Design and Synthesis of Novel Macrocyclic Mer Tyrosine Kinase Inhibitors

Xiaodong Wang,[*,†] Jing Liu,[†,#] Weihe Zhang,[†,Δ] Michael A. Stashko,[†] James Nichols,[‡] Michael J Miley,[§] Jacqueline Norris-Drouin,[†] Zhilong Chen,[†] Mischa Machius,[§] Deborah DeRyckere,[‖] Edgar Wood,[‡] Douglas K. Graham,[‖,‡] H Shelton Earp,[§,⊥,‡] Dmitri Kireev,[†] Stephen V. Frye[†,⊥,‡]

[†]Center for Integrative Chemical Biology and Drug Discovery, Division of Chemical Biology and Medicinal Chemistry, Eshelman School of Pharmacy

[‡]Meryx, Inc., 450 West Dr., Chapel Hill, NC 27599, USA

[§] Department of Pharmacology, University of North Carolina at Chapel Hill, Chapel Hill, NC 27599, USA

[‖]Aflac Cancer and Blood Disorders Center of Children's Healthcare of Atlanta, Department of Pediatrics, School of Medicine, Emory University, Atlanta, GA 30322, USA

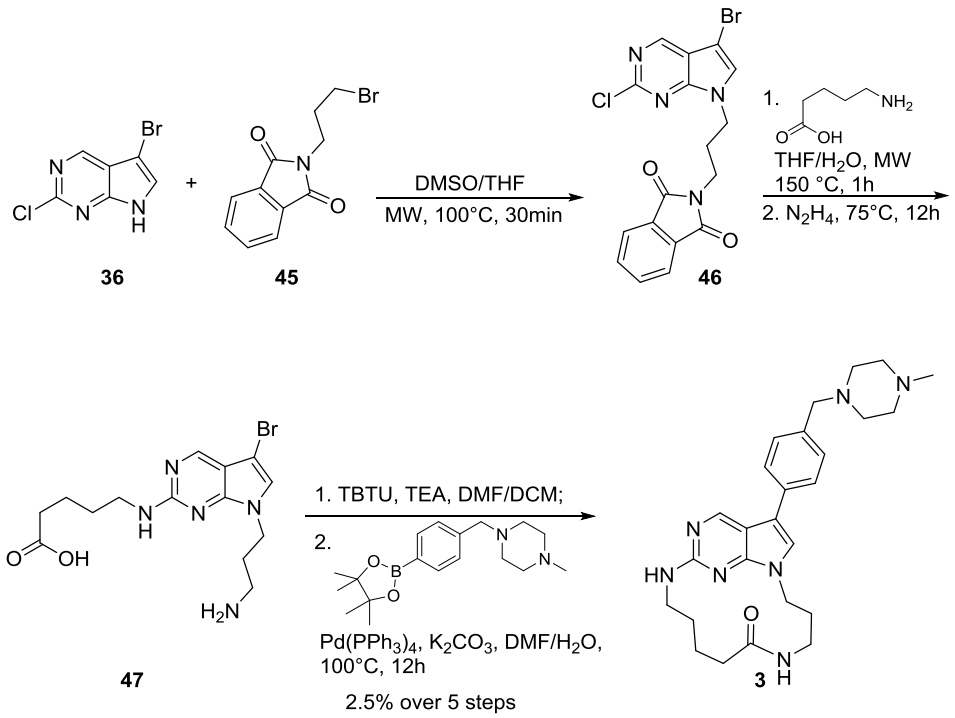
[⊥]Lineberger Comprehensive Cancer Center, Department of Medicine, School of Medicine, University of North Carolina at Chapel Hill, Chapel Hill, NC 27599, USA

Supporting Information

Synthesis of Analogues

Experimental

Microwave reactions were carried out using a CEM Discover-S reactor with a vertically-focused IR external temperature sensor and an Explorer 72 autosampler. The dynamic mode was used to set up the desired temperature and hold time with the following fixed parameters: PreStirring, 1 min; Pressure, 200 psi; Power, 200 W; PowerMax, off; Stirring, high. Flash chromatography was carried out with pre-packed silica gel disposable columns. Preparative HPLC was performed with the UV detection at 220 or 254 nm. Samples were injected onto a 75 x 30 mm, 5 μM, C18(2) column at rt. The flow rate was 30 mL/min. Various linear gradients were used with A being H_2O + 0.5% TFA and B being MeOH. Analytical thin-layer chromatography (TLC) was performed with silica gel 60 F_{254}, 0.25 mm pre-coated TLC plates. TLC plates were visualized using UV_{254} and phosphomolybdic acid with charring. All ^1H NMR spectra were obtained with a 400 MHz spectrometer using chloroform-d ($CDCl_3$, 7.26 ppm), dimethyl sulfoxide-d_6 (DMSO-d_6, 2.50 ppm), or methanol-d_4 (CD_3OD, 3.31 ppm) as an internal reference. Signals are reported as m (multiplet), s (singlet), d (doublet), t (triplet), q (quartet), and bs (broad singlet); and coupling constants are reported in Hertz (Hz). ^{13}C NMR spectra were obtained with a 100 MHz spectrometer using $CDCl_3$ (77.2 ppm), DMSO-d_6 (39.5 ppm), or CD_3OD (49.0 ppm) as the internal standard. LC-MS was performed using an analytical instrument with the UV detector set to 220 nm, 254 nm, and 280 nm, and a single quadrupole mass spectrometer using electrospray ionization (ESI) source. Samples were injected (2 μL) onto a 4.6 x 50 mm, 1.8 μM, C18 column at rt. A linear gradient from 10% to 100% B (MeOH with 0.1% acetic acid) in 5.0 min was followed by pumping 100% B for another 2 or 4 minutes with A being H_2O with 0.1% acetic acid. The flow rate was 1.0 mL/min. High-resolution (positive ion) mass spectra (HRMS) were acquired using a LCMS-TOF mass spectrometer.



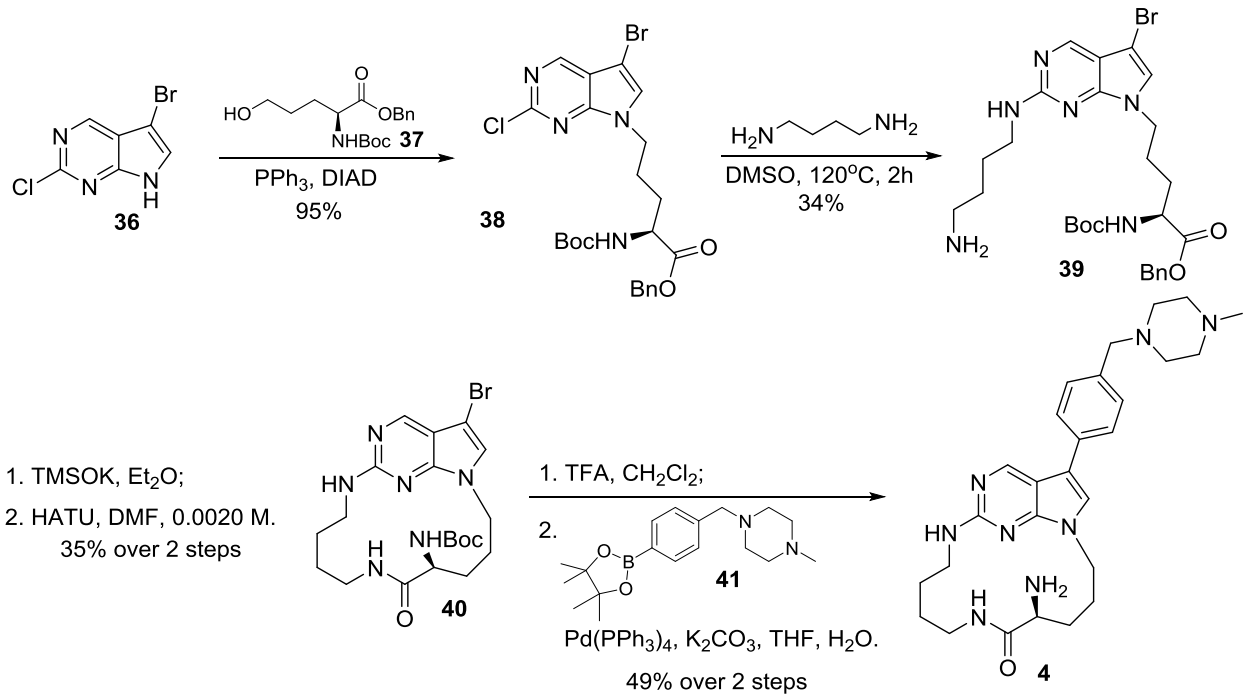
36 **45** **46** **47** **3**

2-(3-(5-bromo-2-chloro-7*H*-pyrrolo[2,3-*d*]pyrimidin-7-yl)propyl)isoindoline-1,3-dione (**46**): To a suspension of 5-bromo-2-chloro-7*H*-pyrrolo[2,3-*d*]pyrimidine (**36**) (100 mg, 0.43 mmol), 2-(3-bromopropyl)isoindoline-1,3-dione (**45**) (173 mg, 0.65 mmol) and potassium carbonate (K_2CO_3), (119 mg, 0.86 mmol) in a mixture of dimethyl sulfoxide (DMSO) and tetrahydrofuran (THF) (8.0 mL, 1:3, v/v) was heated at 100°C under microwave irradiation for 30 min. The mixture was diluted with ethyl acetate (EtOAc) (35 mL), washed with water (3x10 mL) and concentrated to provide the crude 2-(3-(5-bromo-2-chloro-7*H*-pyrrolo[2,3-*d*]pyrimidin-7-yl)propyl)isoindoline-1,3-dione (**46**) (MS *m/z* $[M+H]^+$ calculated for $C_{17}H_{13}BrClN_4O_2$, 420.67; found: 420.05) which was used in next step without further purification.

1^5-(4-((4-Methylpiperazin-1-yl)methyl)phenyl)-1^7*H*-2,8-diaza-1(2,7)-pyrrolo[2,3-*d*]pyrimidinacycloundecaphan-7-one (**3**): A solution of the crude **46** in a mixture of THF and water (10 mL, 3:2, v/v) was added 5-aminopentanoic acid (172.3 mg, 1.47 mmol). The resulting

mixture was heated at 150°C under microwave irradiation for 1h. After the solvent was removed, the residue was dissolved in a mixture of ethanol and water (20 mL, 3:2, v/v) followed by the addition of hydrazine (1.5 mL). Then the reaction mixture was heat at 80°C overnight. The solvent was removed and the residue was purified on HPLC to provide 5-((7-(3-aminopropyl)-5-bromo-7*H*-pyrrolo[2,3-*d*]pyrimidin-2-yl)amino)pentanoic acid (**47**) as an clear oil (MS *m/z* $[M+H]^+$ calculated for $C_{14}H_{21}BrN_5O_2$, 371.26; found: 371.10). A solution of **47** in dimethylformamide (DMF)/dichloromethane (DCM) (120 mL, 2:3, v/v) was added *O*-(benzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium tetrafluoroborate (TBTU) (115 mg) and triethylamine (2.2 mL). The reaction mixture was stirred at room temperature (rt) for overnight. Solvent was removed and the residue (MS *m/z* [M+H]+ calculated for $C_{14}H_{19}BrN_5O^+$ ($[M+H]^+$): 353.24; found: 353.10) was dissolved in dioxane (6.0 mL) followed by the addition of 4-(4-methylpiperazino)methylphenylboronic acid pinacol ester (135 mg, 0.43 mmol), tetrakis(triphenylphosphine)palladium(0) ($Pd(PPh_3)_4$) (12 mg, 0.01 mmol), K_2CO_3 (128 mg, 0.93 mmol) and water (2.0 mL). The resulting mixture was heated at 150°C under microwave irradiation for 15 min, quenched with water (15 mL), extracted with EtOAc (4x15 mL), dried ($MgSO_4$), filtered, and concentrated. The residue was purified on HPLC to give the desired product **3** as a TFA salt. This salt was neutralized with a 7.0 N NH_3 solution in MeOH and was purified by an ISCO silica gel column to provide the desired product **3** as a white solid (5.0 mg, 2.5% over 5 steps). ^{1}H NMR (400 MHz, CD_3OD) δ 8.66 (s, 1H), 7.60 –7.53 (m, 2H), 7.35 (d, J = 8.2 Hz, 2H), 7.31 (s, 1H), 5.47 (s, 2H), 4.27 (t, J = 7.2 Hz, 2H), 3.54 (s, 2H), 3.47–3.40 (m, 2H), 3.19–3.13 (m, 2H), 2.57–2.46 (m, 6H), 2.42–2.38 (m, 2H), 2.27 (s, 3H), 1.96–1.89 (m, 2H), 1.80–1.71 (m, 2H), 1.71–1.61 (m, 2H); MS *m/z* $[M+H]^+$ calculated for $C_{26}H_{36}N_7O$, 462.62; found: 462.30; LC/MS: > 98% purity; retention time (t_R): 2.81 min.

