Supporting Information

Discovery of Selective Transforming Growth Factor β Type II Receptor Inhibitors as Antifibrosis Agents

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1. Abbreviations

The following abbreviations and definitions have been used:

Ac	Acetyl
AcOEt	Ethyl acetate
AcOH	Acetic acid
Bn	Benzyl
Boc	*tert*-Butoxycarbonyl
Bu	Butyl

CDI	1,1'-Carbonyldiimidazole
DMAP	*N*,*N*-Dimethyl-4-aminopyridine
DIPEA	*N*,*N*-Diisopropylethylamine
DME	1,2-Dimethoxyethane
DMF	*N*,*N*-Dimethylformamide
DMSO	Dimethylsulfoxide
dppf	1,1'-Bis(dimethylphosphino)ferrocene
Et	Ethyl
IPE	Diisopropyl ether
Me	Methyl
Ms	Methanesulfonyl
Ph	Phenyl
r.t.	Room temperature
Ruphos Pd G2	Chloro(2-dicyclohexylphosphino-2′,6′-diisopropoxy-1,1′-biphenyl)[2-(2′-amino-1,1′-biphenyl)]palladium(II)
TEA	Triethylamine
TFA	Trifluoroacetic acid
THF	Tetrahydrofuran
TLC	Thin-layer chromatography
Ts	*p*-Toluenesulfonyl
SEM	2-(Trimethylsilyl)ethoxymethyl
δ	Chemical shift (ppm)
J	Coupling constant
s	Singlet
br s	Broad singlet
d	Doublet
t	Triplet
q	Quartet
m	Multiplet
dd	Doublet of doublets
tt	Triplet of triplets
tq	Triplet of quartets

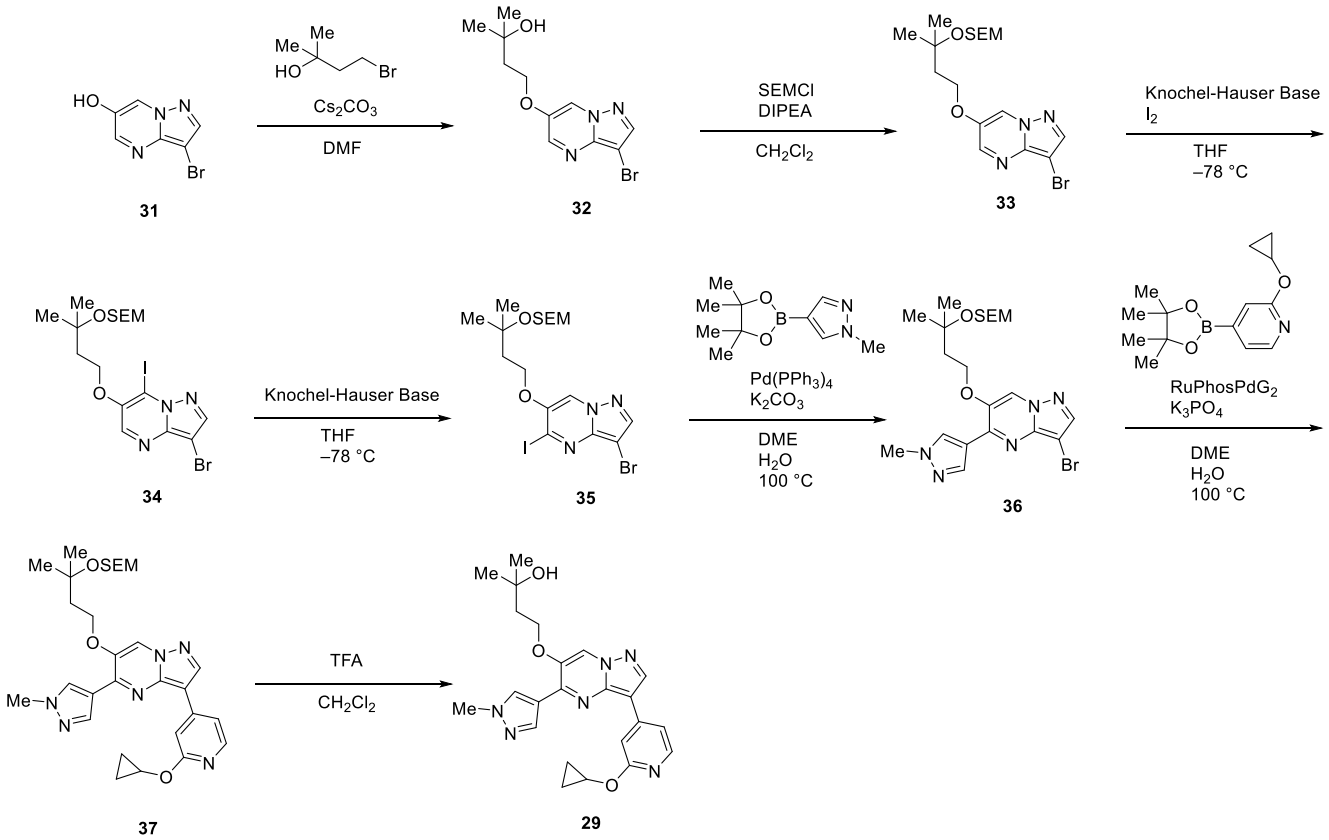
2. Chemistry

General information

Unless otherwise specified, materials were purchased from commercial suppliers and used without further purification. TLC was performed using silica gel 60 F_{254} plates purchased from Merck. Column chromatography

was performed on an Biotage ISO-SPK Isorela Spektra or YAMAZEN EPCLC-Wprep-2XY. Preparative HPLC was performed on a Japan Analytical Industry Co., Ltd. LC-908 instrument. Analytic HPLC was performed on a SHIMADZU Prominence instrument. ^{1}H NMR spectra were recorded on a JEOL ECZ-400S, Bruker AVANCEIII 400, or Agilent 400-MR spectrometer. HRMS spectra were recorded on an LC-MS system composed of Agilent 1290 Infinity LC and Thermo Fisher LTQ-Orbitrap Velos. Microwave reactions were performed using a Biotage Initiatior eight instrument. The purity of inhibitors **1-30** was determined by HPLC [Column: SHIMADZU Shim-pack XR-ODS (3 × 50 mm, 2.2 μm); Mobile phase A: 10 mM H_3PO_4 in water or 0.1% TFA in water; Mobile phase B: MeCN or 0.1% TFA in MeCN; Gradient: 10% B to 90% B from 0 to 5 min, 90% B from 5 to 7 min, 90% B to 10% B from 7 to 7.5 min, 10% B from 7.5 to 10 min; Flow rate: 1.0 mL/min; Detection wavelength: 254 nm. Optical rotation ($[\alpha]_D$) was measured at 25 °C with a Rudolph Research Analytical Autopol V spectrometer.

Synthesis of compound 29



4-((3-Bromopyrazolo[1,5-*a*]pyrimidin-6-yl)oxy)-2-methylbutan-2-ol (32)

To a solution of 3-bromopyrazolo[1,5-*a*]pyrimidin-6-ol (**31**) (1.50 g, 7.01 mmol) in DMF (15 mL) was successively added cesium carbonate (5.71 g, 17.5 mmol) and 4-bromo-2-methylbutan-2-ol (1.43 g, 7.71 mmol) at room temperature. The mixture was warmed to 100 °C and then stirred for 12 h. After cooling to room temperature, the reaction mixture was diluted with water. The precipitated solid was collected by filtration to give the title compound **32** (1.00 g, 48% yield).

^{1}H-NMR (DMSO-d_6) δ: 1.18 (s, 6H), 1.89 (t, J = 7.05 Hz, 2H), 4.18 (t, J = 7.05 Hz, 2H), 4.42 (s, 1H), 8.22 (s, 1H),

8.52 (d, J = 2.77 Hz, 1H), 8.95 (d, J = 2.54 Hz, 1H).

3-Bromo-6-(3-methyl-3-((2-(trimethylsilyl)ethoxy)methoxy)butoxy)pyrazolo[1,5-*a*]pyrimidine (33)

To a solution of compound **32** (850 mg, 2.83 mmol) in CH_2Cl_2 (8.5 mL) was successively added DIPEA (2.0 mL, 11.3 mmol) and 2-(chloromethoxy)ethyltrimethylsilane (2.00 mL, 11.3 mmol) at 0 °C. After stirring at room temperature for 2 h, the reaction mixture was concentrated under reduced pressure. The resulting residue was purified by column chromatography (*n*-hexane/AcOEt) to give the title compound **33** (1.00 g, 83% yield).

^{1}H-NMR ($CDCl_3$) δ: 0.00 (s, 9H), 0.89–0.94 (m, 2H), 1.33 (s, 6H), 2.08 (t, J = 6.82 Hz, 2H), 3.61–3.65 (m, 2H), 4.15 (t, J = 6.82 Hz, 2H), 4.78 (s, 2H), 7.98 (s, 1H), 8.23 (d, J = 2.54 Hz, 1H), 8.41 (d, J = 2.54 Hz, 1H).

3-Bromo-7-iodo-6-(3-methyl-3-((2-(trimethylsilyl)ethoxy)methoxy)butoxy)pyrazolo[1,5-*a*]pyrimidine (34)

Under a nitrogen stream, to a solution of **33** (11.9 g, 27.6 mmol) in THF (120 mL) was added dropwise 1 M Knochel-Hauser Base in THF (68.9 mL, 68.9 mmol) at −78 °C. After stirring at −78 °C for 1 h, a solution of iodine (8.39 g, 33.1 mmol) in THF (20 mL) was added dropwise to the reaction mixture. After stirring at 0 °C for 40 min, to the reaction mixture was added saturated NH_4Cl aq. and AcOEt. The layers were separated and the organic layer was washed with 10% Na_2SO_3 aq. and brine, dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The resulting residue was purified by column chromatography (*n*-hexane/AcOEt) to give the title compound **34** (10.1 g, 66% yield).

^{1}H-NMR ($CDCl_3$) δ: 0.00 (s, 9H), 0.88–0.93 (m, 2H), 1.35 (s, 6H), 2.13 (t, J = 7.06 Hz, 2H), 3.60–3.64 (m, 2H), 4.36 (t, J = 7.06 Hz, 2H), 4.77 (d, J = 0.46 Hz, 2H), 8.15 (d, J = 0.69 Hz, 1H), 8.28 (d, J = 0.69 Hz, 1H).

3-Bromo-5-iodo-6-(3-methyl-3-((2-(trimethylsilyl)ethoxy)methoxy)butoxy)pyrazolo[1,5-*a*]pyrimidine (35)

Under a nitrogen stream, to a solution of **34** (10.1 g, 18.1 mmol) in THF (100 mL) was added dropwise 1M Knochel-Hauser Base in THF (31.7 mL, 31.7 mmol) at −78 °C. After stirring at −78 °C for 1 h, additional 1M Knochel-Hauser Base in THF (9.10 mL, 9.10 mmol) was added dropwise to the reaction mixture. After stirring at −78 °C for 30 min, to the reaction mixture was added 10% AcOH aq. and AcOEt. The layers were separated and the organic layer was washed with saturated $NaHCO_3$ aq. and brine, dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The resulting residue was purified by column chromatography (*n*-hexane/AcOEt) to give the title compound **35** (9.47 g, 94% yield).

^{1}H-NMR ($CDCl_3$) δ: 0.00 (s, 9H), 0.90–0.94 (m, 2H), 1.36 (s, 6H), 2.15 (t, J = 6.76 Hz, 2H), 3.61–3.65 (m, 2H), 4.18 (t, J = 6.76 Hz, 2H), 4.79 (s, 2H), 7.95 (s, 1H), 8.02 (s, 1H).

3-Bromo-5-(1-methyl-1*H*-pyrazol-4-yl)-6-(3-methyl-3-((2-(trimethylsilyl)ethoxy)methoxy)butoxy)pyrazolo[1,5-*a*]pyrimidine (36)

A mixture of compound **35** (500 mg, 0.899 mmol), 1-methyl-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1*H*-pyrazole (224 mg, 1.08 mmol), $Pd(PPh_3)_4$ (51.9 mg, 0.0450 mmol), and K_3PO_4 (572mg, 2.70 mmol) in DME (10 mL) and water (2.5 mL) was stirred at 100 °C for 4 h under an argon atmosphere. After cooling to room temperature, the mixture was diluted with AcOEt and water, and subsequently extracted with AcOEt. The organic layer was

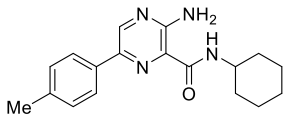
washed with brine, dried over Na_2SO_4 and concentrated under reduced pressure. The resulting residue was purified by column chromatography (*n*-hexane/AcOEt) to give the title compound **36** (267 mg, 58% yield).
^{1}H-NMR ($CDCl_3$) δ: 0.01 (s, 9H), 0.89–0.94 (m, 2H), 1.36 (s, 6H), 2.21 (t, *J* = 7.11 Hz, 2H), 3.62–3.66 (m, 2H), 4.00 (s, 3H), 4.24 (t, *J* = 7.11 Hz, 2H), 4.80 (s, 2H), 7.94 (s, 1H), 8.23 (s, 1H), 8.27 (s, 1H), 8.34 (s, 1H).

3-(2-Cyclopropoxypyridin-4-yl)-5-(1-methyl-1*H*-pyrazol-4-yl)-6-(3-methyl-3-((2-(trimethylsilyl)ethoxy)methoxy)butoxy)pyrazolo[1,5-*a*]pyrimidine (37)
A mixture of **36** (130 mg, 0.255 mmol), 2-cyclopropoxy-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)pyridine (80.0 mg, 0.306 mmol), RuPhosPdG2 (9.89 mg, 0.0130 mmol), and K_3PO_4 (162mg, 0.764 mmol) in DME (20 mL) and water (5.0 mL) was stirred at 100 °C for 1 h under an argon atmosphere. After cooling to room temperature, the mixture was diluted with AcOEt and water, and subsequently extracted with AcOEt. The organic layer was washed with brine, dried over Na_2SO_4 and concentrated under reduced pressure. The resulting residue was purified by column chromatography (*n*-hexane/AcOEt) to give the title compound **37** (37.9 mg, 26% yield).
^{1}H-NMR ($CDCl_3$) δ: 0.01 (s, 9H), 0.85–0.87 (m, 4H), 0.90–0.94 (m, 2H), 1.38 (s, 6H), 2.23 (t, *J* = 7.17 Hz, 2H), 3.63–3.67 (m, 2H), 4.03 (s, 3H), 4.24–4.29 (m, 1H), 4.28 (t, *J* = 7.17 Hz, 2H), 4.81 (s, 2H), 7.58–7.59 (m, 1H), 7.65 (d, *J* = 5.38, 1H), 8.26 (s, 1H), 8.27 (dd, *J* = 5.38, 0.69 Hz, 1H), 8.31 (s, 1H), 8.35 (s, 1H), 8.38 (d, *J* = 0.69 Hz, 1H).

4-((3-(2-Cyclopropoxypyridin-4-yl)-5-(1-methyl-1*H*-pyrazol-4-yl)pyrazolo[1,5-*a*]pyrimidin-6-yl)oxy)-2-methylbutan-2-ol (29)
A solution of **37** (37.9 mg, 0.0670 mmol) in CH_2Cl_2 (380 μL) was treated with TFA (51 μL) at 0 °C. After stirring at 0 °C for 30 min, the solvent was removed under reduced pressure. The residue was diluted with AcOEt and saturated $NaHCO_3$ aq. The mixture was extracted with AcOEt. The organic layer was washed with brine, dried over Na_2SO_4 and concentrated under reduced pressure. The resulting residue was purified by column chromatography (*n*-hexane/AcOEt) to give the title compound **29** (11.6 mg, 40 % yield).
^{1}H-NMR (DMSO-d_6) δ: 0.71–0.82 (m, 4H), 1.22 (s, 6H), 2.06 (t, *J* = 7.05 Hz, 2H), 3.97 (s, 3H), 4.23–4.33 (m, 3H), 4.49 (s, 1H), 7.58–7.61 (m, 1H), 7.87 (dd, *J* = 5.44, 1.39 Hz, 1H), 8.20 (d, *J* = 5.44 Hz, 1H), 8.24 (s, 1H), 8.50 (s, 1H), 8.72 (s, 1H), 8.95 (s, 1H).
^{13}C-NMR (126 MHz, $CDCl_3$) δ: 163.77, 147.11, 146.27, 142.69, 141.04, 139.49, 137.90, 134.91, 129.58, 121.02, 119.44, 115.18, 114.47, 108.66, 69.99, 67.48, 49.13, 42.19, 38.53, 28.72, 28.72, 5.92, 5.92.
HRMS *m/z*: $[M+H]^+$ calcd for $C_{23}H_{26}N_6O_3$, 435.2139; found, 435.2136.
Purity: 97.6%.

Preparation of compounds 1, 2, 3, 4

3-Amino-*N*-cyclohexyl-6-(*p*-tolyl)pyrazine-2-carboxamide (1)



1

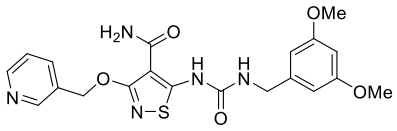
Compound **1** (compound **35** in reference 1) was prepared by previously reported procedure.[1]

^{1}H-NMR (DMSO-d_6) δ: 1.12–1.25 (m, 1H), 1.27–1.41 (m, 2H), 1.44–1.53 (m, 2H), 1.58–1.66 (m, 1H), 1.70–1.78 (m, 2H), 1.78–1.87 (m, 2H), 2.36 (s, 3H), 3.74–3.86 (m, 1H), 7.28 (d, *J* = 7.86 Hz, 2H), 7.58 (br s, 2H), 7.99 (d, *J* = 8.44 Hz, 2H), 8.35 (d, *J* = 8.44 Hz, 1H), 8.79 (s, 1H).

HRMS *m/z*: $[M+H]^+$ calcd for $C_{18}H_{22}N_4O$, 311.1866; found, 311.1869.

Purity: 95.4 %.

5-(3-(3,5-Dimethoxybenzyl)ureido)-3-(pyridin-3-ylmethoxy)isothiazole-4-carboxamide (2)



2

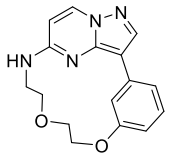
Compound **2** (compound **5** in reference 2) was prepared by previously reported procedure.[2]

^{1}H-NMR (DMSO-d_6) δ: 3.72 (s, 6H), 4.25 (d, *J* = 5.78 Hz, 2H), 5.46 (s, 2H), 6.39 (dd, *J* = 2.03, 2.03 Hz, 1H), 6.45 (d, *J* = 2.03 Hz, 2H), 7.07 (s, 1H), 7.42 (dd, *J* = 7.79, 4.51 Hz, 1H), 7.61 (s, 1H), 7.92 (d, *J* = 7.79 Hz, 1H), 8.55 (d, *J* = 4.51 Hz, 1H), 8.64 (s, 1H), 8.71 (s, 1H), 11.13 (s, 1H).

HRMS *m/z*: $[M+H]^+$ calcd for $C_{20}H_{21}N_5O_5S$, 444.1336; found, 444.1336.

Purity: 97.7 %.

3,6-Dioxa-9-aza-1(3,5)-pyrazolo[1,5-*a*]pyrimidina-2(1,3)-benzenacyclononaphane (3)



3

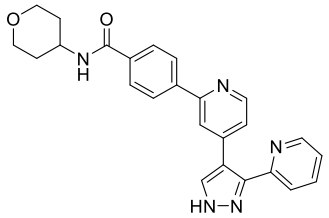
Compound **3** (dechlorinated analogue of OD36 in reference 3) was prepared by previously reported procedure.[4]

^{1}H-NMR (DMSO-d_6) δ: 3.48–3.55 (m, 2H), 3.84–3.91 (m, 2H), 3.94–4.01 (m, 2H), 4.25–4.32 (m, 2H), 6.30 (d, *J* = 7.52 Hz, 1H), 6.59–6.63 (m, 1H), 7.17–7.19 (m, 1H), 7.20–7.24 (m, 1H), 7.81 (t, *J* = 5.20 Hz, 1H), 8.29 (s, 1H), 8.53 (d, *J* = 7.52 Hz, 1H), 8.68 (s, 1H).

HRMS *m/z*: $[M+H]^+$ calcd for $C_{16}H_{16}N_4O_2$, 297.1436; found, 297.1347.

Purity: 97.2 %.

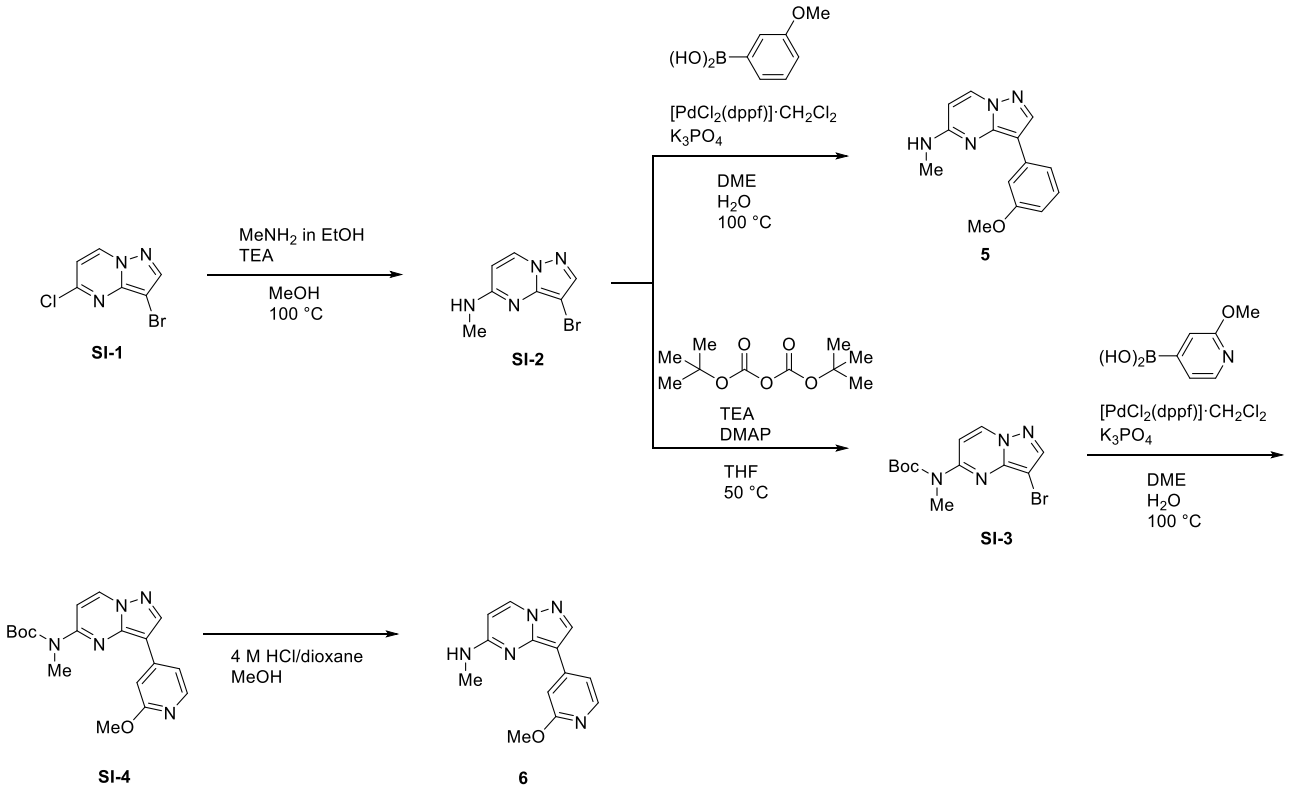
4-(4-(3-(pyridin-2-yl)-1*H*-pyrazol-4-yl)pyridin-2-yl)-*N*-(tetrahydro-2*H*-pyran-4-yl)benzamide (4)



4

Compound **4** was purchased from Selleck Chemicals.

