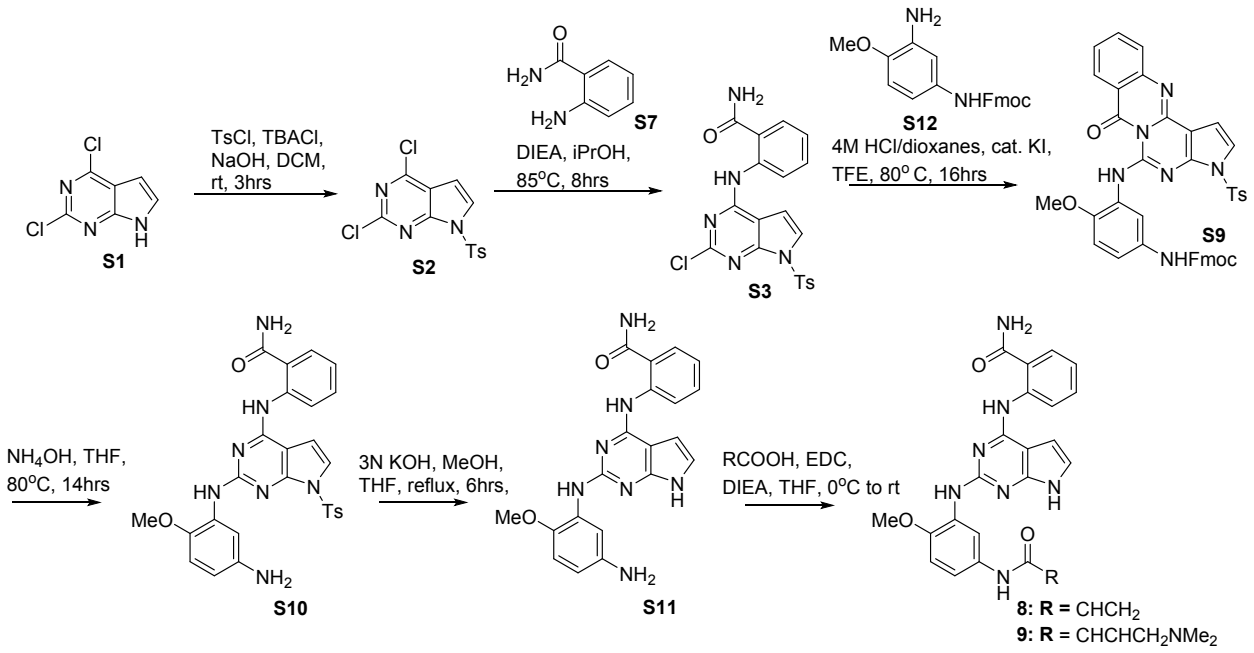
2-((2-((4-amino-2-methoxyphenyl)amino)-7-tosyl-4,7-dihydro-3H-pyrrolo[2,3-d]pyrimidin-4-yl)amino)benzamide (**S5**): To a 50 mL pressure vessel was added crude 5-((4-amino-2-methoxyphenyl)amino)-3-tosylpyrrolo[2',3':4,5]pyrimido[6,1-b]quinazolin-7(3H)-one **S4** (0.264 g, 0.50 mmol), 28% ammonium hydroxide in water (10 mL), and THF (10mL). The sealed reaction was heated to 60 ˚C overnight. After cooling the reaction was diluted with dichloromethane and water. The layers were separated, and the organic layer was washed with NaCl (2x) and then dried over sodium sulfate. The sodium sulfate was filtered off and the filtrate was concentrated. The resulting orange solid was purified using 0-10% MeOH/DCM to give the title compound as a white solid (0.120 g, 0.065 mmol, 44% yield over two steps). 1H NMR (400 MHz, DMSO-d6) δ 12.12 (s, 1H), 8.80 (s, 1H), 8.31 (s, 1H), 7.93 (d, J = 7.8 Hz, 2H), 7.84 – 7.80 (m, 1H), 7.75 (s, 1H), 7.36 (d, J = 8.1 Hz, 3H), 7.33 (d, J = 4.0 Hz, 1H), 7.02 (t, J = 7.6 Hz, 1H), 6.46 (d, J = 4.0 Hz, 1H), 6.38 (d, J = 2.2 Hz, 1H), 6.32 – 6.24 (m, 1H), 5.02 (s, 2H), 3.70 (s, 3H), 2.36 (s, 3H). HPLC (gradient A): retention time = 5.711 min; purity = 94%.

*2-((2-((4-amino-2-methoxyphenyl)amino)-4,7-dihydro-3H-pyrrolo[2,3-d]pyrimidin-4-yl)amino)benzamide (***S6***):* To a 50 mL round bottom flask was added 2-((2-((4-amino-2-methoxyphenyl)amino)-7-tosyl-4,7-dihydro-3H-pyrrolo[2,3-d]pyrimidin-4-yl)amino)benzamide **S5** (0.315 g, 0.579 mmol) followed by THF (10 mL), MeOH (2 mL), and 3N potassium hydroxide (6.0 mL). The reaction was stirred, refluxing, at 65 ˚C overnight. After cooling the organic solvents

were concentrated off and then the reaction was diluted with ethyl acetate. The layers were then separated, and the organic layer was washed with NaCl (1x) and dried over sodium sulfate. The sodium sulfate was then filtered off and the resulting filtrate was concentrated and purified using 0% - 8% MeOH/DCM to give the title compound as a slightly grey solid (0.132 g, 0.339 mmol, 59% yield). 1H NMR (400 MHz, DMSO-*d*6) δ 11.95 (s, 1H),
11.19 (s, 1H), 8.97 (d, *J* = 8.5 Hz, 1H), 8.28 (s, 1H), 7.88 – 7.77 (m, 1H), 7.77 – 7.65 (m, 1H), 7.52 (d, *J* = 8.3 Hz, 1H), 7.41 (t, *J* = 7.9 Hz, 1H), 7.29 (s, 1H), 6.98 (t, *J* = 7.5 Hz, 1H), 6.91 (dd, *J* = 3.4, 2.2 Hz, 1H), 6.33 (d, *J* = 2.3 Hz, 1H), 6.22 (dd, *J* = 3.5, 1.9 Hz, 1H), 6.18 (dd, *J* = 8.4, 2.3 Hz, 1H), 4.91 (s, 2H), 3.71 (s, 3H). HPLC (gradient A): retention time = 4.120 min; purity = 84%.

*2-((2-((4-acrylamido-2-methoxyphenyl)amino)-7H-pyrrolo[2,3-d]pyrimidin-4-yl)amino)benzamide (***5**, **CCG-258903***)*: In a 25 mL flask 2-((2-((4-amino-2-methoxyphenyl)amino)-7H-pyrrolo[2,3-d]pyrimidin-4-yl)amino)benzamide **S6** (0.10 g, 0.257 mmol) was dissolved in THF (6.0 mL). Diisopropylethylamine (0.134 mL, 0.770 mmol) and 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (0.098 g, 0.512 mmol) were then added and the reaction was stirred ten minutes and cooled to 0 ˚C. Acrylic acid (0.02 mL, 0.282 mmol) was then added and the reaction was allowed to warm to room temperature and stir overnight. Water was added to quench the reaction and then ethyl acetate was added to extract the organics. The layers were separated, and the organic layer was washed with $NaHCO_3$ (1x), dried over magnesium sulfate, and concentrated. The resulting residue was purified using flash chromatography (0 – 10% MeOH/DCM) to give a light yellow solid as the desired compound (0.0107 g, 0.024 mmol, 10% yield). 1H NMR (400 MHz, DMSO-*d*6) δ 11.99 (s, 1H), 11.36 (s, 1H), 10.10 (s, 1H), 8.93 (d, *J* = 8.5 Hz, 1H), 8.30 (s, 1H), 8.20 (d, *J* = 8.7 Hz, 1H), 7.83 (d, *J* = 7.7 Hz, 1H), 7.74 (s, 1H), 7.56 – 7.43 (m, 3H), 7.24 – 7.16 (m, 1H), 7.07 – 6.97 (m, 2H), 6.44 (dd, *J* = 17.0, 10.1 Hz, 1H), 6.32 – 6.20 (m, 2H), 5.78 – 5.70 (m, 1H), 3.85 (s, 3H). HPLC (gradient A): retention time = 4.987 min; purity = 95%. Molecular formula: $C_{23}H_{21}N_7O_3$, Mass Calc: 443.17, ESI-MS Found: 444.1626 [M+1]

*(E)-2-((2-((4-(4-(dimethylamino)but-2-enamido)-2-methoxyphenyl)amino)-7H-pyrrolo[2,3-d]pyrimidin-4-yl)amino)benzamide (***6**, **CCG-263045***):* In a 25 mL flask 2-((2-((4-amino-2-methoxyphenyl)amino)-7H-pyrrolo[2,3-d]pyrimidin-4-yl)amino)benzamide **S6** (0.137 g, 0.352 mmol) was dissolved in THF (6.0 mL). Diisopropylethylamine (0.246 mL, 0.1.41 mmol) and 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (0.108 g, 0.563 mmol) were then added and the reaction was stirred ten minutes and cooled to 0 ˚C. *Trans*–4–dimethylaminocrotonic acid hydrochloride (0.070 g, 0.422 mmol) was then added and the reaction was allowed to warm to room temperature and stir overnight. Water was added to quench the reaction and then ethyl acetate was added to extract the organics. The layers were separated, and the organic layer was washed with NaCl (1x), dried over magnesium sulfate, and concentrated. The resulting residue was purified using flash chromatography (5 – 10% MeOH/DCM) to give a light brown solid as the desired compound (0.017 g, 0.034 mmol, 10% yield). 1H NMR (400 MHz, DMSO-*d*6) δ 11.99 (s, 1H), 11.36 (s, 1H), 10.02 (s, 1H), 8.94 (dd, *J* = 8.4, 1.2 Hz, 1H), 8.30 (s, 1H), 8.17 (d, *J* = 8.7 Hz, 1H), 7.83 (dd, *J* = 8.1, 1.6 Hz, 1H), 7.75 (s, 1H), 7.56 – 7.42 (m, 3H), 7.17 (dd, *J* = 8.7, 2.1 Hz, 1H), 7.07 – 6.96 (m, 2H), 6.72 (dt, *J* = 15.4, 5.9 Hz, 1H), 6.31– 6.24 (m, 2H), 3.84 (s, 3H), 3.10 – 3.03 (m, 2H), 2.19 (s, 6H). HPLC (gradient A): retention time = 4.305 min; purity = 97%. Molecular Formula: $C_{26}H_{28}N_8O_3$, Mass Calc: 500.23, ESI-MS Found: 501.2343 [M+1]



Scheme S2: Synthesis of non-homologated *meta*-substituted analogues.

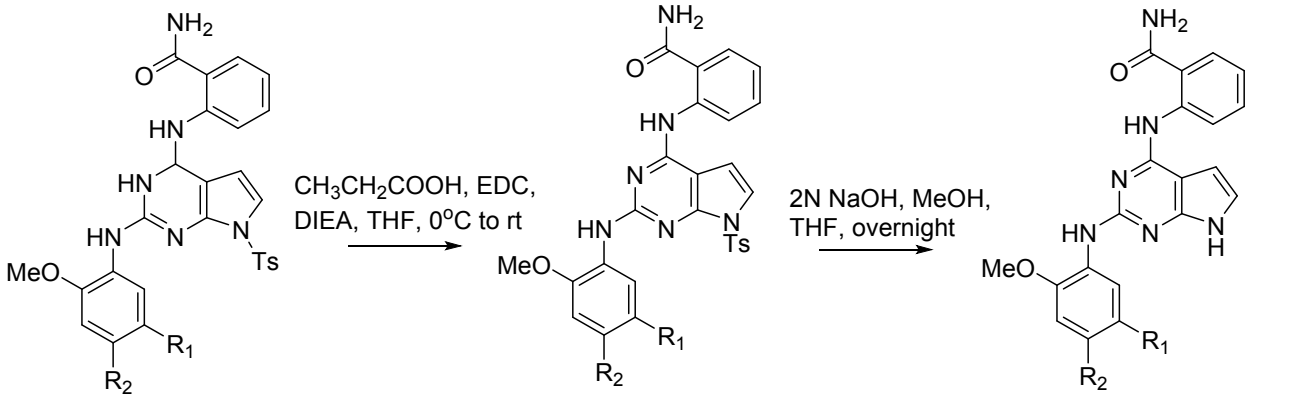
(9H-fluoren-9-yl)methyl (4-methoxy-3-((7-oxo-3-tosyl-3,7-dihydropyrrolo[2',3':4,5]pyrimido[6,1-b]quinazolin-5-yl)amino)phenyl)carbamate (**S9**): To a 100 mL pressure vessel was added 2-((2-chloro-7-tosyl-7H-pyrrolo[2,3-d]pyrimidin-4-yl)amino)benzamide **S3** (0.60 g, 1.36 mmol), (9H-fluoren-9-yl)methyl (3-amino-4-methoxyphenyl)carbamate **S12** (0.515 g, 1.43 mmol), 4M HCl in dioxanes (1.36 mL, 5.44 mmol), potassium iodide (0.04 g, 0.24 mmol), and trifluoroethanol (40 mL). The sealed reaction was then heated overnight at 90 ˚C. The following day the reaction was cooled to room temperature and then diluted with water and an excessive amount of dichloromethane (~100 mL, otherwise an emulsion will form). The layers were separated and the organic was washed with NaCl (1x) and dried with sodium sulfate. The sodium sulfate was then filtered off and the filtrate was concentrated to give a light yellow/orange solid which was taken as is to the next step.

(9H-fluoren-9-yl)methyl (4-methoxy-3-((7-oxo-3-tosyl-3,7-dihydropyrrolo[2',3':4,5]pyrimido[6,1-b]quinazolin-5-yl)amino)phenyl)carbamate (**S10**): To a 100 mL pressure vessel was added (9H-fluoren-9-yl)methyl (4-methoxy-3-((7-oxo-3-tosyl-3,7-dihydropyrrolo[2',3':4,5]pyrimido[6,1-b]quinazolin-5-yl)amino)phenyl)carbamate **S9** (1.02 g, 1.36 mmol), 28% ammonium hydroxide in water (20 mL), and THF (20mL). The reaction was heated in a sealed pressure vessel to 60 ˚C overnight. After cooling the reaction was diluted with ethyl acetate and water. The layers were separated, and the organic layer was washed with NaCl (2x) and then dried of sodium sulfate. The sodium sulfate was filtered off and the filtrate was concentrated. The resulting orange solid was triturated in dichloromethane to give the title compound as an off white solid (0.378 g, 0.70 mmol, 51% yield over two steps). 1H NMR (400 MHz, DMSO-d6) δ 12.13 (s, 1H), 8.76 (dd, J = 8.5, 1.1 Hz, 1H), 8.31 (s, 1H), 8.03 – 7.97 (m, 2H), 7.93 (s, 1H), 7.83 (dd, J = 8.0, 1.5 Hz, 1H), 7.76 (s, 1H), 7.49 (ddd, J = 8.6, 5.7, 1.5 Hz, 2H), 7.41 (d, J = 4.0 Hz, 1H), 7.38 (d, J = 8.2 Hz, 2H), 7.09 – 7.02 (m, 1H), 6.81 (d, J = 8.6 Hz, 1H), 6.53 (d, J = 4.0 Hz, 1H), 6.37 (dd, J = 8.5, 2.7 Hz, 1H), 4.55 (s, 2H), 3.72 (s, 3H), 2.34 (s, 3H). HPLC (gradient A): retention time = 5.850 min; purity = 88%.

2-((2-((5-amino-2-methoxyphenyl)amino)-4,7-dihydro-3H-pyrrolo[2,3-d]pyrimidin-4-yl)amino)benzamide (**S11**): To a 25 mL round bottom flask was added (9H-fluoren-9-yl)methyl (4-methoxy-3-((7-oxo-3-tosyl-3,7-dihydropyrrolo[2',3':4,5]pyrimido[6,1-b]quinazolin-5-yl)amino)phenyl)carbamate **S10** (0.158 g, 0.291 mmol), methanol (2 mL), 3N potassium hydroxide (2 mL) and THF (4.5 mL). The reaction was heated to 65 ˚C and stirred overnight for two days. The reaction was then cooled, and the organic layers were concentrated off. The resulting suspension in the aqueous layer was then filtered off to give adequately pure compound (0.082 mg, 72% yield). 1H NMR (500 MHz, DMSO-d6) δ 11.99 (s, 1H), 11.33 (s, 1H), 8.97 (d, J = 8.1 Hz, 1H), 8.28 (s, 1H), 7.83 (dd, J = 8.0, 1.5 Hz, 1H), 7.72 (s, 1H), 7.59 – 7.49 (m, 2H), 7.39 (s, 1H), 7.06 – 6.97 (m, 2H), 6.73 (d, J = 8.6 Hz, 1H), 6.29 (dd, J = 3.5, 1.9 Hz, 1H), 6.21 (dd, J = 8.5, 2.7 Hz, 1H), 4.47 (s, 2H), 3.74 (s, 3H). HPLC (gradient A): retention time = 4.166 min; purity = 91%.