General procedure A (Path a):



Benzyl (*S*)-5-(5-bromo-2-chloro-7*H*-pyrrolo[2,3-d]pyrimidin-7-yl)-2-((*tert*-butoxycarbonyl) amino)pentanoate (**38**): A solution of **36** (0.23 g, 1.0 mmol) in THF (8.0 mL) was added triphenylphosphine (PPh_3) (0.52 g, 2.0 mmol), followed by a solution of **37** (0.65 g, 2.0 mmol) in THF (2.0 mL) and diisopropyl azodicarboxylate (DIAD) (0.39 mL, 2.0 mmol) at 0°C. The resulting mixture was stirred at rt for 16 h, quenched with brine and extracted with EtOAc (3x10 mL). The combined organic layers were dried (Na_2SO_4), filtered, and concentrated. The residue was purified by an ISCO silica gel column to provide the title compound **38** (0.51 g, 95%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.72 (s, 1H), 7.41–7.28 (m, 5H), 7.13 (s, 1H), 5.16 (dd, J = 4.0, 1.2 Hz, 2H), 5.11 (br, 1H), 4.40 (bs, 1H), 4.21 (t, J = 6.9 Hz, 2H), 1.89–1.74 (m, 3H), 1.68–1.58 (m, 1H), 1.44 (s, 9H).

Benzyl (*S*)-5-(2-((4-aminobutyl)amino)-5-bromo-7*H*-pyrrolo[2,3-d]pyrimidin-7-yl)-2-((*tert*-butoxycarbonyl)amino)pentanoate (**39**): A solution of **38** (0.54 g, 1.0 mmol) in DMSO (15 mL) was added butane-1,4-diamine (0.35 g, 4.0 mmol). The resulting mixture was stirred at 120°C for

2.0 h, quenched with brine and extracted with EtOAc (3x15 mL). The combined organic layers were dried (Na_2SO_4), filtered, and concentrated. The residue was purified by an ISCO silica gel column to provide the title compound **39** (0.20 g, 34%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.37 (s, 1H), 7.37–7.26 (m, 5H), 6.69 (s, 1H), 5.61-5.59 (m, 1H), 5.30–5.24 (m, 1H), 5.13 (dd, J_1= 3.6 Hz, J_2= 1.2 Hz, 2H), 4.39 (br, 1H), 4.06-4.00 (m, 3H), 3.45-3.41 (m, 3H), 2.85 (s, 2H), 1.87–1.57 (m, 8H), 1.43 (s, 9H).

***tert*-Butyl (*S*)-(1^5-bromo-8-oxo-1^7*H*-2,7-diaza-1(2,7)-pyrrolo[2,3-*d*]pyrimidinacyclododecaphane-9-yl)carbamate** (**40**): A solution of **39** (0.15 g, 0.25 mmol) in a mixture of THF (1.0 mL) and ethyl ether (Et_2O) (2.0 mL) was added potassium trimethylsilanolate (KOTMS) (0.038 g, 0.30 mmol). After stirring at rt for 16 h, the precipitate was filtered and washed with Et_2O (5x5 mL). A solution of the solid in a mixture of DCM (15 mL) and DMF (5.0 mL) was added 1-[bis(dimethylamino)methylene]-1*H*-1,2,3-triazolo[4,5-b]pyridinium 3-oxid hexafluorophosphate (HATU) (0.084 g, 0.22 mmol) at 0°C. The resulting reaction mixture was stirred at rt for 16 h, quenched with brine and extracted with EtOAc (3x). The combined organic layers were dried (Na_2SO_4), filtered, and concentrated. The residue was purified by an ISCO silica gel column to provide the title compound **40** (0.032 g, 35%) as a yellow solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.26 (s, 1H), 7.30 (br, 1H), 7.00 (s, 1H), 4.05– 3.87 (m, 3H), 3.65 (bs, 1H), 3.39–3.24 (m, 2H), 3.06 (bs, 3H), 2.94– 2.82 (m, 1H), 2.09– 1.94 (m, 1H), 1.92– 1.56 (m, 6H), 1.49– 1.40 (m, 1H), 1.38 (s, 9H).

(*S*)-9-Amino-1^5-(4-((4-methylpiperazin-1-yl)methyl)phenyl)-1^7*H*-2,7-diaza-1(2,7)-pyrrolo[2,3-*d*]pyrimidinacyclododecaphan-8-one (**4**): A solution of **40** (0.042 g, 0.087 mmol) in a mixture of DCM (2.0 mL) and TFA (1.0 mL) was stirred at rt for 2 h. The solvents were removed under the reduced pressure. The residue was dissolved in a mixture of MeOH and THF

and the solvents were removed under the reduced pressure. A solution of the residue in THF (2.0 mL) was added potassium phosphate tribasic (K_3PO_4) (0.070 g, 0.33 mmol), 1-methyl-4-(4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)benzyl) piperazine **41** (0.10 g, 0.33 mmol), $Pd(PPh_3)_4$ (0.013 g, 0.011 mmol) and H_2O (0.50 mL). The resulting mixture was heated at 70°C for 6.0 h. Silica gel was added to the mixture at rt and the solvents were removed under the reduced pressure. The residue was purified by an ISCO silica gel column to provide the title compound **4** (0.021 g, 49%) as a yellow solid. ^{1}H NMR (400 MHz, CD_3OD) δ 8.81 (s, 1H), 7.68 (s, 1H), 7.64 (d, *J* = 8.2 Hz, 2H), 7.48 (d, *J* = 8.1 Hz, 2H), 4.24–4.16 (m, 2H), 3.90–3.84 (m, 1H), 3.75 (s, 2H), 3.45–3.38 (m, 2H), 2.97– 2.77 (m, 2H), 2.88 (s, 3H), 2.34 (s, 1H), 2.08– 1.79 (m, 5H), 1.78–1.65 (m, 1H), 1.56– 1.42 (s, 1H); MS *m/z* $[M+H]^+$ calculated for $C_{27}H_{39}N_8O$, 491.66; found: 491.40; LC/MS: >95% purity; t_R: 2.75 min.

(*S*)-11-Amino-1^5-(4-((4-methylpiperazin-1-yl)methyl)phenyl)-1^7*H*-2,9-diaza-1(2,7)-pyrrolo[2,3-*d*]pyrimidinacyclotetradecaphan-10-one (**5**): The title compound **5** (0.027 g, 2.8%) was prepared according to general procedure A from **38** (0.54 g, 1.0 mmol), hexane-1,6-diamine (0.46 g, 4.0 mmol) and **41** (0.13 g, 0.40 mmol) as a yellow solid. ^{1}H NMR (400 MHz, CD_3OD) δ 8.79 (s, 1H), 8.40 (br, 1H), 7.72 (s, 1H), 7.66 (d, *J* = 8.2 Hz, 2H), 7.51 (d, *J* = 8.2 Hz, 2H), 4.44–4.35 (m, 1H), 4.21–4.11 (m, 1H), 3.90–3.80 (m, 4H), 3.79–3.70 (m, 1H), 3.55–3.46 (m, 1H), 3.39–3.32 (m, 4H), 3.03–2.80 (m, 8H), 2.09–1.94 (m, 3H), 1.94–1.84 (m, 1H), 1.81–1.72 (m, 2H), 1.59–1.52 (m, 1H), 1.51–1.38 (m, 5H); MS *m/z* $[M+H]^+$ calculated for $C_{29}H_{43}N_8O$, 519.72; found: 519.40; LC/MS: >95% purity; t_R: 2.75 min.

(*S*)-13-Amino-1^5-(4-((4-methylpiperazin-1-yl)methyl)phenyl)-1^7*H*-2,11-diaza-1(2,7)-pyrrolo[2,3-*d*]pyrimidinacyclohexadecaphan-12-one (**6**): The title compound **6** (0.019 g, 3.8%) was prepared according to general procedure A from compound **38** (0.54 g, 1.0 mmol), octane-

1,8-diamine (0.58 g, 4.0 mmol) and **41** (0.13 g, 0.40 mmol) as a yellow solid. ^{1}H NMR (400 MHz, CD_3OD) δ 8.79 (s, 1H), 7.74 (s, 1H), 7.68 (d, *J* = 8.1 Hz, 2H), 7.52 (d, *J* = 8.2 Hz, 2H), 4.46–4.35 (m, 1H), 4.23–4.12 (m, 1H), 3.91 (s, 2H), 3.86 (t, *J* = 5.6 Hz, 1H), 3.82–3.73 (m, 1H), 3.71–3.61 (m, 1H), 3.58–3.49 (m, 1H), 3.38 (bs, 4H), 3.01 (bs, 4H), 2.91 (s, 3H), 2.89–2.81 (m, 1H), 2.03–1.87 (m, 4H), 1.76–1.65 (m, 2H), 1.58–1.47 (m, 2H), 1.46–1.40 (m, 4H), 1.37–1.23 (m, 4H); MS *m/z* $[M+H]^+$ calculated for $C_{31}H_{47}N_8O$, 547.77; found: 547.40; LC/MS: >95% purity; t_R: 3.23 min.

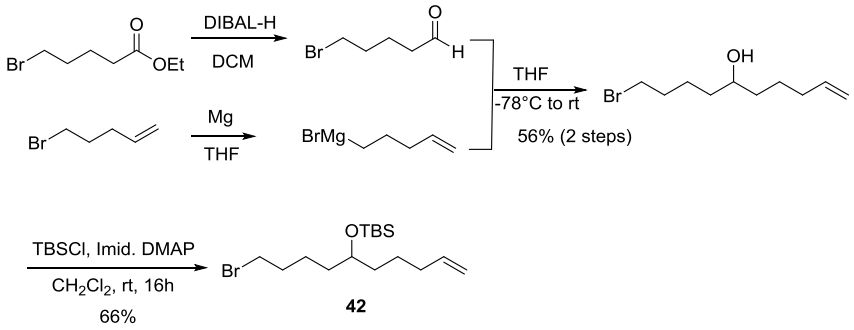
(*S*)-9-Amino-1^5-(4-((4-methylpiperazin-1-yl)methyl)phenyl)-1^7*H*-2,7-diaza-1(2,7)-pyrrolo[2,3-*d*]pyrimidinacyclotridecaphan-8-one (**7**): The title compound **7** (0.031 g, 3.0%) was prepared according to general procedure A from **36** (0.26 g, 1.12 mmol), benzyl (*S*)-2-((*tert*-butoxycarbonyl)amino)-6-hydroxyhexanoate (0.76 g, 2.24 mmol), butane-1,4-diamine (0.098 g, 1.12 mmol), and compound **41** (0.077 g, 0.24 mmol) as a yellow solid. ^{1}H NMR (400 MHz, CD_3OD) δ 8.82 (s, 1H), 7.79 (s, 1H), 7.68 (d, *J* = 8.2 Hz, 2H), 7.52 (d, *J* = 8.2 Hz, 2H), 4.48–4.38 (m, 1H), 4.20 (dt, *J* = 13.9, 4.8 Hz, 1H), 3.90 (s, 2H), 3.88–3.79 (m, 1H), 3.73–3.66 (m, 1H), 3.66–3.59 (m, 1H), 3.43–3.34 (m, 4H), 3.09–2.84 (m, 8H), 2.05–1.80 (m, 4H), 1.80–1.53 (m, 4H), 1.49–1.35 (m, 1H), 1.34–1.21 (m, 1H); MS *m/z* [M+H]+ calculated for $C_{28}H_{41}N_8O$, 505.69; found: 505.30; LC/MS: >95% purity; t_R: 2.75 min.