Synthesis of compounds 5 and 6



3-Bromo-*N*-methylpyrazolo[1,5-*a*]pyrimidin-5-amine (SI-2)

To a solution of 3-bromo-5-chloropyrazolo[1,5-*a*]pyrimidine (**SI-1**) (200 mg, 0.860 mmol) in EtOH (5.0 mL) was added 40 wt% methylamine in MeOH (0.893 mL, 4.30 mmol) at room temperature. After stirring at room temperature for 1 h, the reaction mixture was concentrated under reduced pressure. The resulting residue was diluted with water. The precipitated solid was collected by filtration to give the title compound **SI-2** (169 mg, 86% yield). ^{1}H-NMR ($CDCl_3$) δ: 3.09 (d, *J* = 5.09 Hz, 3H), 4.95 (s, 1H), 6.05 (d, *J* = 7.63 Hz, 1H), 7.81 (s, 1H), 8.16 (d, *J* = 7.63 Hz, 1H).

3-(3-Methoxyphenyl)-*N*-methylpyrazolo[1,5-*a*]pyrimidin-5-amine (5)

A mixture of **SI-2** (20.0 mg, 0.0880 mmol), (3-methoxyphenyl)boronic acid (20.0 mg, 0.152 mmol), $PdCl_2$(dppf)-CH_2Cl_2 (7.20 mg, 0.0310 mmol) in DME (1.5 mL) and 2 M Na_2CO_3 aq. (0.400 mL, 0.800 mmol) was stirred at 100 °C for 6 h under an argon atmosphere. After cooling to room temperature, the reaction mixture was diluted with

AcOEt, filtered through Celite® and concentrated under reduced pressure. The resulting residue was purified by preparative TLC ($CHCl_3$/AcOEt = 3:1 (v/v)) to give the title compound **5** (4.80 mg, 21% yield).

^{1}H-NMR ($CDCl_3$) δ: 3.12 (d, *J* = 4.86 Hz, 3H), 3.89 (s, 3H), 4.86 (s, 1H), 6.04 (d, *J* = 7.63 Hz, 1H), 6.74–6.76 (m, 1H), 7.31 (dd, *J* = 7.98, 7.98 Hz, 1H), 7.55–7.57 (m, 1H), 7.82–7.83 (m, 1H), 8.21 (d, *J* = 7.63 Hz, 1H), 8.21 (s, 1H).

HRMS *m/z*: $[M+H]^+$ calcd for $C_{14}H_{14}N_4O$, 255.1240; found, 255.1241.

Purity: 99.5%.

***tert*-Butyl (3-bromopyrazolo[1,5-*a*]pyrimidin-5-yl)(methyl)carbamate (SI-3)**

To a solution of **SI-2** (658 mg, 2.90 mmol) in THF (7.0 mL) was successively added Boc_2O (696 mg, 3.19 mmol), TEA (485 μL, 3.48 mmol) and DMAP (70.9 mg, 0.580 mmol) at room temperature. After stirring at 50 °C for 4 h, additional Boc_2O (200 mg, 0.917 mmol) was added to the reaction mixture. After stirring at 50 °C for 40 min, the reaction mixture was cooled to room temperature and diluted with AcOEt and water, and subsequently extracted with AcOEt. The organic layer was washed with brine, dried over Na_2SO_4 and concentrated under reduced pressure. The resulting residue was purified by column chromatography (*n*-hexane/AcOEt) to give the title compound **SI-3** (184 mg, 19% yield).

^{1}H-NMR ($CDCl_3$) δ: 1.57 (s, 9H), 3.53 (s, 3H), 7.65 (d, *J* = 7.75 Hz, 1H), 7.98 (s, 1H), 8.40 (d, *J* = 7.75 Hz, 1H).

***tert*-Butyl (3-(2-methoxypyridin-4-yl)pyrazolo[1,5-*a*]pyrimidin-5-yl)(methyl)carbamate (SI-4)**

A mixture of **SI-3** (184 mg, 0.562 mmol), (2-methoxypyridin-4-yl)boronic acid (198 mg, 0.843 mmol), and $PdCl_2$(dppf)-CH_2Cl_2 (138 mg, 0.269 mmol) in DME (3.0 mL) and 2 M K_2CO_3 aq. (1.20 mL, 2.40 mmol) was stirred at 100 °C for 4 h under an argon atmosphere. After cooling to room temperature, the reaction mixture was diluted with AcOEt, filtered through Celite® and concentrated under reduced pressure. The resulting residue was purified by column chromatography (*n*-hexane/AcOEt) to give the title compound **SI-4** (169 mg, 89% yield).

^{1}H-NMR ($CDCl_3$) δ: 1.59 (s, 9H), 3.60 (s, 3H), 3.98 (s, 3H), 7.44–7.46 (m, 1H), 7.52 (d, *J* = 5.38 Hz, 1H), 7.76 (d, *J* = 7.86 Hz, 1H), 8.18 (d, *J* = 5.38 Hz, 1H), 8.40 (s, 1H), 8.49 (d, *J* = 7.86 Hz, 1H).

3-(3-Methoxyphenyl)-*N*-methylpyrazolo[1,5-*a*]pyrimidin-5-amine (6)

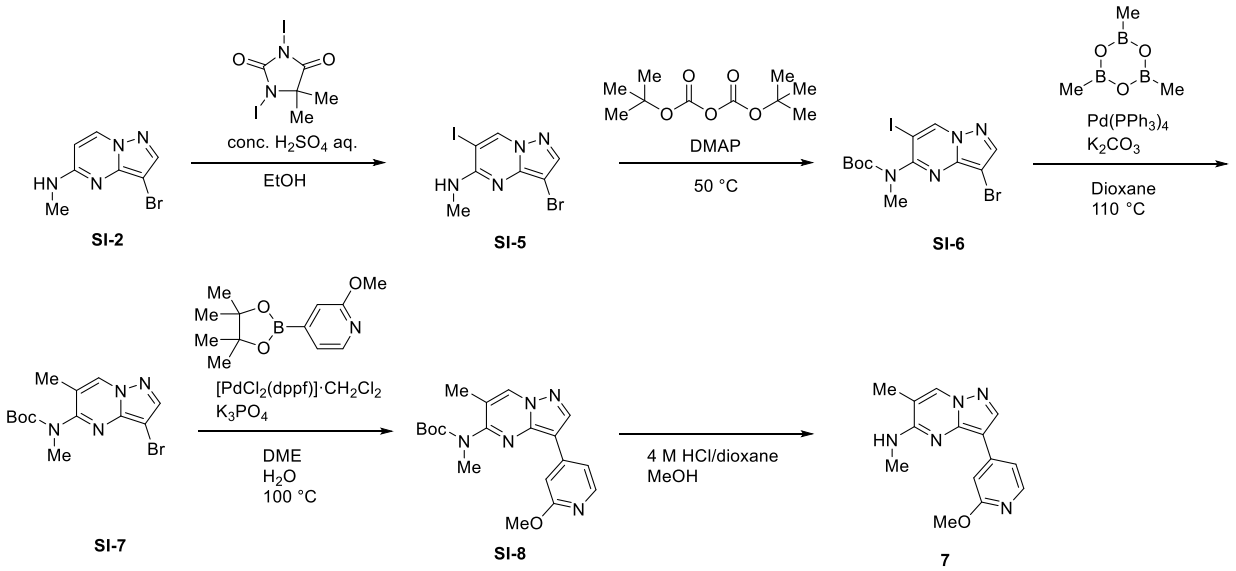
A solution of **SI-4** (139 mg, 0.392 mmol) in MeOH (1.0 mL) and $CHCl_3$ (1.0 mL) was treated with 4 M HCl in dioxane (3.0 mL) at room temperature. After stirring at 50 °C for 1 h, the solvent was removed under reduced pressure. To the resulting residue was carefully added saturated $NaHCO_3$ aq. and the precipitated solid was collected by filtration to give the title compound **6** (80.1 mg, 80% yield).

^{1}H-NMR ($CDCl_3$) δ: 3.15 (d, *J* = 5.09 Hz, 3H), 3.98 (s, 3H), 4.99 (s, 1H), 6.09 (d, *J* = 7.63 Hz, 1H), 7.49–7.50 (m, 1H), 7.55 (d, *J* = 5.43 Hz, 1H), 8.14 (d, *J* = 5.43 Hz, 1H), 8.22 (d, *J* = 7.17 Hz, 1H), 8.24 (s, 1H).

HRMS *m/z*: $[M+H]^+$ calcd for $C_{13}H_{13}N_5O$, 256.1191; found, 256.1191.

Purity: 94.6%.

Synthesis of compound 7



3-Bromo-6-iodo-*N*-methylpyrazolo[1,5-*a*]pyrimidin-5-amine (SI-5)

To a solution of **SI-2** (1.50 g, 6.61 mmol) in EtOH (41 mL) was added 1,3-diiodo-5,5-dimethylimidazolidine-2,4-dione (1.30 g, 3.31 mmol) and conc. H_2SO_4 aq. (1.41 mL, 26.4 mmol) at room temperature. After stirring a at room temperature for 12 h, the reaction mixture was cooled to 0 °C and 2 M NaOH aq. was added. The resulting mixture was diluted with AcOEt and water, and subsequently extracted with AcOEt. The organic layer was washed with brine, dried over Na_2SO_4 and concentrated under reduced pressure. The resulting residue was purified by column chromatography (*n*-hexane/AcOEt) to give the title compound **SI-5** (2.15 g, 92% yield).

^{1}H-NMR ($CDCl_3$) δ: 3.13 (d, *J* = 2.08 Hz, 3H), 5.45 (s, 1H), 7.76 (s, 1H), 8.53 (s, 1H).

***tert*-Butyl (3-bromo-6-iodopyrazolo[1,5-*a*]pyrimidin-5-yl)(methyl)carbamate (SI-6)**

To a solution of **SI-5** (1.94 g, 5.50 mmol) in Boc_2O (13 mL) was added DMAP (33.6 mg, 0.275 mmol) at room temperature. After stirring at 50 °C for 12 h, the reaction mixture was cooled to room temperature and diluted with AcOEt and water, and subsequently extracted with AcOEt. The organic layer was washed with brine, dried over Na_2SO_4 and concentrated under reduced pressure. The resulting residue was purified by column chromatography (*n*-hexane/AcOEt) to give the title compound **SI-6** (847 mg, 34% yield).

^{1}H-NMR ($CDCl_3$) δ: 1.49 (s, 9H), 3.32 (s, 3H), 8.03 (s, 1H), 8.94 (s, 1H).

***tert*-Butyl (3-bromo-6-methylpyrazolo[1,5-*a*]pyrimidin-5-yl)(methyl)carbamate (SI-7)**

A mixture of **SI-6** (174 mg, 0.385 mmol), trimethylboroxine (59.3 mg, 0.424 mmol), $Pd(PPh_3)_4$ (89.0 mg, 0.0770 mmol), and K_2CO_3 (106 mg, 0.770 mmol) in dioxane (4.0 mL) was stirred at 110 °C for 4 h under an argon atmosphere. After cooling to room temperature, the reaction mixture was diluted with AcOEt, filtered through Celite® and concentrated under reduced pressure. The resulting residue was purified by column chromatography (*n*-hexane/AcOEt). The title compound **SI-7** (120 mg) was obtained and used for next step without further purification.

^{1}H-NMR ($CDCl_3$) δ: 1.47 (s, 9H), 2.27 (s, 3H), 3.36 (s, 3H), 8.01 (s, 1H), 8.42 (s, 1H).

***tert*-Butyl (3-(2-methoxypyridin-4-yl)-6-methylpyrazolo[1,5-*a*]pyrimidin-5-yl)(methyl)carbamate (SI-8)**

A mixture of **SI-7** (120 mg, 0.352 mmol), 2-methoxy-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)pyridine (166 mg, 0.704 mmol), $PdCl_2$(dppf)-CH_2Cl_2 (57.5 mg, 0.0704 mmol), and K_3PO_4 (225 mg, 1.06 mmol) in DME (3.0 mL) and water (0.75 mL) was stirred at 100 °C for 1 h under an argon atmosphere. After cooling to room temperature, the reaction mixture was diluted with AcOEt, filtered through Celite® and concentrated under reduced pressure. The resulting residue was purified by column chromatography (*n*-hexane/AcOEt) to give the title compound **SI-8** (43.8 mg, 34% yield for 2 steps).

^{1}H-NMR (CDCl3) δ: 1.50 (s, 9H), 2.30 (s, 3H), 3.43 (s, 3H), 3.99 (s, 3H), 7.45–7.48 (m, 1H), 7.52 (s, 1H), 8.18 (d, J = 5.44 Hz, 1H), 8.41 (s, 1H), 8.48 (s, 1H).

3-(2-Methoxypyridin-4-yl)-*N*,6-dimethylpyrazolo[1,5-*a*]pyrimidin-5-amine (7)

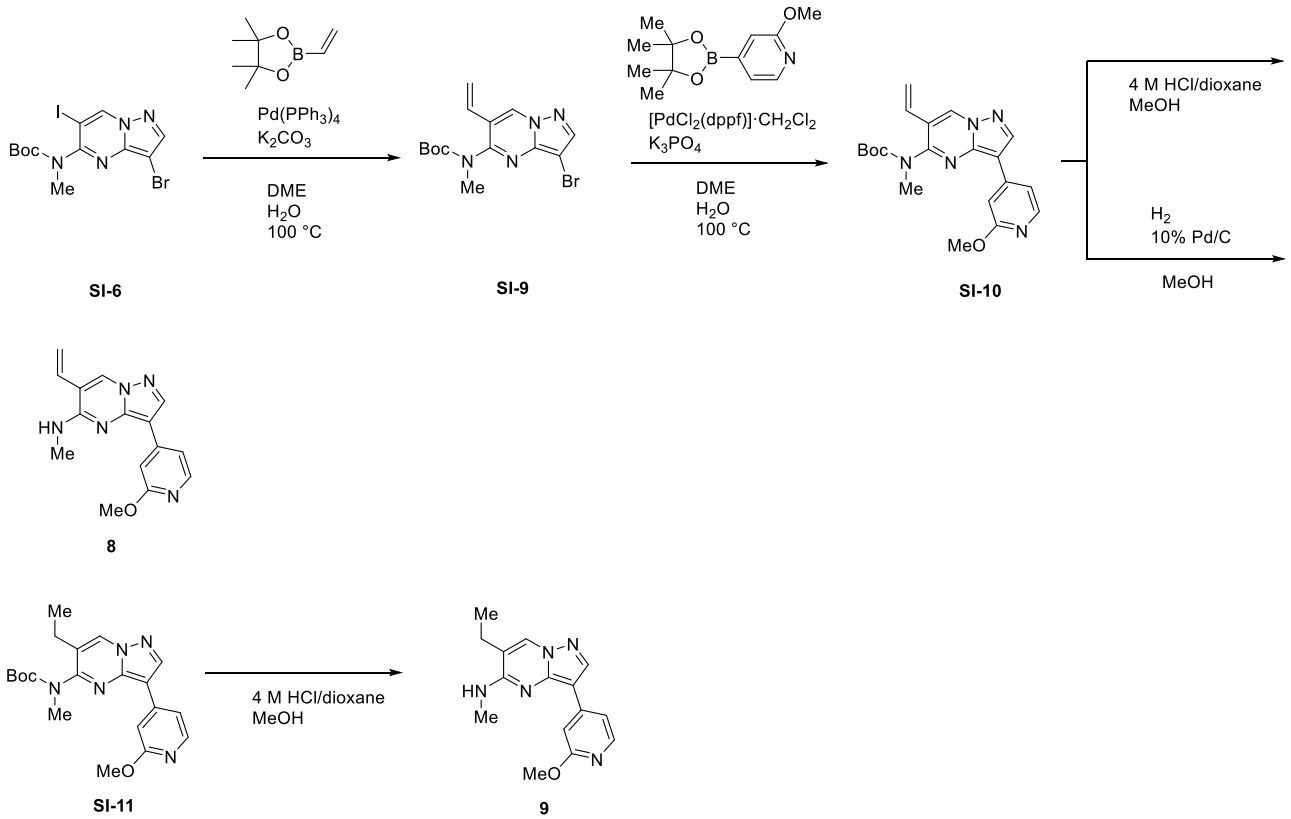
A solution of **SI-8** (43.8 mg, 0.119 mmol) in MeOH (1.0 mL) and $CHCl_3$ (1.0 mL) was treated with 4M HCl in dioxane (3.0 mL) at room temperature. After stirring at room temperature for 30 min, the solvent was removed under reduced pressure. To the resulting residue was carefully added saturated $NaHCO_3$ aq. and the precipitated solid was collected by filtration to give the title compound **6** (4.70 mg, 15% yield).

^{1}H-NMR (DMSO-d_6) δ: 2.13 (s, 3H), 3.03 (d, J = 3.93 Hz, 3H), 4.07 (s, 3H), 7.60 (br s, 1H), 7.90 (br s, 1H), 7.98 (br s, 1H), 8.18 (d, J = 6.24 Hz, 1H), 8.58 (s, 1H), 8.65 (s, 1H).

HRMS *m/z*: $[M+H]^+$ calcd for $C_{14}H_{15}N_5O$, 270.1349; found, 270.1347.

Purity: 94.6%.

Synthesis of compounds 8 and 9



***tert*-Butyl (3-bromo-6-vinylpyrazolo[1,5-*a*]pyrimidin-5-yl)(methyl)carbamate (SI-9)**

A mixture of **SI-6** (233 mg, 0.514 mmol), 4,4,5,5-tetramethyl-2-vinyl-1,3,2-dioxaborolane (95.8 μL, 0.565 mmol), $Pd(PPh_3)_4$ (119 mg, 0.103 mmol), and K_2CO_3 (39.4 mg, 1.03 mmol) in DME (4.0 mL) and water (1.6 mL) was stirred at 100 °C for 4.5 h under an argon atmosphere. After cooling to room temperature, the reaction mixture was diluted with AcOEt, filtered through Celite® and concentrated under reduced pressure. The resulting residue was purified by column chromatography (*n*-hexane/AcOEt) to give the title compound **SI-9** (43.8 mg, 39% yield).

^{1}H-NMR ($CDCl_3$) δ: 1.42 (s, 9H), 3.38 (s, 3H), 5.40 (d, *J* = 10.93 Hz, 1H), 5.73 (d, *J* = 17.63 Hz, 1H), 6.59 (dd, *J* = 17.63, 10.93 Hz, 1H), 8.07 (s, 1H), 8.68 (s, 1H).

***tert*-Butyl (3-(2-methoxypyridin-4-yl)-6-vinylpyrazolo[1,5-*a*]pyrimidin-5-yl)(methyl)carbamate (SI-10)**

A mixture of **SI-9** (115 mg, 0.326 mmol), 2-methoxy-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)pyridine (115 mg, 0.489 mmol) and $PdCl_2$(dppf)-CH_2Cl_2 (53.2 mg, 0.0652 mmol) in DME (3.0 mL) and 2 M K_2CO_3 aq. (1.20 mL, 2.40 mmol) was stirred at 100 °C for 5 h under an argon atmosphere. After cooling to room temperature, the reaction mixture was diluted with AcOEt, filtered through Celite® and concentrated under reduced pressure. The resulting residue was purified by column chromatography (*n*-hexane/AcOEt) to give the title compound **SI-10** (73.7 mg, 59% yield).

^{1}H-NMR ($CDCl_3$) δ: 1.44 (s, 9H), 3.45 (s, 3H), 3.99 (s, 3H), 5.41 (d, *J* = 10.93 Hz, 1H), 5.76 (d, *J* = 17.63 Hz, 1H), 6.63 (dd, *J* = 17.63, 10.93 Hz, 1H), 7.45 (s, 1H), 7.53 (d, *J* = 5.55 Hz, 1H), 8.20 (d, *J* = 5.55 Hz, 1H), 8.46 (s, 1H), 8.75 (s, 1H).

3-(2-Methoxypyridin-4-yl)-*N*-methyl-6-vinylpyrazolo[1,5-*a*]pyrimidin-5-amine (8)

A solution of **SI-10** (71.7 mg, 0.188 mmol) in MeOH (1.0 mL) and $CHCl_3$ (1.0 mL) was treated with 4 M HCl dioxane (3.0 mL) at room temperature. After stirring at 50 °C for 5 h, the solvent was removed under reduced pressure. To the resulting residue was carefully added saturated $NaHCO_3$ aq. and AcOEt. The layers were separated and the organic layer was washed with brine, dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The resulting residue was purified by column chromatography (*n*-hexane/AcOEt) to give the title compound **8** (25.8 mg, 50% yield).

^{1}H-NMR ($CDCl_3$) δ: 3.20 (d, *J* = 4.86 Hz, 3H), 3.98 (s, 3H), 5.10 (br s, 1H), 5.54 (dd, *J* = 10.95, 1.04 Hz, 1H), 5.72 (dd, *J* = 17.20, 1.04 Hz, 1H), 6.52 (ddd, *J* = 17.20, 10.95, 1.01 Hz, 1H), 7.50 (dd, *J* = 1.56, 0.69 Hz, 1H), 7.56 (d, *J* = 5.49 Hz, 1H), 8.14 (dd, *J* = 5.49, 0.69 Hz, 1H), 8.24 (d, *J* = 1.01 Hz, 1H), 8.24 (s, 1H).

HRMS *m/z*: $[M+H]^+$ calcd for C15H15N5O 282.1349; found, 282.1349.

Purity: 95.6%.

***tert*-Butyl (6-ethyl-3-(2-methoxypyridin-4-yl)pyrazolo[1,5-*a*]pyrimidin-5-yl)(methyl)carbamate (SI-11)**

A solution of **SI-10** (39.0 mg, 0.102 mmol) in MeOH (2.0 mL) was treated with 10% palladium on activated carbon (10.0 mg) and stirred under a hydrogen atmosphere (1 atm) at room temperature for 6 h. After removal of the palladium catalyst by Celite® filtration, the filtrate was concentrated and further purified by column chromatography (*n*-hexane/AcOEt) to give the title compound **SI-12** (34.5 mg, 88% yield).