2-((2-((5-acrylamido-2-methoxyphenyl)amino)-7H-pyrrolo[2,3-d]pyrimidin-4-yl)amino)benzamide (**8, CCG-258904**): In a 25 mL flask 2-((2-((5-amino-2-methoxyphenyl)amino)-7H-pyrrolo[2,3-d]pyrimidin-4-yl)amino)benzamide **S11** (0.088 g, 0.226 mmol) was dissolved in THF (6.0 mL). Diisopropylethylamine (0.12 mL, 0.678 mmol) and 1-Ethyl-3-(3 dimethylaminopropyl)carbodiimide hydrochloride (0.087 g, 0.452 mmol) were then added and the reaction was stirred ten minutes and cooled to 0 ˚C. Acrylic acid (0.017 mL, 0.249 mmol) was then added and the reaction was allowed to warm to room temperature and stir overnight. Water was added to quench the reaction and then ethyl acetate was added to extract the organics. The layers were separated, and the organic layer was washed with NaCO3 (1x), dried over magnesium sulfate, and concentrated. The resulting residue was subjected to flash chromatography (5 – 10% MeOH/DCM) and then purified using reverse phase chromatography (30 – 60% Acetonitrile/Water) to give the desired compound as a light yellow solid (0.012 g, 0.026 mmol, 12% yield). 1H NMR (500 MHz, DMSO-d6) δ 12.05 (s, 1H), 11.44 (s, 1H), 10.05 (s, 1H), 8.94 (d, J = 8.4 Hz, 1H), 8.28 (d, J = 2.8 Hz, 2H), 7.82 (dd, J = 7.9, 1.6 Hz, 1H), 7.73 (s, 1H), 7.59 (s, 1H), 7.44 – 7.36 (m, 2H), 6.98 (q, J = 7.1, 5.9 Hz, 3H), 6.45 (dd, J = 17.0, 10.2 Hz, 1H), 6.29 (d, J = 3.5 Hz, 1H), 6.22 (dd, J = 17.0, 2.1 Hz, 1H), 5.71 (dd, J = 10.0, 2.1 Hz, 1H), 3.84 (s, 3H). HPLC (gradient A): retention time = 4.921 min; purity = 93%. Molecular Formula: $C_{23}H_{21}N_7O_3$, Mass Calc: 443.17, ESI-MS Found: 444.1630 [M+1].

(E)-2-((2-((4-(4-(dimethylamino)but-2-enamido)-2-methoxyphenyl)amino)-7H-pyrrolo[2,3-d]pyrimidin-4-yl)amino)benzamide (**9, CCG-263115**): In a 25 mL flask 2-((2-((5-amino-2-methoxyphenyl)amino)-7H-pyrrolo[2,3-d]pyrimidin-4-yl)amino)benzamide **S11** (0.073 g, 0.187 mmol) was dissolved in THF (4.0 mL). Diisopropylethylamine (0.10 mL, 0.561 mmol) and 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (0.047 g, 0.243 mmol) were then added and the reaction was stirred ten minutes and cooled to 0 ˚C. *Trans*–4–dimethylaminocrotonic acid hydrochloride (0.034 g, 0.206 mmol) was then added and the reaction was allowed to warm to room temperature and stir three hours. Water was added to quench the reaction and then ethyl acetate was added to extract the organics. The layers were separated, and the organic layer was washed with NaCl (1x), dried over magnesium sulfate, and concentrated. The resulting residue was purified using flash chromatography (5 – 10% MeOH/DCM) to give a light brown solid as the desired compound (0.012 g, 0.024 mmol, 13% yield). 1H NMR (500 MHz, DMSO-d6) δ 12.07 (s, 1H), 11.34 (s, 1H), 9.88 (s, 1H), 8.94 (dd, J = 8.6, 1.2 Hz, 1H), 8.30 (s, 1H), 8.24 (d, J = 2.5 Hz, 1H), 7.82 (dd, J = 7.9, 1.6 Hz, 1H), 7.74 (s, 1H), 7.60 (s, 1H), 7.43 – 7.34 (m, 2H), 7.05 – 6.94 (m, 3H), 6.75 – 6.65 (m, 1H), 6.30 – 6.22 (m, 2H), 3.83 (s, 3H), 3.04 (dd, J = 6.1, 1.6 Hz, 2H), 2.18 (s, 6H). HPLC (gradient A): retention time = 4.353 min; purity = 93%. Molecular Formula: $C_{26}H_{28}N_8O_3$, Mass Calc: 500.23, ESI-MS Found: 501.2356 [M+1]



S5: R_1 = H, R_2 = NH_2
S10: R_1 = NH_2, R_2 = H

S5': R_1 = H, R_2 = $NHCOCH_2CH_3$
S10': R_1 = $NHCOCH_2CH_3$, R_2 = H

4: R_1 = H, R_2 = $NHCOCH_2CH_3$
7: R_1 = $NHCOCH_2CH_3$, R_2 = H

Synthesis of saturated analogs **4** and **7**.

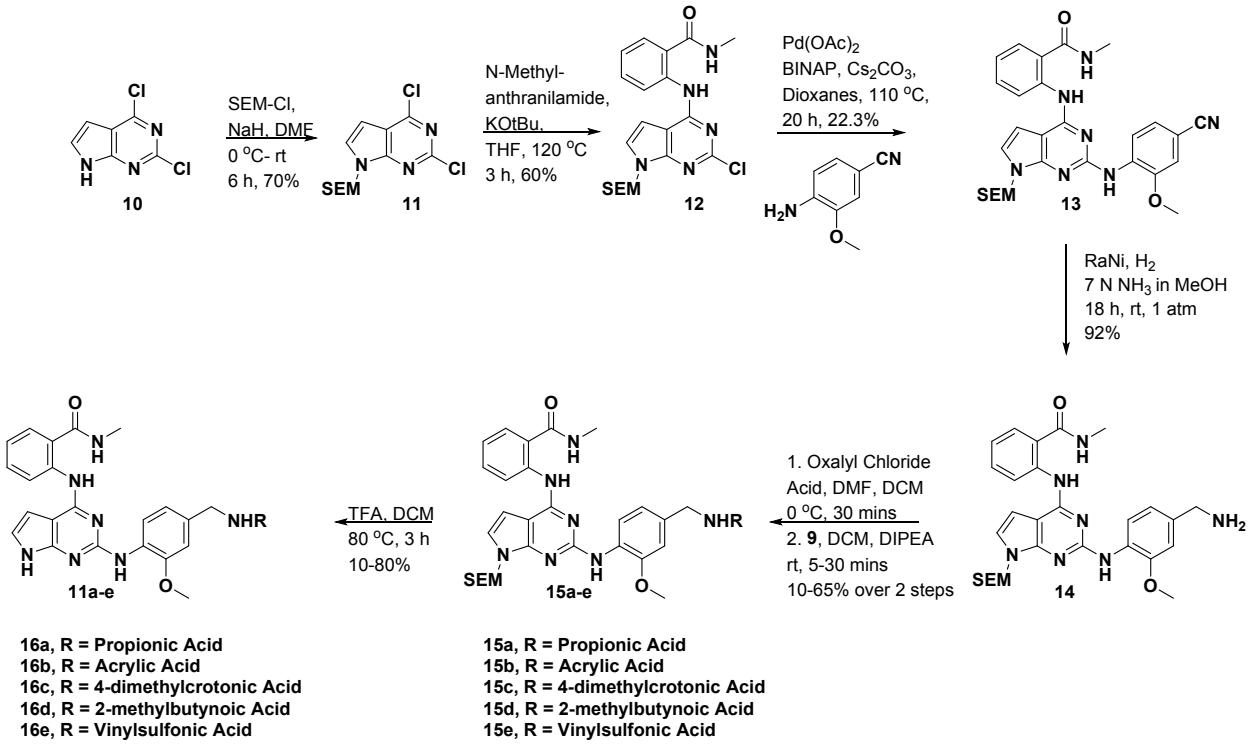
*2-((2-((2-methoxy-4-propionamidophenyl)amino)-7-tosyl-7H-pyrrolo[2,3-d]pyrimidin-4-yl)amino)benzamide (**S5'**):* To a 25 mL round bottom flask was added 2-((2-((4-amino-2-methoxyphenyl)amino)-7-tosyl-4,7-dihydro-3H-pyrrolo[2,3-d]pyrimidin-4-yl)amino)benzamide **S5** (0.070 g, 0.128 mmol), 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (0.049 g, 0.258 mmol), diisopropylethylamine (0.068 mL, 0.387 mmol), and THF (4.0 mL). The reaction was cooled to 0 ˚C and then stirred for ten minutes before adding propionic acid (0.012 mL, 0.155 mmol). The reaction was further stirred overnight. Water was added to quench the reaction and then the reaction was further diluted with ethyl acetate. The two layers were separated. The organic layer was washed with 10% citric acid (1x), NaCl (2x), and then dried with sodium sulfate. The sodium sulfate was then filtered off and the filtrate was concentrated. The resulting residue was purified using 0 – 5% methanol/dichloromethane to give the title compound as a light pink solid (0.022 g, 0.041 mmol, 32% yield). ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.12 (s, 1H), 9.88 (s, 1H), 8.75 (d, *J* = 8.4 Hz, 1H), 8.31 (s, 1H), 8.04 (s, 1H), 7.94 (d, *J* = 8.1 Hz, 3H), 7.82 (dd, *J* = 8.0, 1.6 Hz, 1H), 7.76 (s, 1H), 7.50 (d, *J* = 2.1 Hz, 1H), 7.48 – 7.34 (m, 4H), 7.20 (dd, *J* = 8.6, 2.1 Hz, 1H), 7.10 – 7.02 (m, 1H), 6.52 (d, *J* = 4.0 Hz, 1H), 3.81 (s, 3H), 2.41 – 2.30 (m, 5H), 1.12 (t, *J* = 7.6 Hz, 3H). HPLC (gradient A): retention time = 7.312 min; purity = 94%.

*2-((2-((2-methoxy-5-propionamidophenyl)amino)-7-tosyl-7H-pyrrolo[2,3-d]pyrimidin-4-yl)amino)benzamide (**S10'**)*: To a 25 mL round bottom flask was added (9H-fluoren-9-yl)methyl (4-methoxy-3-((7-oxo-3-tosyl-3,7-dihydropyrrolo[2',3':4,5]pyrimido[6,1-b]quinazolin-5-yl)amino)phenyl)carbamate **S10** (0.100 g, 0.184 mmol), 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (0.071 g, 0.368 mmol), diisopropylethylamine (0.096 mL, 0.552 mmol), and THF (4.0 mL). The reaction was cooled to 0 ˚C and then stirred for ten minutes before adding propionic acid (0.017 mL, 0.221 mmol). The reaction was further stirred overnight. Water was added to quench the reaction and then the reaction was further diluted with ethyl acetate. The two layers were separated. The organic layer was washed with 10% citric acid (1x), NaCl (2x), and then dried with sodium sulfate. The sodium sulfate was then filtered off and the filtrate was concentrated. The resulting residue was purified using 0 – 5% isopropanol/dichloromethane to give the title compound as a white solid (0.065 g, 0.108 mmol, 59% yield). ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.20 (s, 1H), 9.71 (s, 1H), 8.71 (d, *J* = 8.4 Hz, 1H), 8.32 (s, 1H), 8.26 (s, 1H), 7.97 (d, *J* = 8.2 Hz, 2H), 7.93 (d, *J* = 2.5 Hz, 1H), 7.82 (dd, *J* = 8.0,

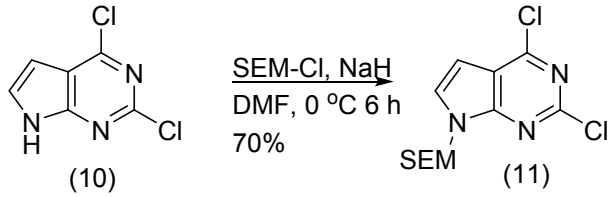
1.5 Hz, 1H), 7.77 (s, 1H), 7.39 (d, *J* = 4.0 Hz, 1H), 7.38 – 7.28 (m, 4H), 7.07 – 6.99 (m, 2H), 6.50 (d, *J* = 4.0 Hz, 1H), 3.79 (s, 3H), 2.34 (s, 3H), 2.29 (q, *J* = 7.6 Hz, 2H), 1.07 (t, *J* = 7.6 Hz, 3H). HPLC (gradient A): retention time = 7.195 min; purity = 93%.

*2-((2-((2-methoxy-4-propionamidophenyl)amino)-7H-pyrrolo[2,3-d]pyrimidin-4-yl)amino)benzamide (***4**, **CCG-262604***):* In a 25 mL round bottom flask a suspension of 2-((2-((2-methoxy-4-propionamidophenyl)amino)-7-tosyl-7H-pyrrolo[2,3-d]pyrimidin-4-yl)amino)benzamide (0.022 g, 0.037 mmol), MeOH (1.0 mL), THF (4.0 mL), and 2N NaOH (4.0mL) was stirred overnight becoming clearer. The reaction was diluted with ethyl acetate and water. The layers were separated and the organic was washed with NaCl (1x), dried over MgSO4, and concentrated. The resulting crude residue was purified using 5% - 10% MeOH/DCM and then further purified by recrystallizing from 15% MeOH/DCM overnight (~ 1.5 mL) to give the title compound as an off white solid (0.013 g, 0.029 mmol, 78% yield). 1H NMR (400 MHz,DMSO-*d*6) δ 11.99 (s, 1H), 11.34 (s, 1H), 9.79 (s, 1H), 8.93 (d, *J* = 8.5 Hz, 1H), 8.30 (s, 1H), 8.13 (d, *J* = 8.6 Hz, 1H), 7.83 (d, *J* = 8.0 Hz, 1H), 7.75 (s, 1H), 7.53 – 7.40 (m, 3H), 7.10 (d, *J* = 8.7 Hz, 1H), 7.05 – 6.95 (m, 2H), 6.28 (s, 1H), 3.82 (s, 3H), 2.31 (q, *J* = 8.5, 7.5 Hz, 2H), 1.09 (t, *J* = 7.5 Hz, 3H). HPLC (gradient A): retention time = 4.906 min; purity = 95%. Molecular Formula: $C_{23}H_{23}N_7O_3$, Mass Calc: 445.19, ESI-MS Found: 446.1926 [M+1]

2-((2-((2-methoxy-5-propionamidophenyl)amino)-7H-pyrrolo[2,3-d]pyrimidin-4-yl)amino)benzamide (**7, CCG-262606**): In a 25 mL flask 2-((2-((2-methoxy-5-propionamidophenyl)amino)-7-tosyl-7H-pyrrolo[2,3-d]pyrimidin-4-yl)amino)benzamide 128 (0.062 g, 0.104 mmol) was suspended in 1,4 – dioxanes (5.0 mL) and 2N NaOH (5.0 mL) and the reaction was stirred vigorously. After 2 hours the reaction was still cloudy and THF (3 mL) and MeOH (1 mL) were then added and the reaction was stirred overnight. The organics were then concentrated off and the pH was lowered with concentrated HCl at which point a new side product formed. Assuming it was the cyclized lactam the compound was then stirred in a sealed reaction vessel with 28% NH4OH (4 mL) and THF (4 mL) at 40 ˚C for four hours, concentrated, and extracted with ethyl acetate (disappearance of the side product was observed confirming likelihood of it being the cyclized lactam). The organic was then washed with NaCl (1x), dried over MgSO4, and concentrated. The resulting residue was then purified on flash chromatography using a gradient of 0 – 5% MeOH/DCM to afford the desired product as a pale yellow solid (0.036 mg, 0.081 mmol, 78% yield). 1H NMR (400 MHz, DMSO-d6) δ 12.07 (s, 1H), 11.33 (s,1H), 9.65 (s, 1H), 8.97 – 8.90 (m, 1H), 8.30 (s, 1H), 8.15 (d, J = 2.5 Hz, 1H), 7.83 (d, J = 7.9 Hz,1H), 7.75 (s, 1H), 7.58 (s, 1H), 7.39 (t, J = 7.8 Hz, 1H), 7.30 (dd, J = 8.8, 2.5 Hz, 1H), 7.04 –6.92 (m, 3H), 6.29 (dd, J = 3.5, 1.9 Hz, 1H), 3.82 (s, 3H), 2.27 (q, J = 7.6 Hz, 2H), 1.07 (t, J =7.6 Hz, 3H). HPLC (gradient A): retention time = 4.927 min; purity = 95% Molecular Formula: $C_{23}H_{23}N_7O_3$, Mass Calc: 445.19, ESI-MS Found: 446.1938 [M+1]

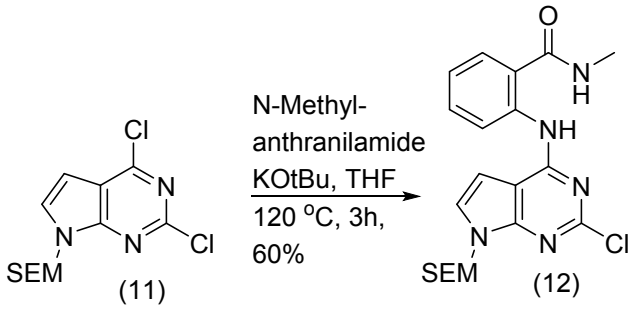


Scheme S3. Synthetic route to *para* substituted homologated probes, **16a-e**.