(*S*)-11-Amino-1^5-(4-((4-methylpiperazin-1-yl)methyl)phenyl)-1^7*H*-2,9-diaza-1(2,7)-pyrrolo[2,3-*d*]pyrimidinacyclopentadecaphan-10-one (**8**): The title compound **8** (0.027 g, 5.4%) was prepared according to general procedure A from **36** (0.26 g, 1.12 mmol), benzyl (*S*)-2-((*tert*-butoxycarbonyl)amino)-6-hydroxyhexanoate (0.76 g, 2.24 mmol), bexane-1,6-diamine (0.13 g, 1.12 mmol), and compound **41** (0.077 g, 0.24 mmol) as a yellow solid. ^{1}H NMR (400 MHz, CD_3OD) δ 8.80 (s, 1H), 7.78 (s, 1H), 7.67 (d, *J* = 8.2 Hz, 2H), 7.51 (d, *J* = 8.2 Hz, 2H), 4.35–4.25 (m, 1H), 4.22–4.12 (m, 1H), 3.89 (s, 2H), 3.86 (t, *J* = 5.8 Hz, 1H), 3.75–3.66 (m, 1H),

3.66–3.58 (m, 1H), 3.48–3.33 (m, 5H), 3.13–2.92 (m, 4H), 2.90 (s, 3H), 2.07–1.85 (m, 4H), 1.85–1.65 (m, 3H), 1.65–1.51 (m, 2H), 1.51–1.38 (m, 5H); MS *m/z* $[M+H]^+$ calculated for $C_{30}H_{45}N_8O$, 533.75; found: 533.40; LC/MS: >95% purity; t_R: 2.99 min.

(*S*)-13-Amino-1^5-(4-((4-methylpiperazin-1-yl)methyl)phenyl)-1^7*H*-2,11-diaza-1(2,7)-pyrrolo[2,3-*d*]pyrimidinacycloheptadecaphan-12-one (**9**): The title compound **9** (0.019 g, 3.7%) was prepared according to general procedure A from **36** (0.26 g, 1.12 mmol), benzyl (*S*)-2-((*tert*-butoxycarbonyl)amino)-6-hydroxyhexanoate (0.76 g, 2.24 mmol), octane-1,8-diamine (0.16 g, 1.12 mmol), and compound **41** (0.066 g, 0.20 mmol) as a yellow solid. ^{1}H NMR (400 MHz, CD_3OD) δ 8.78 (s, 1H), 7.77 (s, 1H), 7.66 (d, *J* = 8.1 Hz, 2H), 7.50 (d, *J* = 8.2 Hz, 2H), 4.20 (t, *J* = 8.1 Hz, 2H), 3.86–3.80 (m, 3H), 3.79–3.71 (m, 1H), 3.67–3.57 (m, 1H), 3.55–3.47 (m, 1H), 3.41–3.32 (bs, 4H), 3.01-2.80 (m, 8H), 2.07–1.90 (m, 4H), 1.78–1.68 (m, 2H), 1.62–1.36 (m, 12H); MS *m/z* $[M+H]^+$ calculated for $C_{32}H_{49}N_8O$, 561.80; found: 561.40; LC/MS: >95% purity; t_R: 3.38 min.

General procedure for the synthesis of bromide side chains:



5-Bromopentanal: A solution of ethyl 5-bromopentanoate (1.58 mL, 10 mmol) in DCM (20 mL) was added a 1.0 M diisobutylaluminium hydride (DIBAL-H) solution in toluene (11 mL, 11 mmol) at -78°C. The reaction mixture was stirred at -78°C for 3h, then diluted with Et_2O, and quenched with brine and a saturated aqueous solution of sodium potassium tartrate. The mixture was warmed

to rt and stirred for 1h, at which time the solution became two clear layers. The aqueous layer was extracted with Et_2O (3x20 mL). The combined organic layers were dried (Na_2SO_4), filtered, and concentrated. The resulting residue was passed through a short silica gel pad and flushed with Et_2O. After concentration the residue was used directly for the next step.

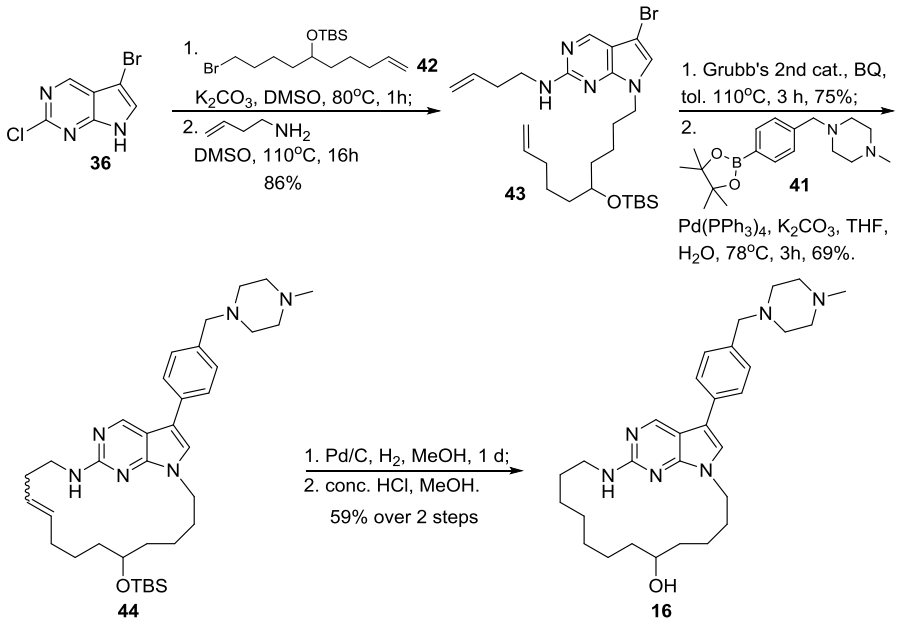
Pent-4-en-1-ylmagnesium bromide: A mixture of Mg tuning (0.58 g, 24 mmol) in THF (15 mL) was added a solution of 5-bromopent-1-ene (2.03 mL, 20 mmol) in THF (3.0 mL) at a flow rate that kept the reaction mixture reflux. After the addition, the reaction mixture was stirred for 1h to provide the title compound.

1-Bromodec-9-en-5-ol: A solution of crude 5-bromopentanal (~10 mmol) in THF (15 mL) was added a solution of pent-4-en-1-ylmagnesium bromide (~20 mmol) in THF (20 mL) at -78°C. The reaction mixture was warmed to 0 °C by itself in 4-5h. The mixture was poured into ice and extracted with EtOAc (3x10 mL). The combined organic layers were dried (Na_2SO_4), filtered, and concentrated. The residue was purified by an ISCO silica gel column to provide the title compound (1.32 g, 56% over 2 steps). ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.90–5.74 (m, 1H), 5.06–4.90 (m, 2H), 3.65–3.55 (m, 1H), 3.42 (t, *J* = 6.8 Hz, 2H), 2.12–2.00 (m, 2H), 1.94–1.82 (m, 2H), 1.62–1.38 (m, 9H).

((1-Bromodec-9-en-5-yl)oxy)(tert-butyl)dimethylsilane (**42**): A solution of 1-bromodec-9-en-5-ol (1.32 g, 5.61 mmol) in DCM (70 mL) was added *tert*-butyldimethylsilyl chloride (TBSCl) (1.06 g, 7.02 mmol), imidazole (0.50 g, 7.30 mmol), and 4-dimethylaminopyridine (DMAP) (0.034 g, 0.28 mmol) at 0°C. The reaction mixture was stirred at rt for 16 h, diluted with Et_2O, quenched with H_2O, and extracted with Et_2O (3x50 mL), The combined organic layers were dried (Na_2SO_4), filtered, and concentrated. The residue was purified by an ISCO silica gel column to provide the

title compound **42** (1.30 g, 66%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.80 (ddt, *J* = 16.9, 10.2, 6.6 Hz, 1H), 5.06–4.90 (m, 2H), 3.71–3.61 (m, 1H), 3.40 (t, *J* = 6.8 Hz, 2H), 2.09–2.00 (m, 2H), 1.91–1.80 (m, 2H), 1.52–1.35 (m, 8H), 0.89 (s, 9H), 0.04 (s, 6H).

General procedure B (Path b):



3-Bromo-*N*-(but-3-en-1-yl)-1-(5-((tert-butyldimethylsilyl)oxy)dec-9-en-1-yl)-1*H*-pyrazolo [3,4-*d*]pyrimidin-6-amine (**43**): To a mixture of **36** (1.11 g, 4.77 mmol) and K_2CO_3 (1.32 g, 9.54 mol) in DMSO (10 mL) was added **42** (2.0 g, 5.72 mmol)). The reaction mixture was stirred at 80°C for 1h, then but-3-en-1-amine (1.75 mL, 19.08 mmol) was added. The resulting reaction mixture was stirred at 110 °C for 16 h, diluted with Et_2O at rt, and washed with brine (2x). The aqueous layers were extracted with a mixture of Et_2O and EtOAc (1:1, 2x). The combined organic layers were dried (Na_2SO_4) and concentrated. The residue was purified by an ISCO silica gel column to provide the title compound **43** (2.20 g, 86%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.41 (s, 1H), 6.79 (s, 1H), 5.95–5.71 (m, 2H), 5.19–4.89 (m, 5H), 4.05 (t, *J* = 7.1 Hz, 2H), 3.59 (tt, *J* = 10.8, 5.4 Hz, 1H), 3.53 (dt, *J* = 12.6, 6.7 Hz, 2H), 2.40 (dt, *J* = 6.8, 6.8 Hz, 2H), 2.02 (dt, *J* = 6.1,

6.1 Hz, 2H), 1.84–1.73 (m, 2H), 1.49–1.29 (m, 8H), 0.84 (s, 9H), 0.01 (s, 3H), -0.03 (s, 3H); MS *m/z* $[M+H]^+$ calculated for $C_{26}H_{44}BrN_4OSi$, 536.65; found: 536.20.

10-((*tert*-Butyldimethylsilyl)oxy)-1^5-(4-((4-methylpiperazin-1-yl)methyl)phenyl)-1^7*H*-2-aza-1(2,7)-pyrrolo[2,3-*d*]pyrimidinacyclotetradecaphan-5-ene (**44**): A solution of **43** (0.54 g, 1.00 mmol) and 1,4-benzoquinone (0.022 g, 0.20 mmol) in toluene (240 mL) was heated to 110 ^oC under Argon atmosphere. A solution of Grubbs 2nd generation catalyst (0.17 g, 0.20 mmol) in toluene (10 mL) was added drop wisely in 15 min. After heated at 110°C for 3.0 h, the reaction mixture was cooled to 60°C. 2-Mercaptonicotinic acid (0.78 g, 5.00 mmol) was added and stirred at 60°C for 2.0 h. Then the reaction mixture was filtered through a Celite pad at rt and washed with EtOAc. The filtrate was concentrated and the residue was purified by an ISCO silica gel column to provide a mixture of E and Z olefin isomers (0.38 g, 75%).