^{1}H-NMR ($CDCl_3$) δ: 1.29 (t, *J* = 7.40 Hz, 3H), 1.48 (br s, 9H), 2.71 (qd, *J* = 7.40, 0.92 Hz, 2H), 3.40 (s, 3H), 3.99 (s, 3H), 7.46 (dd, *J* = 1.45, 0.64 Hz, 1H), 7.53 (dd, *J* = 5.43, 1.45 Hz, 1H), 8.19 (dd, *J* = 5.43, 0.64 Hz, 1H), 8.42 (s, 1H), 8.50 (t, *J* = 0.92 Hz, 1H).

6-Ethyl-3-(2-methoxypyridin-4-yl)-*N*-methylpyrazolo[1,5-*a*]pyrimidin-5-amine (9)

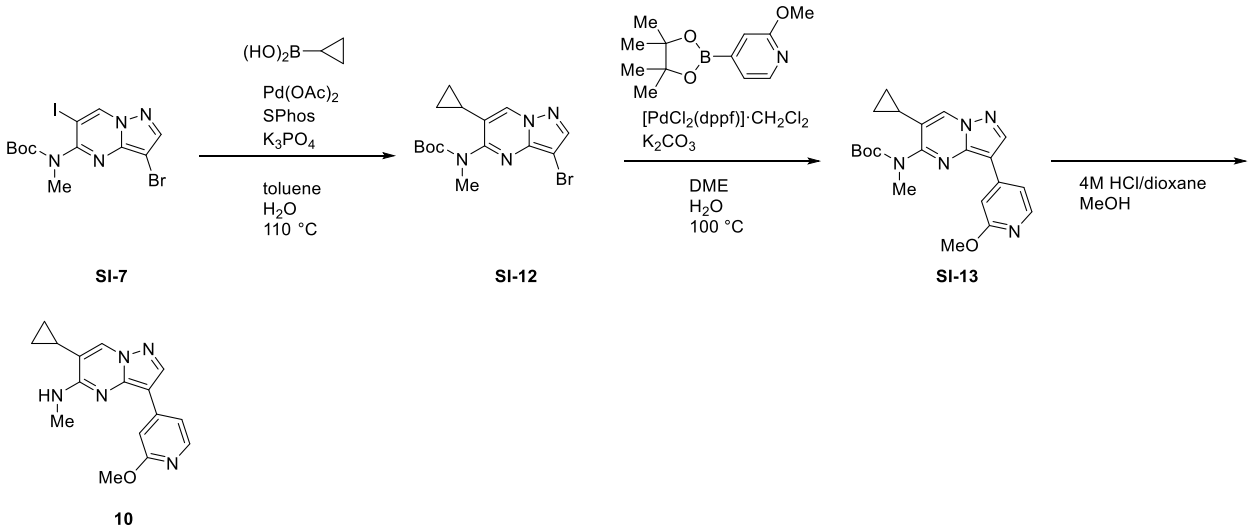
A solution of **SI-11** (34.5 mg, 0.0900 mmol) in MeOH (1.0 mL) and $CHCl_3$ (1.0 mL) was treated with 4 M HCl in dioxane (3.0 mL) at room temperature. After stirring at room temperature for 1.5 h, the solvent was removed under reduced pressure. To the resulting residue was carefully added saturated $NaHCO_3$ aq. and AcOEt. The layers were separated and the organic layer was washed with brine, dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The resulting residue was purified by column chromatography (*n*-hexane/AcOEt) to give the title compound **9** (14.7 mg, 58% yield).

^{1}H-NMR ($CDCl_3$) δ: 1.34 (t, *J* = 7.40 Hz, 3H), 2.47 (qd, *J* = 7.40, 1.22 Hz, 2H), 3.21 (d, *J* = 4.86 Hz, 3H), 3.98 (s, 3H), 4.95 (br s, 1H), 7.51 (dd, *J* = 1.13, 0.64 Hz, 1H), 7.57 (dd, *J* = 5.49, 1.13 Hz, 1H), 8.09 (t, *J* = 1.22 Hz, 1H), 8.14 (dd, *J* = 5.49, 0.64 Hz, 1H), 8.21 (s, 1H).

HRMS *m/z*: $[M+H]^+$ calcd for $C_{15}H_{17}N_5O$ 284.1506; found, 284.1503.

Purity: 99.4%.

Synthesis of compound 10



***tert*-Butyl (3-bromo-6-cyclopropylpyrazolo[1,5-*a*]pyrimidin-5-yl)(methyl)carbamate (SI-12)**

A mixture of **SI-7** (400 mg, 0.883 mmol), cyclopropylboronic acid (83.3 mg, 0.971 mmol), $Pd(OAc)_2$ (39.7 mg, 0.177 mmol), Sphos (72.7 mg, 0.177 mmol) and K_3PO_4 (563 mg, 2.65 mmol) in toluene (10 mL) and water (1.0 mL) was stirred at 110 °C for 4 h under an argon atmosphere. After cooling to room temperature, the reaction mixture was diluted with AcOEt, filtered through Celite® and concentrated under reduced pressure. The resulting residue was purified by column chromatography (*n*-hexane/AcOEt) to give the title compound **SI-12** (114 mg, 35% yield).

^{1}H-NMR ($CDCl_3$) δ: 0.58–0.59 (m, 2H), 0.99–1.01 (m, 2H), 1.47 (s, 9H), 1.95–1.99 (m, 1H), 3.38 (s, 3H), 8.01 (s,

1H), 8.32 (d, *J* = 0.92 Hz, 1H).

***tert*-Butyl (6-cyclopropyl-3-(2-methoxypyridin-4-yl)pyrazolo[1,5-*a*]pyrimidin-5-yl)(methyl)carbamate (SI-13)**

A mixture of **SI-12** (114 mg, 0.310 mmol), 2-methoxy-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)pyridine (146 mg, 0.620 mmol) and $PdCl_2$(dppf)-CH_2Cl_2 (75.9 mg, 0.0930 mmol) in DME (4.0 mL) and 2 M K_2CO_3 aq. (1.60 mL, 3.20 mmol) was stirred at 100 °C for 5 h under an argon atmosphere. After cooling to room temperature, the reaction mixture was diluted with AcOEt, filtered through Celite® and concentrated under reduced pressure. The resulting residue was purified by column chromatography (*n*-hexane/AcOEt) to give the title compound **SI-13** (89.0 mg, 72% yield).

^{1}H-NMR ($CDCl_3$) δ: 0.60–0.61 (m, 2H), 1.01–1.03 (m, 2H), 1.49 (s, 9H), 2.01–2.03 (m, 1H), 3.44 (s, 3H), 3.99 (s, 3H), 7.45 (d, *J* = 1.45, 1H), 7.52 (dd, *J* = 5.43, 1.45 Hz, 1H), 8.18 (dd, *J* = 5.43, 0.64 Hz, 1H), 8.39 (s, 1H), 8.41 (s, 1H).

6-Cyclopropyl-3-(2-methoxypyridin-4-yl)-*N*-methylpyrazolo[1,5-*a*]pyrimidin-5-amine (10)

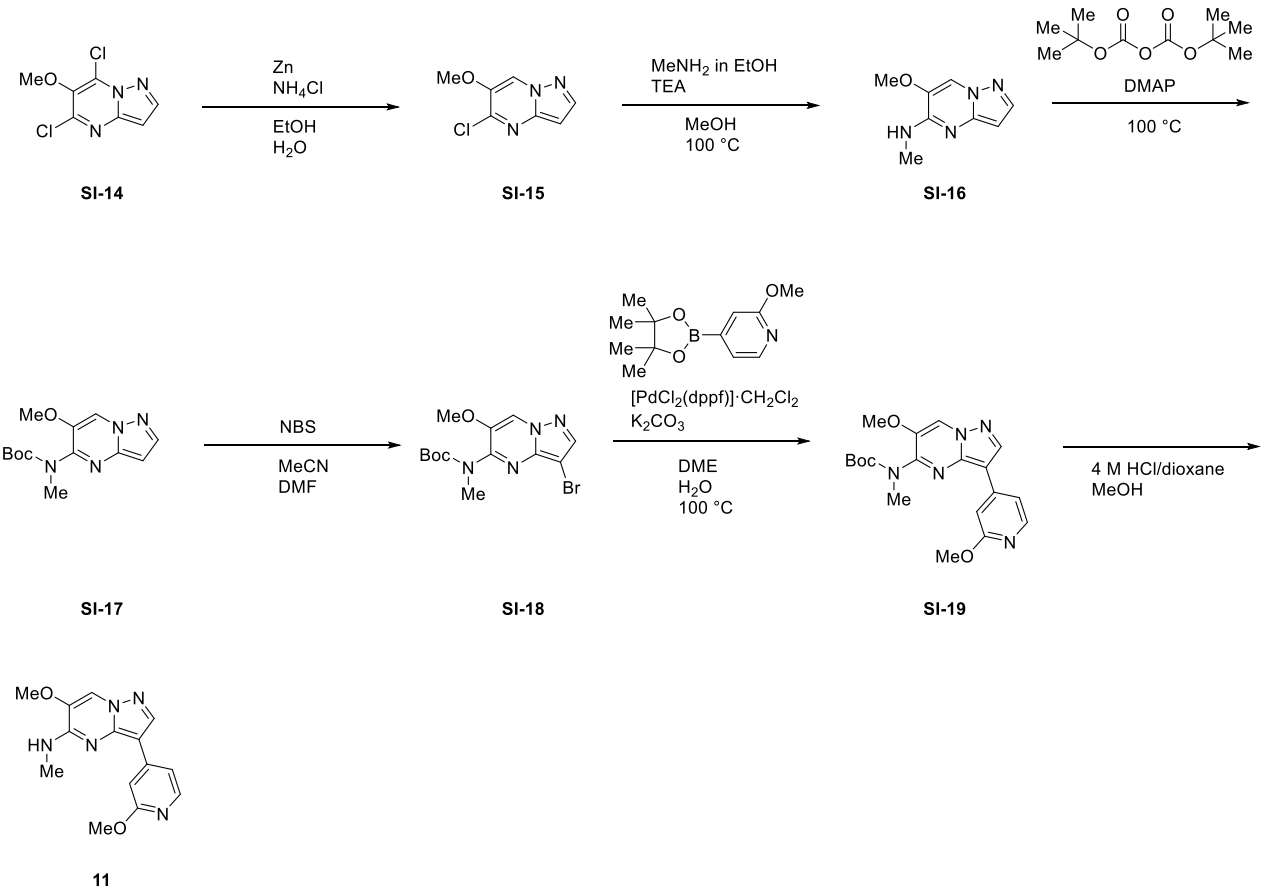
A solution of **SI-13** (44.5 mg, 0.113 mmol) in MeOH (0.50 mL) and $CHCl_3$ (0.50 mL) was treated with 4 M HCl in dioxane (1.0 mL) at room temperature. After stirring at room temperature for 2 h, the solvent was removed under reduced pressure. To the resulting residue was carefully added saturated $NaHCO_3$ aq. and AcOEt. The layers were separated and the organic layer was washed with brine, dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The resulting residue was purified by column chromatography (*n*-hexane/AcOEt) to give the title compound **10** (29.5 mg, 89% yield).

^{1}H-NMR ($CDCl_3$) δ: 0.64–0.66 (m, 2H), 1.00–1.05 (m, 2H), 1.57–1.60 (m, 1H), 3.24 (d, *J* = 4.86 Hz, 3H), 3.98 (s, 3H), 5.54 (br s, 1H), 7.51 (d, *J* = 0.69 Hz, 1H), 7.56 (dd, *J* = 5.32, 0.69 Hz, 1H), 8.07 (d, 1H), 8.14 (d, *J* = 5.32 Hz, 1H), 8.20 (s, 1H).

HRMS *m/z*: $[M+H]^+$ calcd for $C_{16}H_{17}N_5O$ 296.1506; found, 296.1502.

Purity: 100%.

Synthesis of compound 11



5-Chloro-6-methoxypyrazolo[1,5-*a*]pyrimidine (SI-15)

To a solution of 5,7-dichloro-6-methoxypyrazolo[1,5-*a*]pyrimidine (**SI-14**) (41.0 g, 188 mmol) in EtOH (330 mL), THF (330 mL) and water (250 mL) was successively added NH_4Cl (50.3 g, 940 mmol) and Zn (61.5 g, 940 mmol) at room temperature. After stirring at room temperature for 12 h, the reaction mixture was added water and AcOEt. The layers were separated. The organic layer was washed with brine, dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The resulting solid was slurried with Hexane/AcOEt and collected by filtration to give the title compound **SI-15** (26.6 g, 77% yield).

^{1}H-NMR (DMSO-d_6) δ: 3.94 (s, 3H), 6.67 (dd, J = 2.37, 0.75 Hz, 1H), 8.14 (d, J = 2.37 Hz, 1H), 9.05 (d, J = 0.75 Hz, 1H).

6-Methoxy-*N*-methylpyrazolo[1,5-*a*]pyrimidin-5-amine (SI-16)

To a solution of 5-chloro-6-methoxypyrazolo[1,5-*a*]pyrimidine (**SI-15**) (99.6 mg, 0.542 mmol) in MeOH (4.0 mL) was successively added 40 wt% methylamine in MeOH (337 µL, 2.71 mmol) and TEA (378 µL, 2.71 mmol) at room temperature. After stirring at 100 °C for 1 h, the reaction mixture was cooled to room temperature and concentrated under reduced pressure. To the resulting mixture was added water and AcOEt, the layers were separated. The organic layer was washed with brine, dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The resulting residue was purified by column chromatography (*n*-hexane/AcOEt) to give the title compound **SI-16** (96.1 mg, 99% yield).

^{1}H-NMR ($CDCl_3$) δ: 3.09 (d, J = 5.09 Hz, 3H), 3.86 (s, 3H), 5.43 (br s, 1H), 6.14 (d, J = 2.31 Hz, 1H), 7.74 (d, J =

2.31 Hz, 1H), 7.83 (s, 1H).

***tert*-Butyl (6-methoxypyrazolo[1,5-*a*]pyrimidin-5-yl)(methyl)carbamate (SI-17)**

To a solution of **SI-16** (96.1 mg, 0.539 mmol) in Boc_2O (1.26 mL, 5.39 mmol) was added DMAP (3.30 mg, 0.0270 mmol) at room temperature. After stirring at 100 °C for 12 h, the reaction mixture was cooled to room temperature and concentrated under reduced pressure. The resulting residue was purified by column chromatography (*n*-hexane/AcOEt) to give the title compound **SI-17** (150 mg, 100% yield).

^{1}H-NMR ($CDCl_3$) δ: 1.43 (s, 9H), 3.33 (s, 3H), 3.88 (s, 3H), 6.54 (d, J = 2.08 Hz, 1H), 7.97 (d, J = 2.08 Hz, 1H), 8.20 (s, 1H).

***tert*-Butyl (3-bromo-6-methoxypyrazolo[1,5-*a*]pyrimidin-5-yl)(methyl)carbamate (SI-18)**

To a solution of **SI-17** (70.4 mg, 0.253 mmol) in MeCN (1.0 mL) and DMF (1.0 mL) was added NBS (49.5 mg, 0.278 mmol) at room temperature. After stirring at room temperature for 30 min, to the reaction mixture was added water. The precipitated solid was collected by filtration to give the title compound **SI-18** (170 mg, 88% yield).

^{1}H-NMR ($CDCl_3$) δ: 1.43 (s, 9H), 3.37 (s, 3H), 3.88 (s, 3H), 7.95 (s, 1H), 8.13 (s, 1H).

***tert*-Butyl (6-methoxy-3-(2-methoxypyridin-4-yl)pyrazolo[1,5-*a*]pyrimidin-5-yl)(methyl)carbamate (SI-19)**

A mixture of **SI-18** (170 mg, 0.476 mmol), 2-methoxy-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)pyridine (224 mg, 0.952 mmol) and $PdCl_2$(dppf)-CH_2Cl_2 (117 mg, 0.143 mmol) in DME (4.0 mL) and 2 M K_2CO_3 aq. (1.6 mL, 3.20 mmol) was stirred at 100 °C for 5 h under an argon atmosphere. After cooling to room temperature, the reaction mixture was diluted with AcOEt, filtered through Celite® and concentrated under reduced pressure. The resulting residue was purified by column chromatography (*n*-hexane/AcOEt) to give the title compound **SI-19** (137 mg, 75% yield).

^{1}H-NMR ($CDCl_3$) δ: 1.45 (s, 9H), 3.44 (s, 3H), 3.91 (s, 3H), 3.98 (s, 3H), 7.43 (s, 1H), 7.51 (d, J = 5.49 Hz, 1H), 8.18 (d, J = 5.49 Hz, 1H), 8.20 (s, 1H), 8.34 (s, 1H).

6-Methoxy-3-(2-methoxypyridin-4-yl)-*N*-methylpyrazolo[1,5-*a*]pyrimidin-5-amine (11)

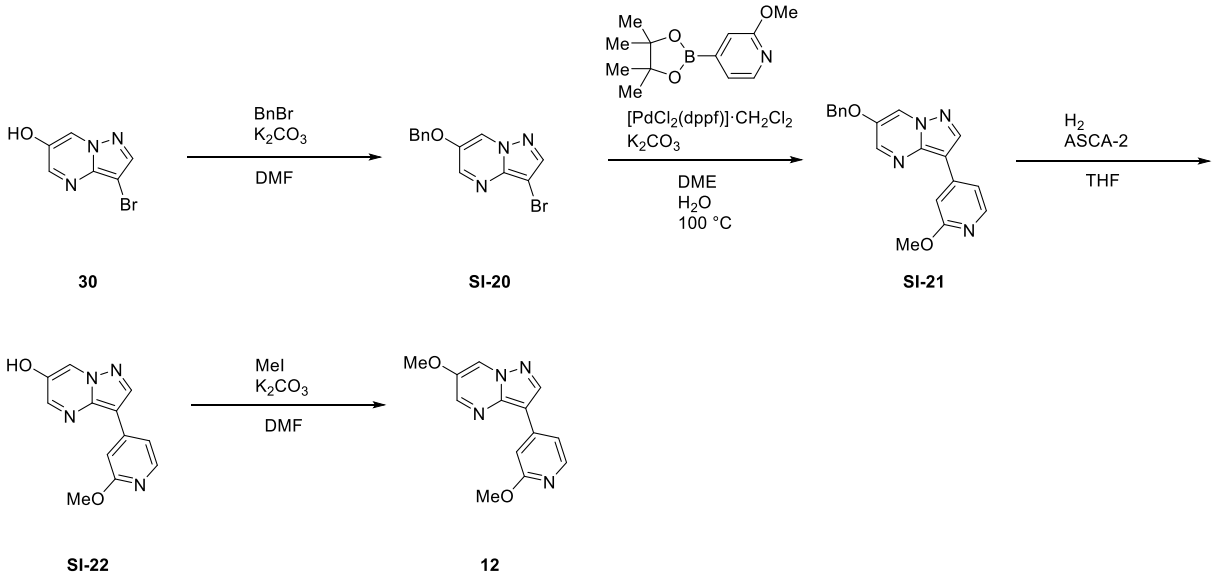
A solution of **SI-19** (68.0 mg, 0.176 mmol) in MeOH (0.50 mL) and $CHCl_3$ (0.50 mL) was treated with 4 M HCl dioxane (1.5 mL) at room temperature. After stirring at room temperature for 2.5 h, the solvent was removed under reduced pressure. To the resulting residue was carefully added saturated $NaHCO_3$ aq. and AcOEt. The layers were separated and the organic layer was washed with brine, dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The resulting residue was purified by column chromatography (*n*-hexane/AcOEt) to give the title compound **11** (43.6 mg, 87% yield).

^{1}H-NMR ($CDCl_3$) δ: 3.20 (d, J = 4.86 Hz, 3H), 3.90 (s, 3H), 3.97 (s, 3H), 5.61 (br s, 1H), 7.49 (dd, J = 1.45, 0.69 Hz, 1H), 7.55 (dd, J = 5.49, 1.45 Hz, 1H), 7.83 (s, 1H), 8.13 (dd, J = 5.49, 0.69 Hz, 1H), 8.15 (s, 1H).

HRMS *m/z*: $[M+H]^+$ calcd for $C_{14}H_{15}N_5O_2$ 286.1299; found, 286.1297.

Purity: 100%.

Synthesis of compound 12



6-(Benzyloxy)-3-bromopyrazolo[1,5-*a*]pyrimidine (SI-20)

To a solution of 3-bromopyrazolo[1,5-*a*]pyrimidin-6-ol (**30**) (1.00 g, 4.67 mmol) in DMF (10 mL) was added K_2CO_3 (1.29 g, 9.34 mmol) and BnBr (610 µL, 5.14 mmol) at 0 °C. After stirring at room temperature for 2 h, to the reaction mixture was added water. The precipitated solid was collected by filtration to give the title compound **SI-20** (1.35 g, 95% yield).

^{1}H-NMR ($CDCl_3$) δ: 5.10 (s, 2H), 7.34–7.45 (m, 5H), 7.99 (s, 1H), 8.22 (d, J = 2.77 Hz, 1H), 8.50 (d, J = 2.77 Hz, 1H).

6-(Benzyloxy)-3-(2-methoxypyridin-4-yl)pyrazolo[1,5-*a*]pyrimidine (SI-21)

A mixture of **SI-20** (1.35 g, 4.44 mmol), 2-methoxy-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)pyridine (1.36 g, 5.77 mmol) and $PdCl_2$(dppf)-CH_2Cl_2 (363 mg, 0.444 mmol) in DME (14 mL) and 2 M K_2CO_3 aq. (11.2 mL, 22.4 mmol) was stirred at 100 °C for 5 h under an argon atmosphere. After cooling to room temperature, the reaction mixture was diluted with AcOEt and water, filtered through Celite® . The layers were separated and the organic layer was washed with brine, dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The resulting residue was purified by column chromatography (*n*-hexane/AcOEt) to give the title compound **SI-21** (1.42 g, 96% yield).

^{1}H-NMR ($CDCl_3$) δ: 3.98 (s, 3H), 5.13 (s, 2H), 7.37–7.48 (m, 6H), 7.54 (dd, J = 5.38, 1.50 Hz, 1H), 8.19 (d, J = 5.38 Hz, 1H), 8.29 (d, J = 2.66 Hz, 1H), 8.37 (s, 1H), 8.56 (d, J = 2.66 Hz, 1H).