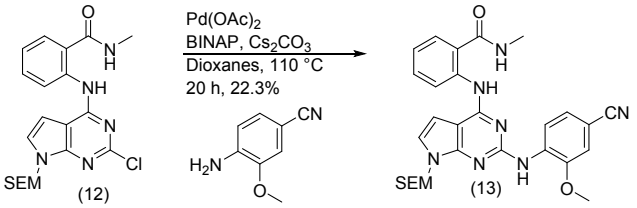
*2,4-Dichloro-7-((2-(trimethylsilyl)ethoxy)methyl)-7H-pyrrolo[2,3-d]pyrimidine (**11**)*

To a flame dried flask were added 5.0002 g (30 mmol) of 2,4-dichloro-7H-pyrrolo[2,3-d]pyrimidine (**5**), dissolved in 60 mL of DMF. The solution was cooled to 0 ˚C, and 666.3 mg of NaH (60% dispersion in mineral oil, 27.76 mmol, 0.9 equiv) were added portion wise. The solution was allowed to stir at room temperature for 30 min, then cooled back to 0 ˚C and 6.0 mL of SEM-Cl (30 mmol) then added. The solution was then allowed to warm to rt and stirred for 6 h. The reaction was quenched with 500 mL of brine and then extracted with EtOAc (3 x 150 mL). The combined organic layers were washed with brine (1 x 150 mL) and then dried over $MgSO_4$. The solvent was removed under pressure, and the yellow residue was taken up in hexanes. The solution was run through a silica plug, eluting with a gradient of hexanes to 5% EtOAc/hexanes. The solvent was removed under pressure to yield a white solid. Yield: 6.9200 g, 70% HPLC: 8.948 min, Molecular Formula: $C_{12}H_{17}Cl_2N_3OSi$, Mass Calc: 317.05, ESI MS-Found: 318 [M+1], purity: 99% ^{1}H NMR (500 MHz, Chloroform-d) δ 7.38 (d, J = 3.7 Hz, 1H), 6.68 (d, J = 3.7 Hz, 1H), 5.61 (s, 2H), 3.67 – 3.59 (m, 2H), 1.00 – 0.88 (m, 2H), -0.03 (s, 9H). ^{13}C NMR (126 MHz, cdcl3) δ 160.97, 152.57, 152.11, 129.20, 116.16, 100.82, 94.05, 66.74, 26.96, -1.72.



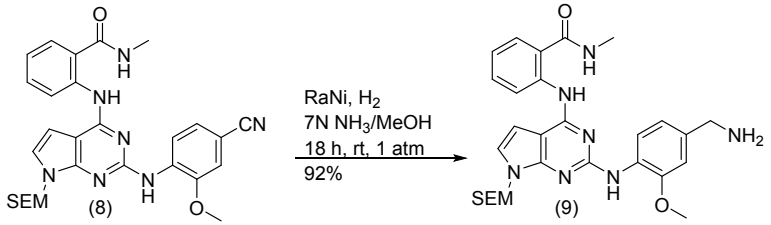
*2-((2-Chloro-7-((2-(trimethylsilyl)ethoxy)methyl)-7H-pyrrolo[2,3-d]pyrimidin-4-yl)amino)-N-methylbenzamide (**12**)*

A flask was charged with 1.000 g of **11** (3 mmol, 1 equiv.), 501.1 mg of 2-amino-N-methylbenzamide (3 mmol, 1 equiv.), and 16 mL of THF. The resulting solution was cooled to 0 ˚C and 702.1 mg of KOtBu (6.0 mmol, 2 equiv.) were added to form an orange solution which was heated to 120 ˚C for 3 h. The solution was cooled to rt and quenched with water, extracted with EtOAc (3 x 100 mL) and the combined organic layers were washed with brine (1 x 50mL). The organic layer was dried over Na_2SO_4 , filtered and concentrated in vacuo and purified by column chromatography eluting with 60% EtOAc/Hexanes to afford an off white solid. Yield: 802.0 mg, 60% HPLC: 8.96 min, Molecular Formula: $C_{20}H_{26}ClN_5O_2Si$, Mass Calc: 431.15, ESI-MS-Found: 432 [M+1], purity: 92% ^{1}HNMR (500 MHz, DMSO-d6) δ 12.06 (s, 1H), 8.80 (d, J = 5.0 Hz, 1H), 8.63 (d, J = 8.4 Hz, 1H), 7.79 (d, J = 7.9 Hz, 1H), 7.58 (t, J = 7.9 Hz, 1H), 7.51 (d, J = 3.5 Hz, 1H), 7.16 (t, J = 7.6 Hz, 1H), 6.56 (d, J = 3.5 Hz, 1H), 5.51 (s, 2H), 3.52 (t, J = 8.0 Hz, 2H), 2.82 (d, J = 4.4 Hz, 3H), 0.84 (t, J = 8.1 Hz, 2H), -0.08 (s, 9H). ^{13}CNMR (126 MHz, dmso) δ 169.60, 153.90, 152.61, 151.34, 140.04, 132.42, 128.62, 127.74, 122.56, 121.28, 121.13, 103.84, 98.70, 72.98, 66.03, 40.50, 40.34, 40.17, 40.00, 39.83, 39.67, 39.50, 26.82, 17.53, -0.96.



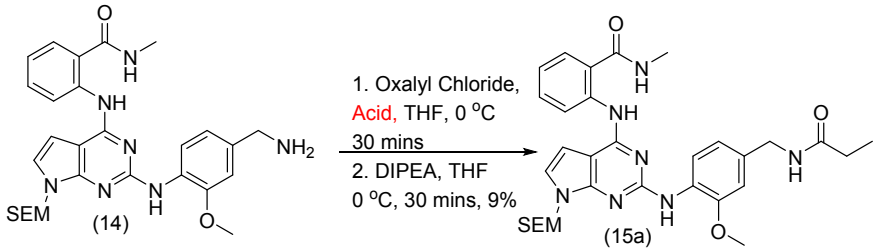
*2-((2-((4-Cyano-2-methoxyphenyl)amino)-7-((2-(trimethylsilyl)ethoxy)methyl)-7H-pyrrolo[2,3-d]pyrimidin-4-yl)amino)-N-methylbenzamide (**13**)*

Compound **13** was prepared using protocols described in the literature.[2] A flame dried three neck flask was charged with 201.1 mg of **12** (0.463 mmol,1 equiv), 795.6 mg of Cs_2CO_3 (2.32 mmol,5 equiv.), 60.3 mg of BINAP, racemic (0.00927 mmol, 0.2 equiv.), $Pd(OAc)_2$ (0.1 equiv., 5 mol%), and 84.1 mg of 4-amino-3-methoxybenzonitrile (0.555 mmol 1.2 equiv.). The mixture was degassed with three cycles of evacuation and back filled with nitrogen and 4 mL of dioxanes added. The solution was further degassed with an additional 4 cycles of evacuation and back filled with nitrogen, then heated to 110 ˚C for 20 h. The reaction mixture was passed through a pad of celite, washing with EtOAc and purified by column chromatography eluting with 50% EtOAc/Hexanes to afford a dark red solid 56.2 mg, 22.3% HPLC: 8.912 min, Molecular Formula: $C_{28}H_{33}N_7O_3Si$, Mass Calc: 543.24 ESI-MS-Found: 544.17 [M+1], purity: 90% ^{1}H NMR (500 MHz, Chloroform-d) δ 11.19 (s, 1H), 8.86 (dd, J = 8.5, 5.7 Hz, 2H), 7.78 (s, 1H), 7.56 – 7.49 (m, 2H), 7.10 – 7.05 (m, 2H), 7.01 (d, J = 3.6 Hz, 1H), 6.64 (d, J = 3.7 Hz, 1H), 5.56 (s, 2H), 3.62 – 3.57 (m, 2H), 3.05 (d, J = 4.8 Hz, 3H), 0.98 – 0.92 (m, 2H), -0.07 (d, J = 1.0 Hz, 9H).^{13}CNMR (126 MHz, dmso) δ 169.62, 163.07, 158.90, 153.91, 152.63, 151.36, 140.05, 132.42, 128.63, 127.73, 123.88, 122.57, 118.65, 117.79, 103.85, 98.72, 72.99, 66.05, 26.83, 17.53, -0.96.



*2-((2-((4-(Aminomethyl)-2-methoxyphenyl)amino)-7-((2-(trimethylsilyl)ethoxy)methyl)-7H-pyrrolo[2,3-d]pyrimidin-4-yl)amino)-N-methylbenzamide (**14**)*

To a dried flask were added 270.2 mg of **13** and10 mL (13 mmol) of 7N methanolic ammonia.[3] The solution was degassed with three rounds of evacuation and back filled with nitrogen. RaNi (slurry in water) was added and the mixture was further degassed with three rounds of evacuation and back filled with nitrogen. The atmosphere was then replaced with hydrogen and allowed to stir under H_2 for 6 h. Once complete, H_2 atmosphere was removed and the solution was passed through a pad of celite, washing with methanolic ammonia. The solvent was removed under pressure to give a light green solid, 250 mg, 92%. HPLC: 6.331 min, Molecular Formula: $C_{28}H_{37}N_7O_3Si$, Mass Calc: 547.27 ESI-MS-Found: 548.2719 [M+1], purity: 97%. ^{1}HNMR (500 MHz, DMSO-d6) δ 11.76 (s, 1H), 8.82 (dq, J = 30.6, 7.9 Hz, 2H), 8.41 (dd, J = 32.4, 24.4 Hz, 1H), 7.81 (q, J = 15.7, 10.9 Hz, 1H), 7.70 – 7.59 (m, 1H), 7.54 (dt, J = 25.5, 12.4 Hz, 1H), 7.21 (s, 1H), 7.15 – 7.05 (m, 2H), 6.93 (dt, J = 22.3, 10.2 Hz, 1H), 6.46 (s, 1H), 5.49 (d, J = 18.2 Hz, 2H), 4.08 – 4.04 (m, 2H), 3.90 (d, J = 25.2 Hz, 3H), 3.62 – 3.54 (m, 3H), 2.86 (s, 3H), 0.93 – 0.87 (m, 2H), -0.08 (d, J = 10.4 Hz, 9H).^{13}CNMR (126 MHz, dmso) δ 170.66, 169.78, 155.97, 153.45, 152.36, 148.46, 141.03, 134.79, 132.16, 128.36, 126.19, 124.07, 121.24, 121.13, 119.91, 119.23, 117.65, 113.43, 108.23, 99.45, 98.55, 72.40, 65.67, 60.11, 56.03, 26.68, 17.46, -1.09.

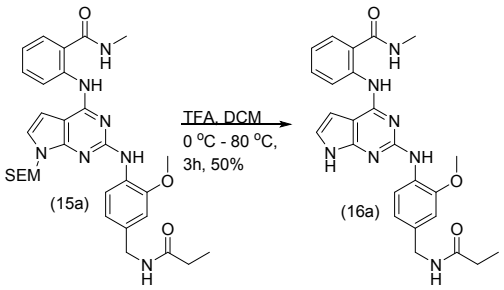


Acid = propionic acid

*2-((2-((2-methoxy-4-(propionamidomethyl)phenyl)amino)-7-((2-(trimethylsilyl)ethoxy)methyl)-7H-pyrrolo[2,3-d]pyrimidin-4-yl)amino)-N-methylbenzamide (**15a**)*

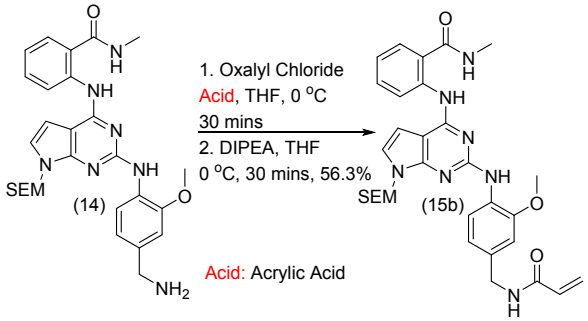
A dried flask was charged with 0.02 mL of propionic acid (0.0913 mmol, 1 equiv), 2 drops of DMF, and 2 mL of DCM (40 mmol). The mixture was cooled to 0 ˚C and 0.04 mL of oxalyl chloride (5 equiv., 0.5 mmol) were added. The mixture was allowed to warm to rt and stir for 1 h. Once complete, the solvent was removed under pressure and the light green residue was rinsed with DCM (3 x 10 mL). The green residue was then taken up in DCM (2 mL, 40 mmol), and the mixture was cooled back to 0 ˚C. At 0 ˚C were added 0.1 mL of DIPEA (0.0913 mmol,1.0 equiv.), and 50.4 mg of **14** (1 equiv, 0.09 mmol). The solution was warmed to rt for 3 h. Once complete the solvent was removed. The dark yellow residue was purified by preparatory TLC plate (EtOAc/1% MeOH). The band containing the desired material was collected, and the material was eluted off silica gel with acetone. The solvent was removed to give a light yellow solid, 5 mg, 9%. HPLC: 7.04 min, Molecular Formula: $C_{31}H_{41}N_7O_4Si$, Mass Calc: 603.30 ESI-MS-Found: 604 [M+1], purity: 90%. ^{1}HNMR (700 MHz, DMSO-d6) δ 11.69 (s, 1H), 8.82 – 8.78 (m, 1H), 8.75 (d, J = 4.8 Hz, 1H), 8.33 (d, J = 8.1 Hz, 1H), 8.22 (t, J = 5.8 Hz, 1H), 7.95 (s, 1H), 7.75 (dd, J = 7.9, 1.6 Hz, 1H), 7.63

(s, 1H), 7.52 – 7.46 (m, 1H), 7.18 (d, J = 3.6 Hz, 1H), 7.07 (td, J = 7.7, 1.2 Hz, 1H), 6.94 (d, J = 1.8 Hz, 1H), 6.83 (dd, J = 8.2, 1.8 Hz, 1H), 6.41 (d, J = 3.6 Hz, 1H), 5.47 (s, 2H), 4.24 (d, J = 5.9 Hz, 2H), 3.87 (s, 3H), 3.57 – 3.51 (m, 2H), 2.82 (d, J = 4.5 Hz, 3H), 2.15 (q, J = 7.6 Hz, 2H), 1.04 (t, J = 7.6 Hz, 3H), 0.89 – 0.84 (m, 2H), -0.10 (s, 9H).^{13}CNMR (176 MHz, dmso) δ 173.25, 169.89, 162.79, 156.06, 153.56, 152.46, 148.64, 141.08, 133.36, 132.27, 128.68, 124.24, 121.39, 120.11, 119.49, 118.29, 110.20, 99.53, 98.65, 72.54, 65.79, 56.15, 42.47, 29.04, 26.81, 17.58, 10.55, -0.95.