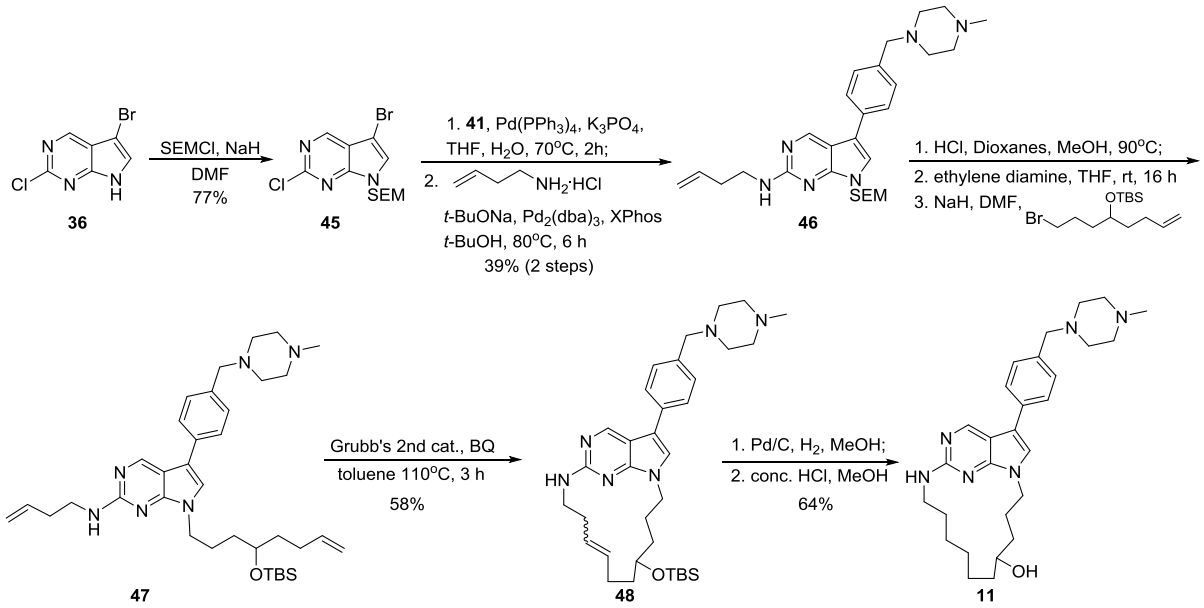
A solution of this mixture (0.11 g, 0.20 mmol) in THF (4.0 mL) was added K_2CO_3 (0.14 g, 1.00 mmol), **41** (0.19 g, 0.60 mmol), $Pd(PPh_3)_4$ (0.046 g, 0.04 mmol) and H_2O (1.0 mL). The resulting mixture was stirred at 78°C for 2.0 h and then was added silica gel at rt. The solvents were removed under reduced pressure and the residue was purified by silica gel column to provide the desired intermediates **44** (a mixture of E and Z isomers) (0.080 g, 69%) which was used in the next step without further purification.

1^5-(4-((4-Methylpiperazin-1-yl)methyl)phenyl)-1^7*H*-2-aza-1(2,7)-pyrrolo[2,3-*d*]pyrimidinacyclotetradecaphan-10-ol (**16**): A solution of **44** (0.17 g, 0.28 mmol) in methanol (5.0 mL) was added Pd/C (0.034 g, 5 wt% Pd/C, 20 wt%) and stirred under H_2 atmosphere at rt for 1 d. The reaction mixture was filtered through a short Celite pad and concentrated. The filtrate was concentrated and purified by reverse phase HPLC to provide a mixture of desired product and

des-TBS by-product due to the presence of TFA in HPLC solvents. A solution of the mixture in MeOH (5.0 mL) was added concentrated HCl solution (0.50 mL). The reaction mixture was stirred at rt for 1 h. The solvents were removed under the reduced pressure. The residue was azeotroped three times with MeOH to provide the desired product **16** as a HCl salt (UNC3133A) (0.084 g, 59% over two steps). ^{1}H NMR (400 MHz, CD_3OD) δ 8.82 (s, 1H), 7.87 (s, 1H), 7.78 (d, *J* = 8.3 Hz, 2H), 7.69 (d, *J* = 8.3 Hz, 2H), 4.44–4.31 (m, 3H), 4.20–4.09 (m, 1H), 3.85–3.38 (m, 11H), 3.00 (s, 3H), 2.09–1.84 (m, 2H), 1.82–1.66 (m, 2H), 1.65–1.57 (m, 1H), 1.56–1.32 (m, 13H); ^{13}C NMR (500 MHz, D_2O): δ 157.6, 153.7, 141.1, 135.3, 134.1 (2C), 132.9, 131.2, 129.2 (2C), 118.9, 112.1, 72.5, 63.0, 54.2 (2C), 51.4 (2C), 46.5, 45.4, 43.1, 36.9 (2C), 30.9, 29.8, 29.2, 29.0, 27.2, 25.8, 24.1. MS *m/z* $[M+H]^+$ calculated for $C_{30}H_{45}N_6O^+$, 505.73; found: 505.40; LC/MS: >95% purity; t_R: 4.48 min.

1^5-(4-((4-Methylpiperazin-1-yl)methyl)phenyl)-1^7*H*-2-aza-1(2,7)-pyrrolo[2,3-*d*]pyrimidinacycloundecaphan-8-ol (**10**): The title compound **10** (0.030 g, 23%) was prepared according to general procedure B from **36** (0.065 g, 0.28 mmol), ((7-bromohept-1-en-4-yl)oxy)(*tert*-butyl)dimethylsilane (0.10 g, 0.34 mmol), but-3-en-1-amine (0.099 mL, 1.1 mmol), and **41** (0.19 g, 0.60 mmol) as a yellow solid. ^{1}H NMR (400 MHz, CD_3OD) δ 8.88 (s, 1H), 7.88 (s, 1H), 7.80 (d, *J* = 8.4 Hz, 2H), 7.76 (d, *J* = 8.4 Hz, 2H), 4.57 (s, 2H), 4.45–4.36 (m, 1H), 4.15–4.05 (m, 1H), 4.03–3.94 (m, 1H), 3.90–3.59 (m, 9H), 3.45–3.34 (m, 1H), 3.03 (s, 3H), 2.10–2.00 (m, 1H), 1.99–1.78 (m, 3H), 1.77–1.68 (m, 1H), 1.68–1.59 (m, 3H), 1.58–1.46 (m, 3H), 1.44–1.33 (m, 1H); MS *m/z* $[M+H]^+$ calculated for $C_{27}H_{39}N_6O$, 463.65; found: 463.30 $[M+H]^+$; LC/MS: >95% purity; t_R: 4.06 min.

General procedure C:



5-Bromo-2-chloro-7-((2-(trimethylsilyl)ethoxy)methyl)-7*H*-pyrrolo[2,3-d]pyrimidine (**45**): To a solution of **36** (2.32 g, 10 mmol) and 2-(trimethylsilyl)ethoxymethyl chloride (SEMCl) (2.11 mL, 12 mmol) in DMF (50 mL) was added NaH (0.60 g, 60% in mineral oil, 15 mmol) at 0°C. The resulting mixture was stirred at rt for16 h, quenched with water, and extraction with EtOAc (3x50 mL). The combined organic layers were dried (Na_2SO_4) and concentrated. The residue was purified by an ISCO silica gel column to provide the title compound **45** (2.8 g, 77%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.77 (s, 1H), 7.38 (s, 1H), 5.59 (s, 2H), 3.60–3.49 (m, 2H), 0.98–0.87 (m, 2H), -0.03 (s, 10H).

***N*-(But-3-en-1-yl)-5-(4-((4-methylpiperazin-1-yl)methyl)phenyl)-7-((2-(trimethylsilyl) ethoxy)methyl)-7*H*-pyrrolo[2,3-d]pyrimidin-2-amine** (**46**): A solution of **45** (5.4 g, 15 mmol) in a mixture of THF (100 mL) and H_2O (20 mL) was added K_2CO_3 (6.3 g, 30 mmol), **41** (6.3 g, 19.5 mmol), and $Pd(PPh_3)_4$ (1.38 g, 1.13 mmol). The resulting mixture was refluxed for 3 h. Silica gel was added at rt and the solvents were removed under reduced pressure. The residue was purified by an ISCO silica gel column to provide the desired compound which was contaminated

with small amount of impurities (6.19 g). A solution of the compound (2.36 g, 5.0 mmol) in *t*-BuOH (50 mL) was added tris(dibenzylideneacetone)dipalladium(0) ($Pd_2(dba)_3$) (0.23 g, 0.25 mmol), 2-dicyclohexylphosphino-2′,4′,6′-triisopropylbiphenyl (XPhos) (0.24 g, 0.5 mmol), sodium *tert*-butoxide (NaO-*t*Bu) (1.68 g, 17.5 mmol), and but-3-en-1-amine hydrochloride salt (1.08 g, 10 mmol) at rt. The resulting mixture was stirred at 85°C for 6 h, quenched with saturated ammonium chloride (NH_4Cl) solution at rt, and extracted with EtOAc (3x50 mL). The combined organic layers were dried (Na_2SO_4), filtered, and concentrated. The residue was purified by an ISCO silica gel column to provide the title compound **46** (1.14 g, 39%). ^{1}H NMR (400 MHz, CD_3OD) δ 8.81 (s, 1H), 7.75 (s, 1H), 7.66 (d, *J* = 8.0 Hz, 2H), 7.48 (d, *J* = 8.1 Hz, 2H), 5.91 (ddt, *J* = 17.2, 10.3, 6.9 Hz, 1H), 5.59 (s, 2H), 5.13 (dd, *J* = 23.3, 13.7 Hz, 2H), 3.73 (s, 2H), 3.68 (t, *J* = 7.9 Hz, 2H), 3.62 (t, *J* = 7.0 Hz, 2H), 3.50–3.13 (m, 8H), 2.87 (s, 3H), 2.52–2.43 (m, 2H), 0.95 (t, *J* = 7.9 Hz, 2H), -0.03 (s, 9H).

***N*-(But-3-en-1-yl)-7-(4-((tert-butyldimethylsilyl)oxy)oct-7-en-1-yl)-5-(4-((4-methylpiperazin-1-yl)methyl)phenyl)-7*H*-pyrrolo[2,3-d]pyrimidin-2-amine** (**47**): A solution of **46** (1.14 g, 2.25 mmol) in MeOH (5.0 mL) was added a 4.0 M HCl solution in dioxanes (5.6 mL, 22.5 mmol) at rt. The reaction mixture was heated at 90°C for 4 h. Then the solvents were removed under reduced pressure. A solution of the residue in THF (10 mL) was added ethylenediamine (1.5 mL, 22.5 mmol). The resulting mixture was stirred at rt for 16 h. The solvent was removed under reduced pressure. The residue was purified by an ISCO silica gel column to provide *N*-(but-3-en-1-yl)-5-(4-((4-methylpiperazin-1-yl)methyl)phenyl)-7*H*-pyrrolo[2,3-d] pyrimidin-2-amine (0.67 g, 79%). ^{1}H NMR (400 MHz, CD_3OD) δ 8.74 (s, 1H), 7.63 (d, *J* = 8.1 Hz, 2H), 7.41 (d, *J* = 8.1 Hz, 2H), 7.30 (s, 1H), 5.90 (ddt, *J* = 17.1, 10.3, 6.8 Hz, 1H), 5.17–5.03 (m, 2H), 3.71 (s, 2H), 3.49 (t, *J* = 7.0 Hz, 2H), 3.17 (bs, 4H), 2.78 (bs, 7H), 2.46–2.36 (m, 2H).

A suspension of NaH (0.012 g, 60% in mineral oil, 0.3 mmol) in DMF (4.0 mL) was added *N*-(but-3-en-1-yl)-5-(4-((4-methylpiperazin-1-yl)methyl)phenyl)-7*H*-pyrrolo[2,3-d]pyrimidin-2-amine (0.076 g, 0.2 mmol) at 0°C. After stirring at rt for 30 min, the mixture was cooled to 0°C and was added ((1-bromooct-7-en-4-yl)oxy)(tert-butyl)dimethylsilane (0.096 g, 0.3 mmol). The resulting mixture was stirred at rt for 4 h, quenched with saturated NH_4Cl solution, and extracted with EtOAc (3x50 mL). The combined organic layers were dried (Na_2SO_4), filtered, and concentrated. The residue was purified by an ISCO silica gel column to provide the title compound **47** which was contaminated with small amount of impurities (0.049 g, 40%).

9-((*tert*-Butyldimethylsilyl)oxy)-1^5-(4-((4-methylpiperazin-1-yl)methyl)phenyl)-1^7*H*-2-aza-1(2,7)-pyrrolo[2,3-*d*]pyrimidinacyclododecaphan-5-ene (**48**): To a solution of **47** (0.049 g, 0.079 mmol) and benzoquinone (0.0017 g, 0.016 mmol) in toluene (50 mL) was added a solution of Grubbs 2nd generation catalyst (0.013 g, 0.016 mmol) in toluene (2.0 mL) slowly (15 min) at 110°C. The reaction mixture was refluxed for 3 h, then 2-mercaptonicotinic acid (0.050 g, 0.32 mmol) was added at 60°C. The resulting mixture was heated at 60°C for 2 h, then was passed through a pad of Celite at rt and washed with DCM. The filtrate was concentrated and purified by ISCO silica gel column to provide the title compound **48** (0.027 g, 58%) which was a mixture of *E*- and Z-isomer.