3-(2-Methoxypyridin-4-yl)pyrazolo[1,5-*a*]pyrimidin-6-ol (SI-22)

A solution of **SI-21** (400 mg, 1.20 mmol) in THF (8.0 mL) was treated with ASCA-2 (80.0 mg) and stirred under a hydrogen atmosphere (1 atm) at room temperature overnight. After removal of the palladium catalyst by Celite® filtration, the filtrate was concentrated. The resulting solid was slurried with $CHCl_3$ and collected by filtration to give the title compound **SI-22** (197 mg, 68% yield).

^{1}H-NMR (DMSO-d_6) δ: 3.87 (s, 3H), 7.57 (dd, J = 1.45, 0.64 Hz, 1H), 7.69 (dd, J = 5.43, 1.45 Hz, 1H), 8.15 (dd, J

= 5.43, 0.64 Hz, 1H), 8.58 (d, *J* = 2.66 Hz, 1H), 8.60 (d, *J* = 2.66 Hz, 1H), 8.73 (s, 1H), 10.45 (br s, 1H).

6-Methoxy-3-(2-methoxypyridin-4-yl)pyrazolo[1,5-*a*]pyrimidine (12)

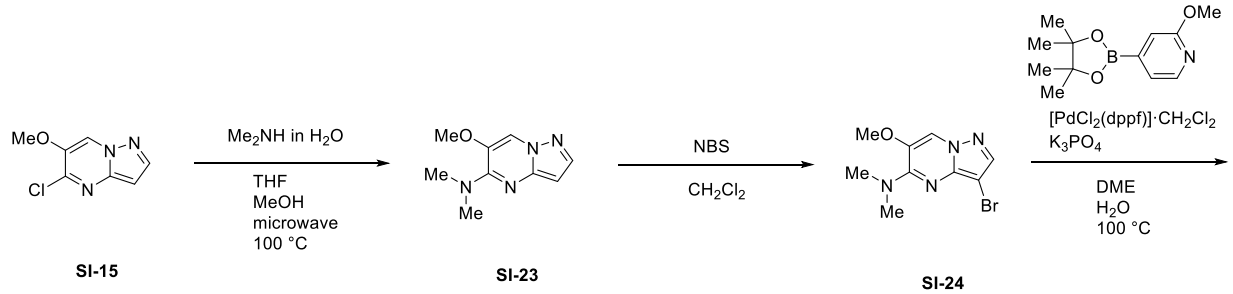
To a solution of **SI-22** (15.0 mg, 0.0619 mmol) in DMF (1.0 mL) was added K_2CO_3 (25.7 mg, 0.186 mmol) and MeI (11.6 μL, 0.186 mmol) at room temperature. After stirring at room temperature for 12 h, to the reaction mixture was added water. The precipitated solid was collected by filtration to give the title compound **12** (8.20 mg, 52% yield).

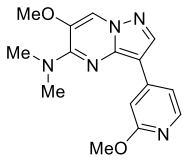
^{1}H-NMR ($CDCl_3$) δ: 3.92 (s, 3H), 3.99 (s, 3H), 7.47 (dd, *J* = 1.45, 0.58 Hz, 1H), 7.55 (dd, *J* = 5.49, 1.45 Hz, 1H), 8.19 (dd, *J* = 5.49, 0.58 Hz, 1H), 8.25 (d, *J* = 2.77 Hz, 1H), 8.37 (s, 1H), 8.50 (d, *J* = 2.77 Hz, 1H).

HRMS *m/z*: $[M+H]^+$ calcd for $C_{13}H_{12}N_4O_2$ 257.1033; found, 257.1032.

Purity: 91.5%.

Synthesis of compound 13





13

3-Bromo-6-methoxy-*N*,*N*-dimethylpyrazolo[1,5-*a*]pyrimidin-5-amine (SI-24)

A microwave vial was charged with 5-chloro-6-methoxypyrazolo[1,5-*a*]pyrimidine (**SI-15**) (300 mg, 1.63 mmol), 9.5 M dimethylamine aq. (428 μL, 8.17 mmol), MeOH (3.0 mL) and THF (1.0 mL). The vial was heated to 100 °C in the microwave for 40 min. After removal of solvent under reduced pressure, then crude **SI-23** was ready for the next reaction. To a solution of **SI-23** in CH_2Cl_2 (9.0 mL) was added NBS (291 mg, 1.63 mmol) at 0 °C. After stirring at 0 °C for 30 min, to the reaction mixture was added 10% Na_2SO_3 aq. and $CHCl_3$. The layers were separated and the organic layer was washed with brine, dried over Na_2SO_4, filtered, and concentrated under reduced pressure to give the title compound **SI-24** (337 mg, 76% yield for 2 steps).

^{1}H-NMR ($CDCl_3$) δ: 3.23 (s, 6H), 3.83 (s, 3H), 7.73 (s, 1H), 7.85 (s, 1H).

6-Methoxy-3-(2-methoxypyridin-4-yl)-*N*,*N*-dimethylpyrazolo[1,5-*a*]pyrimidin-5-amine (13)

A microwave vial was charged with **SI-24** (30.0 mg, 0.111 mmol), 2-methoxy-4-(4,4,5,5-tetramethyl-1,3,2-

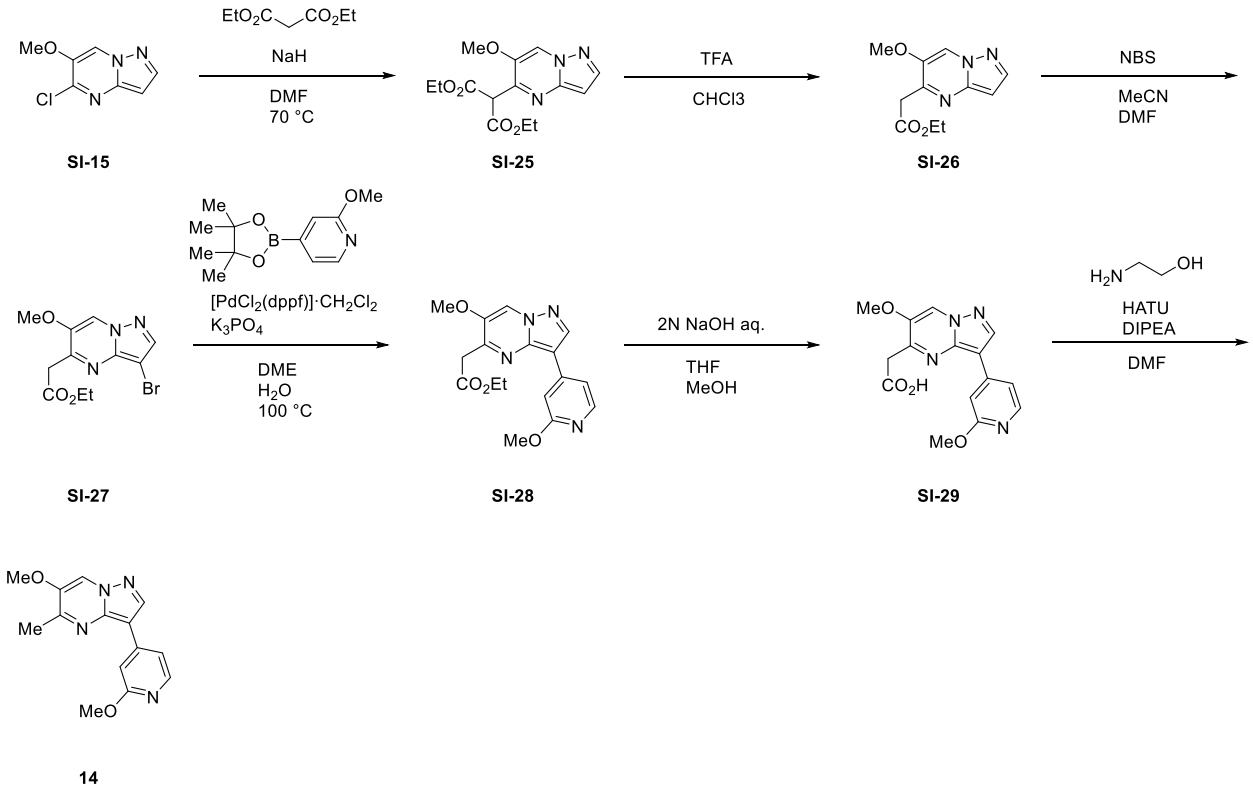
dioxaborolan-2-yl)pyridine (28.6 mg, 0.122 mmol), $PdCl_2$(dppf)-CH_2Cl_2 (9.04 mg, 0.0110 mmol) , K_3PO_4 (70.5 mg, 0.332 mmol) , DME (1.0 mL) and water (0.40 mL). The vial was heated to 100 °C in the microwave for 45 min. After removal of solvent under reduced pressure, the resulting residue was purified by column chromatography (*n*-hexane/AcOEt) to give the title compound **13** (8.30 mg, 25% yield).

^{1}H-NMR ($CDCl_3$) δ: 3.31 (s, 6H), 3.87 (s, 3H), 3.97 (s, 3H), 7.45–7.46 (m, 1H), 7.52 (dd, *J* = 5.49, 1H), 7.92 (s, 1H), 8.13 (d, *J* = 5.49 Hz, 1H), 8.18 (s, 1H).

HRMS *m/z*: $[M+H]^+$ calcd for $C_{15}H_{17}N_5O_2$ 300.1455; found, 300.1452.

Purity: 94.1%.

Synthesis of compound 14



Diethyl 2-(6-methoxypyrazolo[1,5-*a*]pyrimidin-5-yl)malonate (SI-25)

Under a nitrogen stream, to a solution of diethyl malonate (0.46 mL, 2.45 mmol) in DMF (6.0 mL) was added NaH (60% oil suspension) (98.0 mg, 2.45 mmol) at 0 °C. After stirring at room temperature for 1 h, 5-chloro-6-methoxypyrazolo[1,5-*a*]pyrimidine (**SI-15**) (300 mg, 1.63 mmol) was added to the reaction mixture at 0 °C. The mixture was stirred at 70 °C for 5 h. After cooling to room temperature, to the reaction mixture was added 10% $KHSO_4$ aq. and AcOEt. The layers were separated and the organic layer was washed with brine, dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The resulting residue was purified by column chromatography (*n*-hexane/AcOEt) to give the title compound **SI-25** (286 mg, 52% yield).

^{1}H-NMR ($CDCl_3$) δ: 1.30 (t, *J* = 7.05 Hz, 6H), 3.87 (s, 3H), 4.26–4.34 (m, 4H), 4.98 (s, 1H), 6.61 (d, *J* = 2.43 Hz, 1H), 7.98 (d, *J* = 2.43 Hz, 1H), 8.21 (s, 1H).

Ethyl 2-(6-methoxypyrazolo[1,5-*a*]pyrimidin-5-yl)acetate (SI-26)

A solution of **SI-25** (286 mg, 0.851 mmol) in $CHCl_3$ (6.0 mL) was treated with TFA (3.0 mL) at room temperature. After stirring at room temperature for 12 h, the solvent was removed under reduced pressure. To the resulting residue was carefully added saturated $NaHCO_3$ aq. and AcOEt. The layers were separated and the organic layer was washed with brine, dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The resulting residue was purified by column chromatography (*n*-hexane/AcOEt) to give the title compound **SI-26** (185 mg, 93% yield).

^{1}H-NMR ($CDCl_3$) δ: 1.26 (t, *J* = 7.11 Hz, 3H), 3.88 (s, 3H), 3.91 (s, 2H), 4.20 (q, *J* = 7.11 Hz, 2H), 6.60 (d, *J* = 2.31, 1H), 7.98 (d, *J* = 2.31 Hz, 1H), 8.20 (s, 1H).

Ethyl 2-(3-bromo-6-methoxypyrazolo[1,5-*a*]pyrimidin-5-yl)acetate (SI-27)

To a solution of **SI-26** (785 mg, 3.34 mmol) in MeCN (4.0 mL) and DMF (1.0 mL) was added NBS (653 mg, 3.67 mmol) at 0 °C. After stirring at room temperature for 1 h, to the reaction mixture was added water. The precipitated solid was collected by filtration to give the title compound **SI-27** (916 mg, 87% yield).

^{1}H-NMR ($CDCl_3$) δ: 1.27 (t, *J* = 7.11 Hz, 3H), 3.89 (s, 3H), 3.97 (s, 2H), 4.20 (q, *J* = 7.11 Hz, 2H), 7.96 (s, 1H), 8.15 (s, 1H).

Ethyl 2-(6-methoxy-3-(2-methoxypyridin-4-yl)pyrazolo[1,5-*a*]pyrimidin-5-yl)acetate (SI-28)

A mixture of **SI-27** (281 mg, 0.894 mmol), 2-methoxy-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)pyridine (315 mg, 1.34 mmol), $PdCl_2$(dppf)-CH_2Cl_2 (73.0 mg, 0.0894 mmol) and K_3PO_4 (569 mg, 2.68 mmol) in DME (6.0 mL) and water (2.4 mL) was stirred at 100 °C for 2.5 h under an argon atmosphere. After cooling to room temperature, the reaction mixture was diluted with AcOEt and water, filtered through Celite® . The layers were separated and the organic layer was washed with brine, dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The resulting residue was purified by column chromatography (*n*-hexane/AcOEt) to give the title compound **SI-28** (138 mg, 45% yield).

^{1}H-NMR ($CDCl_3$) δ: 1.29 (t, *J* = 7.13 Hz, 3H), 3.91 (s, 3H), 3.98 (s, 3H), 3.99 (s, 2H), 4.24 (q, *J* = 7.13 Hz, 2H), 7.50 (d, *J* = 1.45 Hz, 1H), 7.53 (dd, *J* = 5.43, 1.45 Hz, 1H), 8.17 (dd, *J* = 5.43, 0.64 Hz, 1H), 8.21 (s, 1H), 8.35 (s, 1H).

2-(6-Methoxy-3-(2-methoxypyridin-4-yl)pyrazolo[1,5-*a*]pyrimidin-5-yl)acetic acid (SI-29)

To a solution of **SI-28** (136 mg, 0.397 mmol) in MeOH (2.0 mL) and THF (2.0 mL) was added 2N NaOH aq. (2.00 mL, 4.00 mmol) at 0 °C. After stirring at room temperature for 2 h, To the reaction mixture was added 2 M HCl aq. and water at 0 °C. The precipitated solid was collected by filtration to give the title compound **SI-29** (121 mg, 97% yield).

^{1}H-NMR (DMSO-d_6) δ: 3.88 (d, *J* = 0.90 Hz, 3H), 3.90 (s, 2H), 3.92 (s, 3H), 7.57 (s, 1H), 7.72 (d, *J* = 5.46 Hz, 1H), 8.15 (d, *J* = 5.46 Hz, 1H), 8.77 (d, *J* = 0.90 Hz, 1H), 8.96 (s, 1H), 12.73 (br s, 1H).

6-Methoxy-3-(2-methoxypyridin-4-yl)-5-methylpyrazolo[1,5-*a*]pyrimidine (14)

To a solution of **SI-29** (80.0 mg, 0.279 mmol), 2-aminoethan-1-ol (25.1 μL, 0.419 mmol) in DMF (2.0 mL) were

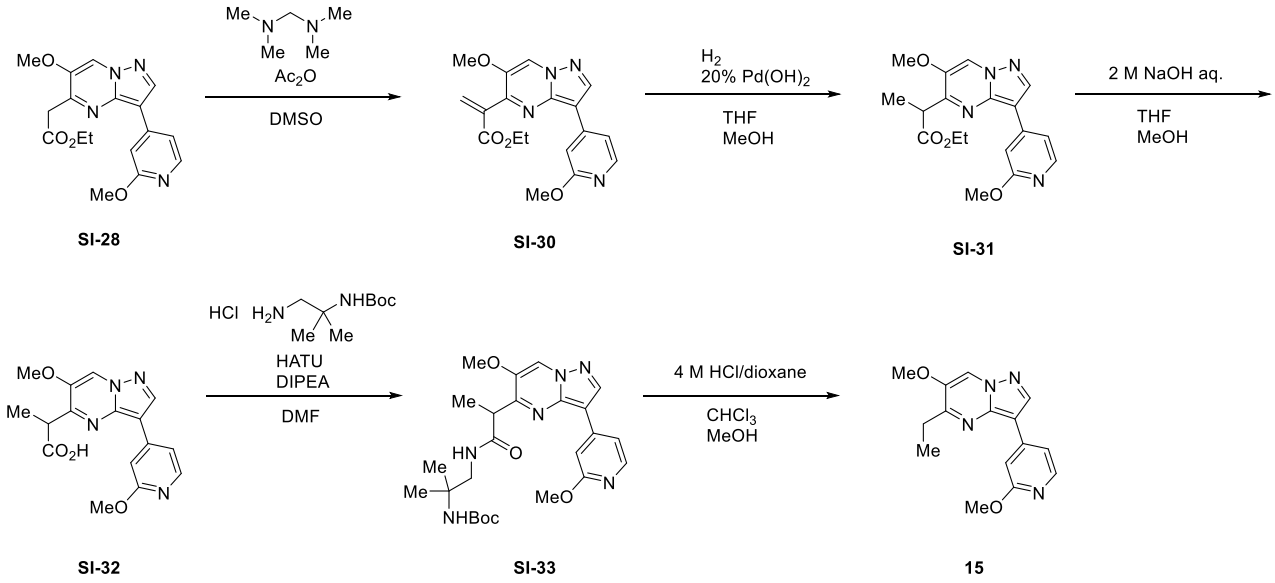
added successively DIPEA (73.0 μL, 0.419 mmol) and HATU (159 mg, 0.419 mmol) at room temperature. After stirring at room temperature for 12 h, to the reaction mixture was added AcOEt and saturated $NaHCO_3$ aq.. The layers were separated and the organic layer was washed with brine, dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The resulting residue was purified by preparative TLC (MeOH/AcOEt = 1:4 (v/v)) to give the title compound **14** (32.3 mg, 32% yield).

^{1}H-NMR ($CDCl_3$) δ: 2.62 (s, 3H), 3.91 (s, 3H), 3.99 (s, 3H), 7.52 (dd, *J* = 1.50, 0.64 Hz, 1H), 7.56 (dd, *J* = 5.38, 1.50 Hz, 1H), 8.13 (s, 1H), 8.18 (dd, *J* = 5.38, 0.64 Hz, 1H), 8.31 (s, 1H).

HRMS *m/z*: $[M+H]^+$ calcd for $C_{14}H_{14}N_4O_2$ 271.1190; found, 271.1189.

Purity: 92.9%.

Synthesis of compound 15



Ethyl 2-(6-methoxy-3-(2-methoxypyridin-4-yl)pyrazolo[1,5-*a*]pyrimidin-5-yl)acrylate (SI-30)

To a solution of **SI-28** (1.16 g, 3.39 mmol) in DMSO (15 mL) was added successively Ac_2O (1.12 mL, 11.9 mmol) and *N*,*N*,*N*′,*N*′-tetramethylmethanediamine (0.785 mL, 5.76 mmol) at room temperature. After stirring at room temperature for 30 min, to the reaction mixture was added water at 0 °C. The precipitated solid was collected by filtration to give the title compound **SI-30** (800 mg, 67% yield).

^{1}H-NMR ($CDCl_3$) δ: 1.30 (t, *J* = 7.17 Hz, 3H), 3.88 (s, 3H), 3.98 (s, 3H), 4.29 (q, *J* = 7.17 Hz, 2H), 6.31 (d, *J* = 1.04 Hz, 1H), 6.58 (d, *J* = 1.04 Hz, 1H), 7.48–7.49 (m, 1H), 7.57 (dd, *J* = 5.49, 1.50 Hz, 1H), 8.18 (d, *J* = 5.49 Hz, 1H), 8.25 (s, 1H), 8.39 (s, 1H).

Ethyl 2-(6-methoxy-3-(2-methoxypyridin-4-yl)pyrazolo[1,5-*a*]pyrimidin-5-yl)propanoate (SI-31)

A solution of **SI-30** (400 mg, 1.13 mmol) in MeOH (3.0 mL) and THF (3.0 mL) was treated with 20% palladium hydroxide on activated carbon (40.0 mg) and stirred under a hydrogen atmosphere (1 atm) at room temperature overnight. After removal of the palladium catalyst by Celite® filtration, the filtrate was concentrated and further purified by column chromatography (*n*-hexane/AcOEt) to give the title compound **SI-31** (353 mg, 88% yield).

^{1}H-NMR ($CDCl_3$) δ: 1.23 (t, *J* = 7.07 Hz, 3H), 1.67 (d, *J* = 7.17 Hz, 3H), 3.91 (s, 3H), 3.98 (s, 3H), 4.16–4.26 (m, 3H), 7.50–7.51 (m, 1H), 7.58 (d, *J* = 5.44 Hz, 1H), 8.18 (d, *J* = 5.44 Hz, 1H), 8.20 (s, 1H), 8.37 (s, 1H).

2-(6-Methoxy-3-(2-methoxypyridin-4-yl)pyrazolo[1,5-*a*]pyrimidin-5-yl)acetic acid (SI-32)

To a solution of **SI-31** (353 mg, 0.99 mmol) in MeOH (3.0 mL) and THF (3.0 mL) was added 2 M NaOH aq. (3.00 mL, 6.00 mmol) at 0 °C. After stirring at 50 °C for 2 h, To the reaction mixture was added 2 M HCl aq. and water at 0 °C. The precipitated solid was collected by filtration to give the title compound **SI-32** (286 mg, 88% yield).

^{1}H-NMR (DMSO-d_6) δ: 1.53 (d, *J* = 7.17 Hz, 3H), 3.88 (s, 3H), 3.93 (s, 3H), 4.17 (q, *J* = 7.17 Hz, 1H), 7.61 (s, 1H), 7.72 (d, *J* = 5.49 Hz, 1H), 8.16 (d, *J* = 5.49 Hz, 1H), 8.79 (s, 1H), 8.97 (s, 1H), 12.61 (br s, 1H).

***tert*-Butyl (2-(2-(6-methoxy-3-(2-methoxypyridin-4-yl)pyrazolo[1,5-*a*]pyrimidin-5-yl)propanamido)ethyl)carbamate (SI-33)**

To a solution of **SI-32** (50.0 mg, 0.152 mmol), *tert*-butyl (1-amino-2-methylpropan-2-yl)carbamate hydrochloride (68.4 mg, 0.305 mmol) in DMF (2.0 mL) were added successively DIPEA (0.106 mL, 0.609 mmol) and HATU (87.0 mg, 0.228 mmol) at room temperature. After stirring at room temperature for 12 h, to the reaction mixture was added water at 0 °C. The precipitated solid was collected by filtration. Title compound **SI-33** (47.4 mg) was obtained and used for next step without further purification.