*2-((2-((2-methoxy-4-(propionamidomethyl)phenyl)amino)-7H-pyrrolo[2,3-d]pyrimidin-4-yl)amino)-N-methylbenzamide (**16a, CCG-264561**)*

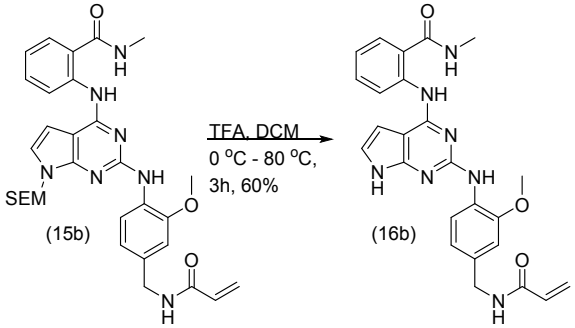
A dried flask was charged with 5 mg of **15a** (8 µmol, 1.0 equiv), and 2 mL of DCM. The solution was cooled to 0 ˚C, and 0.08 mL of TFA (25 equiv., 0.2 mmol) were added in one portion. The solution was warmed to 80 ˚C and stirred until complete. Once complete, the solvent was removed, and the residue was rinsed with DCM (3 x 5 mL). The yellow residue was then purified by preparatory TLC plate (1% MeOH/EtOAc). The band containing the desired material was collected, and the material was washed off silica gel with MeOH. The solvent was removed to give a light yellow oil, 2.0 mg, 50%. HPLC: 4.99 min, Molecular Formula: $C_{25}H_{27}N_7O_3$, Mass Calc: 473.22 ESI-MS-Found: 474 [M+1], purity: 92% ^{1}HNMR (700 MHz, DMSO-d6) δ 11.68 (s, 1H), 8.80 (d, J = 8.3 Hz, 1H), 8.75 (d, J = 5.1 Hz, 1H), 8.28 (d, J = 7.9 Hz, 1H), 8.23 (s, 1H), 7.75 (d, J = 7.7 Hz, 1H), 7.60 (s, 1H), 7.47 (t, J = 7.9 Hz, 1H), 7.13 (d, J = 3.6 Hz, 1H), 7.06 (t, J = 7.5 Hz, 1H), 6.93 (s, 1H), 6.84 (d, J = 8.1 Hz, 1H), 6.46 (t, J = 7.3 Hz, 1H), 6.38 (d, J = 3.6 Hz, 1H), 4.24 (d, J = 5.9 Hz, 2H), 3.86 (d, J = 4.3 Hz, 3H), 2.82 (d, J = 4.5 Hz, 3H), 2.16 (dd, J = 10.7, 4.7 Hz, 2H), 1.05 (t, J = 7.6 Hz, 3H). ^{13}CNMR (176 MHz, dmso) δ 173.25, 169.89, 162.79, 156.06, 153.56, 152.46, 148.64, 141.08, 133.36, 132.27, 128.68, 124.24, 121.39, 120.11, 119.49, 118.29, 110.20, 99.53, 92.08, 56.15, 42.47, 29.04, 26.81,10.55.



*2-((2-((4-(acrylamidomethyl)-2-methoxyphenyl)amino)-7-((2-(trimethylsilyl)ethoxy)methyl)-7H-pyrrolo[2,3-d]pyrimidin-4-yl)amino)-N-methylbenzamide (**15b**)*

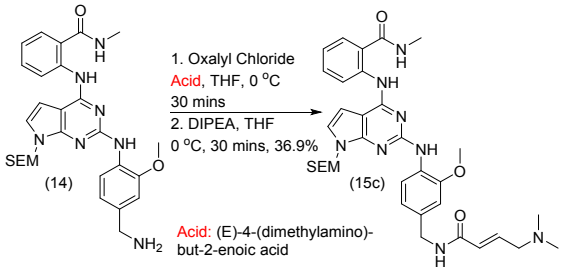
A dried flask was charged with 0.05 mL of acrylic acid (0.219 mmol, 1 equiv), 2 drops of DMF and 2 mL of THF (0.1 molar).The mixture was cooled to 0 ˚C and 0.1 mL of oxalyl chloride (1.14 mmol, 5.1 equiv) were added. The solution was allowed to warm to rt for 1 h. Once complete, the solvent

was removed under pressure and the light green residue was rinsed with DCM (3 x 10 mL). The light green residue was then taken up in DCM (5 mL, 40 mmol) and cooled to 0 ˚C. At 0 ˚C, 120 mg of **14** (0.219 mmol, 1 equiv) and 0.1 mL of DIPEA (0.57 mmol, 2.6 equiv) were added. The solution warmed to rt and stirred until reaction was complete. Once complete, the solvent was removed, evaporating product onto silica gel. The material was purified by column chromatography (EtOAc/Hex). The major product was eluted in MeOH and collected to give a light yellow solid, 70.6 mg, 53.6%. HPLC: 7.24 min, Molecular Formula: $C_{31}H_{39}N_7O_4Si$, Mass Calc: 601.28 ESI-MS-Found: 674 [M+Acrylic Acid], purity: 90%. ^{1}HNMR (700 MHz, DMSO-d6) δ 11.71 (d, J = 4.9 Hz, 1H), 8.80 (dt, J = 8.5, 3.0 Hz, 1H), 8.75 (q, J = 4.4 Hz, 1H), 8.55 (t, J = 5.9 Hz, 1H), 8.35 (d, J = 8.0 Hz, 1H), 7.95 (s, 1H), 7.76 (dt, J = 8.0, 2.0 Hz, 1H), 7.64 (s, 1H), 7.49 (ddd, J = 8.5, 7.1, 1.5 Hz, 1H), 7.20 – 7.17 (m, 1H), 7.10 – 7.04 (m, 1H), 6.97 (d, J = 1.8 Hz, 1H), 6.86 (dd, J = 8.2, 1.8 Hz, 1H), 6.42 (dd, J = 3.6, 1.6 Hz, 1H), 6.33 – 6.27 (m, 1H), 6.17 – 6.12 (m, 1H), 5.47 (s, 2H), 4.34 (t, J = 6.0 Hz, 2H), 3.87 (d, J = 4.5 Hz, 3H), 3.58 – 3.51 (m, 2H), 2.82 (d, J = 4.4 Hz, 3H), 0.88 – 0.84 (m, 2H), -0.10 (s, 9H).^{13}CNMR (176 MHz, dmso) δ 169.38, 164.43, 162.26, 155.53, 153.05, 151.94, 148.15, 140.58, 132.31, 131.76, 128.36, 127.95, 125.19, 123.73, 120.87, 120.76, 119.57, 119.28, 119.10, 109.93, 99.06, 98.14, 72.04, 65.29, 55.67, 42.21, 26.29, 17.08, -1.46.



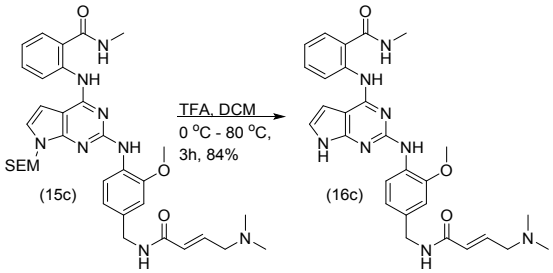
*2-((2-((4-(acrylamidomethyl)-2-methoxyphenyl)amino)-7H-pyrrolo[2,3-d]pyrimidin-4-yl)amino)-N-methylbenzamide (**16b, CCG-264099**)*

A dried flask was charged with 10 mg of **15b** (0.008 mmol, 1.0 equiv), and 2 mL of DCM (4 mmol). The solution was cooled to 0 ˚C, and 0.20 mL of TFA (150 equiv.) were added in one portion. The dark red solution warmed to 80 ˚C for 3 h. Once complete, the mixture was cooled to rt and quenched with saturated K_2CO_3 in MeOH. The mixture was then extracted with DCM (3 x 30 mL) and the combined organic layers were washed with brine (1 x 30 mL). The organic layer was dried over $MgSO_4$ and then purified by preparatory TLC plate (50% EtOAc/Hex with 5% MeOH) to yield a white solid, 4.7 mg, 60%. HPLC: 5.750 min, Molecular Formula: $C_{25}H_{25}N_7O_3$, Mass Calc: 471.20 ESI-MS-Found: 472 [M+1], purity: 96%. ^{1}HNMR (700 MHz, DMSO-d6) δ 11.43 (s, 1H), 10.72 (s, 1H), 8.78 (d, J = 8.8 Hz, 1H), 8.55 (t, J = 5.8 Hz, 1H), 8.22 (d, J = 8.1 Hz, 1H), 7.97 (dd, J = 8.0, 1.6 Hz, 1H), 7.61 – 7.56 (m, 2H), 7.10 (t, J = 7.9 Hz, 1H), 7.03 (dd, J = 3.5, 2.2 Hz, 1H), 6.96 (d, J = 1.8 Hz, 1H), 6.85 (dd, J = 8.2, 1.8 Hz, 1H), 6.40 (dd, J = 3.5, 1.9 Hz, 1H), 6.33 – 6.24 (m, 1H), 6.14 (dd, J = 17.1, 2.3 Hz, 1H), 5.62 (dd, J = 10.2, 2.2 Hz, 1H), 4.33 (d, J = 5.9 Hz, 2H), 3.86 (d, J = 8.8 Hz, 6H).. ^{13}CNMR (176 MHz, dmso) δ 168.98, 164.94, 155.90, 153.28, 152.97, 149.14, 142.41, 134.50, 133.02, 132.25, 131.05, 128.99, 125.74, 121.55, 121.40, 121.11, 119.83, 116.30, 110.55, 99.56, 97.82, 56.17, 42.78, 26.29, 17.08.



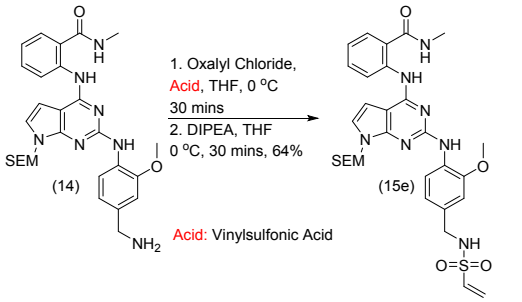
*(E)-2-((2-((4-((4-(dimethylamino)but-2-enamido)methyl)-2-methoxyphenyl)amino)-7-((2-(trimethylsilyl)ethoxy)methyl)-7H-pyrrolo[2,3-d]pyrimidin-4-yl)amino)-N-methylbenzamide (**15c**)*

A dried flask was charged with 29.7 mg of (E)-4-(dimethylamino)but-2-enoic acid (1.0 equiv., 0.219 mmol), 3 drops of DMF, and 2 mL of THF (0.1 molar). . The mixture was cooled to 0 ˚C and 0.02 mL of oxalyl chloride (1.1 equiv., 0.241 mmol) were added. The solution was warmed to rt and allowed for 1 h. Once complete, the solvent was removed under pressure and the light green residue was rinsed with DCM (3 x 10 mL). The light green residue was then taken up in THF (5 mL, 40 mmol) and cooled to 0 ˚C. At 0 ˚C were added 120.0 mg of **14** (0.219 mmol, 1.0 equiv) and 0.1 mL of DIPEA (2.6 equiv., 0.57 mmol). The mixture was warmed to rt and stirred until reaction was complete. Once complete, the solvent was removed, evaporating product onto silica gel. The material was purified by column chromatography (30-50% EtOAc/Hex). The major product was collected, and the solvent was removed under pressure to give a light yellow solid, 53.2 mg, 36.9% HPLC: 7.288 min, Molecular Formula: $C_{34}H_{46}N_8O_4Si$, Mass Calc: 658.34 ESI-MS-Found: 659.3460 [M+H], purity: 80%. ^{1}HNMR (700 MHz, Chloroform-d) δ 8.91 (dd, J = 11.7, 8.4 Hz, 2H), 8.64 – 8.60 (m, 2H), 8.10 (dd, J = 20.4, 7.9 Hz, 1H), 7.66 (s, 1H), 7.64 (d, J = 8.1 Hz, 2H), 7.28 (d, J = 1.9 Hz, 1H), 7.20 (t, J = 7.3 Hz, 2H), 7.00 (s, 1H), 6.99 (d, J = 2.0 Hz, 1H), 6.98 (s, 1H), 6.83 (d, J = 1.7 Hz, 1H), 6.32 (d, J = 7.7 Hz, 1H), 5.97 (d, J = 15.3 Hz, 1H), 5.53 (s, 2H), 4.47 (d, J = 5.6 Hz, 2H), 3.91 (s, 3H), 3.55 (dt, J = 8.3, 2.2 Hz, 3H), 3.07 (dd, J = 6.2, 1.6 Hz, 2H), 3.02 (d, J = 4.7 Hz, 3H), 2.18 (s, 6H), 0.88 (t, J = 6.9 Hz, 2H), -0.09 (s, 9H). ^{13}CNMR (176 MHz, cdcl3) δ 168.97, 167.63, 166.29, 155.12, 151.63, 147.90, 143.92, 143.34, 132.23, 130.15, 128.90, 122.70, 121.88, 120.48, 118.89, 117.88, 117.18, 116.44, 111.80, 104.18, 100.23, 87.02, 66.28, 63.24, 55.93, 49.42, 44.04, 26.99, 23.95, -1.29.



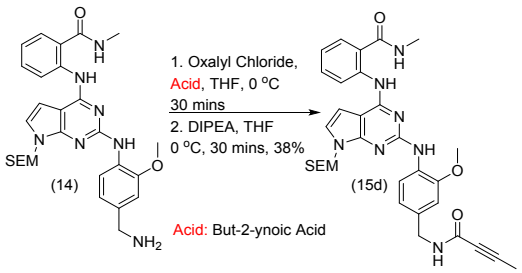
*(E)-2-((2-((4-((4-(dimethylamino)but-2-enamido)methyl)-2-methoxyphenyl)amino)-7H-pyrrolo[2,3-d]pyrimidin-4-yl)amino)-N-methylbenzamide (**16c**):*

Compound **16c** was synthesized using the protocol described for **16b**. Yields a bright yellow solid, 40.5 mg, 84%. HPLC: 4.72 min, Molecular Formula: $C_{28}H_{32}N_8O_3$, Mass Calc: 528.26 ESI-MS-Found: 559 [M+MeOH], purity: 95%. ^{1}HNMR (700 MHz, DMSO-d6) δ 11.69 (s, 2H), 8.81 (d, J = 8.4 Hz, 1H), 8.75 (s, 1H), 8.32 – 8.28 (m, 2H), 7.76 (d, J = 8.0 Hz, 1H), 7.60 (s, 1H), 7.48 (t, J = 7.7 Hz, 1H), 7.14 (d, J = 3.3 Hz, 1H), 7.07 (t, J = 7.3 Hz, 1H), 6.94 (s, 1H), 6.85 (d, J = 8.1 Hz, 1H), 6.46 (s, 1H), 6.39 (d, J = 3.4 Hz, 1H), 4.25 – 4.21 (m, 2H), 3.87 (s, 3H), 3.06 (m, 2H), 2.83 (d, J = 4.4 Hz, 3H), 2.18 (s, 6H).^{13}CNMR (176 MHz, dmso) δ 168.73, 167.68, 166.40, 155.97, 150.40, 147.03, 144.02, 143.35, 132.93, 130.30, 128.36, 122.80, 121.68, 120.05, 118.89, 117.96, 117.13, 116.36, 111.84, 99.30, 91.72, 63.80, 60.57, 55.97, 46.71, 43.98, 26.73.



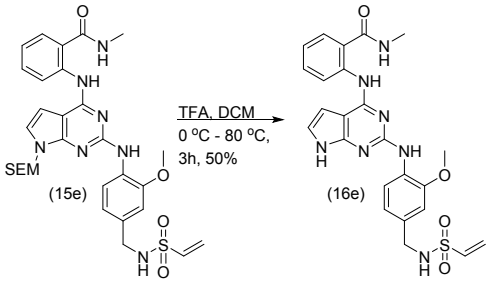
*2-((2-((2-methoxy-4-(vinylsulfonamidomethyl)phenyl)amino)-7-((2-(trimethylsilyl)ethoxy)methyl)-7H-pyrrolo[2,3-d]pyrimidin-4-yl)amino)-N-methylbenzamide (**15e**)*

Compound **15e** was synthesized using the protocol described for **15a**. Yields a yellow solid, 15 mg, 64%. HPLC: 7.163 min, Molecular Formula: $C_{30}H_{39}N_7O_5SSi$, Mass Calc: 637.25, ESI-MS-Found: 638 [M+1], purity: 95% ^{1}HNMR (700 MHz, Chloroform-d) δ 11.61 (s, 1H), 8.93 (d, J = 8.5 Hz, 1H), 8.29 (s, 1H), 8.10 (d, J = 7.2 Hz, 1H), 8.05 (s, 2H), 7.75 (m, 1H), 7.54 (m, 1H), 7.42 (m, 1H), 7.36 (s, 1H), 7.22 (d, J = 8.2 Hz, 1H), 7.05 (d, J = 9.2 Hz, 2H), 6.97 (m, 2H), 6.25 (m, 2H), 6.05 (d, J = 9.0 Hz, 1H), 5.80 (d, J = 10.0 Hz, 1H), 5.57 (s, 2H), 3.87 (d, J = 13.2 Hz, 3H), 3.54 (m, 2H), 3.36 (s, 2H), 3.04 (d, J = 5.1 Hz, 3H), 0.85 (m, 2H), -0.08 (s, 9H).^{13}CNMR (176 MHz, cdcl3) δ 168.42, 167.74, 154.59, 151.23, 147.52, 144.10, 135.80, 133.05, 132.47, 130.63, 128.35, 123.63, 119.80, 119.02, 117.86, 117.16, 116.42, 110.90, 103.45, 100.62, 86.55, 66.35, 56.77, 45.81, 26.52, 23.88, -1.64.