1^5-(4-((4-Methylpiperazin-1-yl)methyl)phenyl)-1^7*H*-2-aza-1(2,7)-pyrrolo[2,3-*d*]pyrimidinacyclododecaphan-9-ol (**11**): A solution of **48** (0.027 g, 0.046 mmol) in MeOH (3.0 mL) was added Pd/C (0.0054 g, 5 wt% Pd/C, 20 wt%). The reaction mixture was stirred under hydrogen atmosphere for 1 d, then was passed through a pad of Celite, and washed with MeOH. The filtrate was concentrated and purified by reverse phase HPLC. After concentration a mixture

of the desired product and des-TBS by-product was obtained due to the presence of 0.5% of TFA in the elute of HPLC.

A solution of this mixture in MeOH (3.0 mL) was added concentrated HCl (0.30 mL). The reaction mixture was stirred at rt for 1 h and was concentrated. The residue was azeotroped three times with MeOH to provide the HCl salt of the desired product **11** as a yellow solid (0.014 g, 64% over 2 steps). ^{1}H NMR (400 MHz, CD_3OD) δ 8.66 (s, 1H), 7.60 (d, J = 8.0 Hz, 2H), 7.39 (d, *J* = 8.0 Hz, 2H), 7.32 (s, 1H), 4.53–4.41 (m, 1H), 4.01–3.85 (m, 3H), 3.27–3.19 (m, 3H), 2.79 –2.41 (bs, 8H), 2.36 (s, 3H), 2.16–1.97 (m, 2H), 1.79–1.67 (m, 2H), 1.64–1.52 (m, 2H), 1.50–1.42 (m, 2H), 1.41–1.21 (m, 6H); MS *m/z* $[M+H]^+$ calculated for $C_{28}H_{41}N_6O$, 477.68; found: 477.30; LC/MS: >95% purity; t_R: 3.86 min.

1^5-(4-((4-Methylpiperazin-1-yl)methyl)phenyl)-1^7*H*-2-aza-1(2,7)-pyrrolo[2,3-*d*]pyrimidinacyclotridecaphan-10-ol (**12**): The title compound **12** (0.017 g, 4.1%) was prepared according to general procedure C from **36** (0.19 g, 0.84 mmol), **41** (0.27 g, 0.84 mmol), but-3-en-1-amine hydrochloride salt (0.18 g, 1.7 mmol), and ((1-bromonon-8-en-4-yl)oxy)(*tert*-butyl)dimethylsilane (0.096 g, 0.30 mmol) as a yellow solid. ^{1}H NMR (400 MHz, CD_3OD) δ 8.86 (s, 1H), 7.88 (s, 1H), 7.80 (d, *J* = 8.3 Hz, 2H), 7.75 (d, *J* = 8.4 Hz, 2H), 4.54 (s, 2H), 4.35–4.24 (m, 1H), 4.21–4.12 (m, 1H), 3.89–3.57 (m, 10H), 3.55–3.46 (m, 1H), 3.02 (s, 3H), 1.99–1.87 (m, 2H), 1.75–1.64 (m, 2H), 1.64–1.56 (m, 2H), 1.55–1.44 (m, 6H), 1.44–1.28 (m, 4H); MS *m/z* $[M+H]^+$ calculated for $C_{29}H_{43}N_6O$, 491.70; found: 491.40; LC/MS: >95% purity; t_R: 4.24 min.

1^5-(4-((4-Methylpiperazin-1-yl)methyl)phenyl)-1^7*H*-2-aza-1(2,7)-pyrrolo[2,3-*d*]pyrimidinacyclotetradecaphan-11-ol (**13**): The title compound **13** (0.065 g, 43%) was prepared according to general procedure B from **36** (0.070 g, 0.30 mmol), ((1-bromodec-9-en-4-

yl)oxy)(*tert*-butyl)dimethylsilane (0.13 g, 0.36 mmol), but-3-en-1-amine (0.11 mL, 1.2 mmol), and **41** (0.19 g, 0.60 mmol) as a yellow solid. ^{1}H NMR (400 MHz, CD_3OD) δ 8.84 (s, 1H), 7.88 (s, 1H), 7.79 (d, *J* = 8.3 Hz, 2H), 7.73 (d, *J* = 8.3 Hz, 2H), 4.48 (s, 2H), 4.41–4.32 (m, 1H), 4.21–4.11 (m, 1H), 3.81–3.46 (m, 11H), 3.01 (s, 3H), 2.12–1.92 (m, 2H), 1.81–1.69 (m, 2H), 1.55–1.35 (m, 14H); MS *m/z* $[M+H]^+$ calculated for $C_{30}H_{45}N_6O$, 505.73; found: 505.40; LC/MS: >95% purity; t_R: 4.69 min.

1^5-(4-((4-Methylpiperazin-1-yl)methyl)phenyl)-1^7*H*-2-aza-1(2,7)-pyrrolo[2,3-*d*]pyrimidinacyclododecaphan-8-ol (**14**): The title compound **14** (0.056 g, 38%) was prepared according to general procedure B from **36** (0.070 g, 0.30 mmol), ((8-bromooct-1-en-4-yl)oxy)(*tert*-butyl)dimethylsilane (0.12 g, 0.36 mmol), but-3-en-1-amine (0.11 mL, 1.2 mmol), and **41** (0.19 g, 0.60 mmol) as a yellow solid. ^{1}H NMR (400 MHz, CD_3OD) δ 8.86 (s, 1H), 7.90 (s, 1H), 7.81 (d, *J* = 8.4 Hz, 2H), 7.77 (d, *J* = 8.4 Hz, 2H), 4.58 (s, 2H), 4.44–4.35 (m, 1H), 4.22–4.13 (m, 1H), 3.91–3.59 (m, 11H), 3.03 (s, 3H), 2.14–1.82 (m, 3H), 1.80–1.66 (m, 2H), 1.63–1.33 (m, 8H), 1.16 (d, *J* = 8.7 Hz, 1H); MS *m/z* $[M+H]^+$ calculated for $C_{28}H_{41}N_6O$, 477.68; found: 477.40; LC/MS: >95% purity; t_R: 4.24 min.

1^5-(4-((4-Methylpiperazin-1-yl)methyl)phenyl)-1^7*H*-2-aza-1(2,7)-pyrrolo[2,3-*d*]pyrimidinacyclotridecaphan-9-ol (**15**): The title compound **15** (0.013 g, 3.2%) was prepared according to general procedure C from **36** (0.19 g, 0.84 mmol), **41** (0.27 g, 0.84 mmol), but-3-en-1-amine hydrochloride salt (0.18 g, 1.7 mmol), and ((9-bromonon-1-en-5-yl)oxy)(*tert*-butyl)dimethylsilane (0.10 g, 0.3 mmol) as a yellow solid. ^{1}H NMR (400 MHz, CD_3OD) δ 8.86 (s, 1H), 7.88 (s, 1H), 7.81 (d, *J* = 8.4 Hz, 2H), 7.76 (d, *J* = 8.4 Hz, 2H), 4.56 (s, 2H), 4.38–4.27 (m, 1H), 4.23–4.13 (m, 1H), 3.92–3.58 (m, 10H), 3.57–3.47 (m, 1H), 3.03 (s, 3H), 2.05–1.94 (m, 1H), 1.94–1.85 (m, 1H), 1.83–1.74 (m, 1H), 1.72–1.54 (m, 4H), 1.53–1.43 (m, 7H), 1.42–1.33 (m, 2H);

MS *m/z* $[M+H]^+$ calculated for $C_{29}H_{43}N_6O$, 491.70; found: 491.40; LC/MS: >95% purity; t_R: 4.26 min.

1^5-(4-((4-Methylpiperazin-1-yl)methyl)phenyl)-1^7*H*-2-aza-1(2,7)-pyrrolo[2,3-*d*]pyrimidinacyclopentadecaphan-11-ol (**17**): The title compound **17** (0.046 g, 29%) was prepared according to general procedure B from **36** (0.071 g, 0.31 mmol), ((1-bromoundec-10-en-5-yl)oxy)(*tert*-butyl)dimethylsilane (0.14 g, 0.37 mmol), but-3-en-1-amine (0.11 mL, 1.2 mmol), and **41** (0.13 g, 0.40 mmol) as a yellow solid. ^{1}H NMR (400 MHz, CD_3OD) δ 8.86 (s, 1H), 7.90 (s, 1H), 7.81 (d, *J* = 8.4 Hz, 2H), 7.76 (d, *J* = 8.4 Hz, 2H), 4.56 (s, 2H), 4.31–4.21 (m, 2H), 3.86–3.54 (m, 11H), 3.03 (s, 3H), 2.00–1.90 (m, 2H), 1.81–1.71 (m, 2H), 1.56–1.34 (m, 16H); MS *m/z* $[M+H]^+$ calculated for $C_{31}H_{47}N_6O$, 519.76; found: 519.40; LC/MS: >95% purity; t_R: 4.82 min.

1^5-(4-((4-Methylpiperazin-1-yl)methyl)phenyl)-1^7*H*-2-aza-1(2,7)-pyrrolo[2,3-*d*]pyrimidinacyclotridecaphan-8-ol (**18**): The title compound **18** (0.058 g, 40%) was prepared according to general procedure B from **36** (0.070 g, 0.30 mmol), ((9-bromonon-1-en-4-yl)oxy)(*tert*-butyl)dimethylsilane (0.12 g, 0.36 mmol), but-3-en-1-amine (0.11 mL, 1.2 mmol), and **41** (0.19 g, 0.60 mmol) as a yellow solid. ^{1}H NMR (400 MHz, CD_3OD) δ 8.88 (s, 1H), 7.90 (s, 1H), 7.81 (d, *J* = 8.4 Hz, 2H), 7.77 (d, *J* = 8.4 Hz, 2H), 4.58 (s, 2H), 4.36–4.16 (m, 2H), 3.90–3.62 (m, 10H), 3.59–3.50 (m, 1H), 3.03 (s, 3H), 2.07–1.78 (m, 3H), 1.66–1.37 (m, 13H); MS *m/z* $[M+H]^+$ calculated for $C_{29}H_{43}N_6O$, 491.70; found: 491.40; LC/MS: >95% purity; t_R: 4.34 min.

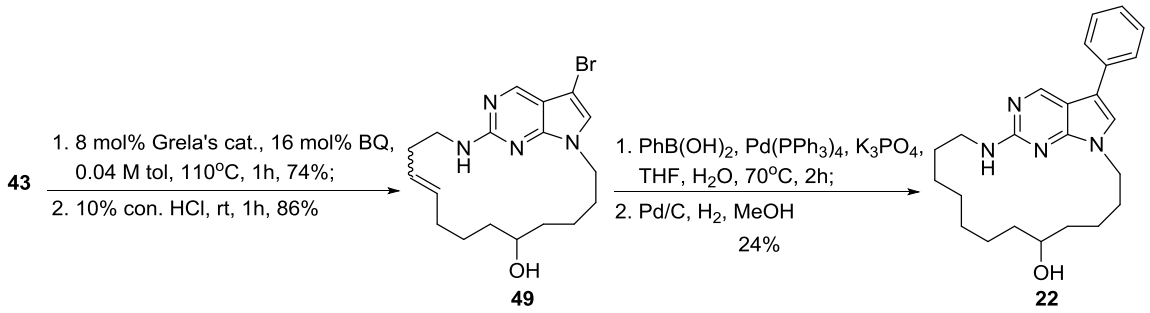
1^5-(4-((4-Methylpiperazin-1-yl)methyl)phenyl)-1^7*H*-2-aza-1(2,7)-pyrrolo[2,3-*d*]pyrimidinacyclotetradecaphan-9-ol (**19**): The title compound **19** (0.052 g, 32%) was prepared according to general procedure B from **36** (0.076 g, 0.33 mmol), ((10-bromodec-1-en-5-yl)oxy)(*tert*-butyl)dimethylsilane (0.14 g, 0.40 mmol), but-3-en-1-amine (0.12 mL, 1.3 mmol), and

41 (0.19 g, 0.60 mmol) as a yellow solid. ^{1}H NMR (400 MHz, CD_3OD) δ 8.86 (s, 1H), 7.90 (s, 1H), 7.81 (d, *J* = 8.3 Hz, 2H), 7.76 (d, *J* = 8.3 Hz, 2H), 4.55 (s, 2H), 4.33–4.17 (m, 2H), 3.91–3.59 (m, 10H), 3.57–3.48 (m, 1H), 3.02 (s, 3H), 2.05–1.90 (m, 2H), 1.81–1.69 (m, *J* = 13.7, 7.0 Hz, 2H), 1.60–1.35 (m, 14H); MS *m/z* $[M+H]^+$ calculated for $C_{30}H_{45}N_6O$, 505.73; found: 505.40; LC/MS: >95% purity; t_R: 4.47 min.