^{1}H-NMR ($CDCl_3$) δ: 1.21 (s, 9H), 1.22 (s, 6H), 1.73 (d, *J* = 7.13 Hz, 3H), 3.42 (d, *J* = 5.78 Hz, 2H), 3.91 (s, 3H), 3.99 (s, 3H), 4.27 (q, *J* = 7.13 Hz, 1H), 4.71 (br s, 1H), 7.30 (br s, 1H), 7.54 (s, 1H), 7.56 (d, *J* = 5.67 Hz, 1H), 8.18 (d, *J* = 5.67 Hz, 1H), 8.20 (s, 1H), 8.36 (s, 1H).

5-Ethyl-6-methoxy-3-(2-methoxypyridin-4-yl)pyrazolo[1,5-*a*]pyrimidine (15)

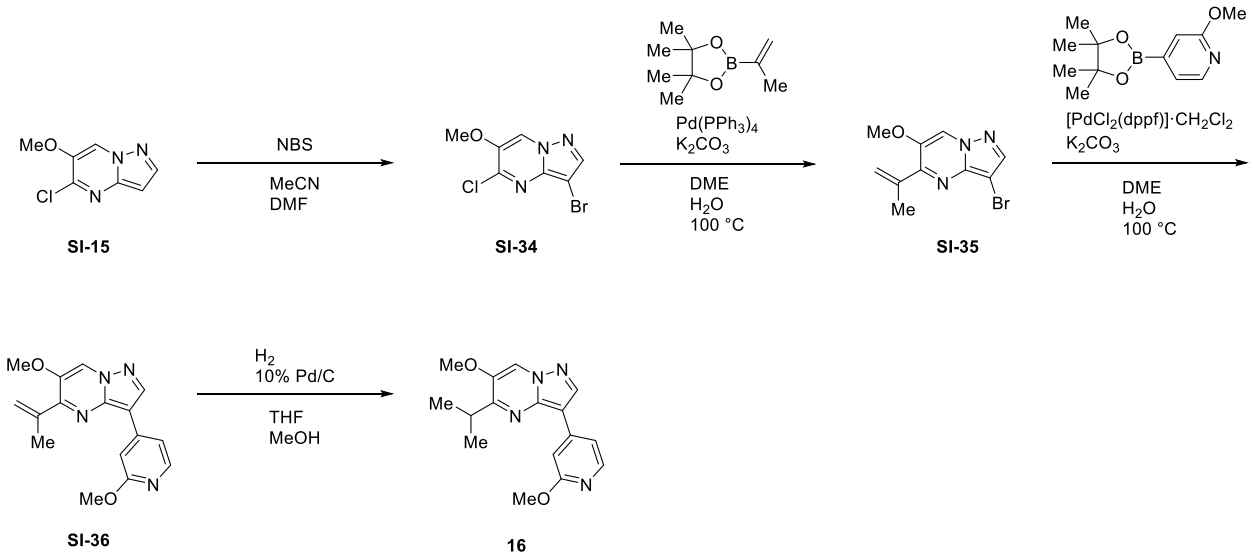
A solution of **SI-33** (47.4 mg, 0.0950 mmol) in MeOH (1.0 mL) and $CHCl_3$ (1.0 mL) was treated with 4 M HCl in dioxane (2.00 mL, 8.00 mmol) at room temperature. After stirring at room temperature for 5 h, the solvent was removed under reduced pressure. The resulting residue was purified by column chromatography (*n*-hexane/AcOEt) to give the title compound **15** (5.00 mg, 12% yield for 2 steps).

^{1}H-NMR ($CDCl_3$) δ: 1.40 (t, *J* = 7.40 Hz, 3H), 2.96 (q, *J* = 7.40 Hz, 2H), 3.91 (s, 3H), 3.99 (s, 3H), 7.55–7.55 (m, 1H), 7.61 (d, *J* = 5.49 Hz, 1H), 8.13 (s, 1H), 8.18 (d, *J* = 5.49 Hz, 1H), 8.33 (s, 1H).

HRMS *m/z*: $[M+H]^+$ calcd for $C_{15}H_{16}N_4O_2$ 285.1346; found, 285.1345.

Purity: 100%.

Synthesis of compound 16



3-Bromo-5-chloro-6-methoxypyrazolo[1,5-*a*]pyrimidine (SI-34)

To a solution of **SI-15** (505 mg, 2.75 mmol) in MeCN (4.0 mL) and DMF (1.0 mL) was added NBS (539 mg, 3.03 mmol) at room temperature. After stirring at room temperature for 1 h, to the reaction mixture was added water. The precipitated solid was collected by filtration to give the title compound **SI-34** (687 mg, 95% yield).

^{1}H-NMR ($CDCl_3$) δ: 3.96 (s, 3H), 7.99 (s, 1H), 8.19 (s, 1H).

3-Bromo-6-methoxy-5-(prop-1-en-2-yl)pyrazolo[1,5-*a*]pyrimidine (SI-35)

A mixture of **SI-34** (100 mg, 0.381 mmol), 4,4,5,5-tetramethyl-2-vinyl-1,3,2-dioxaborolane (78.8 μL, 0.419 mmol), $Pd(PPh_3)_4$ (44.0 mg, 0.0381 mmol), and K_2CO_3 (29.1 mg, 0.762 mmol) in DME (3.0 mL) and water (1.2 mL) was stirred at 100 °C for 1 h under an argon atmosphere. After cooling to room temperature, the reaction mixture was diluted with AcOEt, filtered through Celite® and concentrated under reduced pressure. The resulting residue was purified by column chromatography (*n*-hexane/AcOEt) to give the title compound **SI-35** (57.7 mg, 57% yield).

^{1}H-NMR ($CDCl_3$) δ: 2.25–2.28 (m, 3H), 3.89 (s, 3H), 5.63–5.66 (m, 1H), 5.92–5.94 (m, 1H), 7.96 (s, 1H), 8.17 (s, 1H).

6-Methoxy-3-(2-methoxypyridin-4-yl)-5-(prop-1-en-2-yl)pyrazolo[1,5-*a*]pyrimidine (SI-36)

A mixture of **SI-35** (57.7mg, 0.215 mmol), 2-methoxy-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)pyridine (65.8 mg, 0.280 mmol) and $PdCl_2$(dppf)-CH_2Cl_2 (35.1 mg, 0.0430 mmol) in DME (2.0 mL) and 2 M K_2CO_3 aq. (0.800 mL, 1.60 mmol) was stirred at 100 °C for 5 h under an argon atmosphere. After cooling to room temperature, the reaction mixture was diluted with AcOEt, filtered through Celite® and concentrated under reduced pressure. The resulting residue was purified by column chromatography (*n*-hexane/AcOEt) to give the title compound **SI-36** (47.2 mg, 74% yield).

^{1}H-NMR ($CDCl_3$) δ: 2.34 (d, J = 0.69 Hz, 3H), 3.93 (s, 3H), 3.98 (s, 3H), 5.74–5.76 (m, 1H), 6.12–6.14 (m, 1H), 7.52 (s, 1H), 7.59 (d, J = 5.44 Hz, 1H), 8.18 (d, J = 5.44 Hz, 1H), 8.24 (s, 1H), 8.37 (s, 1H).

5-Isopropyl-6-methoxy-3-(2-methoxypyridin-4-yl)pyrazolo[1,5-*a*]pyrimidine (16)

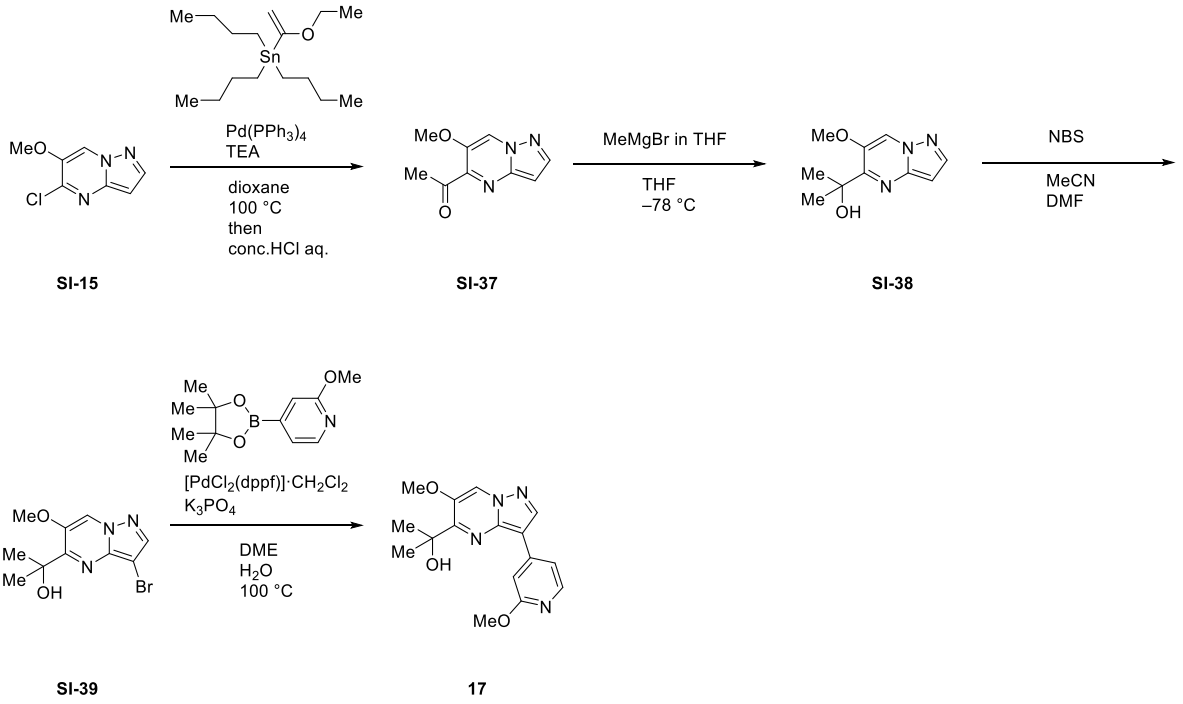
A solution of **SI-36** (45.0 mg, 0.152 mmol) in MeOH (1.0 mL) and THF (1.0 mL) was treated with 10% palladium on activated carbon (9.00 mg) and stirred under a hydrogen atmosphere (1 atm) at room temperature overnight. After removal of the palladium catalyst by Celite® filtration, the filtrate was concentrated and further purified by column chromatography (*n*-hexane/AcOEt). The resulting solid was slurried with *n*-hexane/IPE=3/1 and collected by filtration to give the title compound **16** (22.7 mg, 50 % yield).

^{1}H-NMR ($CDCl_3$) δ: 1.37 (d, J = 6.70 Hz, 6H), 3.45–3.57 (m, 1H), 3.91 (s, 3H), 3.99 (s, 3H), 7.55 (dd, J = 1.45, 0.69 Hz, 1H), 7.62 (dd, J = 5.49, 1.45 Hz, 1H), 8.14 (s, 1H), 8.18 (dd, J = 5.49, 0.69 Hz, 1H), 8.33 (s, 1H).

HRMS *m/z*: $[M+H]^+$ calcd for $C_{16}H_{18}N_4O_2$ 299.1503; found, 299.1500.

Purity: 100%.

Synthesis of compound 17



1-(6-Methoxypyrazolo[1,5-*a*]pyrimidin-5-yl)ethan-1-one (SI-37)

A mixture of **SI-15** (1.00 g, 5.45 mmol), tributyl(1-ethoxyvinyl)stannane (2.02 mL, 5.99 mmol) and $Pd(PPh_3)_4$ (629 mg, 0.545 mmol) in dioxane (10 mL) was stirred at 100 °C for 3 h under an argon atmosphere. After cooling in a water bath, to the reaction mixture was added conc. HCl aq. (0.934 mL, 7.08 mmol). The reaction mixture was stirred at room temperature for 2.5 h. After cooling in a water bath, to the reaction mixture was added TEA (1.14 mL, 8.17 mmol). The resulting mixture was diluted with $CHCl_3$, filtered through Celite®. The organic layer was washed with water and brine, dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The resulting residue was diluted with MeCN and Hexane. The layers were separated and the MeCN layer was washed five times with Hexane, and concentrated under reduced pressure. The resulting residue was purified by column chromatography (*n*-hexane/AcOEt) to give the title compound **SI-37** (787 mg, 68% yield).

^{1}H-NMR ($CDCl_3$) δ: 2.71 (s, 3H), 3.93 (s, 3H), 6.80 (dd, J = 2.49, 0.75 Hz, 1H), 8.10 (d, J = 2.49 Hz, 1H), 8.38 (d,

J = 0.75 Hz, 1H).

2-(6-Methoxypyrazolo[1,5-*a*]pyrimidin-5-yl)propan-2-ol (SI-38)

Under a nitrogen stream, to a solution of **SI-37** (100 mg, 0.523 mmol) in THF (4.0 mL) was added dropwise 0.95 M MeMgBr in THF (0.826 mL, 0.785 mmol) at −78 °C. The reaction mixture was stirred at room temperature for 4.5 h. After cooling to 0 °C, to the reaction mixture was added saturated NH_4Cl aq. and AcOEt. The layers were separated and the organic layer was washed with brine, dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The resulting residue was purified by column chromatography (*n*-hexane/AcOEt) to give the title compound **SI-38** (59.9 mg, 55% yield).

^{1}H-NMR ($CDCl_3$) δ: 1.63 (s, 6H), 3.93 (s, 3H), 5.24 (s, 1H), 6.61 (dd, J = 2.31, 0.81 Hz, 1H), 8.00 (d, J = 2.31 Hz, 1H), 8.26 (d, J = 0.81 Hz, 1H).

2-(3-Bromo-6-methoxypyrazolo[1,5-*a*]pyrimidin-5-yl)propan-2-ol (SI-39)

To a solution of **SI-38** (53.3 mg, 0.257 mmol) in MeCN (1.0 mL) and DMF (1.0 mL) was added NBS (50.4 mg, 0.283 mmol) at 0 °C. After stirring at room temperature for 12 h, to the reaction mixture was added water. The precipitated solid was collected by filtration to give the title compound **SI-39** (80.0 mg, quant.).

^{1}H-NMR ($CDCl_3$) δ: 1.64 (s, 6H), 3.94 (s, 3H), 5.23 (s, 1H), 7.98 (s, 1H), 8.20 (s, 1H).

2-(6-Methoxy-3-(2-methoxypyridin-4-yl)pyrazolo[1,5-*a*]pyrimidin-5-yl)propan-2-ol (17)

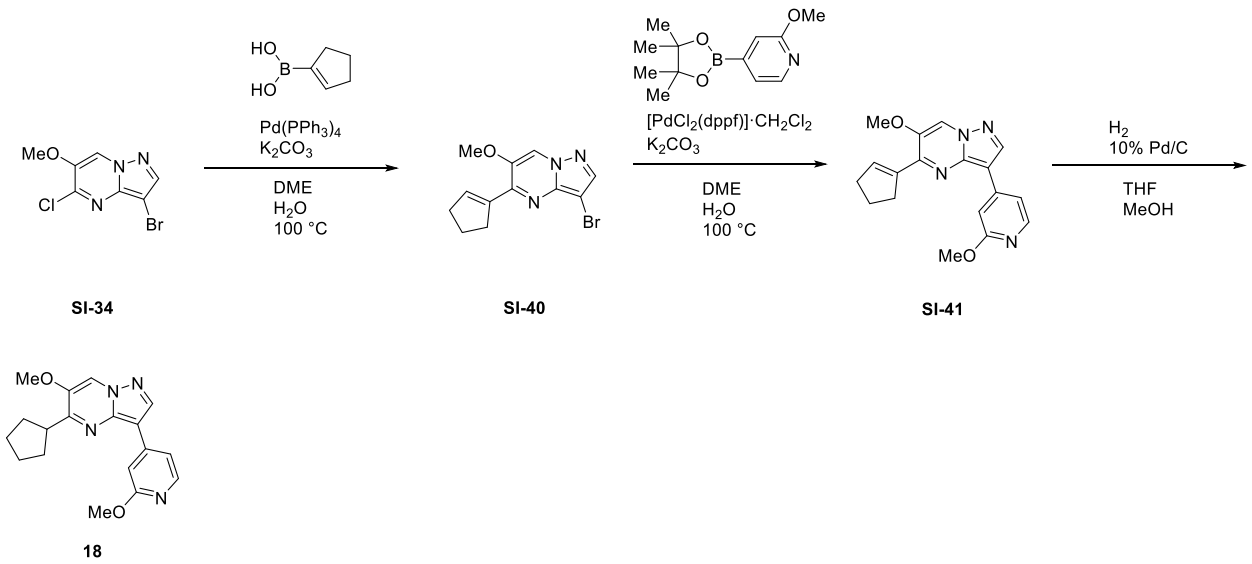
A mixture of **SI-39** (80.0 mg, 0.280 mmol), 2-methoxy-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)pyridine (99.0 mg, 0.419 mmol), $PdCl_2$(dppf)-CH_2Cl_2 (22.8 mg, 0.028 mmol) and K_3PO_4 (178 mg, 0.839 mmol) in DME (3.0 mL) and water (1.2 mL) was stirred at 100 °C for 3 h under an argon atmosphere. After cooling to room temperature, the reaction mixture was diluted with AcOEt, filtered through Celite® and concentrated under reduced pressure. The resulting residue was purified by column chromatography (*n*-hexane/AcOEt). The resulting solid was slurried with *n*-hexane/AcOEt=1/2 and collected by filtration to give the title compound **17** (41.3 mg, 47% yield).

^{1}H-NMR ($CDCl_3$) δ: 1.69 (s, 6H), 3.98 (s, 3H), 3.98 (s, 3H), 4.92 (s, 1H), 7.35–7.37 (m, 1H), 7.51 (dd, J = 5.49, 1.50 Hz, 1H), 8.20 (d, J = 5.49 Hz, 1H), 8.29 (s, 1H), 8.37 (s, 1H).

HRMS *m/z*: $[M+H]^+$ calcd for $C_{16}H_{18}N_4O_3$ 315.1452; found, 315.1449.

Purity: 100%.

Synthesis of compound 18



18

3-Bromo-5-(cyclopent-1-en-1-yl)-6-methoxypyrazolo[1,5-*a*]pyrimidine (SI-40)

A mixture of **SI-34** (100 mg, 0.381 mmol), cyclopent-1-en-1-ylboronic acid (46.9 mg, 0.419 mmol), $Pd(PPh_3)_4$ (44.0 mg, 0.0381 mmol), and K_2CO_3 (29.1 mg, 0.762 mmol) in DME (3.0 mL) and water (1.2 mL) was stirred at 100 °C for 1 h under an argon atmosphere. After cooling to room temperature, the reaction mixture was diluted with THF and MeOH, filtered through Celite® and concentrated under reduced pressure. The resulting residue was purified by column chromatography (*n*-hexane/AcOEt) to give the title compound **SI-40** (73.2 mg, 65% yield).

^{1}H-NMR ($CDCl_3$) δ: 1.93–2.00 (m, 2H), 2.64–2.70 (m, 2H), 2.98–3.04 (m, 2H), 3.92 (s, 3H), 7.09–7.11 (m, 1H), 7.93 (s, 1H), 8.12 (s, 1H).

5-(Cyclopent-1-en-1-yl)-6-methoxy-3-(2-methoxypyridin-4-yl)pyrazolo[1,5-*a*]pyrimidine (SI-41)

A mixture of **SI-40** (73.2 mg, 0.249 mmol), 2-methoxy-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)pyridine (76.2 mg, 0.324 mmol) and $PdCl_2$(dppf)-CH_2Cl_2 (40.7 mg, 0.0498 mmol) in DME (2.0 mL) and 2 M K_2CO_3 aq. (0.800 mL, 1.60 mmol) was stirred at 100 °C for 8 h under an argon atmosphere. After cooling to room temperature, the reaction mixture was diluted with AcOEt, filtered through Celite® and concentrated under reduced pressure. The resulting residue was purified by column chromatography (*n*-hexane/AcOEt) to give the title compound **SI-41** (47.4 mg, 59% yield).

^{1}H-NMR ($CDCl_3$) δ: 1.97–2.05 (m, 2H), 2.69–2.75 (m, 2H), 3.06–3.12 (m, 2H), 3.95 (s, 3H), 3.99 (s, 3H), 7.17–7.20 (m, 1H), 7.54 (dd, J = 1.45, 0.69 Hz, 1H), 7.61 (dd, J = 5.38, 1.45 Hz, 1H), 8.18 (dd, J = 5.38, 0.69 Hz, 1H), 8.19 (s, 1H), 8.35 (s, 1H).

5-Cyclopentyl-6-methoxy-3-(2-methoxypyridin-4-yl)pyrazolo[1,5-*a*]pyrimidine (18)

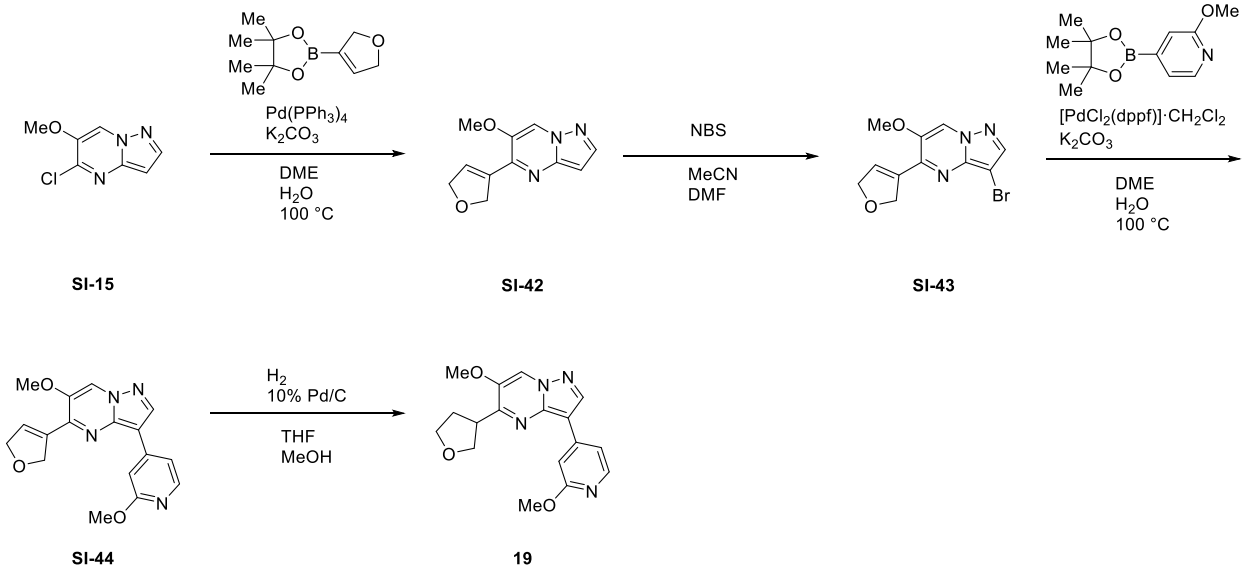
A solution of **SI-41** (42.6 mg, 0.132 mmol) in MeOH (1.0 mL) and THF (1.0 mL) was treated with 10% palladium on activated carbon (9.00 mg) and stirred under a hydrogen atmosphere (1 atm) at room temperature overnight. After removal of the palladium catalyst by Celite® filtration, the filtrate was concentrated and further purified by column chromatography (*n*-hexane/AcOEt). The resulting solid was slurried with *n*-hexane/IPE=2/1 and collected by filtration to give the title compound **18** (1.50 mg, 4% yield).