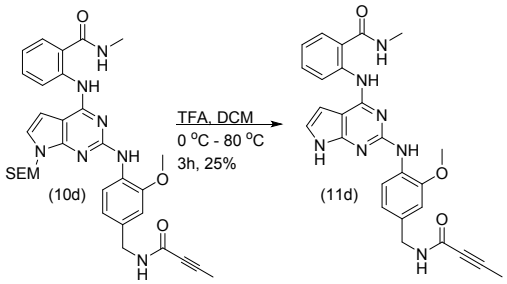
*2-((2-((4-(but-2-ynamidomethyl)-2-methoxyphenyl)amino)-7-((2-(trimethylsilyl)ethoxy)methyl)-7H-pyrrolo[2,3-d]pyrimidin-4-yl)amino)-N-methylbenzamide (**15d**)*

Compound **15d** was synthesized using the protocol described for **15a**. Yields a light yellow solid, 15 mg, 38%. HPLC: 7.15 min, Molecular Formula: $C_{32}H_{39}N_7O_4Si$, Mass Calc: 613.28, ESI-MS-Found: 614 [M+1], purity: 80% ^{1}HNMR (700 MHz, Chloroform-d) δ 11.09 (s, 1H), 8.91 (d, J = 8.4 Hz, 1H), 8.64 (d, J = 8.2 Hz, 1H), 8.21 (s, 1H), 7.51 (d, J = 7.7 Hz, 1H), 7.49 (t, J = 6.2 Hz, 1H), 7.01 (t, J = 7.5 Hz, 1H), 6.94 (d, J = 3.6 Hz, 1H), 6.87 (dd, J = 8.3, 1.8 Hz, 1H), 6.82 (d, J = 1.9 Hz, 1H), 6.60 (d, J = 3.5 Hz, 1H), 6.49 (d, J = 5.6 Hz, 1H), 6.39 (d, J = 5.4 Hz, 1H), 5.53 (s, 2H), 4.42 (d, J = 5.7 Hz, 1H), 3.92 (s, 3H), 3.51 – 3.43 (m, 3H), 2.99 (s, 3H), 1.93 (s, 3H), 0.94 (t, J = 8.2 Hz, 2H), -0.08 (s, 9H). ^{13}CNMR (176 MHz, cdcl3) δ 170.35, 156.89, 155.81, 153.79, 152.77, 149.75, 147.90, 141.29, 132.28, 130.27, 129.29, 126.79, 122.71, 121.86, 121.11, 120.53, 118.22, 109.83, 99.59, 83.75, 66.30, 55.94, 49.28, 27.01, 17.83, 3.84, -1.29.



*2-((2-((2-methoxy-4-(vinylsulfonamidomethyl)phenyl)amino)-7H-pyrrolo[2,3-d]pyrimidin-4-yl)amino)-N-methylbenzamide (**16e**)*

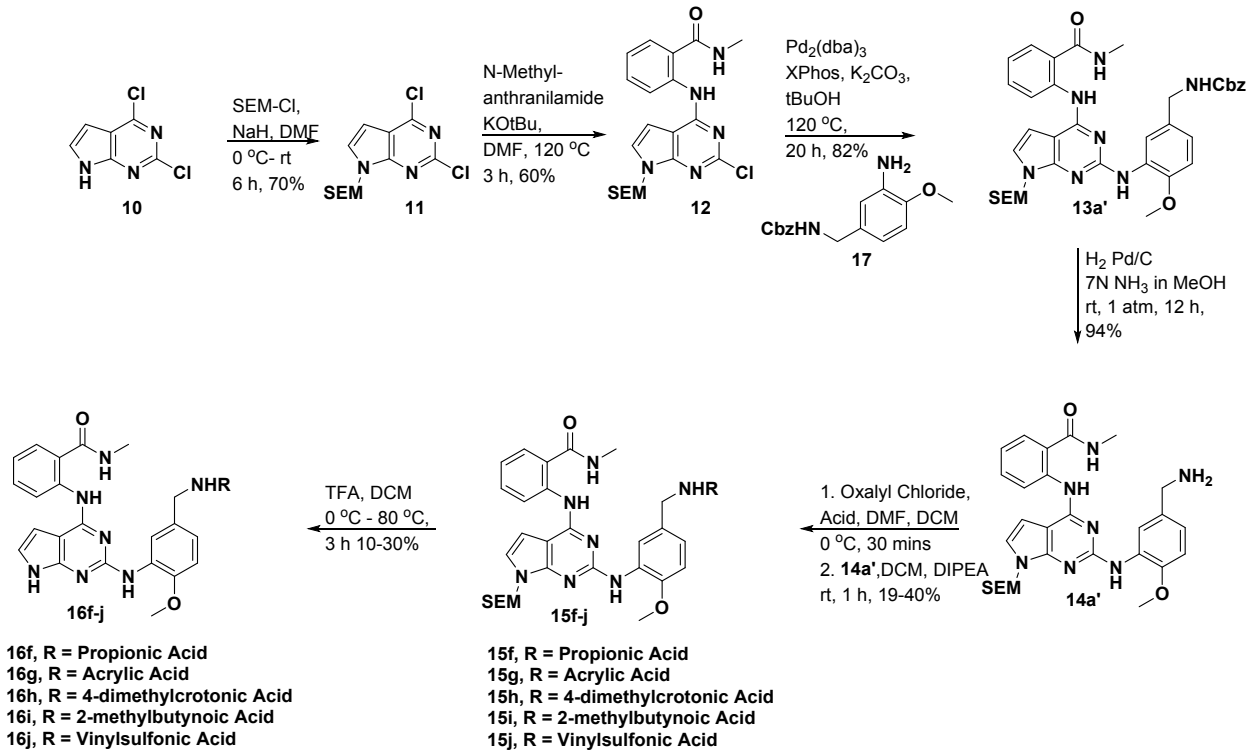
Compound **16e** was synthesized using the protocol described for **16b**. Yields a yellow solid, 6 mg, 50% HPLC: 5.7 min, Molecular Formula: $C_{24}H_{25}N_7O_4S$, Mass Calc: 507.17 ESI-MS-Found: 508 [M+1], purity: 95%^{1}H NMR (700 MHz, Chloroform-d) δ 11.60 (d, J = 18.4 Hz, 2H), 9.47 (d, J = 2.3 Hz, 1H), 9.10 (s, 1H), 8.91 (d, J = 9.1 Hz, 1H), 8.44 (d, J = 7.7 Hz, 1H), 7.85 (s, 1H), 7.80 (d, J = 8.6 Hz, 1H), 7.56 (s, 1H), 7.50 – 7.47 (m, 1H), 7.45 (s, 1H), 7.19 (s, 2H), 7.14 (s, 2H), 7.10 (s, 1H), 7.06 (s, 2H), 6.67 (d, J = 19.8 Hz, 1H), 6.28 (s, 1H), 5.65 (s, 1H), 3.86 (s, 3H), 3.35 (d, J = 2.7 Hz, 2H), 3.04 (d, J = 4.4 Hz, 3H).^{13}CNMR (176 MHz, dmso) δ 168.35, 167.93, 155.05, 150.76, 147.84, 143.92, 135.24, 132.83, 132.37, 130.53, 128.35, 121.68, 119.90, 118.68, 117.80, 117.14, 116.46, 111.78, 99.28, 91.81, 56.08, 47.41, 25.80.



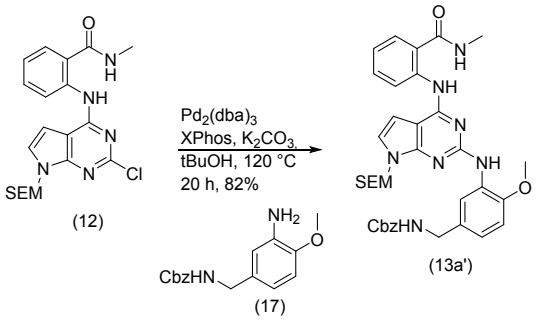
*2-((2-((4-(but-2-ynamidomethyl)-2-methoxyphenyl)amino)-7H-pyrrolo[2,3-d]pyrimidin-4-yl)amino)-N-methylbenzamide (**16d**)*

Compound **16d** was synthesized using the protocol described for **16b**. Yields a white solid, 10 mg, 25% HPLC: 5.3 min, Molecular Formula: $C_{26}H_{25}N_7O_3$, Mass Calc: 483.20 ESI-MS-Found: 484 [M+1], purity: 95%.^{1}HNMR (700 MHz, DMSO-d6) δ 11.64 (s, 1H), 11.37 (s, 1H), 8.95 (t, J = 6.2 Hz, 1H), 8.84 (d, J = 8.4 Hz, 1H), 8.73 (d, J = 4.6 Hz, 1H), 8.23 (d, J = 8.1 Hz, 1H), 7.75 (d, J = 7.8 Hz, 1H), 7.53 (s, 1H), 7.49 (t, J = 7.9 Hz, 1H), 7.05 (t, J = 7.5 Hz, 1H), 7.01 (d, J = 3.3 Hz, 1H), 6.93 (s, 1H), 6.81 (d, J = 8.1 Hz, 1H), 6.33 (s, 1H), 4.25 (d, J = 6.1 Hz, 2H), 3.86 (s, 3H), 2.83 (d, J = 4.4 Hz, 3H), 1.97 (d, J = 1.1 Hz, 3H). ^{13}CNMR (176 MHz, cdcl3) δ 170.17, 156.71, 155.63, 153.61, 153.23, 152.59, 149.57, 147.72, 141.11, 132.10, 130.09, 129.11, 126.61, 122.53, 121.68, 120.93, 120.35, 120.02, 118.04, 109.65, 99.41, 55.76, 44.03, 26.83, 3.66.

*Synthesis of **16f-j***



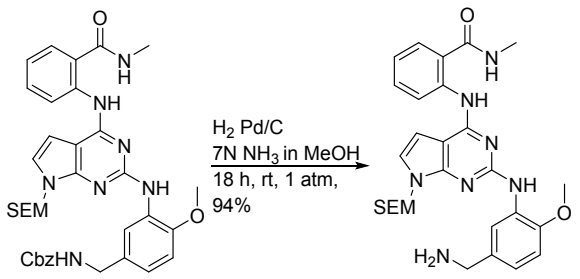
Scheme S4. Synthetic route for *meta* homologated compounds **16f-j**.



*Benzyl-(4-methoxy-3-((4-((2-(methylcarbamoyl)phenyl)amino)-7-((2-(trimethylsilyl)ethoxy)methyl)-7H-pyrrolo[2,3-d]pyrimidin-2-yl)amino)benzyl)carbamate (**13a**')*

A flame dried three neck flask was charged with 90.0 mg of **12** (0.21 mmol, 1.0 equiv), 68.2 mg of K_2CO_3 (0.42 mmol, 2 equiv.), 5.5 mg of XPhos (0.012 mmol, 0.055 equiv), 2.1 mg of $Pd_2(dba)_3$ (0.002 mmol,0.01 equiv) and 4 mL of tBuOH. The mixture was degassed with 8 cycles of evacuation and back filled with nitrogen. The solution was heated to 110 ˚C for 20 h. Once complete, the solution was passed through a pad of celite, and purified by column chromatography (eluting with 50% EtOAc/Hexanes). The solvent was removed under pressure to give a dark red solid, 120.0 mg, 82% HPLC: 8.109 min, Molecular Formula: $C_{36}H_{43}N_7O_5Si$, Mass Calc: 681.31 ESI-MS-Found: 682 [M+1], purity: 97% ^{1}HNMR (700 MHz, $CDCl_3$-d) δ 11.03 (s, 1H), 8.84 (d, J = 8.4 Hz, 1H), 8.69 (s, 1H), 7.55 (s, 1H), 7.46 (t, J = 8.1 Hz, 1H), 7.42 (d, J = 7.8 Hz, 1H), 7.33 (dd, J = 13.1, 5.8 Hz, 4H), 7.31 – 7.27 (m, 1H), 6.96 – 6.91 (m, 2H), 6.82 (q, J = 8.5 Hz, 2H), 6.59 (d, J = 3.7 Hz, 1H), 6.41 (q, J = 4.6 Hz, 1H), 5.49 (s, 2H), 5.13 (s, 2H), 4.33 (d, J = 5.7 Hz, 2H), 3.90 (s, 3H), 3.59 – 3.54 (m, 2H), 2.96 (d, J = 4.8 Hz, 3H), 0.93 (t, J = 8.2 Hz, 2H), -0.09 (s, 9H). ^{13}CNMR (176 MHz, $CDCl_3$) δ 170.45, 156.52, 155.88, 153.86, 152.88, 147.15, 141.32,

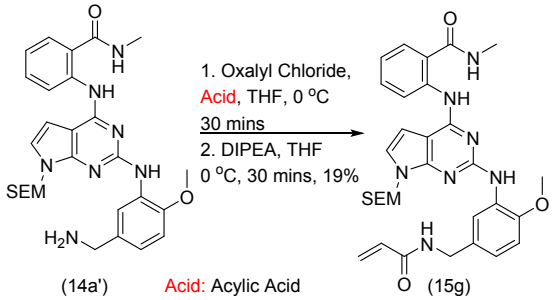
136.93, 132.19, 130.89, 128.72, 128.28, 128.23, 127.02, 122.90, 121.81, 121.24, 120.55, 119.57, 117.59, 109.96, 100.38, 99.59, 66.33, 56.06, 45.65, 27.03, 17.88, -1.21.



(13a') (14a')

2-((2-((5-(aminomethyl)-2-methoxyphenyl)amino)-7-((2-(trimethylsilyl)ethoxy)methyl)-7H-pyrrolo[2,3-d]pyrimidin-4-yl)amino)-N-methylbenzamide (**14a**')

A dried flask was charged with 120 mg of **13a**' (0.176 mmol, 1.0 equiv) and 3 mL of 7N NH_3 in MeOH. The solution was degassed with three rounds of evacuation and back filled with nitrogen, and then RaNi (slurry in water), was added. The solution was further degassed by three cycles of evacuation and back fill with nitrogen. The atmosphere was replaced with hydrogen, and the mixture was stirred for 12 hours. Once complete, the atmosphere was removed and then solution was passed through a pad of celite. The solvent was removed to yield a green solid, 91.8 mg, 93.5%. Molecular Formula: $C_{28}H_{37}N_7O_3Si$, Mass Calc: 547.27 ESI-MS-Found: 548 [M+1], HPLC: 6.241min, purity: 98.2%. ^{1}HNMR (500 MHz, Chloroform-d) δ 10.61 (s, 1H), 8.55 – 8.48 (m, 1H), 8.44 (d, J = 8.0 Hz, 1H), 8.36 (s, 1H), 7.56 (d, J = 10.3 Hz, 1H), 7.50 (s, 1H), 7.42 (d, J = 8.6 Hz, 1H), 7.10 (d, J = 7.5 Hz, 1H), 7.03 (s, 1H), 7.01 – 6.96 (m, 1H), 6.91 (dd, J = 10.3, 7.4 Hz, 2H), 6.80 (d, J = 8.1 Hz, 1H), 6.71 (t, J = 9.6 Hz, 1H), 6.47 (d, J = 9.9 Hz, 1H), 5.51 (s, 2H), 3.89 – 3.84 (m, 3H), 3.55 (t, J = 8.4 Hz, 2H), 3.03 – 2.93 (m, 3H), 0.96 – 0.89 (m, 2H), -0.07 (d, J = 2.7 Hz, 9H). ^{13}CNMR (176 MHz, cdcl3) δ 169.94, 155.53, 153.31, 152.31, 140.77, 131.64, 126.52, 122.13, 121.28, 120.51, 119.78, 117.79, 109.92, 99.67, 99.06, 77.03, 77.00, 76.84, 76.82, 76.66, 76.64, 72.21, 65.78, 55.36, 26.43, 17.33, 13.86, -0.33, -1.75, -1.78. (126 MHz, cdcl3) δ 168.39, 167.61, 155.35, 153.84, 147.88, 144.63, 140.11, 133.00, 131.50, 130.70, 128.22, 121.92, 120.22, 119.20, 118.07, 116.49, 114.73, 103.48, 99.40, 86.60, 66.10, 55.72, 41.14, 26.69, 17.66, -1.46.