1^5-(4-((4-Methylpiperazin-1-yl)methyl)phenyl)-1^7*H*-2-aza-1(2,7)-pyrrolo[2,3-*d*]pyrimidinacyclopentadecaphan-10-ol (**20**): The title compound **20** (0.051 g, 27%) was prepared according to general procedure B from **36** (0.085 g, 0.37 mmol), ((11-bromoundec-1-en-6-yl)oxy)(*tert*-butyl)dimethylsilane (0.17 g, 0.44 mmol), but-3-en-1-amine (0.14 mL, 1.5 mmol), and **41** (0.19 g, 0.60 mmol) as a yellow solid. ^{1}H NMR (400 MHz, CD_3OD) δ 8.87 (s, 1H), 7.89 (s, 1H), 7.80 (d, *J* = 8.4 Hz, 2H), 7.75 (d, *J* = 8.4 Hz, 2H), 4.53 (s, 2H), 4.32–4.17 (m, 2H), 3.86–3.49 (m, 11H), 3.02 (s, 3H), 2.01–1.89 (m, 2H), 1.79–1.68 (m, 2H), 1.54–1.34 (m, 16H); MS *m/z* $[M+H]^+$ calculated for $C_{31}H_{47}N_6O$, 519.76; found: 519.40; LC/MS: >95% purity; t_R: 4.72 min.

1^5-(4-((4-Methylpiperazin-1-yl)methyl)phenyl)-1^7*H*-2-aza-1(2,7)-pyrrolo[2,3-*d*]pyrimidinacyclohexadecaphan-11-ol (**21**): The title compound **21** (0.073 g, 47%) was prepared according to general procedure B from **36** (0.070 g, 0.30 mmol), ((1-bromododec-11-en-64-yl)oxy)(*tert*-butyl)dimethylsilane (0.13 g, 0.36 mmol), but-3-en-1-amine (0.11 mL, 1.2 mmol), and **41** (0.13 g, 0.40 mmol) as a yellow solid. ^{1}H NMR (400 MHz, CD_3OD) δ 8.85 (s, 1H), 7.89 (s, 1H), 7.81 (d, *J* = 8.4 Hz, 2H), 7.76 (d, *J* = 8.4 Hz, 2H), 4.57 (s, 2H), 4.34–4.15 (m, 2H), 3.96–3.46 (m, 11H), 3.03 (s, 3H), 2.02–1.90 (m, 2H), 1.80–1.69 (m, 2H), 1.59–1.25 (m, 18H); MS *m/z* $[M+H]^+$ calculated for $C_{32}H_{49}N_6O$, 533.79; found: 533.50; LC/MS: >95% purity; t_R: 5.02 min.

General procedure D:



1^5-Bromo-1^7*H*-2-aza-1(2,7)-pyrrolo[2,3-*d*]pyrimidinacyclotetradecaphan-5-en-10-ol (**49**): A solution of **43** (2.2 g, 4.11 mmol) in toluene (100 mL) was vacuumed and refilled with Argon (3x), and then was added a solution of Grela's catalyst (0.22 g, 0.32 mmol) in toluene (5.0 mL) in 30 min at 110°C. The resulting solution was stirred at 110°C for 1 h, then 2-mercaptonicotinic acid (0.13 g, 0.82 mmol) was added at 60°C. The resulting mixture was heated at 60°C for 2 h, then was passed through a pad of Celite at rt and washed with DCM. The filtrate was concentrated and purified by ISCO silica gel column to provide the desired ring–close product (1.55 g, 74%).

A solution of this compound (1.55 g, 3.06 mmol) in MeOH (20 mL) was added concentrated HCl (1.0 mL) and stirred at rt for 1 h. The solvents were removed under reduced pressure. The resulting residue was dissolved in EtOAc, neutralized with aqueous sodium bicarbonate ($NaHCO_3$) solution, and extracted with EtOAc (3x50 mL). The combined organic layers were dried (Na_2SO_4) and concentrated. The residue was purified by ISCO silica gel column to provide the title compound as a mixture of *E* and *Z* isomers (1.03 g, 86%). The *E* and *Z* isomers were separated by a reverse phase HPLC. One isomer: ^{1}H NMR (400 MHz, CD_3OD) δ 8.27 (s, 1H), 6.78 (s, 1H), 5.36–5.24 (m, 2H), 4.14–4.04 (m, 1H), 3.92–3.83 (m, 1H), 3.52–3.41 (m, 2H), 3.39–3.30 (m, 1H), 2.31–2.16 (m, 2H), 2.02–1.85 (m, 2H), 1.77–1.55 (m, 2H), 1.49–1.25 (m, 5H), 1.22–1.13 (m, 2H), 1.11–1.04 (m, 1H); MS *m/z* $[M+H]^+$ calculated for $C_{18}H_{26}BrN_4O$, 394.34; found: 394.10; LC/MS: >95% purity. The other isomer: ^{1}H NMR (400 MHz, CD_3OD) δ 8.25 (s, 1H), 6.81 (s, 1H), 5.42–5.25 (m,

2H), 4.24–4.12 (m, 1H), 3.85–3.78 (m, 1H), 3.50–3.32 (m, 3H), 2.43–2.31 (m, 1H), 2.27–2.13 (m, 1H), 2.08–1.96 (m, 1H), 1.95–1.86 (m, 1H), 1.85–1.73 (m, 1H), 1.71–1.53 (m, 2H), 1.45–1.37 (m, 2H), 1.36–1.25 (m, 4H), 1.24–1.11 (m, 1H); MS *m/z* $[M+H]^+$ calculated for $C_{18}H_{26}BrN_4O$, 394.34; found: 394.20; LC/MS: >95% purity.

1^5-Phenyl-1^7*H*-2-aza-1(2,7)-pyrrolo[2,3-*d*]pyrimidinacyclotetradecaphan-10-ol (**22**): A mixture of **49** (0.039 g, 0.10 mmol), K_2CO_3 (0.055 g, 0.40 mmol), phenylboronic acid (0.024 g, 0.20 mmol), and $Pd(PPh_3)_4$ (0.023 g, 0.02 mmol) in a mixture of THF (4.0 mL) and H_2O (1.0 mL) was refluxed for 3 h. Silica gel was then added at rt. The solvents were removed under reduced pressure. The residue was purified by ISCO silica gel column to provide the Suzuki coupling product.

A solution of this compound in MeOH (3.0 mL) was added Pd/C (4.0 mg, 10 wt% Pd/C, 10 wt%). The reaction mixture was stirred under hydrogen atmosphere for 1 d. then passed through a pad of Celite and washed with MeOH. The filtrate was concentrated and purified by reverse phase HPLC. After concentration the residue was dissolved in a 7.0 M ammonium solution in MeOH (1.0 mL). The reaction mixture was stirred at rt for 2 h. The solvent was removed under reduced pressure. The residue was purified by ISCO silica gel column to provide the title compound as a TFA salt. A solution of the TFA salt in MeOH (1.0 mL) was added a 4.0 M HCl solution in dioxanes (0.10 mL). The resulting mixture was stirred for 2 h and concentrated to provide the HCl salt of the title product **22** as a yellow solid (9.5 mg, 24%).^{1}H NMR (400 MHz, CD_3OD) δ 8.76 (s, 1H), 7.75 (s, 1H), 7.67–7.62 (m, 2H), 7.47 (dd, *J* = 10.5, 4.8 Hz, 2H), 7.39–7.32 (m, 1H), 4.41–4.30 (m, 1H), 4.18–4.08 (m, 1H), 3.76–3.66 (m, 1H), 3.64–3.56 (m, 1H), 3.54–3.45 (m, 1H), 2.09–1.96 (m, 1H), 1.95–1.85 (m, 1H), 1.81–1.68 (m, 2H), 1.65–1.56 (m, 1H), 1.54–1.39 (m, 13H); MS *m/z* $[M+H]^+$ calculated for $C_{24}H_{33}N_4O$, 393.56; found: 393.30; LC/MS: >95% purity; t_R: 5.54 min.

1^5-(4-Fluorophenyl)-1^7*H*-2-aza-1(2,7)-pyrrolo[2,3-*d*]pyrimidinacyclotetradecaphan-10-ol (**23**): The title compound **23** (0.012 g, 29%) was prepared according to general procedure D from **49** (0.039 g, 0.10 mmol) and 4-fluorophenylboronic acid (0.028 g, 0.20 mmol) as a yellow solid. ^{1}H NMR (400 MHz, CD_3OD) δ 8.76 (s, 1H), 7.73 (s, 1H), 7.70–7.62 (m, 2H), 7.21 (t, *J* = 8.8 Hz, 2H), 4.35 (dt, *J* = 14.5, 7.4 Hz, 1H), 4.12 (dt, *J* = 13.6, 6.7 Hz, 1H), 3.71 (dt, *J* = 14.6, 7.4 Hz, 1H), 3.64–3.56 (m, 1H), 3.53–3.44 (m, 1H), 2.07–1.95 (m, 1H), 1.95–1.85 (m, 1H), 1.81–1.67 (m, 2H), 1.64–1.57 (m, 1H), 1.54–1.38 (m, 13H); MS *m/z* $[M+H]^+$ calculated for $C_{24}H_{32}FN_4O$, 411.55; found: 411.30; LC/MS: >95% purity; t_R: 5.61 min.

1^5-(4-Methoxyphenyl)-1^7*H*-2-aza-1(2,7)-pyrrolo[2,3-*d*]pyrimidinacyclotetradecaphan-10-ol (**24**): The title compound **24** (0.028 g, 66%) was prepared according to general procedure D from **49** (0.039 g, 0.10 mmol) and 4-methoxyphenylboronic acid (0.030 g, 0.20 mmol) as a yellow solid. ^{1}H NMR (400 MHz, CD_3OD) δ 8.71 (s, 1H), 7.62 (s, 1H), 7.58–7.52 (m, 2H), 7.05–6.98 (m, 2H), 4.39–4.27 (m, 1H), 4.10 (dt, *J* = 13.6, 6.7 Hz, 1H), 3.83 (s, 3H), 3.74–3.64 (m, 1H), 3.63–3.55 (m, 1H), 3.48 (ddd, *J* = 13.7, 8.0, 6.0 Hz, 1H), 2.05–1.94 (m, 1H), 1.94–1.84 (m, 1H), 1.82–1.66 (m, 2H), 1.65–1.55 (m, 1H), 1.54–1.39 (m, 13H); MS *m/z* $[M+H]^+$ calculated for $C_{25}H_{35}N_4O_2$, 423.58; found: 423.30; LC/MS: >95% purity; t_R: 5.45 min.

1^5-(3-Methoxyphenyl)-1^7*H*-2-aza-1(2,7)-pyrrolo[2,3-*d*]pyrimidinacyclotetradecaphan-10-ol (**25**): The title compound **25** (0.028 g, 66%) was prepared according to general procedure D from **49** (0.039 g, 0.10 mmol) and 3-methoxyphenylboronic acid (0.030 g, 0.20 mmol) as a yellow solid. ^{1}H NMR (400 MHz, CD_3OD) δ 8.74 (s, 1H), 7.76 (s, 1H), 7.37 (t, *J* = 8.0 Hz, 1H), 7.23–7.18 (m, 1H), 7.17–7.12 (m, 1H), 6.94–6.88 (m, 1H), 4.35 (dt, *J* = 14.5, 7.4 Hz, 1H), 4.11 (dt, *J* = 13.6, 6.8 Hz, 1H), 3.86 (s, 3H), 3.75–3.66 (m, 1H), 3.63–3.55 (m, 1H), 3.49 (ddd, *J* = 13.8, 8.0, 6.1 Hz, 1H), 2.07–1.95 (m, 1H), 1.94–1.85 (m, 1H), 1.82–1.67 (m, 2H), 1.64–1.55 (m, 1H), 1.55–1.37 (m, 13H);

MS *m/z* $[M+H]^+$ calculated for $C_{25}H_{35}N_4O_2$, 423.58; found: 423.30; LC/MS: >95% purity; t_R: 5.57 min.