^{1}H-NMR ($CDCl_3$) δ: 1.69–1.80 (m, 2H), 1.84–1.95 (m, 2H), 1.96–2.10 (m, 4H), 3.57–3.65 (m, 1H), 3.91 (s, 3H), 3.99 (s, 3H), 7.51–7.53 (m, 1H), 7.61 (dd, *J* = 5.44, 1.16 Hz, 1H), 8.12 (s, 1H), 8.18 (d, *J* = 5.44 Hz, 1H), 8.32 (s, 1H).

HRMS *m/z*: $[M+H]^+$ calcd for $C_{18}H_{20}N_4O_2$ 325.1659; found, 325.1657.

Purity: 91.7%.

Synthesis of compound 19



3-Bromo-5-(cyclopent-1-en-1-yl)-6-methoxypyrazolo[1,5-*a*]pyrimidine (SI-42)

A mixture of **SI-15** (100 mg, 0.545 mmol), 2-(2,5-dihydrofuran-3-yl)-4,4,5,5-tetramethyl-1,3,2-dioxaborolane (322 mg, 1.64 mmol) and $Pd(PPh_3)_4$ (89.0 mg, 0.109 mmol) in DME (3.0 mL) and 2 M K_2CO_3 aq. (1.20 mL, 2.40 mmol) was stirred at 100 °C for 0.5 h under an argon atmosphere. After cooling to room temperature, the reaction mixture was diluted with THF and MeOH, filtered through Celite® and concentrated under reduced pressure. The resulting residue was purified by column chromatography (*n*-hexane/AcOEt). The title compound **SI-42** (216 mg) was obtained and used for next step without further purification.

^{1}H-NMR ($CDCl_3$) δ: 3.96 (s, 3H), 4.96 (td, *J* = 4.74, 2.08 Hz, 2H), 5.19 (td, *J* = 4.74, 2.08 Hz, 2H), 6.60 (d, *J* = 2.43 Hz, 1H), 7.10–7.12 (m, 1H), 7.99 (d, *J* = 2.43 Hz, 1H), 8.26 (s, 1H).

3-Bromo-5-(2,5-dihydrofuran-3-yl)-6-methoxypyrazolo[1,5-*a*]pyrimidine (SI-43)

To a solution of **SI-42** (216 mg) in MeCN (1.0 mL) and DMF (1.0 mL) was added NBS (97.0 mg, 0.545 mmol) at room temperature. After stirring at room temperature for 2 h, to the reaction mixture was added water. The precipitated solid was collected by filtration to give the title compound **SI-43** (133 mg, 82% yield for 2 steps).

1H-NMR (CDCl3) δ: 3.96 (s, 3H), 4.97 (td, *J* = 4.74, 2.08Hz, 2H), 5.23 (td, *J* = 4.74, 2.08 Hz, 2H), 7.14–7.17 (m, 1H), 7.96 (s, 1H), 8.19 (s, 1H).

5-(2,5-Dihydrofuran-3-yl)-6-methoxy-3-(2-methoxypyridin-4-yl)pyrazolo[1,5-*a*]pyrimidine (SI-44)

A mixture of **SI-43** (133 mg, 0.448 mmol), 2-methoxy-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)pyridine

(158 mg, 0.672 mmol) and $PdCl_2$(dppf)-CH_2Cl_2 (73.2 mg, 0.0896 mmol) in DME (3.0 mL) and 2 M K_2CO_3 aq. (1.20 mL, 2.40 mmol) was stirred at 100 °C for 8 h under an argon atmosphere. After cooling to room temperature, the reaction mixture was diluted with AcOEt, filtered through Celite® and concentrated under reduced pressure. The resulting residue was purified by column chromatography (*n*-hexane/AcOEt) to give the title compound **SI-44** (86.6 mg, 66% yield).

^{1}H-NMR ($CDCl_3$) δ: 3.99 (s, 3H), 3.99 (s, 3H), 5.01 (td, *J* = 4.74, 2.08 Hz, 2H), 5.30 (td, *J* = 4.74, 2.08 Hz, 2H), 7.18–7.21 (m, 1H), 7.43–7.46 (m, 1H), 7.53 (dd, *J* = 5.55, 1.39 Hz, 1H), 8.18 (d, *J* = 5.55 Hz, 1H), 8.26 (s, 1H), 8.37 (s, 1H).

6-Methoxy-3-(2-methoxypyridin-4-yl)-5-(tetrahydrofuran-3-yl)pyrazolo[1,5-*a*]pyrimidine (19)

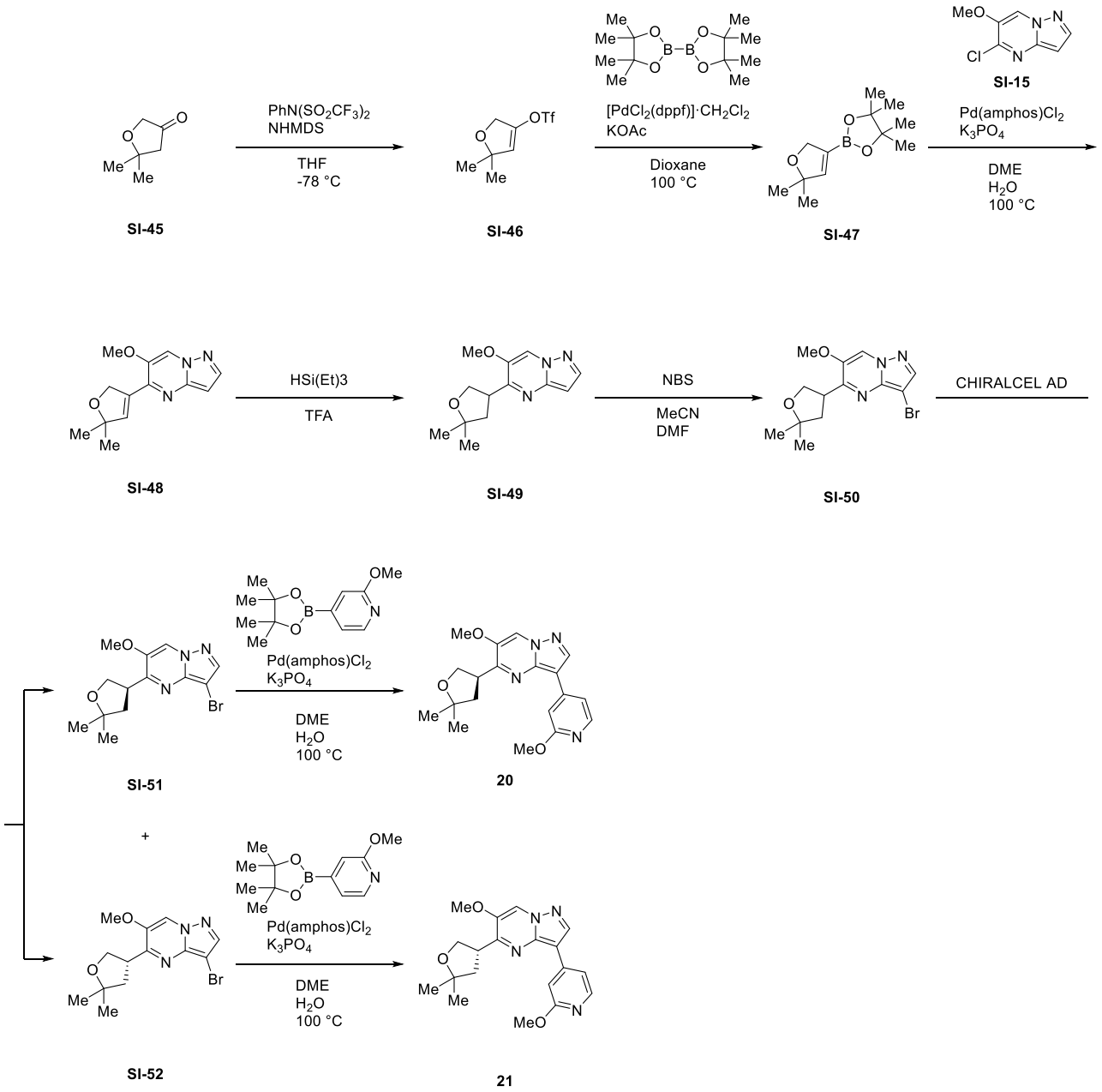
A solution of **SI-44** (30.0 mg, 0.0919 mmol) in MeOH (1.0 mL) and THF (1.0 mL) was treated with 10% palladium on activated carbon (10.0 mg) and stirred under a hydrogen atmosphere (1 atm) at room temperature overnight. After removal of the palladium catalyst by Celite® filtration, the filtrate was concentrated and further purified by preparative TLC (*n*-hexane /AcOEt = 1:2 (v/v)) to give the title compound **19** (10.5 mg, 35% yield).

^{1}H-NMR ($CDCl_3$) δ: 2.34 (dd, *J* = 12.41, 6.13 Hz, 1H), 2.56 (dd, *J* = 13.15, 7.17 Hz, 1H), 3.91–4.07 (m, 3H), 3.93 (s, 3H), 3.99 (s, 3H), 4.10–4.17 (m, 1H), 4.26 (t, *J* = 7.77 Hz, 1H), 7.48 (s, 1H), 7.58 (d, *J* = 5.23 Hz, 1H), 8.18 (s, 1H), 8.19 (d, *J* = 5.23 Hz, 1H), 8.35 (s, 1H).

HRMS *m/z*: $[M+H]^+$ calcd for $C_{17}H_{18}N_4O_3$ 327.1452; found, 327.1450.

Purity: 91.4%.

Synthesis of compounds 20 and 21



5,5-Dimethyl-2,5-dihydrofuran-3-yl trifluoromethanesulfonate (SI-46)

Under a nitrogen stream, to a solution of 1 M NHMDS in THF (4.82 mL, 4.82 mmol) in THF (2.5 mL) was added dropwise a solutoin of 5,5-dimethyldihydrofuran-3(2*H*)-one (**SI-45**) (500 mg, 4.38 mmol) in THF (2.5 mL) at −78 °C. After stirring at −78 °C for 1 h, a solution of 1,1,1-trifluoro-*N*-phenyl-*N*-((trifluoromethyl)sulfonyl)methanesulfonamide (1.88 g, 5.26 mmol) in THF (2.5 mL) was added dropwise to the reaction mixture. After stirring at −78 °C for 1 h, to the reaction mixture was added saturated NH_4Cl aq. and AcOEt. The layers were separated and the organic layer was washed with brine, dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The title compound **SI-46** (2.96 g) was obtained and used for next step without further purification.

^{1}H-NMR ($CDCl_3$) δ: 1.39 (s, 6H), 4.63 (d, J = 2.14 Hz, 2H), 5.76 (t, J = 2.14 Hz, 1H).

2-(5,5-Dimethyl-2,5-dihydrofuran-3-yl)-4,4,5,5-tetramethyl-1,3,2-dioxaborolane (SI-47)

A mixture of **SI-46** (1.83 g, 2.68 mmol), 4,4,4',4',5,5,5',5'-octamethyl-2,2'-bi(1,3,2-dioxaborolane) (883 mg, 3.48 mmol), $PdCl_2$(dppf)-CH_2Cl_2 (109 mg, 0.134 mmol) and KOAc (525 mg, 5.35 mmol) in dioxane (13 mL) was stirred at 80 °C for 2 h under an argon atmosphere. After cooling to room temperature, the reaction mixture was diluted with AcOEt, filtered through Celite®. The organic layer was washed with saturated NH_4Cl aq., brine, dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The resulting residue was purified by column chromatography (*n*-hexane/AcOEt). The title compound **SI-47** (1.30 g) was obtained and used for next step without further purification.

^{1}H-NMR ($CDCl_3$) δ: 1.28 (s, 12H), 1.30 (s, 6H), 4.75 (d, *J* = 2.31 Hz, 2H), 6.38 (t, *J* = 2.31 Hz, 1H).

5-(5,5-Dimethyl-2,5-dihydrofuran-3-yl)-6-methoxypyrazolo[1,5-*a*]pyrimidine (SI-48)

A mixture of **SI-47** (8.54 g), **SI-15** (1.40 g, 7.63 mmol), Pd(amphos)Cl_2 (256 mg, 0.381 mmol), K_3PO_4 (3.24 g, 15.3 mmol) in DME (14 mL) and water (2.8 mL) was stirred at 100 °C for 3 h under an argon atmosphere. After cooling to room temperature, the reaction mixture was diluted with AcOEt and water. The layers were separated. The organic layer was washed with brine, dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The resulting residue was purified by column chromatography (*n*-hexane/AcOEt) to give the title compound **SI-48** (699 mg, 37 %).

^{1}H-NMR ($CDCl_3$) δ: 1.43 (s, 6H), 3.95 (s, 3H), 5.17 (s, 2H), 6.60 (s, 1H), 6.95 (s, 1H), 7.98 (s, 1H), 8.23 (s, 1H).

5-(5,5-Dimethyltetrahydrofuran-3-yl)-6-methoxypyrazolo[1,5-*a*]pyrimidine (SI-49)

To a solution of **SI-48** (699 mg, 2.85 mmol) in Et_3SiH (6.99 mL, 43.9 mmol) was added TFA (7.0 mL) at 0 °C. After stirring at room temperature for 20 h, the solvent was removed under reduced pressure. To the resulting residue was carefully added saturated $NaHCO_3$ aq. and AcOEt. The layers were separated and the organic layer was washed with brine, dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The resulting residue was purified by column chromatography (*n*-hexane/AcOEt). The title compound **SI-49** (1.13 g) was obtained and used for next step without further purification.

^{1}H-NMR ($CDCl_3$) δ: 1.37 (s, 3H), 1.42 (s, 3H), 2.16–2.21 (m, 1H), 2.28–2.33 (m, 1H), 3.93 (s, 3H), 4.02–4.16 (m, 2H), 4.29–4.35 (m, 1H), 6.64 (d, *J* = 2.54 Hz, 1H), 7.99 (d, *J* = 2.54 Hz, 1H), 8.66 (s, 1H).

3-Bromo-5-(5,5-dimethyltetrahydrofuran-3-yl)-6-methoxypyrazolo[1,5-*a*]pyrimidine (SI-50)

To a solution of **SI-49** (1.13 g) in MeCN (6.8 mL) and DMF (1.4 mL) was added NBS (537 mg, 3.02 mmol) at room temperature. After stirring at room temperature for 1 h, to the reaction mixture was added saturated Na_2SO_3 aq. and AcOEt. The layers were separated and the organic layer was washed with brine, dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The resulting residue was purified by column chromatography (*n*-hexane/AcOEt) to give the title compound **SI-50** (645 mg, 69% yield for 2 steps).

1H-NMR (CDCl3) δ: 1.35 (s, 3H), 1.43 (s, 3H), 2.10–2.17 (m, 1H), 2.38–2.45 (m, 1H), 3.89 (s, 3H), 3.95–4.16 (m, 2H), 4.25–4.31 (m, 1H), 7.93 (s, 1H), 8.08 (s, 1H).

(R)-3-Bromo-5-(5,5-dimethyltetrahydrofuran-3-yl)-6-methoxypyrazolo[1,5-*a*]pyrimidine (SI-51), (S)-3-

bromo-5-(5,5-dimethyltetrahydrofuran-3-yl)-6-methoxypyrazolo[1,5-*a*]pyrimidine (SI-52)

SI-50 (400 mg, 1.23 mmol) was subjected to chiral preparative HPLC (Column: DAICEL CHIRALPAK AD (0.46 cm φ x 15 cm); Mobile phase: *n*-hexane : IPA = 90 : 10; Flow rate: 0.6 mL/min; Detection wavelength: 254 nm) to afford two
enantiomers:
(R)-3-bromo-5-(5,5-dimethyltetrahydrofuran-3-yl)-6-methoxypyrazolo[1,5-*a*]pyrimidine (**SI-51**) (90.7 mg, 23% yield) and
(S)-3-bromo-5-(5,5-dimethyltetrahydrofuran-3-yl)-6-methoxypyrazolo[1,5-*a*]pyrimidine (**SI-52**) (91.8 mg, 23% yield).

5-(5,5-Dimethyltetrahydrofuran-3-yl)-6-methoxy-3-(2-methoxypyridin-4-yl)pyrazolo[1,5-*a*]pyrimidine (20)

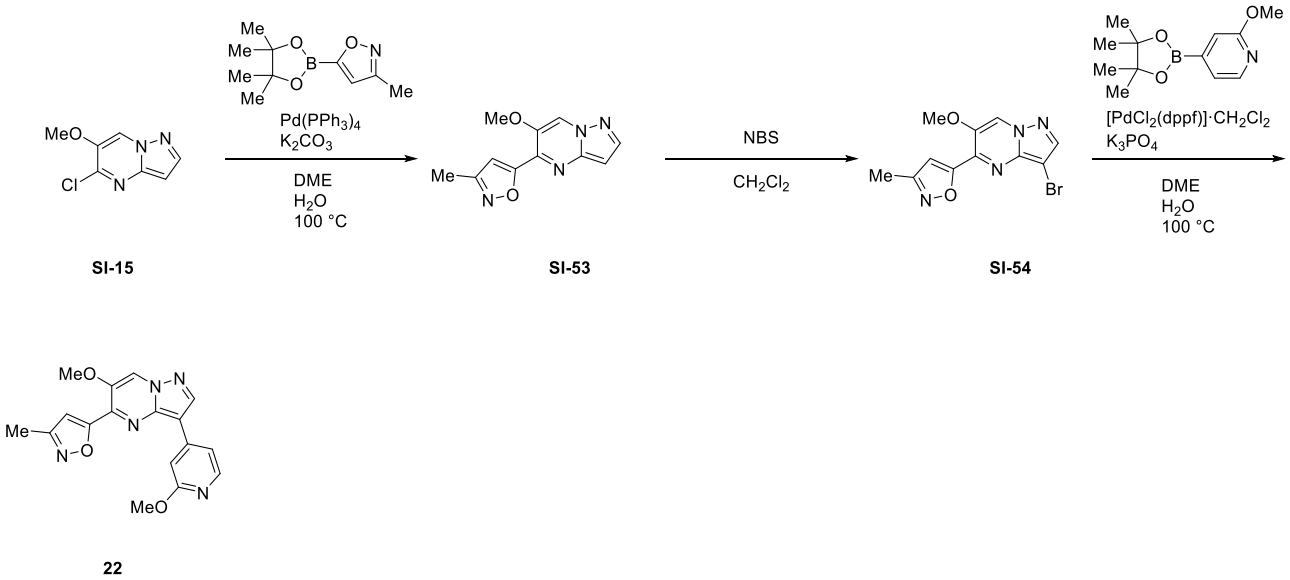
A mixture of **SI-51** (79.0 mg, 0.242 mmol), 2-methoxy-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)pyridine (114 mg, 0.484 mmol), Pd(amphos)Cl_2 (8.15 mg, 0.012 mmol) and K_3PO_4 (154 mg, 0.727 mmol) in DME (0.79 mL) and water (0.16 mL) was stirred at 100 °C for 1.5 h under an argon atmosphere. After cooling to room temperature, the reaction mixture was diluted with AcOEt and water. The layers were separated. The organic layer was washed with brine, dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The resulting residue was purified by preparative TLC (Hexane/AcOEt = 1:3 (v/v)) to give to give the title compound **20** (75.8 mg, 84% yield)

^{1}H-NMR (DMSO-d_6) δ: 1.28 (s, 3H), 1.33 (s, 3H), 2.15–2.31 (m, 2H), 3.88 (s, 3H), 3.93 (s, 3H), 3.97–4.07 (m, 2H), 4.16–4.24 (m, 1H), 7.62 (s, 1H), 7.71 (d, J = 5.44 Hz, 1H), 8.16 (d, J = 5.44 Hz, 1H), 8.74 (s, 1H), 8.90 (s, 1H).
HRMS *m/z*: $[M+H]^+$ calcd for $C_{19}H_{22}N_4O_3$ 355.1765; found, 355.1761.
Optical purity: >99% ee [HPLC condition; Column: DAICEL CHIRALPAK OJ (0.46 cm φ x 15 cm); Mobile phase: 0.1 % TFA in water : 0.1 % TFA in MeCN = 85 : 15; Flow rate: 1 mL/min; Detection wavelength: 254 nm; Retention time: 12.0 min].
Purity: 100%.
$[\alpha]^{25}_D$ 6.25 (*c* 0.08, $CHCl_3$).

Compound **21** was synthesized in a similar manner to that of compound **20**.

^{1}H-NMR (DMSO-d_6) δ: 1.28 (s, 3H), 1.33 (s, 3H), 2.15–2.31 (m, 2H), 3.88 (s, 3H), 3.93 (s, 3H), 3.97–4.07 (m, 2H), 4.16–4.24 (m, 1H), 7.62 (s, 1H), 7.71 (d, J = 5.44 Hz, 1H), 8.16 (d, J = 5.44 Hz, 1H), 8.74 (s, 1H), 8.90 (s, 1H).
HRMS *m/z*: $[M+H]^+$ calcd for $C_{19}H_{22}N_4O_3$ 355.1765; found, 355.1761.
Optical purity: >99% ee [HPLC condition; Column: DAICEL CHIRALPAK OJ (0.46 cm φ x 15 cm); Mobile phase: 0.1 % TFA in water : 0.1 % TFA in MeCN = 85 : 15; Flow rate: 1 mL/min; Detection wavelength: 254 nm; Retention time: 13.8 min].
Purity: 100%.
$[\alpha]^{25}_D$ –5.0 (*c* 0.08, $CHCl_3$).

Synthesis of compound 22



5-(3-Bromo-6-methoxypyrazolo[1,5-*a*]pyrimidin-5-yl)-3-methylisoxazole (SI-54)

A mixture of **SI-15** (400 mg, 2.18 mmol), 3-methyl-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)isoxazole (478 mg, 2.29 mmol) and $Pd(PPh_3)_4$ (252 mg, 0.218 mmol), K_3PO_4 (925 mg, 4.36 mmol) in DME (6.0 mL) and water (2.4 mL) was stirred at 100 °C for 1 h under an argon atmosphere. After cooling to room temperature, the reaction mixture was purified by column chromatography (*n*-hexane/AcOEt), then crude **SI-53** was ready for the next reaction. To a solution of **SI-53** in CH_2Cl_2 (6.0 mL) was added NBS (346 mg, 1.94 mmol) at 0 °C. After stirring at room temperature for 30 min, to the reaction mixture was added 10% Na_2SO_3 aq. and $CHCl_3$. The layers were separated and the organic layer was washed with brine, dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The resulting residue was purified by column chromatography (*n*-hexane/AcOEt). The title compound **SI-54** (119 mg) was obtained and used for next step without further purification.