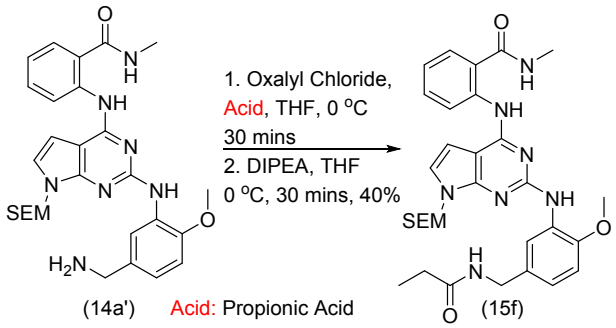


(14a') Acid: Acylic Acid (15g)

*2-((2-((5-(acrylamidomethyl)-2-methoxyphenyl)amino)-7-((2-(trimethylsilyl)ethoxy)methyl)-7H-pyrrolo[2,3-d]pyrimidin-4-yl)amino)-N-methylbenzamide (**15g**)*

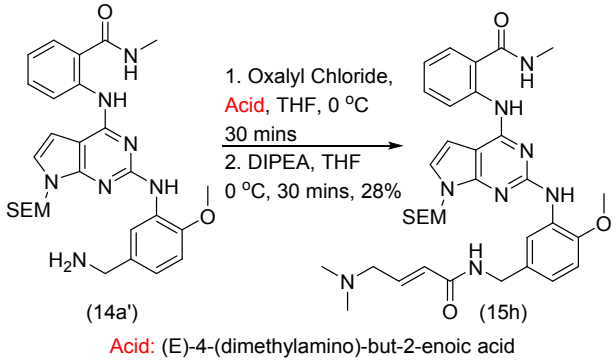
Compound **15g** was synthesized using the protocol described for **15a**. Yields a light yellow residue, 13.9 mg, 19%. HPLC: 7.104 min, Molecular Formula: $C_{31}H_{39}N_7O_4Si$, Mass Calc: 601.28 ESI-MS-Found: 602.2900 [M+1], purity: 74.4%. ^{1}HNMR (700 MHz, $CDCl_3$) δ 11.03 (s, 1H), 8.81 (s, 1H), 8.65 (s, 1H), 7.50 – 7.45 (m, 2H), 7.01 (q, J = 8.1, 7.6 Hz, 1H), 6.93 (t, J = 3.7 Hz, 1H),

6.85 (d, J = 8.5 Hz, 1H), 6.83 (d, J = 8.2 Hz, 1H), 6.60 – 6.55 (m, 2H), 6.31 (dd, J = 3.0, 1.8 Hz, 1H), 6.28 (dd, J = 2.8, 1.8 Hz, 1H), 5.66 (dd, J = 10.5, 2.0 Hz, 1H), 5.62 (dd, J = 10.3, 1.6 Hz, 1H), 5.51 (s, 2H), 4.44 (dd, J = 9.8, 5.6 Hz, 2H), 3.91 (d, J = 2.7 Hz, 3H), 3.59 – 3.55 (m, 2H), 3.01 (d, J = 3.7 Hz, 3H), 0.96 – 0.89 (m, 2H), -0.08 (d, J = 9.7 Hz, 9H). ^{13}CNMR (176 MHz, cdcl3) δ 170.30, 165.28, 155.82, 153.81, 150.34, 147.14, 143.85, 132.16, 131.02, 130.18, 127.82, 127.71, 126.58, 122.87, 121.85, 119.99, 118.04, 117.18, 116.36, 110.04, 99.67, 86.17, 66.30, 56.00, 44.21, 27.00, -1.29.



*2-((2-((2-methoxy-5-(propionamidomethyl)phenyl)amino)-7-((2-(trimethylsilyl)ethoxy)methyl)-7H-pyrrolo[2,3-d]pyrimidin-4-yl)amino)-N-methylbenzamide (**15f**)*

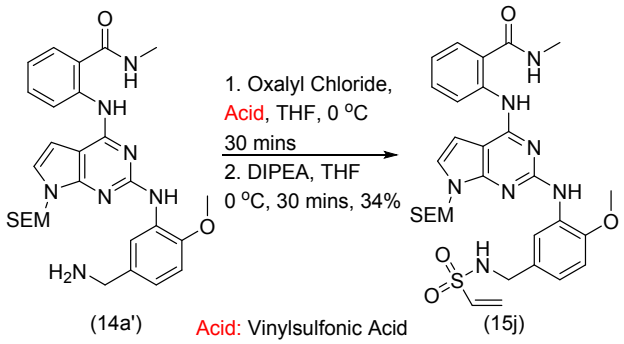
Compound **15f** was synthesized using the protocol described for **15a**. Yields aa light yellow residue,20 mg, 40%. HPLC: 6.781 min, Molecular Formula: $C_{31}H_{41}N_7O_4Si$, Mass Calc: 603.30 ESI-MS-Found: 603.3219 [M+1], purity: 99% ^{1}HNMR (500 MHz, Chloroform-d) δ 11.13 (s, 1H), 8.88 (d, J = 8.4 Hz, 1H), 8.61 (d, J = 8.2 Hz, 1H), 7.99 (s, 1H), 7.54 (s, 1H), 7.50 (d, J = 7.9 Hz, 2H), 7.00 (t, J = 7.6 Hz, 1H), 6.94 (d, J = 3.6 Hz, 1H), 6.87 (d, J = 8.3 Hz, 1H), 6.82 (s, 1H), 6.71 (d, J = 8.0 Hz, 1H), 6.64 (d, J = 1.9 Hz, 1H), 6.62 (s, 1H), 6.60 (t, J = 3.8 Hz, 2H), 6.53 (d, J = 5.7 Hz, 1H), 5.53 (s, 2H), 4.28 (d, J = 5.5 Hz, 3H), 3.91 (s, 3H), 3.59 (t, J = 8.2 Hz, 2H), 3.01 (d, J = 4.7 Hz, 3H), 2.80 (s, 2H), 2.24 (dq, J = 18.6, 7.6 Hz, 2H), 1.18 (dt, J = 15.0, 7.6 Hz, 3H), 0.94 (t, J = 8.2 Hz, 2H), -0.07 (s, 9H).^{13}CNMR (126 MHz, cdcl3) δ 173.53, 170.13, 162.56, 153.57, 152.35, 147.76, 146.72, 140.95, 136.35, 132.00, 130.94, 130.38, 129.69, 126.73, 122.55, 121.67, 121.03, 120.14, 118.19, 117.76, 114.49, 110.29, 109.65, 100.00, 99.45, 72.58, 66.11, 55.52, 43.36, 26.78, 17.64, 9.83, -1.49.



*(E)-2-((2-((5-((4-(dimethylamino)but-2-enamido)methyl)-2-methoxyphenyl)amino)-7-((2-(trimethylsilyl)ethoxy)methyl)-7H-pyrrolo[2,3-d]pyrimidin-4-yl)amino)-N-methylbenzamide (**15h**)*

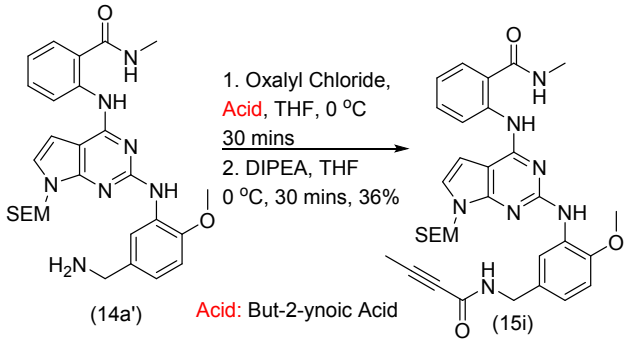
Compound **15h** was synthesized using the protocol described for **15a**. Yields a light yellow residue, 20 mg, 28%. HPLC: 7.65 min, Molecular Formula: $C_{34}H_{46}N_8O_4Si$, Mass Calc: 658.34 ESI-MS-Found: 662 [M+4], purity: 85%. ^{1}HNMR (700 MHz, Chloroform-d) δ 10.87 (s, 1H), 8.78 (d, J

= 8.3 Hz, 1H), 8.70 – 8.65 (m, 1H), 7.57 (s, 1H), 7.49 (d, J = 7.7 Hz, 2H), 7.23 (d, J = 6.5 Hz, 1H), 7.03 (t, J = 7.6 Hz, 1H), 6.96 (d, J = 3.6 Hz, 1H), 6.85 (d, J = 3.0 Hz, 2H), 6.60 (d, J = 3.5 Hz, 1H), 6.44 (d, J = 5.5 Hz, 1H), 5.54 (s, 2H), 4.40 (d, J = 5.8 Hz, 2H), 3.93 (s, 3H), 3.60 – 3.55 (m, 2H), 3.03 (d, J = 4.8 Hz, 3H), 0.94 (ddd, J = 10.9, 7.6, 3.2 Hz, 2H), -0.08 (s, 9H).^{13}CNMR (176 MHz, cdcl3) δ 169.99, 160.06, 156.21, 155.36, 153.45, 152.51, 146.93, 140.68, 131.63, 130.59, 128.68, 126.62, 122.54, 121.86, 120.99, 120.81, 119.65, 117.58, 109.60, 99.95, 99.16, 65.90, 55.63, 44.10, 26.61, 21.35, 17.43, -1.66.



*2-((2-((2-methoxy-5-(vinylsulfonamidomethyl)phenyl)amino)-7-((2-(trimethylsilyl)ethoxy)methyl)-7H-pyrrolo[2,3-d]pyrimidin-4-yl)amino)-N-methylbenzamide (**15j**)*

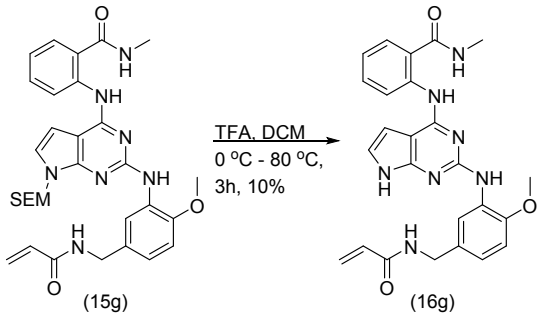
Compound **15j** was synthesized using the protocol described for **15a**. Yields a light yellow residue, 10 mg, 34%. HPLC: 7.534 min, Molecular Formula: $C_{30}H_{39}N_7O_5SSi$, Mass Calc: 637.25 ESI-MS-Found: 638 [M+1], purity: 85%. ^{1}HNMR (400 MHz, Chloroform-*d*) δ 8.37 (s, 1H), 8.04 (s, 1H), 7.50 (s, 1H), 6.89 (s, 3H), 6.50 (s, 1H), 6.22 (s, 1H), 5.91 (s, 1H), 5.57 (s, 2H), 4.23 (m, 2H), 3.95 (s, 3H), 3.61 (s, 2H), 3.37 (d, *J* = 16.6 Hz, 2H), 2.96 – 2.81 (m, 3H), 0.88 (d, *J* = 24.2 Hz, 2H), -0.07 (s, 9H). ^{13}CNMR (176 MHz, cdcl3) δ 169.98, 165.69, 155.06, 150.31, 147.80, 143.19, 135.08, 133.85, 131.70, 128.59, 122.86, 121.52, 120.55, 118.62, 117.86, 116.77, 115.07, 110.45, 103.85, 100.40, 74.30, 68.65, 55.73, 44.36, 25.17, 22.46, 17.58, -1.56.



*2-((2-((5-(but-2-ynamidomethyl)-2-methoxyphenyl)amino)-7-((2-(trimethylsilyl)ethoxy)methyl)-7H-pyrrolo[2,3-d]pyrimidin-4-yl)amino)-N-methylbenzamide (**15i**)*

Compound **15i** was synthesized using the protocol described for **15a**. Yields a light yellow residue, 10 mg, 36%. HPLC: 8.610 min, Molecular Formula: $C_{32}H_{39}N_7O_4Si$, Mass Calc: 613.28 ESI-MS-Found: 623, purity: 65%. ^{1}HNMR (500 MHz, Chloroform-d) δ 11.10 (s, 1H), 8.92 (d, J = 8.4 Hz, 1H), 8.65 (d, J = 8.3 Hz, 1H), 7.52 (d, J = 7.1 Hz, 1H), 7.49 (d, J = 8.2 Hz, 1H), 7.02 (t, J = 7.7 Hz, 1H), 6.95 (d, J = 3.6 Hz, 1H), 6.88 (d, J = 8.4 Hz, 1H), 6.83 (s, 1H), 6.72 (d, J = 8.2 Hz, 1H), 6.65 (d, J = 2.0 Hz, 1H), 6.63 (d, J = 2.3 Hz, 1H), 6.62 – 6.60 (m, 2H), 5.55 (d, J = 7.9 Hz, 2H), 4.32 (d, J = 5.7 Hz, 3H), 3.93 (s, 2H), 3.60 (t, J = 8.1 Hz, 2H), 3.03 (d, J = 4.7 Hz, 3H), 1.93

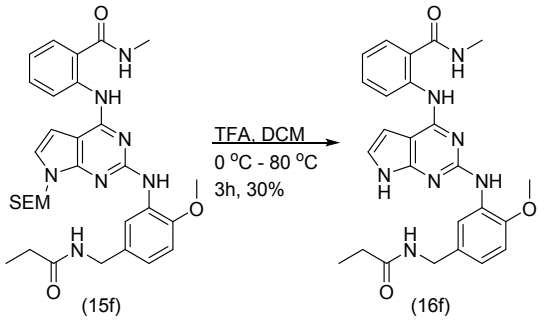
(s, 4H), 0.95 (t, J = 8.1 Hz, 2H), -0.06 (d, J = 4.0 Hz, 9H). ^{13}CNMR (126 MHz, cdcl3) δ 170.42, 155.90, 153.88, 153.48, 152.86, 148.01, 147.13, 141.38, 136.74, 132.34, 130.24, 129.42, 126.86, 122.79, 121.95, 121.19, 120.60, 120.30, 118.34, 118.09, 114.70, 110.58, 109.93, 100.33, 99.66, 83.71, 72.81, 66.38, 55.81, 43.86, 27.07, 17.92, 3.86, -1.21.



(15g) (16g)

*2-((2-((5-(acrylamidomethyl)-2-methoxyphenyl)amino)-7-((2-(trimethylsilyl)ethoxy)methyl)-7H-pyrrolo[2,3-d]pyrimidin-4-yl)amino)-N-methylbenzamide (**16g**)*

Compound **16g** was synthesized using the protocol described for **16b**. Yields a light yellow solid, 2 mg, 10%. HPLC: 5.114 min, Molecular Formula: $C_{25}H_{25}N_7O_3$, Mass Calc: 471.20 ESI-MS-Found: 471 [M], purity: 90%. ^{1}HNMR (700 MHz, DMSO-d6) δ 11.70 (s, 1H), 8.80 (dd, J = 8.0, 3.3 Hz, 1H), 8.75 (t, J = 4.6 Hz, 1H), 8.57 (t, J = 6.0 Hz, 1H), 8.40 (d, J = 2.1 Hz, 1H), 7.76 (dd, J = 8.0, 1.5 Hz, 1H), 7.63 (d, J = 3.9 Hz, 1H), 7.52 – 7.49 (m, 1H), 7.16 (d, J = 3.5 Hz, 1H), 7.06 (t, J = 7.6 Hz, 1H), 6.99 (d, J = 4.5 Hz, 1H), 6.87 – 6.83 (m, 1H), 6.51 (t, J = 7.3 Hz, 1H), 6.40 (d, J = 3.6 Hz, 1H), 5.60 (ddd, J = 10.3, 4.3, 2.1 Hz, 1H), 4.31 (d, J = 6.0 Hz, 2H), 3.87 (d, J = 2.5 Hz, 3H), 2.82 (d, J = 4.5 Hz, 3H).^{13}CNMR (176 MHz, dmso) δ 169.63, 167.25, 164.75, 155.43, 153.21, 147.32, 143.98, 134.27, 132.08, 131.91, 128.25, 125.52, 121.04, 120.71, 119.73, 118.62, 117.83, 116.41, 110.45, 99.58, 90.85, 56.10, 42.56, 26.55.