1^5-(2-Methoxyphenyl)-1^7*H*-2-aza-1(2,7)-pyrrolo[2,3-*d*]pyrimidinacyclotetradecaphan-10-ol (**26**): The title compound **26** (0.028 g, 66%) was prepared according to general procedure D from **49** (0.039 g, 0.10 mmol) and 2-methoxyphenylboronic acid (0.030 g, 0.20 mmol) as a yellow solid. ^{1}H NMR (400 MHz, CD_3OD) δ 8.55 (s, 1H), 7.66 (s, 1H), 7.49 (dd, *J* = 7.6, 1.6 Hz, 1H), 7.40–7.30 (m, 1H), 7.12 (d, *J* = 7.8 Hz, 1H), 7.04 (td, *J* = 7.5, 0.9 Hz, 1H), 4.34 (dt, *J* = 14.4, 7.4 Hz, 1H), 4.11 (dt, *J* = 13.6, 6.7 Hz, 1H), 3.89 (s, 3H), 3.70 (dt, *J* = 17.6, 7.5 Hz, 1H), 3.64–3.56 (m, 1H), 3.54–3.43 (m, 1H), 2.05–1.94 (m, 1H), 1.93–1.84 (m, 1H), 1.81–1.66 (m, 2H), 1.65–1.56 (m, 1H), 1.54–1.35 (m, 13H); MS *m/z* $[M+H]^+$ calculated for $C_{25}H_{35}N_4O_2$, 423.58; found: 423.30; LC/MS: >95% purity; t_R: 5.38 min.

1^5-(4-(Morpholinomethyl)phenyl)-1^7*H*-2-aza-1(2,7)-pyrrolo[2,3-*d*]pyrimidinacyclotetradecaphan-10-ol (**27**): The title compound **27** (0.021 g, 44%) was prepared according to general procedure D from **49** (0.039 g, 0.10 mmol) and 4-(4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)benzyl)morpholine (0.061 g, 0.20 mmol) as a yellow solid. ^{1}H NMR (400 MHz, CD_3OD) δ 8.87 (s, 1H), 7.90 (s, 1H), 7.80 (d, *J* = 8.2 Hz, 2H), 7.72 (d, *J* = 8.2 Hz, 2H), 4.44 (s, 2H), 4.41–4.31 (m, 1H), 4.14 (dt, *J* = 13.6, 6.7 Hz, 1H), 4.05 (dd, *J* = 13.0, 3.1 Hz, 2H), 3.87 (t, *J* = 11.6 Hz, 2H), 3.76–3.66 (m, 1H), 3.64–3.56 (m, 1H), 3.54–3.45 (m, 1H), 3.41 (d, *J* = 12.5 Hz, 2H), 3.26 (td, *J* = 12.3, 3.6 Hz, 2H), 2.08–1.96 (m, 1H), 1.96–1.85 (m, 1H), 1.83–1.67 (m, 2H), 1.66–1.56 (m, 1H), 1.54–1.32 (m, 13H); MS *m/z* $[M+H]^+$ calculated for $C_{29}H_{42}N_5O_2$, 492.69; found: 492.40; LC/MS: >95% purity; t_R: 4.54 min.

(4-(10-Hydroxy-1^7*H*-2-aza-1(2,7)-pyrrolo[2,3-*d*]pyrimidinacyclotetradecaphane-15-yl)phenyl)(morpholino)methanone (**28**): The title compound **28** (0.033 g, 65%) was prepared according to general procedure D from **49** (0.039 g, 0.10 mmol) and morpholino(4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl)methanone (0.063 g, 0.20 mmol) as a yellow solid. ^{1}H NMR (400 MHz, CD_3OD) δ 8.77 (s, 1H), 7.74–7.66 (m, 3H), 7.51 (d, *J* = 8.1 Hz, 2H), 4.41–4.29 (m, 1H), 4.10 (dt, *J* = 13.5, 6.6 Hz, 1H), 3.86–3.41 (m, 11H), 2.06–1.94 (m, 1H), 1.93–1.83 (m, 1H), 1.80–1.65 (m, 2H), 1.64–1.55 (m, 1H), 1.53–1.32 (m, 13H); MS *m/z* $[M+H]^+$ calculated for $C_{29}H_{40}N_5O_3$, 506.67; found: 506.30; LC/MS: >95% purity; t_R: 5.28 min.

1^5-(4-(Morpholinosulfonyl)phenyl)-1^7*H*-2-aza-1(2,7)-pyrrolo[2,3-*d*]pyrimidinacyclotetradecaphan-10-ol (**29**): The title compound **29** (0.034 g, 62%) was prepared according to general procedure D from **49** (0.039 g, 0.10 mmol) and (4-(morpholinosulfonyl)phenyl)boronic acid (0.054 g, 0.20 mmol) as a yellow solid. ^{1}H NMR (400 MHz, CD_3OD) δ 8.87 (s, 1H), 7.91–7.78 (m, 5H), 4.43–4.30 (m, 1H), 4.13 (dt, *J* = 13.4, 6.6 Hz, 1H), 3.80–3.71 (m, 4H), 3.71–3.66 (m, 1H), 3.64–3.56 (m, 1H), 3.52–3.42 (m, 1H), 3.07–2.95 (m, 4H), 2.07–1.96 (m, 1H), 1.96–1.85 (m, 1H), 1.81–1.66 (m, 2H), 1.63–1.56 (m, 1H), 1.54–1.32 (m, 13H); MS *m/z* $[M+H]^+$ calculated for $C_{28}H_{40}N_5O_4S$, 542.72; found: 542.30; LC/MS: >95% purity; t_R: 5.58 min.

***N*-(4-(10-Hydroxy-1^7*H*-2-aza-1(2,7)-pyrrolo[2,3-*d*]pyrimidinacyclotetradecaphane-15-yl)phenyl)morpholine-4-carboxamide** (**30**): The title compound **30** (0.020 g, 39%) was prepared according to general procedure D from **49** (0.039 g, 0.10 mmol) and *N*-(4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl)morpholine-4-carboxamide (0.066 g, 0.20 mmol) as a yellow solid. ^{1}H NMR (400 MHz, CD_3OD) δ 8.71 (s, 1H), 7.59 (s, 1H), 7.51 (s, 4H), 4.33 (dt, *J* = 14.3, 7.3 Hz, 1H), 4.08 (dt, *J* = 13.5, 6.7 Hz, 1H), 3.76–3.71 (m, 4H), 3.71–3.65 (m, 1H), 3.64–3.57 (m,

1H), 3.57–3.51 (m, 4H), 3.46 (ddd, *J* = 13.8, 8.0, 5.9 Hz, 1H), 2.06–1.94 (m, 1H), 1.93–1.83 (m, 1H), 1.80–1.66 (m, 2H), 1.64–1.54 (m, 1H), 1.53–1.34 (m, 13H); MS *m/z* $[M+H]^+$ calculated for $C_{29}H_{41}N_6O_3$, 521.69; found: 521.40; LC/MS: >95% purity; t_R: 5.19 min.

1^5-(4-Morpholinophenyl)-1^7*H*-2-aza-1(2,7)-pyrrolo[2,3-*d*]pyrimidinacyclotetradecaphan-10-ol (**31**): The title compound **31** (0.027 g, 58%) was prepared according to general procedure D from **49** (0.039 g, 0.10 mmol) and (4-morpholinophenyl)boronic acid (0.041 g, 0.20 mmol) as a yellow solid. ^{1}H NMR (400 MHz, CD_3OD) δ 8.90 (s, 1H), 7.97 (s, 1H), 7.93 (s, 4H), 4.37 (dt, *J* = 14.2, 7.3 Hz, 1H), 4.23–4.17 (m, 4H), 4.17–4.10 (m, 1H), 3.83–3.76 (m, 4H), 3.76–3.66 (m, 1H), 3.65–3.56 (m, 1H), 3.55–3.45 (m, 1H), 2.09–1.97 (m, 1H), 1.97–1.86 (m, 1H), 1.83–1.66 (m, 2H), 1.65–1.55 (m, 1H), 1.54–1.32 (m, 13H); MS *m/z* $[M+H]^+$ calculated for $C_{28}H_{40}N_5O_2$, 478.66; found: 478.30; LC/MS: >95% purity; t_R: 5.42 min.

1^5-(Pyridin-2-yl)-1^7*H*-2-aza-1(2,7)-pyrrolo[2,3-*d*]pyrimidinacyclotetradecaphan-10-ol (**32**): The title compound **32** (0.011 g, 30%) was prepared according to general procedure D from **49** (0.039 g, 0.10 mmol) and 2-(tributylstannyl)pyridine (0.073 g, 0.2 mmol) as a yellow solid. ^{1}H NMR (400 MHz, CD_3OD) δ 9.17 (s, 1H), 8.70 (d, *J* = 5.4 Hz, 1H), 8.25 (s, 1H), 8.19 (dd, *J* = 13.8, 6.3 Hz, 1H), 8.06 (d, *J* = 8.0 Hz, 1H), 7.61–7.54 (m, 1H), 4.40 (dt, *J* = 14.2, 7.3 Hz, 1H), 4.19 (dt, *J* = 13.7, 6.8 Hz, 1H), 3.78–3.67 (m, 1H), 3.64–3.56 (m, 1H), 3.55–3.47 (m, 1H), 2.10–2.00 (m, 1H), 1.99–1.89 (m, 1H), 1.84–1.68 (m, 2H), 1.66–1.56 (m, 2H), 1.54–1.39 (m, 11H), 1.38–1.31 (m, 1H); MS *m/z* $[M+H]^+$ calculated for $C_{23}H_{32}N_5O$, 394.54; found: 394.30; LC/MS: >95% purity; t_R: 5.23 min.

1^5-(Pyridin-3-yl)-1^7*H*-2-aza-1(2,7)-pyrrolo[2,3-*d*]pyrimidinacyclotetradecaphan-10-ol (**33**): The title compound **33** (0.040 g, 99%) was prepared according to general procedure D from **49**

(0.039 g, 0.10 mmol) and 3-pyridyl boronic acid (0.032 g, 0.20 mmol) as a yellow solid. ^{1}H NMR (400 MHz, CD_3OD) δ 9.31 (d, *J* = 1.6 Hz, 1H), 9.14 (s, 1H), 9.01 (d, *J* = 8.3 Hz, 1H), 8.83 (d, *J* = 5.6 Hz, 1H), 8.34 (s, 1H), 8.21 (dd, *J* = 8.2, 5.8 Hz, 1H), 4.48–4.35 (m, 1H), 4.21 (dt, *J* = 13.6, 6.7 Hz, 1H), 3.81–3.68 (m, 1H), 3.66–3.57 (m, 1H), 3.52 (ddd, *J* = 13.7, 7.9, 6.1 Hz, 1H), 2.12–2.00 (m, 1H), 1.99–1.89 (m, 1H), 1.84–1.68 (m, 2H), 1.66–1.56 (m, 1H), 1.55–1.32 (m, 13H); MS *m/z* $[M+H]^+$ calculated for $C_{23}H_{32}N_5O$, 394.54; found: 394.30; LC/MS: >95% purity; t_R: 5.30 min.