^{1}H-NMR ($CDCl_3$) δ: 2.43 (s, 3H), 4.03 (s, 3H), 7.00 (s, 1H), 8.06 (s, 1H), 8.36 (s, 1H).

5-(6-Methoxy-3-(2-methoxypyridin-4-yl)pyrazolo[1,5-*a*]pyrimidin-5-yl)-3-methylisoxazole (22)

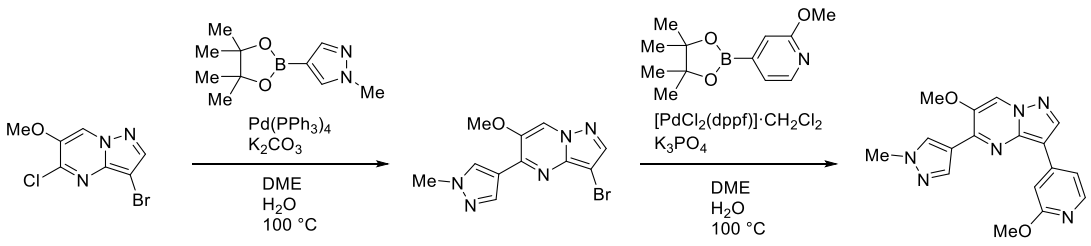
A mixture of **SI-54** (38.5 mg), 2-methoxy-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)pyridine (32.2 mg, 0.137 mmol), $PdCl_2$(dppf)-CH_2Cl_2 (10.2 mg, 0.0120 mmol) and K_3PO_4 (79.0 mg, 0.374 mmol) in DME (1.5 mL) and water (0.60 mL) was stirred at 100 °C for 1 h under an argon atmosphere. After cooling to room temperature, the reaction mixture was purified by column chromatography (MeOH/AcOEt). The resulting residue wad purified by preparative TLC ($CHCl_3$/MeOH = 10:1 (v/v)) to give to give the title compound **22** (5.30 mg).

^{1}H-NMR ($CDCl_3$) δ: 2.45 (s, 3H), 4.00 (s, 3H), 4.05 (s, 3H), 7.03 (s, 1H), 7.50–7.51 (m, 1H), 7.72 (d, J = 5.43 Hz, 1H), 8.24 (d, J = 5.43 Hz, 1H), 8.41 (s, 1H), 8.45 (s, 1H).

HRMS *m/z*: $[M+H]^+$ calcd for $C_{17}H_{15}N_5O_3$ 338.1248; found, 338.1246.

Purity: 94.0%.

Synthesis of compound 23



SI-34 SI-55 23

3-Bromo-6-methoxy-5-(1-methyl-1*H*-pyrazol-4-yl)pyrazolo[1,5-*a*]pyrimidine (SI-55)

A mixture of **SI-34** (85.0 mg, 0.324 mmol), 1-methyl-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1*H*-pyrazole (70.7 mg, 0.340 mmol) and $Pd(PPh_3)_4$ (37.4 mg, 0.0324 mmol), K_2CO_3 (24.8mg, 0.648 mmol) in DME (4.0 mL) and water (1.6 mL) was stirred at 100 °C for 3.5 h under an argon atmosphere. After cooling to room temperature, the reaction mixture was diluted with AcOEt, filtered through Celite® and concentrated under reduced pressure. The resulting residue was purified by column chromatography (*n*-hexane/AcOEt) to give the title compound **SI-55** (77.9 mg, 78% yield).

^{1}H-NMR ($CDCl_3$) δ: 3.99 (s, 3H), 3.99 (s, 3H), 7.94 (s, 1H), 8.18 (s, 1H), 8.24 (s, 1H), 8.32 (s, 1H).

5-(6-Methoxy-3-(2-methoxypyridin-4-yl)pyrazolo[1,5-*a*]pyrimidin-5-yl)-3-methylisoxazole (23)

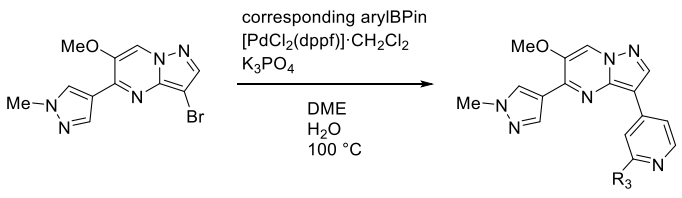
A mixture of **SI-55** (20.0 mg, 0.0649 mmol), 2-methoxy-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)pyridine (16.0 mg, 0.0681 mmol), $PdCl_2$(dppf)-CH_2Cl_2 (16.0 mg, 0.0195 mmol) and K_3PO_4 (41.4 mg, 0.195 mmol) in DME (2.0 mL) and water (0.80 mL) was stirred at 100 °C for 5 h under an argon atmosphere. After cooling to room temperature, the reaction mixture was purified by column chromatography (*n*-hexane/AcOEt). The resulting residue was purified by preparative TLC ($CHCl_3$/MeOH = 10:1 (v/v)) to give to give the title compound **23** (8.10 mg, 37% yield).

^{1}H-NMR ($CDCl_3$) δ: 4.00 (s, 3H), 4.02 (s, 3H), 4.03 (s, 3H), 7.53–7.55 (m, 1H), 7.61 (d, J = 5.38 Hz, 1H), 8.20 (d, J = 5.38 Hz, 1H), 8.25 (s, 1H), 8.25 (s, 1H), 8.35 (s, 1H), 8.37 (s, 1H).

HRMS *m/z*: $[M+H]^+$ calcd for $C_{17}H_{16}N_6O_2$ 337.1408; found, 337.1403.

Purity: 95.0%.

Synthesis of compounds 24, 25, 26 and 27

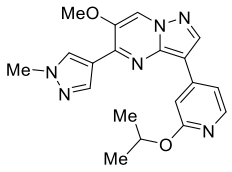


SI-55 24, 25, 26, 27

A microwave vial was charged with **SI-55** (1 equiv), corresponding arylBpin (2 equiv), $PdCl_2$(dppf)-CH_2Cl_2 (0.1 equiv), K_3PO_4 (3.0 equiv), DME (0.13 M) and water (0.17 M). The vial was heated to 100 °C in the microwave for 50 min. The reaction mixture was purified by column chromatography (*n*-hexane/AcOEt). The resulting residue

was purified by preparative TLC ($CHCl_3$/MeOH = 10:1 (v/v)) to give compounds **24**, **25**, **26** and **27**.

3-(2-Isopropoxypyridin-4-yl)-6-methoxy-5-(1-methyl-1*H*-pyrazol-4-yl)pyrazolo[1,5-*a*]pyrimidine (24)



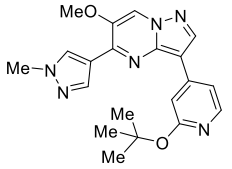
Compound **24** was prepared by coupling of **SI-55** with 2-isopropoxy-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)pyridine which was commercially available.

^{1}H-NMR (DMSO-d_6) δ: 1.33 (d, J = 6.47 Hz, 6H), 3.97 (s, 3H), 4.03 (s, 3H), 5.26–5.33 (m, 1H), 7.43 (s, 1H), 7.87 (d, J = 5.49 Hz, 1H), 8.17 (d, J = 5.49 Hz, 1H), 8.25 (s, 1H), 8.56 (s, 1H), 8.73 (s, 1H), 8.95 (s, 1H).

HRMS *m/z*: $[M+H]^+$ calcd for $C_{19}H_{20}N_6O_2$ 365.1721; found, 365.1719.

Purity: 98.5%.

3-(2-(*tert*-Butoxy)pyridin-4-yl)-6-methoxy-5-(1-methyl-1*H*-pyrazol-4-yl)pyrazolo[1,5-*a*]pyrimidine (25)



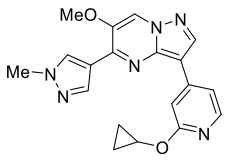
Compound **25** was prepared by coupling of **SI-55** with 2-(tert-butoxy)-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)pyridine which was commercially available.

^{1}H-NMR (DMSO-d_6) δ: 1.58 (s, 9H), 3.97 (s, 3H), 4.03 (s, 3H), 7.38–7.40 (m, 1H), 7.84 (d, J = 5.38 Hz, 1H), 8.15 (d, J = 5.38 Hz, 1H), 8.24 (s, 1H), 8.55 (s, 1H), 8.72 (s, 1H), 8.95 (s, 1H).

HRMS *m/z*: $[M+H]^+$ calcd for $C_{20}H_{22}N_6O_2$ 379.1877; found, 379.1877.

Purity: 91.0%.

3-(2-Cyclopropoxypyridin-4-yl)-6-methoxy-5-(1-methyl-1*H*-pyrazol-4-yl)pyrazolo[1,5-*a*]pyrimidine (26)



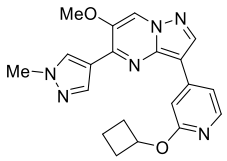
Compound **26** was prepared by coupling of **SI-55** with 2-cyclopropoxy-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)pyridine which was commercially available.

^{1}H-NMR (DMSO-d_6) δ: 0.69–0.76 (m, 2H), 0.76–0.83 (m, 2H), 3.97 (s, 3H), 4.03 (s, 3H), 4.23–4.29 (m, 1H), 7.61 (s, 1H), 7.89 (d, J = 5.38 Hz, 1H), 8.21 (d, J = 5.38 Hz, 1H), 8.25 (s, 1H), 8.56 (s, 1H), 8.74 (s, 1H), 8.96 (s, 1H).

HRMS *m/z*: $[M+H]^+$ calcd for $C_{19}H_{18}N_6O_2$ 363.1564; found, 363.1559.

Purity: 94.9%.

3-(2-Cyclobutoxypyridin-4-yl)-6-methoxy-5-(1-methyl-1*H*-pyrazol-4-yl)pyrazolo[1,5-*a*]pyrimidine (27)



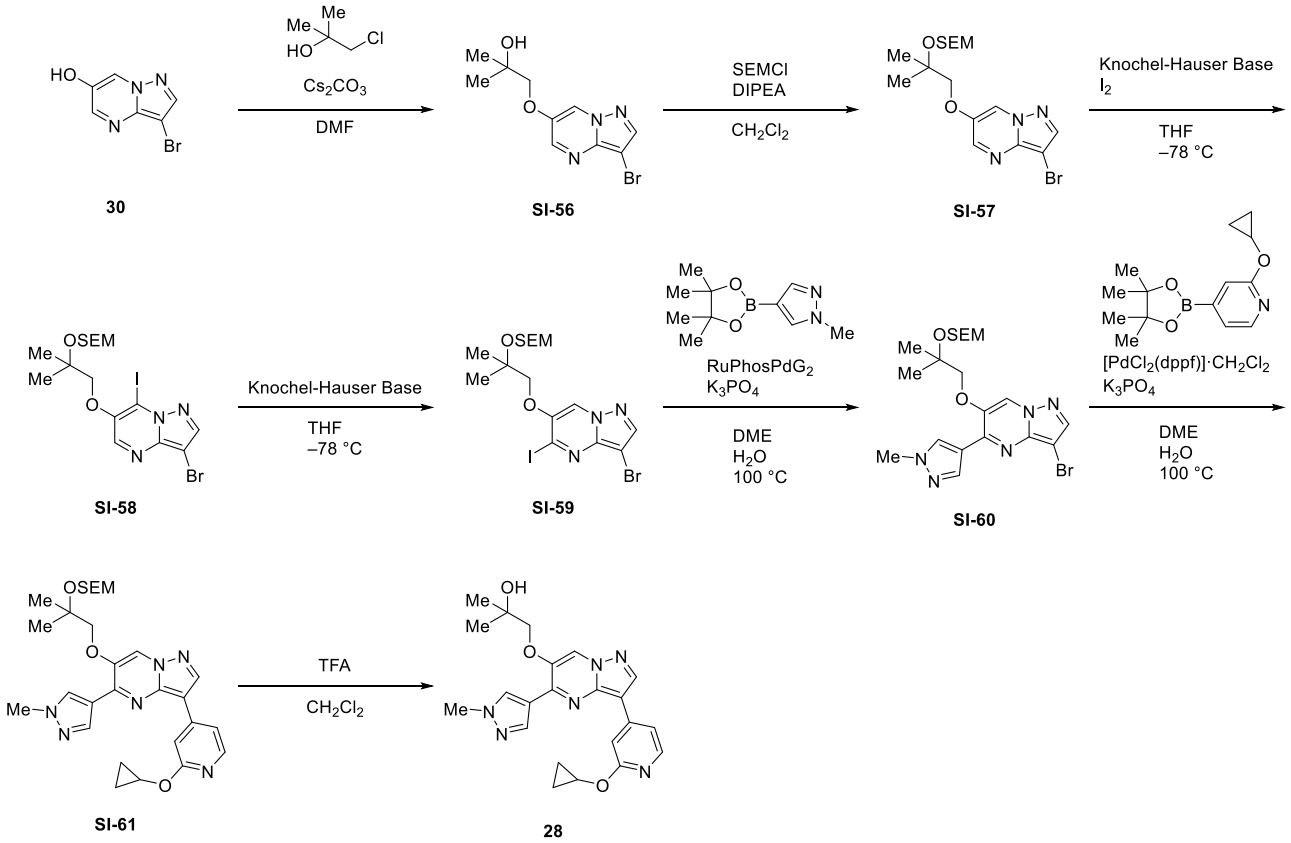
Compound **27** was prepared by coupling of **SI-55** with 2-cyclobutoxy-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)pyridine which was commercially available.

^{1}H-NMR (DMSO-d_6) δ: 1.60–1.71 (m, 1H), 1.76–1.84 (m, 1H), 2.04–2.15 (m, 2H), 2.39–2.51 (m, 2H), 3.98 (s, 3H), 4.03 (s, 3H), 5.15–5.22 (m, 1H), 7.48–7.50 (m, 1H), 7.85 (d, J = 5.21 Hz, 1H), 8.15 (d, J = 5.21 Hz, 1H), 8.26 (s, 1H), 8.56 (s, 1H), 8.74 (s, 1H), 8.96 (s, 1H).

HRMS *m/z*: $[M+H]^+$ calcd for $C_{20}H_{20}N_6O_2$ 377.1721; found, 377.1719.

Purity: 98.7%.

Synthesis of compound 28



1-((3-Bromopyrazolo[1,5-*a*]pyrimidin-6-yl)oxy)-2-methylpropan-2-ol (SI-56)

To a solution of 3-bromopyrazolo[1,5-*a*]pyrimidin-6-ol (**30**) (10.0 g, 46.7 mmol) in DMF (200 mL) was successively added cesium carbonate (60.9g, 187 mmol) and 1-chloro-2-methylpropan-2-ol (9.81 mL, 98.0 mmol) at room temperature. The mixture was warmed to 100 °C and then stirred for 12 h. After cooling to room temperature, the reaction mixture was diluted with AcOEt and water. The layers were separated and the organic layer was washed with brine, dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The resulting residue was purified

by column chromatography (*n*-hexane/AcOEt) to give the title compound **SI-56** (6.84 g, 51% yield).
^{1}H-NMR ($CDCl_3$) δ: 1.40 (s, 6H), 3.84 (s, 2H), 8.00 (s, 1H), 8.24 (d, J = 2.77 Hz, 1H), 8.49 (d, J = 2.77 Hz, 1H).

3-Bromo-6-(2-methyl-2-((2-(trimethylsilyl)ethoxy)methoxy)propoxy)pyrazolo[1,5-*a*]pyrimidine (SI-57)
To a solution of compound **SI-56** (7.00 g, 24.5 mmol) in CH_2Cl_2 (70 mL) was successively added DIPEA (17.1 mL, 98.0 mmol) and 2-(chloromethoxy)ethyltrimethylsilane (13.0 mL, 73.4 mmol) at 0 °C. After stirring at room temperature for 7.5 h, additional 2-(chloromethoxy)ethyltrimethylsilane (4.34 mL, 24.5 mmol) was added to the reaction mixture at 0 °C. After stirring at room temperature for 4.5 h, the reaction mixture was purified by column chromatography (*n*-hexane/AcOEt) to give the title compound **SI-57** (10.9 g, quant.).
^{1}H-NMR ($CDCl_3$) δ: 0.00 (s, 9H), 0.85–0.91 (m, 2H), 1.42 (s, 6H), 3.62–3.67 (m, 2H), 3.90 (s, 2H), 4.85 (s, 2H), 8.01 (s, 1H), 8.24 (d, *J* = 2.54 Hz, 1H), 8.49 (d, *J* = 2.54 Hz, 1H).

3-Bromo-7-iodo-6-(2-methyl-2-((2-(trimethylsilyl)ethoxy)methoxy)propoxy)pyrazolo[1,5-*a*]pyrimidine (SI-58)
Under a nitrogen stream, to a solution of **SI-57** (10.9 g, 26.2 mmol) in THF (110 mL) was added dropwise 1 M Knochel-Hauser Base in THF (39.3 mL, 39.3 mmol) at −78 °C. After stirring at −78 °C for 1.5 h, a solution of iodine (7.97 g, 31.4 mmol) in THF (20 mL) was added dropwise to the reaction mixture. After stirring at 0 °C for 5.5 h, additional iodine (3.00 g, 11.8 mmol) in THF (20 mL) was added to the reaction mixture at 0 °C. After stirring at 0 °C for 1 h, to the reaction mixture was added saturated NH_4Cl aq. and AcOEt. The layers were separated and the organic layer was washed with 10% Na_2SO_3 aq. and brine, dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The resulting residue was purified by column chromatography (*n*-hexane/AcOEt) to give the title compound **SI-58** (5.00 g, 35% yield).
^{1}H-NMR ($CDCl_3$) δ: 0.00 (s, 9H), 0.86–0.93 (m, 2H), 1.48 (s, 6H), 3.62–3.67 (m, 2H), 4.10 (s, 2H), 4.87 (s, 2H), 8.17 (s, 1H), 8.31 (s, 1H).

3-Bromo-5-iodo-6-(2-methyl-2-((2-(trimethylsilyl)ethoxy)methoxy)propoxy)pyrazolo[1,5-*a*]pyrimidine (SI-59)
Under a nitrogen stream, to a solution of **SI-58** (5.00 g, 9.22 mmol) in THF (50 mL) was added dropwise 1 M Knochel-Hauser Base in THF (27.7 mL, 27.7 mmol) at −78 °C. After stirring at −78 °C for 30 min, to the reaction mixture was added 10% AcOH aq. and AcOEt. The layers were separated and the organic layer was washed with saturated $NaHCO_3$ aq. and brine, dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The resulting residue was purified by column chromatography (*n*-hexane/AcOEt). The title compound **SI-59** (3.56 g) was obtained and used for next step without further purification.
^{1}H-NMR ($CDCl_3$) δ: 0.00 (s, 9H), 0.85–0.90 (m, 2H), 1.48 (s, 6H), 3.63–3.68 (m, 2H), 3.92 (s, 2H), 4.88 (s, 2H), 7.97 (s, 1H), 7.99 (s, 1H).

3-Bromo-5-(1-methyl-1*H*-pyrazol-4-yl)-6-(2-methyl-2-((2-(trimethylsilyl)ethoxy)methoxy)propoxy)pyrazolo[1,5-*a*]pyrimidine (SI-60)

A mixture of **SI-59** (200 mg, 0.369 mmol), 1-methyl-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1*H*-pyrazole (92.0 mg, 0.443 mmol), RuPhosPdG2 (14.3 mg, 0.0180 mmol), and K_3PO_4 (235 mg, 1.11 mmol) in DME (4.0 mL) and water (1.0 mL) was stirred at 100 °C for 2 h under an argon atmosphere. After cooling to room temperature, the mixture was purified by column chromatography (*n*-hexane/AcOEt) to give the title compound **SI-60** (78.2 mg).

^{1}H-NMR ($CDCl_3$) δ: 0.07 (s, 9H), 0.88–0.99 (m, 2H), 1.53 (s, 6H), 3.66–3.73 (m, 2H), 4.04 (s, 2H), 4.05 (s, 3H), 4.93 (s, 2H), 8.00 (s, 1H), 8.27 (s, 1H), 8.44 (s, 1H), 8.48 (s, 1H).

3-(2-Cyclopropoxypyridin-4-yl)-5-(1-methyl-1*H*-pyrazol-4-yl)-6-(2-methyl-2-((2-(trimethylsilyl)ethoxy)methoxy)propoxy)pyrazolo[1,5-*a*]pyrimidine (SI-61)

A mixture of **SI-60** (78.2 mg, 0.158 mmol), 2-cyclopropoxy-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)pyridine (61.7 mg, 0.236 mmol), RuPhosPdG2 (12.2 mg, 0.0160 mmol), and K_3PO_4 (100 mg, 0.473 mmol) in DME (3.0 mL) and water (1.2 mL) was stirred at 100 °C for 2 h under an argon atmosphere. After cooling to room temperature, the mixture was purified by column chromatography (*n*-hexane/AcOEt) to give the title compound **SI-61** (41.2 mg, 48% yield).

^{1}H-NMR ($CDCl_3$) δ: 0.07 (s, 9H), 0.89–0.94 (m, 6H), 1.55 (s, 6H), 3.67–3.72 (m, 2H), 4.09 (s, 2H), 4.09 (s, 3H), 4.30–4.36 (m, 1H), 4.95 (s, 2H), 7.65–7.66 (m, 1H), 7.71 (dd, J = 5.32, 1.39 Hz, 1H), 8.34 (dd, J = 5.32, 0.31 Hz, 1H), 8.34 (s, 1H), 8.42 (s, 1H), 8.46 (s, 1H), 8.51 (d, J = 0.46 Hz, 1H).