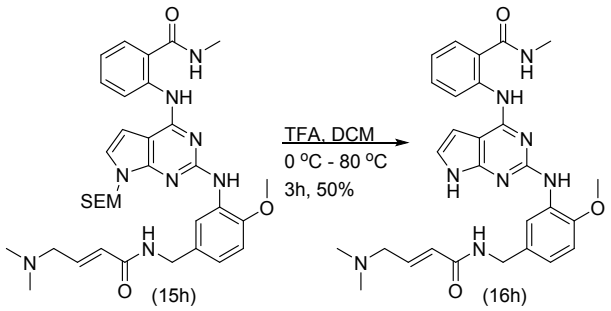


(15f) (16f)

*2-((2-((2-methoxy-5-(propionamidomethyl)phenyl)amino)-7-((2-(trimethylsilyl)ethoxy)methyl)-7H-pyrrolo[2,3-d]pyrimidin-4-yl)amino)-N-methylbenzamide (**16f**)*

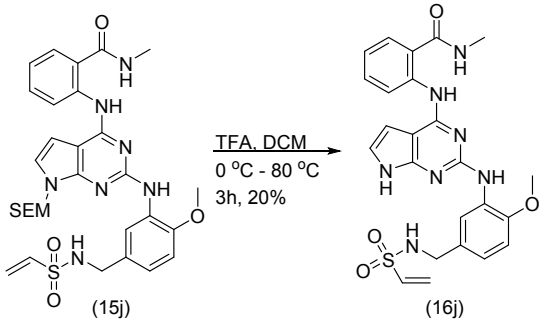
Compound **16f** was synthesized using the protocol described for **16b**. Yields a light yellow residue, 5 mg, 30%. HPLC: 4.99 min, Molecular Formula: $C_{25}H_{27}N_7O_3$, Mass Calc: 473.22 ESI-MS-Found: 474 [M+1], purity: 95% ^{1}HNMR (700 MHz, DMSO-d6) δ 11.64 (s, 1H), 11.37 (s, 1H), 8.85 (d, J = 8.6 Hz, 1H), 8.74 (s, 1H), 8.26 – 8.19 (m, 2H), 8.11 – 8.04 (m, 2H), 7.75 (d, J = 8.1 Hz, 1H), 7.53 (s, 1H), 7.50 – 7.44 (m, 1H), 7.08 – 7.03 (m, 1H), 7.03 – 6.98 (m, 1H), 6.93 (s, 1H), 6.82 (d, J = 8.4 Hz, 1H), 4.24 (d, J = 6.6 Hz, 2H), 3.86 (d, J = 5.5 Hz, 3H), 2.83 (d, J = 4.6 Hz, 3H), 2.16 (q, J = 7.7 Hz, 2H), 1.05 (t, J = 7.6 Hz, 3H). ^{13}CNMR(176 MHz, dmso) δ 172.87, 169.81, 163.15, 161.80, 159.33, 155.89, 153.30, 152.61, 148.82, 145.71, 141.27, 137.80, 133.24, 132.28,

128.79, 128.34, 120.96, 119.90, 119.67, 119.40, 115.42, 113.37, 110.68, 99.47, 97.73, 55.73, 42.24, 28.85, 26.69, 10.39.



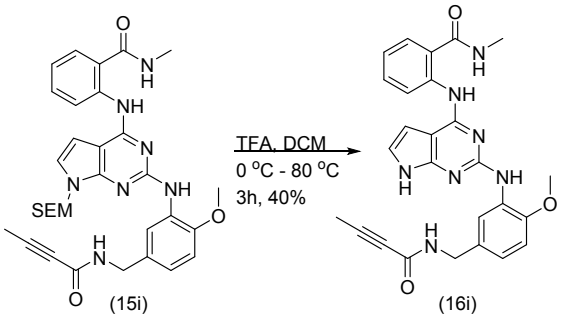
*(E)-2-((2-((5-((4-(dimethylamino)but-2-enamido)methyl)-2-methoxyphenyl)amino)-7-((2-(trimethylsilyl)ethoxy)methyl)-7H-pyrrolo[2,3-d]pyrimidin-4-yl)amino)-N-methylbenzamide (**16h**)*

Compound **16h** synthesized using the protocol described for **16b**. Yields a bright yellow solid, 8 mg, 50%. HPLC: 5.485 min, Molecular Formula: $C_{28}H_{32}N_8O_3$, Mass Calc: 528.26 ESI-MS-Found: 562 [M+H+MeOH], purity: 95%. ^{1}HNMR (700 MHz, DMSO-d6) δ 11.71 (s, 1H), 9.34 (t, J = 6.3 Hz, 1H), 8.80 (d, J = 8.4 Hz, 1H), 8.78 – 8.71 (m, 1H), 8.39 – 8.36 (m, 1H), 7.76 (dd, J = 7.9, 1.6 Hz, 1H), 7.64 (s, 1H), 7.53 – 7.48 (m, 1H), 7.16 (d, J = 3.5 Hz, 1H), 7.08 – 7.04 (m, 1H), 6.99 (d, J = 8.3 Hz, 1H), 6.86 (dd, J = 8.2, 2.2 Hz, 1H), 6.42 (t, J = 7.3 Hz, 1H), 6.40 (d, J = 3.6 Hz, 1H), 5.48 (d, J = 7.2 Hz, 2H), 4.29 (d, J = 6.3 Hz, 2H), 3.87 (s, 3H), 2.83 (d, J = 4.4 Hz, 3H), 1.26 (dd, J = 6.3, 3.7 Hz, 6H).^{13}CNMR (176 MHz, dmso) δ 169.63, 160.56, 157.59, 155.46, 153.20, 147.55, 143.59, 140.93, 132.65, 132.09, 130.54, 128.22, 123.46, 121.00, 120.71, 119.67, 118.96, 118.06, 116.65, 114.47, 110.47, 99.57, 97.95, 66.48, 56.07, 42.76, 41.57, 26.54.



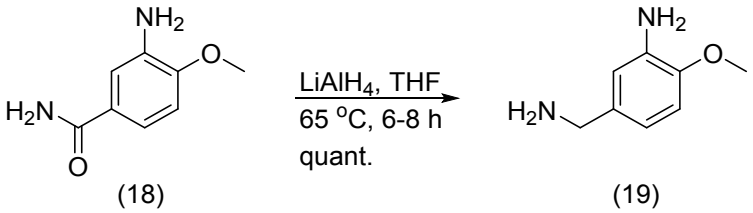
*2-((2-((2-methoxy-5-(vinylsulfonamidomethyl)phenyl)amino)-7H-pyrrolo[2,3-d]pyrimidin-4-yl)amino)-N-methylbenzamide (**16j**)*

Compound **16j** was synthesized using the protocol described for **16a**. Yields a white residue, 2 mg, 20%. HPLC: 6.13 min, Molecular Formula: $C_{24}H_{25}N_7O_4S$, Mass Calc: 507.17 ESI-MS-Found: 508 [M+1], purity: 95%. ^{1}HNMR (700 MHz, Chloroform-d) δ 10.82 (s, 1H), 8.63 (s, 1H), 8.51 (s, 1H), 7.58 (s, 1H), 7.53 (s, 1H), 7.47 (s, 1H), 6.92 (s, 1H), 6.83 (s, 1H), 6.58 (s, 1H), 5.81 (s, 1H), 4.17 – 4.07 (m, 2H), 3.92 (s, 3H), 3.03 (s, 3H). ^{13}CNMR (176 MHz, cdcl3) δ 169.98, 165.69, 155.06, 150.31, 147.80, 143.19, 135.08, 133.85, 131.70, 128.59, 122.86, 121.52, 120.55, 118.62, 117.86, 116.77, 115.07, 110.45, 103.85, 100.40, 55.73, 44.36, 25.17.



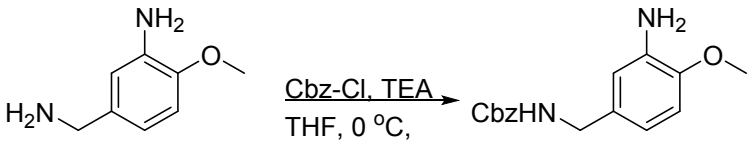
*2-((2-((5-(but-2-ynamidomethyl)-2-methoxyphenyl)amino)-7H-pyrrolo[2,3-d]pyrimidin-4-yl)amino)-N-methylbenzamide (**16i**)*

Compound **16i** was synthesized using the protocol described for **16b**. Yields a white residue, 4 mg, 40%. HPLC: 5.67 min, Molecular Formula: $C_{26}H_{25}N_7O_3$, Mass Calc: 483.20 ESI-MS-Found: 484 [M+1], 506 [M+Na], purity: 92% 1H NMR (700 MHz, DMSO-d6) δ 12.05 (s, 1H), 11.68 (s, 1H), 8.96 (s, 1H), 8.83 (s, 3H), 8.74 (s, 1H), 8.62 (d, J = 8.1 Hz, 1H), 8.28 (s, 1H), 7.75 (s, 1H), 7.61 (s, 1H), 7.51 (t, J = 3.4 Hz, 1H), 7.07 (s, 2H), 6.94 (s, 1H), 4.25 (d, J = 5.8 Hz, 2H), 3.86 (d, J = 3.4 Hz, 3H), 2.84 – 2.80 (m, 7H), 1.95 – 1.94 (m, 12H). ^{13}CNMR (176 MHz, dmso) δ 162.92, 162.46, 161.19, 156.25, 152.67, 147.87, 145.77, 137.82, 134.02, 131.84, 131.51, 127.00, 122.23, 121.19, 121.07, 119.79, 119.58, 118.60, 116.58, 115.41, 113.24, 110.65, 55.73, 42.37, 26.70, 3.40.



*5-(aminomethyl)-2-methoxyaniline (**19**)*

A flame dried flask was charged with 995.4 mg of 3-amino-4-methoxybenzamide (**18**) (6 mmol, 1.0 equiv) and 30 mL of dry THF (0.1 molar). The solution was cooled to 0 °C and 6.1 mL of LAH (10 mmol, 2 equiv) were added dropwise over 5 min. The bright yellow solution was allowed to continue stirring at 0 °C for 30 min. After 30 min, the solution was warmed to 65 °C for 6-8 hours. Once complete, the reaction was cooled back to 0 °C and worked up with the Feiser Method: 0.457 mL water, followed by 0.457 mL of freshly prepared 15% NaOH, and 1.371 mL of water. The resultant brown solution was allowed to stir at rt for 15 min. Then $MgSO_4$ was added and the solution was allowed to stir for additional 15 min. The $MgSO_4$ was filtered off and the solvent was removed to yield a yellow oil, 0.900 g, 100%. Molecular Formula: $C_8H_{12}N_2O$, Mass Calc: 152.09 ESI-MS-Found: 152 [M] HPLC: 0.96 min, purity: > 95% ^{1}HNMR (400 MHz, DMSO-d6) δ 6.69 (d, *J* = 8.1 Hz, 1H), 6.59 (d, *J* = 2.1 Hz, 1H), 6.46 (dd, *J* = 8.1, 2.1 Hz, 1H), 4.59 (s, 2H), 3.72 (s, 3H), 3.52 (s, 2H), 1.63 (s, 2H).



(19) (17)

*benzyl (3-amino-4-methoxybenzyl)carbamate (**17**)*

A flame dried flask was charged with 900.0 mg of **18** (5.91 mmol, 1.0 equiv) and 40 mL of dry THF (0.19 molar). The solution was cooled to 0 °C and 0.92 mL of TEA (6.6 mmol, 1.1 equiv), and 0.94 mL of Cbz-Cl (6.6 mmol, 1.1 equiv) were added in one portion, respectively. Once complete, the reaction was quenched with water and then extracted with EtOAc (3 x 50 mL). The combined organic layers were washed with brine (1 x 30 mL) and then dried over Na_2SO_4. The crude oil was purified by column chromatography (0-100% EtOAc/Hex) to give a light yellow oil, 1.14 g, 67.3%. HPLC: 4.871 min, Molecular Formula: $C_{16}H_{18}N_2O_3$ Mass Calc: 286.13 ESI-MS-Found: 287, purity: 85% 1HNMR (500 MHz, Chloroform-d) δ 7.34 (dd, J = 21.0, 4.5 Hz, 5H), 6.72 (d, J = 8.1 Hz, 1H), 6.65 (s, 1H), 6.62 (d, J = 7.8 Hz, 1H), 5.13 (s, 2H), 4.25 (d, J = 5.9 Hz, 2H), 3.83 (s, 3H), 3.79 (s, 2H).

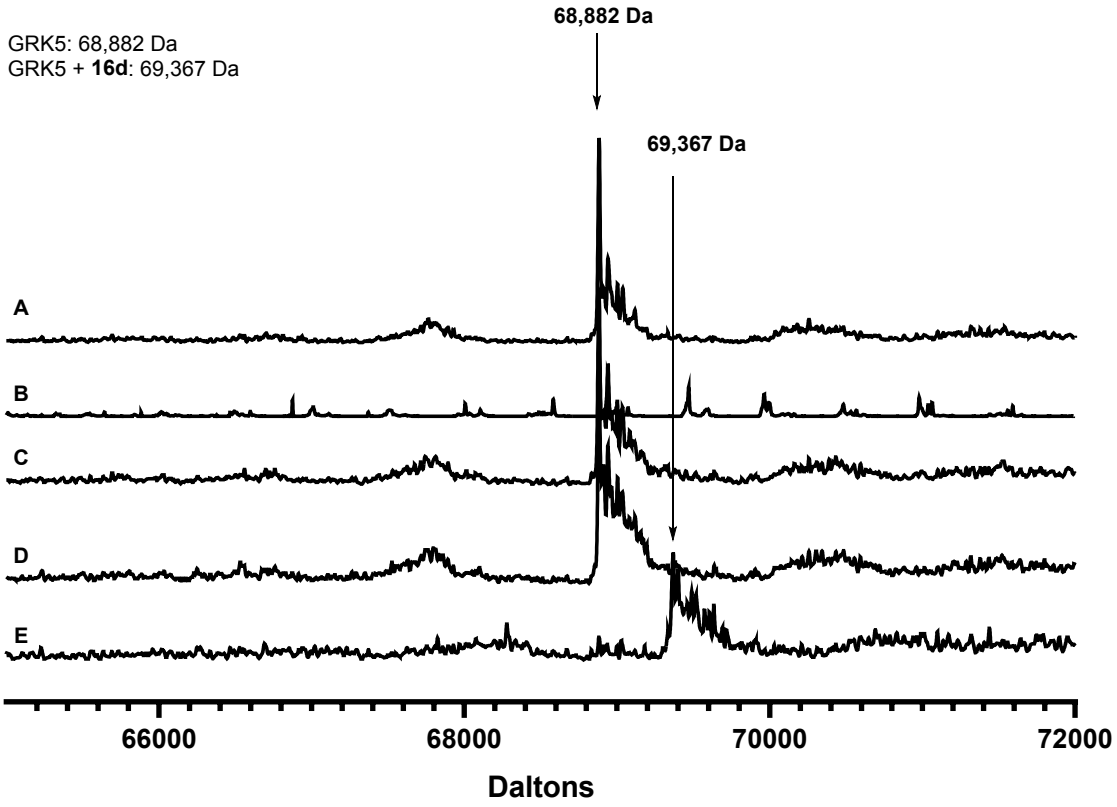


Figure S1. Intact protein MS of GRK5 incubated with **16b-e**. (A) MS trace for hGRK5 shows the predicted weight of 68,882 Da. (B) MS trace GRK5 incubated with **16b**, shows that only a small portion of compound labels GRK5. (C) MS trace for GRK5 incubated with **16c** indicating no covalent interaction. (D) MS trace for GRK5 incubated with **16e** indicating no covalent interaction. (E) MS trace for GRK5 incubated with **16d** indicating that covalent interaction has taken place, with the predicted adduct appearing at 69,367 Da.