1^5-(Pyridin-4-yl)-1^7*H*-2-aza-1(2,7)-pyrrolo[2,3-*d*]pyrimidinacyclotetradecaphan-10-ol (**34**): The title compound **34** (0.035 g, 90%) was prepared according to general procedure D from **49** (0.039 g, 0.10 mmol) and 4-pyridyl boronic acid (0.032 g, 0.20 mmol) as a yellow solid. ^{1}H NMR (400 MHz, CD_3OD) δ 9.26 (s, 1H), 8.85–8.79 (m, 2H), 8.75 (s, 1H), 8.41 (d, *J* = 5.7 Hz, 2H), 4.43 (dt, *J* = 14.6, 7.4 Hz, 1H), 4.24 (dt, *J* = 13.7, 6.8 Hz, 1H), 3.74 (dt, *J* = 14.6, 7.5 Hz, 1H), 3.65–3.57 (m, 1H), 3.53 (ddd, *J* = 13.8, 8.0, 6.1 Hz, 1H), 2.12–2.01 (m, 1H), 2.01–1.91 (m, 1H), 1.84–1.68 (m, 2H), 1.66–1.56 (m, 1H), 1.56–1.34 (m, 13H); MS *m/z* $[M+H]^+$ calculated for $C_{23}H_{32}N_5O$, 394.54; found: 394.30; LC/MS: >95% purity; t_R: 4.96 min.

1^5-(Tetrahydro-2*H*-pyran-4-yl)-1^7*H*-2-aza-1(2,7)-pyrrolo[2,3-*d*]pyrimidinacyclotetradecaphan-10-ol (**35**): The title compound **35** (0.011 g, 27%) was prepared according to general procedure D from **49** (0.039 g, 0.10 mmol) and 2-(3,6-dihydro-2H-pyran-4-yl)-4,4,5,5-tetramethyl-1,3,2-dioxaborolane (0.042 g, 0.20 mmol) as a white solid. ^{1}H NMR (400 MHz, CD_3OD) δ 8.65 (s, 1H), 7.24 (s, 1H), 4.26 (dt, *J* = 14.3, 7.4 Hz, 1H), 4.07–3.98 (m, 3H), 3.72–3.65 (m, 1H), 3.64–3.55 (m, 3H), 3.45 (ddd, *J* = 13.7, 7.9, 6.0 Hz, 1H), 3.04 (tt, *J* = 11.7, 3.6 Hz, 1H), 1.99–1.94 (m, 1H), 1.94–1.88 (m, 2H), 1.87–1.80 (m, 1H), 1.80–1.74 (m, 2H), 1.74–1.67 (m, 2H), 1.61–1.54 (m, 1H), 1.52–1.34 (m, 13H); MS *m/z* $[M+H]^+$ calculated for $C_{23}H_{37}N_4O_2$, 401.58; found: 401.30; LC/MS: >95% purity; t_R: 4.90 min.

Cell-based ELISA Assay

A cell-based ELISA was used to measure biological activity of compounds against Mer phosphorylation. Briefly, Lipofectamine (Promega) was used to transiently transfect HEK293 cells with a chimeric EGFR-Mer receptor encoding rat-EGFR ligand-binding domain amino-acids 1 to 2010 fused to human Mer intracellular domain, amino acids 1585 to 3000. The pcDNA3-EGFR-Mer was custom prepared by Aldevron. Dose-response curves of compounds were prepared in 384-well plates (Greiner 781091). Transfected cells were added and allowed to incubate with compound for 30 min at 37°C. Cells were exposed to EGF (100 ng/mL) for 15 min at 37°C, followed by lysis on ice with 50 mM Tris, pH 8, 30 mM NaCl, 1% NP40 containing HALT protease/phosphatase inhibitor (ThermoFisher 78441). After 1.0 h on ice, lysates were transferred to detection plates (Greiner 781074) previously coated with anti-rat EGFR (Sino Biological). Following overnight incubation at 4°C, these plates were washed and treated with anti-phosphotyrosine-HRP (antibodies-online.com ABIN135060) for 2.0 h at 4°C. The amount of Mer phosphorylation was determined by washing the plates, adding Super Signal ELISA Pico Substrate (Thermo Scientific PI37070), and reading the luminescence in an Envision (PerkinElmer). The data analysis program Screenable was used to calculate IC_{50} values.

Selectivity Profiling for 10 (UNC2250) (Carna Biosciences)

Compound preparation: Test compound was dissolved in and diluted with dimethylsulfoxide (DMSO) to achieve 100-fold higher concentration as specified for the compound. The solution was further diluted 25-fold with assay buffer to make the final test compound solution. Reference compounds for assay control were prepared similarly.

Assay reagents and procedures

Off-chip Mobility Shift Assay (MSA)

1) 5 uL of 4x compound solution, 5 uL of 4x Substrate/ATP/Metal solution, and 10 uL of 2x kinase solution were prepared with assay buffer (20 mM HEPES, 0.01% Triton X-100, 2 mM DTT, pH7.5) and mixed and incubated in a polypropylene 384 well microplate for 1 or 5 hour(s) at rt, depending on kinase)

2) 60 uL of Termination Buffer (QuickScout Screening Assist MSA; Cama Biosciences) was added to the well.

3) The reaction mixture was applied to a LabChip3000 system (Caliper Life Science), and the product and substrate peptide peaks were separated and quantitated.

4) The kinase reaction was evaluated by the product ratio calculated from peak heights of product(P) and substrate(S) peptides (P/(P+S)).

Data Analysis

The readout value of reaction control (complete reaction mixture) was set as 0% inhibition, and the readout value of background (Enzyme(-)) was set as 100% inhibition, then the percent inhibition of each test solution was calculated.

Table 1. % Inhibition Data of 16.

Kinase	**16** @ 300 nM
ABL	28.9
CSK	15.9
EGFR	8.1
FGFR1	84.1
IGF1R	12.9
ITK	91.2
JAK3	34.0
KDR	79.4
LCK	62.2
MER	100.2
MET	54.3
PDGFRα	92.0

SRC	21.6
SYK	22.7
TRKA	101.3
AKT1	-3.5
AurA	84.4
CaMK4	1.3
CDK2/CycA2	-5.2
DAPK1	6.6
DYRK1B	35.9
Erk2	-7.2
IKKβ	-5.7
IRAK4	70.6
JNK2	-3.5
MST1	56.6
p38α	3.6
p70S6K	86.0
PKCα	11.5
ROCK1	2.9

X-Ray Crystallography

Crystallization

Crystals of Mer in complex with macrocycle **3** were obtained by vapor diffusion from sitting drops at 12 °C. Protein at 32.5 mg/mL in crystallization buffer (20 mM Tris pH 8.0, 500 mM sodium chloride, 2 mM β-mercaptoethanol) was incubated with **3** (dissolved in DMSO) to give a final concentration of 2.5 mM and slowly rocked overnight. This solution was mixed 1:1 with and equilibrated against crystallization solution containing 27-33% (v/v) Peg 400, 200 mM magnesium chloride, 100 mM Tris pH 8.5. Plate-like crystals grew to final dimensions of up to 1000 x 400 x 50 µm over 10 days. Prior to diffraction data collection, crystals were vitrified by plunging into liquid nitrogen. The Mer/**3** crystals displayed the symmetry of space group $P2_1$ with

cell parameters a = 51.1 Å, b = 91.3 Å, c = 69.2 Å, β = 100.42°, contained two molecules in the asymmetric unit, and diffracted X-rays to a minimum Bragg spacing of about 2.55 Å (Figure 2S).

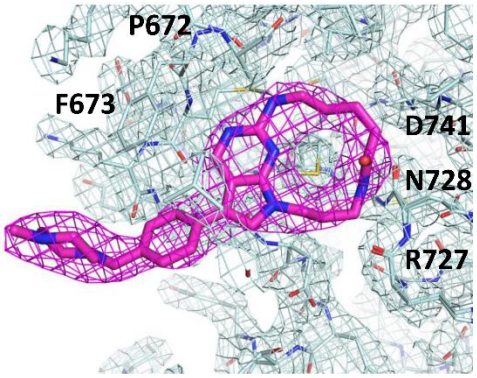


Figure 2S. X-ray structure of macrocyclic compound **3** complexed with Mer protein (kinase domain) (PDB code 5K0K).

Structure Determination

Data were collected at the Southeast Regional Collaborative Access Team (SER-CAT) 22-BM beamline at the Advanced Photon Source, Argonne National Laboratory at a wavelength of 1.0000Å and a temperature of 100K. Data were processed using the program HKL2000. The diffraction quality of our crystals was non-uniform: when the thin edge was exposed to X-rays, the resulting diffraction spots were radially smeared, which was accompanied by a loss of resolution and a somewhat reduced completeness in the high-resolution shells.

The structure of the Mer/**3** complex was determined by molecular replacement with the program Phaser. The search model was generated from the coordinates of Mer in complex with ADP (PDB entry 3BRB) with all non-protein atoms removed. Refinement was carried out using the program Phenix, interspersed with manual revisions of the model using the program Coot. Refinement consisted of conjugate-gradient minimization and calculation of individual atomic

displacement and translation/libration/screw (TLS) parameters. To avoid any model bias, coordinates for **3** were not included until the remainder of the model (including water molecules and ions) was completed. Inclusion of high-resolution, albeit weaker, data increased the stability and convergence of the refinement process. For data collection and refinement statistics see Table 2. The current model contains two molecules of the Mer kinase domain bound to compound **3**, 6 chloride ions, 1 magnesium ion, and 28 water molecules. Residues that could not be identified in the electron density were: chain A, 596-598, 659-664, 745-762; chain B, 597-598, 621-636, 743-762. The model exhibits excellent geometry as determined by MolProbity. Ramachandran analysis identified 97.6% favored, 2.4% allowed, and 0.0% disallowed residues. The coordinates and structure factors have been deposited in the RCSB Protein Data Bank under accession number 5K0K.

Table 2. Data collection and refinement statistics

Data collection	
Space group	$P2_1$
Cell dimensions *a*, *b*, *c* (Å), β(°)	51.1, 91.3, 69.2, 100.4
Resolution (Å)	34.55–2.55 (2.57–2.55)
R_{merge} (%)[a]	8.6 (68.6)
$I/\sigma I$	7.9 (1.9)
Cut-off (σ)	-3
Unique reflections	20,516 (479)
Completeness (%)	99.8 (96.0)
Redundancy	4.2 (3.8)
Wilson B-factor (Å^2)	35.7

Refinement	
Resolution (Å)	34.5470–2.545(2.61–2.54)
No. of reflections (work/free)	15,942/1,772 (617/69)
Cut-off (σ)	0
R_{work} / R_{free}	19.8/25.4 (23.9/27.2)
No. of atoms	
Protein	8,272
UNC2434	138
Ions	7
Water	28
B-factors (Å^2)	
Protein	56.6
UNC2434	57.6
Ions	47.5
Water	36.3
R.m.s. deviations	
Bond lengths (Å)	0.004
Bond angles (°)	0.720
Ramachandran[b]	
Favored (%)	97.6
Generally Allowed (%)	2.4
Disallowed (%)	0.0
Missing residues	A: 596-598, 659-664, 745-762 B: 597-598, 621-636, 743-762

Values in parentheses denote highest resolution shell

[a]R_{merge} = 100 $\Sigma_h\Sigma_i|I_{h,\,i} - \langle I_h\rangle|/\Sigma_h\Sigma_i I_{h,\,i}$, where the outer sum (h) is over the unique reflections and the inner sum (i) is over the set of independent observations of each unique reflection.

[b]As defined by the validation suite MolProbity.

PK Study

A group of 12 male Swiss albino mice (group I) were dosed intravenously (IV) with solution formulation of **16** in normal saline (0.9% w/v NaCl solution). Another group of 12 male Swiss albino mice (group II) were dosed orally (PO) with formulation of **16** in normal saline. From each mouse, two blood samples (200 μL/sample) were collected from retro orbital sinus such that samples were obtained at 0.08, 0.25, 0.5, 1, 2, 4, 8 & 24 h (iv) & 0.25, 0.5, 1, 2, 4, 6, 8 & 24 h (po) post dose. At each time point blood samples were collected from three mice. Immediately after collection, plasma was harvested by centrifugation of blood and was stored below -70ºC until analysis. All samples were processed for analysis by precipitation using Albendazole as internal standard and analyzed with partially validated HPLC/MS/MS method (LLOQ was 1.0 ng/mL). Pharmacokinetic parameters were calculated from mean plasma drug concentration versus time data using Phoenix® WinNonlin® Enterprise software (version 6.3).