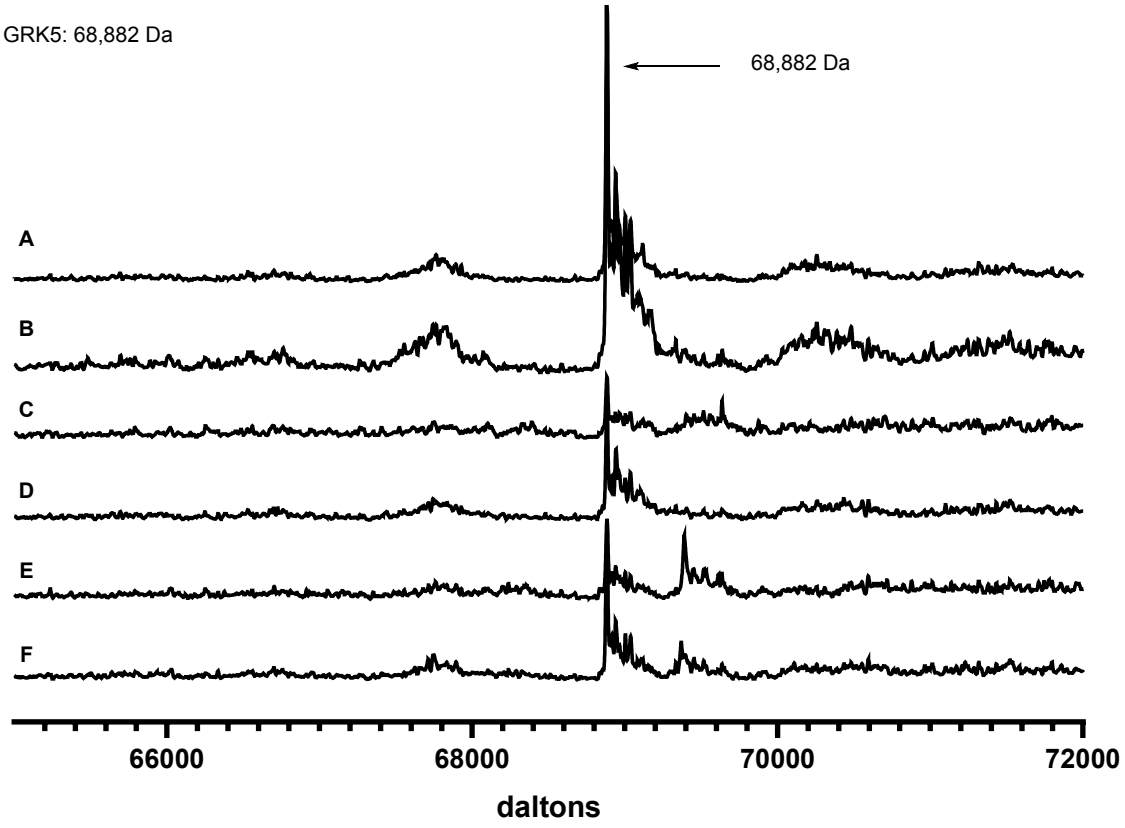


Figure S2. Intact protein MS of GRK5 incubated with **16f-j**. (A) MS trace for hGRK5shows the predicted weight of 68,882 Da. (B) MS trace GRK5 incubated with **16f**. (C) MS trace for GRK5 incubated with **16g**. (D) MS trace for GRK5 incubated with **16h**. (E) MS trace for GRK5 incubated with **16i**. (F) MS trace for GRK5 incubated with **16j**. None showed strong evidence for covalent adduct formation.

A

Accession	Description	Coverage	# Peptides	# PSMs	# Unique Pept	# AAs	MW [kDa]	calc. pI	Score A2 Sequest HT	# Peptides A2 Sequest HT
P34947	G protein-coupled receptor kinase 5 OS=Homo sapiens OX=9606 GN=GRK5 PE=1 SV=1	85.25423729	75	1749	75	590	67.7	8.1	5696.33	75
SSPA_STAAU	COMMON CONTAMINANT!	69.64285714	38	438	38	336	36.3	5.19	1322.15	38
TRFE_HUMAN	COMMON CONTAMINANT!	5.014326648	1	5	1	698	77	7.12	19.24	1
KCRM_HUMAN	COMMON CONTAMINANT!	8.398950131	1	4	1	381	43.1	7.25	9.62	1
CO5_HUMAN	COMMON CONTAMINANT!	3.818615752	2	4	2	1676	188.2	6.52	8.3	2
ANT3_HUMAN	COMMON CONTAMINANT!	16.59482759	2	2	2	464	52.6	6.71	6.2	2
PEPA_PIG	COMMON CONTAMINANT!	11.13989637	1	2	1	386	41.3	4.23	3.61	1
TAU_HUMAN	COMMON CONTAMINANT!	4.887714663	1	1	1	757	78.7	6.71	3.38	1
KRHB2_HUMAN	COMMON CONTAMINANT!	10.13645224	2	2	2	513	56.6	6.74	3.16	2
TRY2_BOVIN	COMMON CONTAMINANT!	14.17004049	1	1	1	247	26.3	4.92	2.81	1
RASH_HUMAN	COMMON CONTAMINANT!	14.81481481	1	1	1	189	21.3	5.31	2.73	1
K1H6_HUMAN	COMMON CONTAMINANT!	7.280513919	1	1	1	467	52.2	4.94	2.64	1
KRHB1_HUMAN	COMMON CONTAMINANT!	5.148514851	1	1	1	505	54.9	5.55	2.34	1
CAS2_BOVIN	COMMON CONTAMINANT!	4.054054054	1	1	1	222	26	8.43	1.95	1
KRHB6_HUMAN	COMMON CONTAMINANT!	9.670781893	1	5	1	486	53.5	5.66	0	1

B

Accession	Description	Coverage	# Peptides	# PSMs	# Unique Peptide	# AAs	MW [kDa]	calc. pI	Score A2 Sequest HT	# Peptides A2 Seq
P34947	G protein-coupled receptor kinase 5	85.25423729	75	1749	75	590	67.7	8.1	5696.33	
Checked	Confidence	Annotated Sequence	Modifications	Modifications	# Protein Groups	# Proteins	# PSMs	Master Protein Accessions	Positions in Master	# Missed Cleavag
FALSE	High	[D].YCSLCDKQPIGRLLFRQFCE.[T]	1xDeamidated [Q]; 1xRowland [C2]; 2xCarbamidomethyl [C5; C19]		1	1	3	P34947	P34947 [53-72]	
FALSE	High	[E].AGMLDPPFVPDPRAVYCKDVLDIE.[Q]	1xOxidation [M3]; 1xRowland [C17]		1	1	1	P34947	P34947 [458-481]	

C

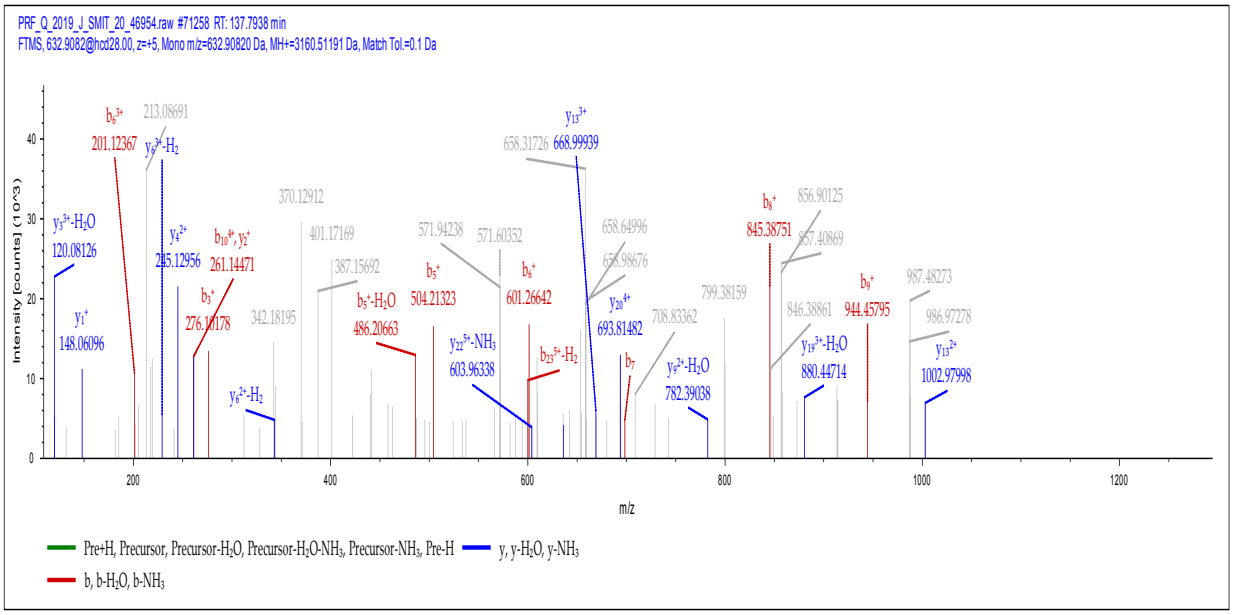


Figure S3. Tandem MS/MS table & details for hGRK5 incubated with **16d**. (A) Tandem MS/MS table of proteins detected in the GRK5·**16d** sample, indicating that 85% of the peptides belong to GRK5. (B) GRK5·**16d** analysis table showing only the modification made to GRK5 by **16d**. Our desired modification (denoted "Rowland") is observed at Cys474 with high confidence. Additional modification is observed at Cys54, which likely occurs via a non-selective mechanism. (C) Fragmentation of peptide labeled "Rowlands [C17]".

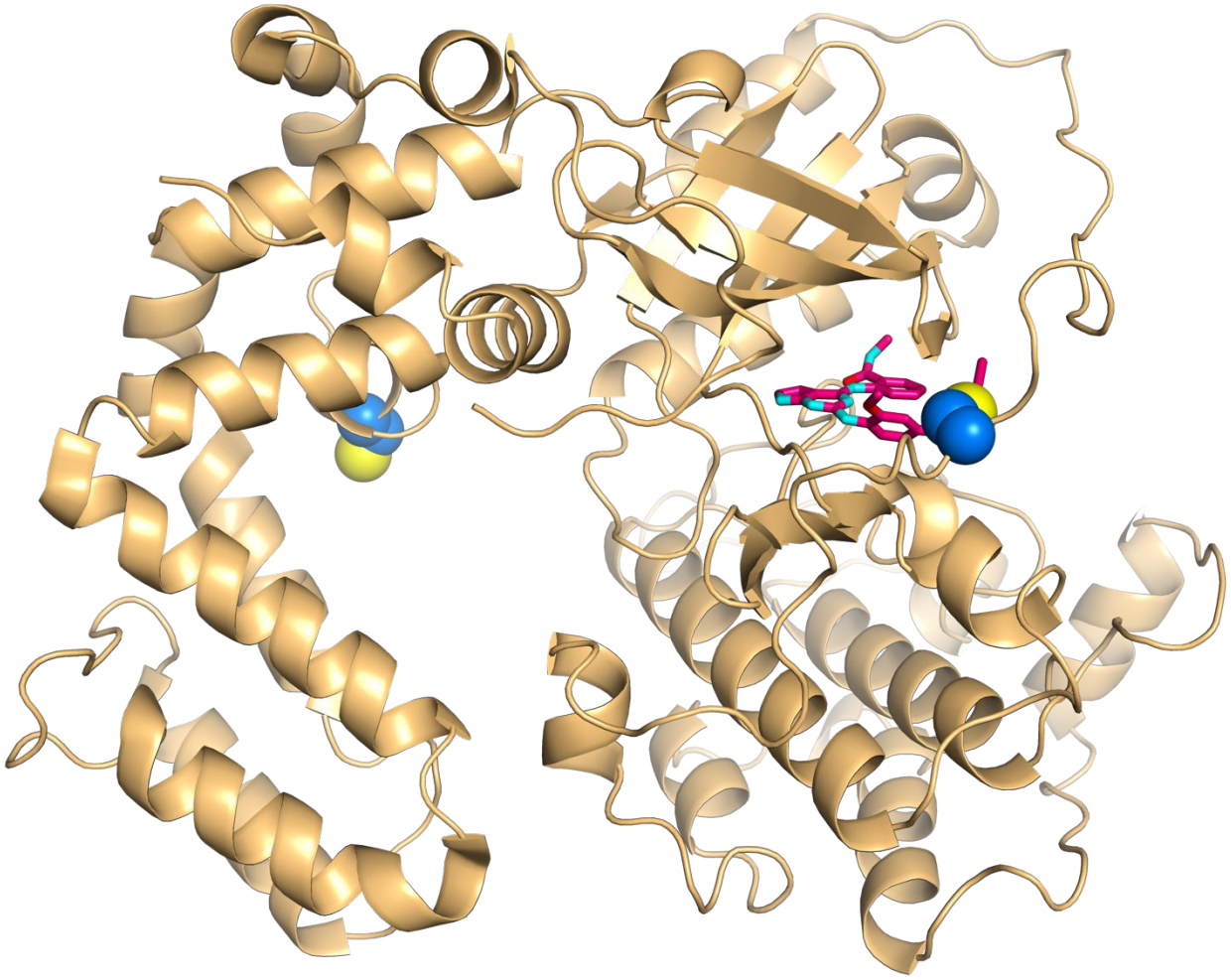


Figure S4. Structural rationalization of tandem MS/MS results. Wild-type GRK5 (blue ribbon diagram) shown with **16d** (gold carbons) docked into active site. Cysteine sites of modification identified by tandem MS/MS are shown with sphere side chains (blue carbon,yellow sulfur). Cys54 is in a highly solvent exposed region, suggesting non-selective labelling.[5]

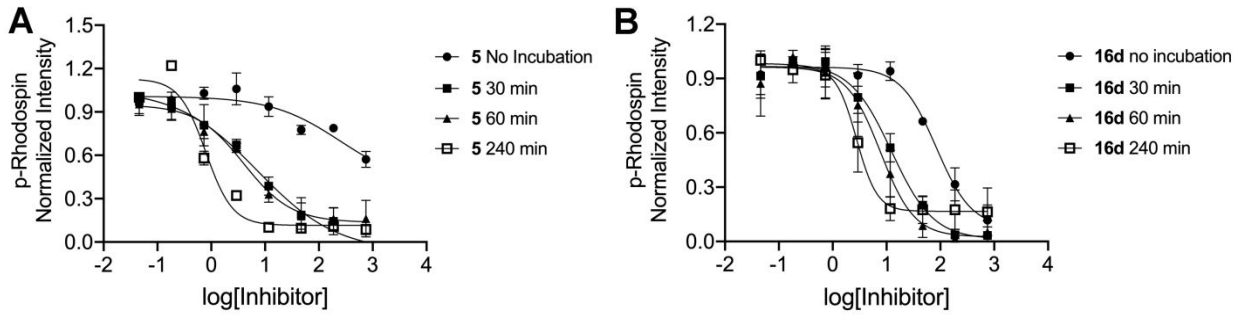


Figure S5. Time dependent inhibition of GRK5 by **5** and **16d** using light activated ROS as substrate. Compounds were preincubated for times of 0 mins (solid circle), 30 min (solid square), 60 min (solid triangle) and 240 min (open square). Both compounds demonstrate the expected leftward shift in IC_{50} as preincubation time increase, similar to when tubulin is used as substrate (Figure 2). (A) IC_{50} values of **5** at each preincubation time: $[IC_{50}]_{0\ min}$ >100 μM, $[IC_{50}]_{30\ min}$ =4.2 ± 2 μM, $[IC_{50}]_{60\ min}$ =3.4 ± 2 μM and $[IC_{50}]_{240\ min}$ =0.9 ± 0.2 μM. (B) IC_{50} values of **16d** at each preincubation time: $[IC_{50}]_{0\ min}$ =91 ± 20 μM, $[IC_{50}]_{30\ min}$ =12 ± 3 μM, $[IC_{50}]_{60\ min}$ =7.6 ± 4 μM and $[IC_{50}]_{240\ min}$ =2.7 ± 0.8 μM. Errors correspond to standard deviation from 2 (panel A) or 3 (panel B) replicates.

Supplementary References

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(2) Gray, N. S.; Hatcher, J.; Choi, H. G. Lrrk2 Inhibitors and Methods of Making and Using the Same. WO2016130920A2, August 18, 2016.

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(4) Kim, M.-S.; Zhong, J.; Pandey, A. Common Errors in Mass Spectrometry-Based Analysis of Post-Translational Modifications. *Proteomics* **2016**, *16* (5), 700–714. https://doi.org/10.1002/pmic.201500355.

(5) Gehringer, M.; Laufer, S. A. Emerging and Re-Emerging Warheads for Targeted Covalent Inhibitors: Applications in Medicinal Chemistry and Chemical Biology. *J. Med. Chem.* **2018**. https://doi.org/10.1021/acs.jmedchem.8b01153.