1-((3-(2-Cyclopropoxypyridin-4-yl)-5-(1-methyl-1*H*-pyrazol-4-yl)pyrazolo[1,5-*a*]pyrimidin-6-yl)oxy)-2-methylpropan-2-ol (28)

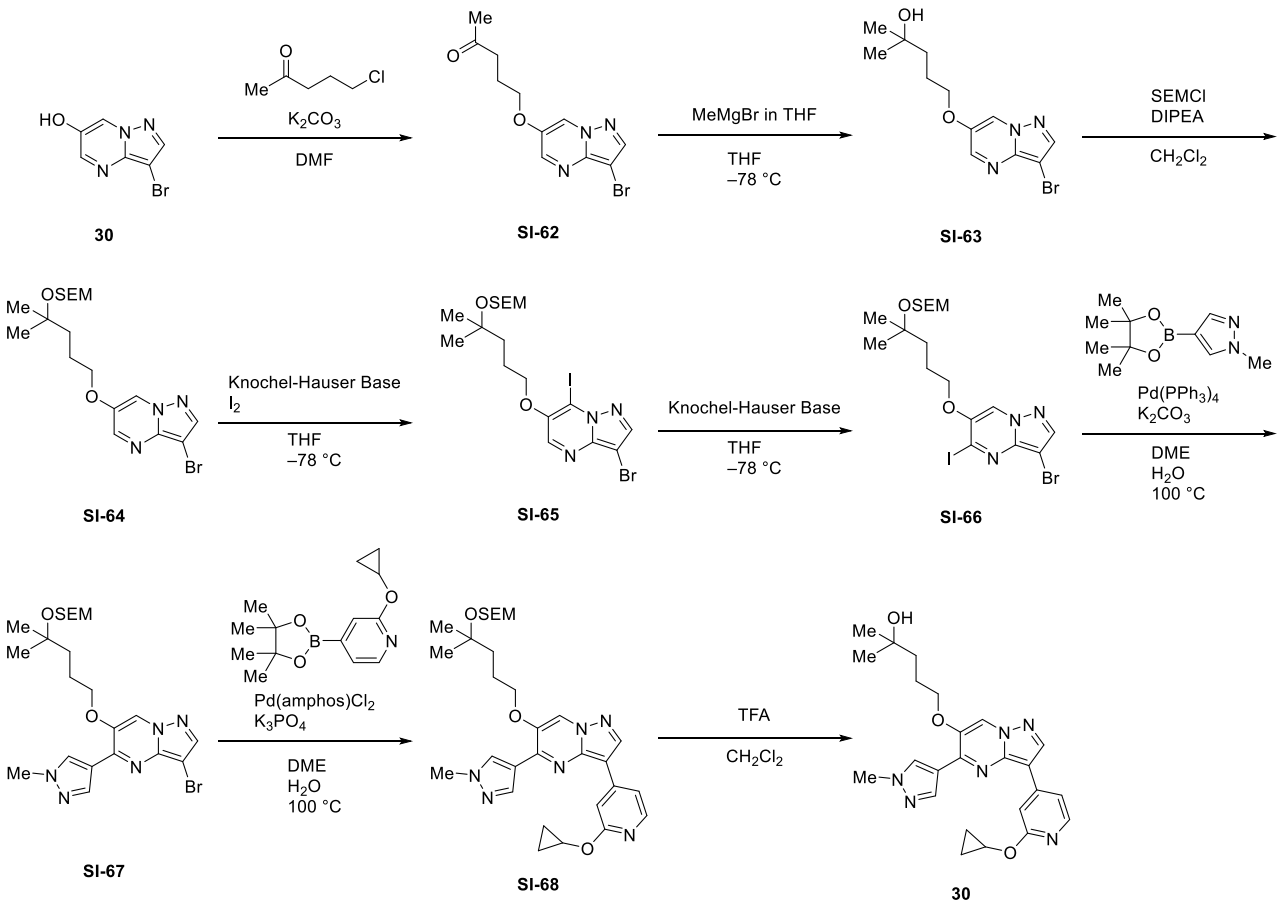
A solution of **SI-61** (41.2 mg, 0.0750 mmol) in CH_2Cl_2 (410 µL) was treated with TFA (58 µl) at 0 °C. After stirring at 0 °C for 1.5 h, the solvent was removed under reduced pressure. The residue was diluted with $CHCl_3$ and saturated $NaHCO_3$ aq. The mixture was extracted with $CHCl_3$. The organic layer was washed with brine, dried over Na_2SO_4 and concentrated under reduced pressure. The resulting residue was purified by column chromatography (*n*-hexane/AcOEt) to give the title compound **28** (12.1 mg, 38% yield).

^{1}H-NMR (DMSO-d_6) δ: 0.71–0.80 (m, 4H), 1.30 (s, 6H), 3.97 (s, 3H), 4.01 (s, 2H), 4.25–4.28 (m, 1H), 4.91 (s, 1H), 7.59–7.61 (m, 1H), 7.89 (dd, J = 5.32, 1.39 Hz, 1H), 8.21 (d, J = 5.32 Hz, 1H), 8.36 (s, 1H), 8.60 (s, 1H), 8.73 (s, 1H), 8.96 (s, 1H).

HRMS *m/z*: $[M+H]^+$ calcd for $C_{22}H_{24}N_6O_3$ 421.1983; found, 421.1978.

Purity: 100%.

Synthesis of compound 30



5-((3-Bromopyrazolo[1,5-*a*]pyrimidin-6-yl)oxy)pentan-2-one (SI-62)

To a solution of 3-bromopyrazolo[1,5-*a*]pyrimidin-6-ol (**30**) (10.0 g, 46.7 mmol) in DMF (50 mL) was successively added K_2CO_3 (9.69 g, 70.1 mmol) and 5-chloropentan-2-one (8.05 mL, 70.1 mmol) at room temperature. The mixture was warmed to 80 °C and then stirred for 12 h. After cooling to room temperature, The reaction mixture was diluted with water. The precipitated solid was collected by filtration to give the title compound **SI-62** (7.60 g, 55% yield).

^{1}H-NMR ($CDCl_3$) δ: 2.10–2.17 (m, 2H), 2.20 (s, 3H), 2.70 (t, J = 6.82 Hz, 2H), 4.02 (t, J = 6.13 Hz, 2H), 7.99 (s, 1H), 8.20 (d, J = 2.77 Hz, 1H), 8.41 (d, J = 2.77 Hz, 1H).

5-((3-Bromopyrazolo[1,5-*a*]pyrimidin-6-yl)oxy)-2-methylpentan-2-ol (SI-63)

Under a nitrogen stream, to a solution of **SI-62** (7.60 g, 25.5 mmol) in THF (70 mL) was added dropwise 1.07 M MeMgBr in THF (28.6 mL, 30.6 mmol) at −78 °C. After stirring at −78 °C for 10 min, to the reaction mixture was added saturated water and AcOEt. The layers were separated and the organic layer was washed with brine, dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The resulting residue was purified by column chromatography (*n*-hexane/AcOEt) to give the title compound **SI-63** (5.80 g, 72% yield).

^{1}H-NMR ($CDCl_3$) δ: 1.29 (s, 6H), 1.65–1.69 (m, 2H), 1.93–2.01 (m, 2H), 4.02 (t, J = 6.36 Hz, 2H), 7.99 (s, 1H), 8.19 (d, J = 2.66 Hz, 1H), 8.44 (d, J = 2.66 Hz, 1H).

3-Bromo-6-((4-methyl-4-((2-(trimethylsilyl)ethoxy)methoxy)pentyl)oxy)pyrazolo[1,5-*a*]pyrimidine (SI-64)

To a solution of compound **SI-63** (5.80 g, 18.5 mmol) in CH_2Cl_2 (60 mL) was successively added DIPEA (6.45 mL, 36.9 mmol) and 2-(chloromethoxy)ethyltrimethylsilane (6.22 mL, 35.1 mmol) at room temperature. After stirring at 40 °C for 30 min, the reaction mixture concentrated under reduced pressure. The resulting residue was diluted with AcOEt and water. The layers were separated and the organic layer was washed with brine, dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The resulting residue was purified by column chromatography (*n*-hexane/AcOEt) to give the title compound **SI-64** (2.54 g, 31% yield).

^{1}H-NMR ($CDCl_3$) δ: 0.00 (s, 9H), 0.88–0.97 (m, 2H), 1.26 (s, 6H), 1.64–1.70 (m, 2H), 1.89–1.97 (m, 2H), 3.60–3.64 (m, 2H), 3.98 (t, *J* = 6.36 Hz, 2H), 4.75 (s, 2H), 7.97 (s, 1H), 8.16 (d, *J* = 2.54 Hz, 1H), 8.42 (d, *J* = 2.54 Hz, 1H).

3-Bromo-7-iodo-6-((4-methyl-4-((2-(trimethylsilyl)ethoxy)methoxy)pentyl)oxy)pyrazolo[1,5-*a*]pyrimidine (SI-65)

Under a nitrogen stream, to a solution of **SI-64** (2.54 g, 5.71 mmol) in THF (25 mL) was added dropwise 1 M Knochel-Hauser Base in THF (14.3 mL, 14.3 mmol) at −78 °C . After stirring at −78 °C for 1.5 h, a solution of iodine (1.74 g, 6.86 mmol) in THF (8.0 mL) was added dropwise to the reaction mixture. After stirring at 0 °C for 1.5 h, to the reaction mixture was added saturated NH_4Cl aq. and AcOEt. The layers were separated and the organic layer was washed with 10% Na_2SO_3 aq. and brine, dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The resulting residue was purified by column chromatography (*n*-hexane/AcOEt) to give the title compound **SI-65** (2.23 g, 69% yield).

^{1}H-NMR ($CDCl_3$) δ: 0.00 (s, 9H), 0.89–0.93 (m, 2H), 1.27 (s, 6H), 1.71–1.76 (m, 2H), 1.91–1.99 (m, 2H), 3.60–3.64 (m, 2H), 4.19 (t, *J* = 6.43 Hz, 2H), 4.76 (s, 2H), 8.15 (s, 1H), 8.24 (s, 1H).

3-Bromo-5-iodo-6-((4-methyl-4-((2-(trimethylsilyl)ethoxy)methoxy)pentyl)oxy)pyrazolo[1,5-*a*]pyrimidine (SI-66)

Under a nitrogen stream, to a solution of **SI-65** (2.23 g, 3.93 mmol) in THF (22 mL) was added dropwise 1 M Knochel-Hauser Base in THF (9.81 mL, 9.81 mmol) at −78 °C. After stirring at −78 °C for 30 min, to the reaction mixture was added 10% AcOH aq. and AcOEt. The layers were separated and the organic layer was washed with saturated $NaHCO_3$ aq. and brine, dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The resulting residue was purified by column chromatography (*n*-hexane/AcOEt). The title compound **SI-66** (2.24 g) was obtained and used for next step without further purification.

^{1}H-NMR ($CDCl_3$) δ: 0.00 (s, 9H), 0.89–0.93 (m, 2H), 1.28 (s, 6H), 1.71–1.75 (m, 2H), 1.94–2.02 (m, 2H), 3.60–3.65 (m, 2H), 4.02 (t, *J* = 6.28 Hz, 2H), 4.76 (s, 2H), 7.94 (s, 1H), 7.94 (s, 1H).

3-Bromo-5-(1-methyl-1*H*-pyrazol-4-yl)-6-((4-methyl-4-((2-(trimethylsilyl)ethoxy)methoxy)pentyl)oxy)pyrazolo[1,5-*a*]pyrimidine (SI-67)

A mixture of **SI-66** (556 mg, 0.975 mmol), 1-methyl-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1*H*-pyrazole (243 mg, 1.17 mmol), $Pd(PPh_3)_4$ (113 mg, 0.0970 mmol), and K_3PO_4 (414 mg, 1.95 mmol) in DME (5.6 mL) and water (1.1 mL) was stirred at 100 °C for 4.5 h under an argon atmosphere. After cooling to room temperature, the

mixture was purified by column chromatography (*n*-hexane/AcOEt). The title compound **SI-67** (325 mg) was obtained and used for next step without further purification.

^{1}H-NMR ($CDCl_3$) δ: 0.00 (s, 9H), 0.89–0.94 (m, 2H), 1.31 (s, 6H), 1.71–1.76 (m, 2H), 2.02–2.10 (m, 2H), 3.62–3.67 (m, 2H), 3.99 (s, 3H), 4.08 (t, *J* = 6.59 Hz, 2H), 4.78 (s, 2H), 7.93 (s, 1H), 8.16 (s, 1H), 8.28 (s, 1H), 8.34 (s, 1H).

3-(2-Cyclopropoxypyridin-4-yl)-5-(1-methyl-1*H*-pyrazol-4-yl)-6-((4-methyl-4-((2-(trimethylsilyl)ethoxy)methoxy)pentyl)oxy)pyrazolo[1,5-*a*]pyrimidine (SI-68)

A mixture of **SI-67** (168 mg), 2-cyclopropoxy-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)pyridine (167 mg, 0.641 mmol), Pd(amphos)Cl_2 (21.5 mg, 0.0320 mmol), and K_3PO_4 (204 mg, 0.961 mmol) in DME (2.5 mL) and water (0.80 mL) was stirred at 100 °C for 40 min under an argon atmosphere. After cooling to room temperature, the mixture was purified by column chromatography (*n*-hexane/AcOEt) to give the title compound **SI-68** (64.5 mg).

^{1}H-NMR ($CDCl_3$) δ: 0.00 (s, 9H), 0.84–0.87 (m, 4H), 0.90–0.94 (m, 2H), 1.31 (s, 6H), 1.73–1.78 (m, 2H), 2.05–2.12 (m, 2H), 3.63–3.67 (m, 2H), 4.02 (s, 3H), 4.12 (t, *J* = 6.47 Hz, 2H), 4.23–4.28 (m, 1H), 4.79 (s, 2H), 7.58–7.59 (m, 1H), 7.69 (d, *J* = 6.24 Hz, 1H), 8.23 (s, 1H), 8.26 (d, *J* = 6.24 Hz, 1H), 8.28 (s, 1H), 8.34 (s, 1H), 8.38 (s, 1H).

5-((3-(2-Cyclopropoxypyridin-4-yl)-5-(1-methyl-1*H*-pyrazol-4-yl)pyrazolo[1,5-*a*]pyrimidin-6-yl)oxy)-2-methylpentan-2-ol (30)

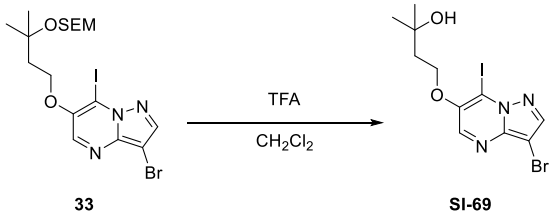
A solution of **SI-68** (63.9 mg, 0.110 mmol) in CH_2Cl_2 (2.0 mL) was treated with TFA (84 μL) at 0 °C. After stirring at 0 °C for 40 min, the solvent was removed under reduced pressure and then azeotroped with toluene. The resulting residue was purified by column chromatography (*n*-hexane/AcOEt) to give the title compound **30** (54.0 mg, quant.).

^{1}H-NMR (DMSO-d_6) δ: 0.71–0.82 (m, 4H), 1.16 (s, 6H), 1.55–1.61 (m, 2H), 1.91–1.99 (m, 2H), 3.98 (d, *J* = 0.23 Hz, 3H), 4.20 (t, *J* = 6.36 Hz, 2H), 4.24–4.29 (m, 1H), 4.31 (s, 1H), 7.60–7.61 (m, 1H), 7.88 (dd, *J* = 5.49, 1.27 Hz, 1H), 8.21 (d, *J* = 5.49 Hz, 1H), 8.26 (s, 1H), 8.50 (s, 1H), 8.74 (s, 1H), 8.93 (s, 1H).

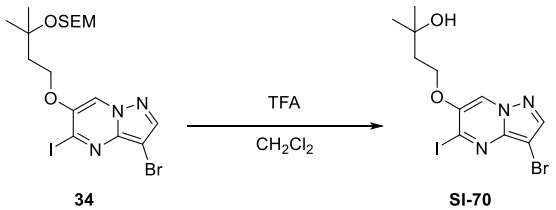
HRMS *m/z*: $[M+H]^+$ calcd for $C_{24}H_{28}N_6O_3$ 449.2296; found, 449.2295.

Purity: 95.6%.

Synthesis of SI-69 and SI-70



33 **SI-69**



34 **SI-70**

4-((3-Bromo-7-iodopyrazolo[1,5-*a*]pyrimidin-6-yl)oxy)-2-methylbutan-2-ol (SI-69)

A solution of **33** (50.0 mg, 0.0900 mmol) in CH_2Cl_2 (0.59 mL) was treated with TFA (69 μL) at 0 °C. After stirring at 0 °C for 2.5 h, the solvent was removed under reduced pressure and then azeotroped with toluene. The resulting residue was purified by column chromatography (*n*-hexane/AcOEt) to give the title compound **SI-69** (26.8 mg, 70% yield).

^{1}H-NMR ($CDCl_3$) δ: 1.38 (s, 6H), 2.12 (t, *J* = 6.59 Hz, 2H), 4.42 (t, *J* = 6.59 Hz, 2H), 8.17 (s, 1H), 8.30 (s, 1H).

HRMS *m/z*: $[M+H]^+$ calcd for $C_{11}H_{13}BrIN_3O_2$ 425.9309; found, 425.9311.

4-((3-Bromo-5-iodopyrazolo[1,5-*a*]pyrimidin-6-yl)oxy)-2-methylbutan-2-ol (SI-70)

SI-70 was synthesized in a similar manner to that of **SI-69**

^{1}H-NMR ($CDCl_3$) δ: 1.37 (s, 6H), 2.14 (t, *J* = 6.30 Hz, 2H), 4.24 (t, *J* = 6.30 Hz, 2H), 7.97 (s, 1H), 8.03 (s, 1H).

HRMS *m/z*: $[M+H]^+$ calcd for $C_{11}H_{13}BrIN_3O_2$ 425.9309; found, 425.9312.

3. Co-crystal X-ray structure analysis

The TGF-βRII kinase domain (residues 237-549aa) with 6 amino acids mutation to enhance crystallization was produced in insect cells and purified according to reference.[5]

Crystals of the TGF-βRII /compound complex were obtained at 4 °C in sitting drops by using the vapor diffusion against a reservoir containing 12−22% PEG4000 , 3% Ethylene Glycol, 16% glycerol, 160 mM $MgCl_2$ and 80 mM Tris·HCl at pH 7.5-9.3 . The 9.2 mg/mL of TGF-βRII protein was incubated with 1mM compound at 4 °C for 1-2 hr. Drops consisting of 0.15 μL TGF-βRII /compound complex solution and 0.15 μL reservoir solution were equilibrated against 50 μl reservoir solution.

Diffraction data were collected at X06SA (Swiss Light Source). The crystals were mounted using a Micro mount loop (MiTeGen, LLC), frozen in a stream of liquid nitrogen and cooled to 100 K during data collection. The intensity data were integrated with XDS[6] and scaled using aimless.[7] The TGF-βRII /compound complex structure was solved by molecular replacement with MOLREP in CCP4[8,9] suite using the structure of the TGF-βRII kinase domain (PDB ID 5E8V[4]). The structural model was built in Coot[10] and refined using REFMAC5 in CCP4 suite.[11] Figure was created using PyMOL.[12] Crystallization data and refinement statics are summarized in supplemental Table S1. The structure has been deposited in the RCSB Protein Data Bank database (PDB code: 7DV6).

Table S1. Crystallization data and refinement statics for compound 20.

Space group P212121
Unit-cell parameters
a, b, c (Å) 61.97 76.01 77.98
α, β, γ (˚) 90.00 90.00 90.00
Resolution range(Å) 48.53 – 2.39 (2.48–2.39)
Total reflections 100876
Unique reflections 15148

Completeness (%) 99.9 (99.6)
Redundancy 6.7 (6.9)
I/δ (%l) 19.0 (7.6)
Rmerge (%) 8.2 (27.0)
Refinement statistics
Resolution range (Å) 28.81–2.39
No. of reflections 14345
Rcryst (Rfree) 16.28 (22.39)
No. of atoms
Protein 2425
Ligand 26
Water 202
B-factors
Protein 36.8
Ligand or Ion 46.5
Water 61.2
R.m.s. deviations
Bond length (Å) 0.012
Bond angles (˚) 2.150

4. Modeling study

Glide docking and MM-GBSA simulation were examined to predict the binding poses of compound **29** using the co-crystal structure of compound **20**. These calculations were set up and run using Maestro 2019-3. Docking was run using Extra Precision Mode with flexible ligand sampling. H-bond constraint to His328 was also applied. MM-GBSA simulations were run using Prime with the VGSB model for solvation, the OPLS3e force field, and minimization for sampling. We confirmed the predicted binding mode of compound **29** was similar to that of compound **20**.

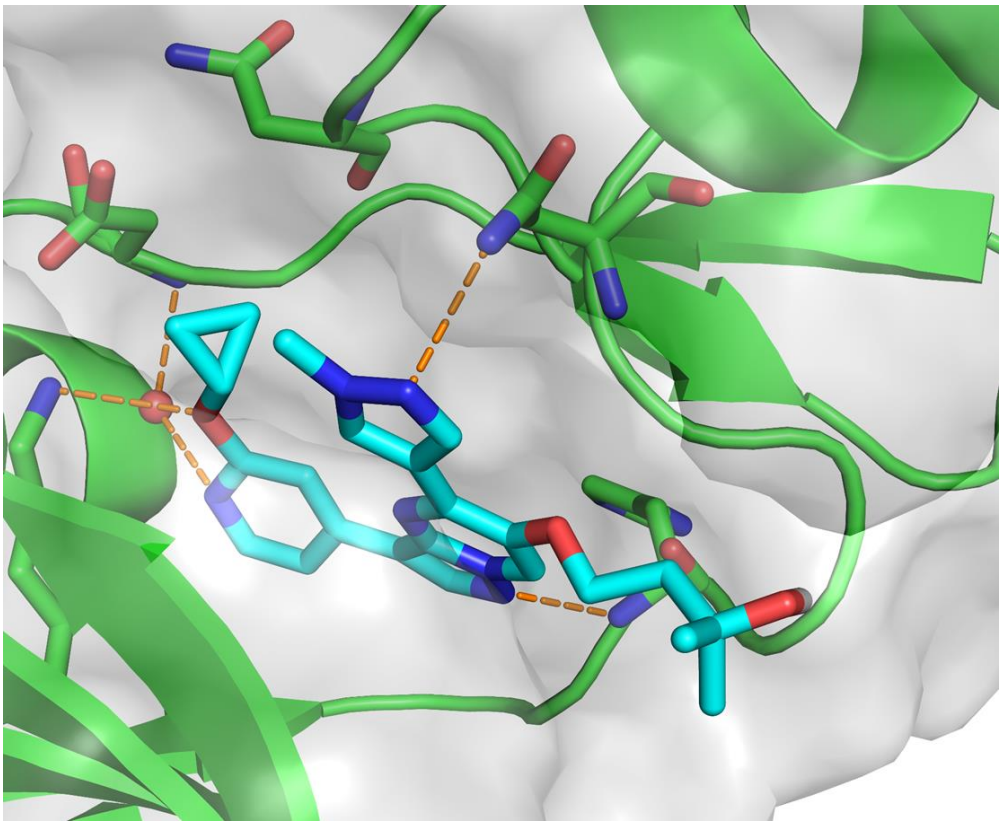


Figure S1. Docking result of compound 29.

5. Single crystal X-ray structure analysis

The single crystal X-ray structures of compound **29**, **SI-69** and **SI-70** have been deposited in the Cambridge Crystallographic Data Centre (CCDC Numbers: 1989276-1989278). Crystallization data and refinement statics are summarized in Table S2-S4.

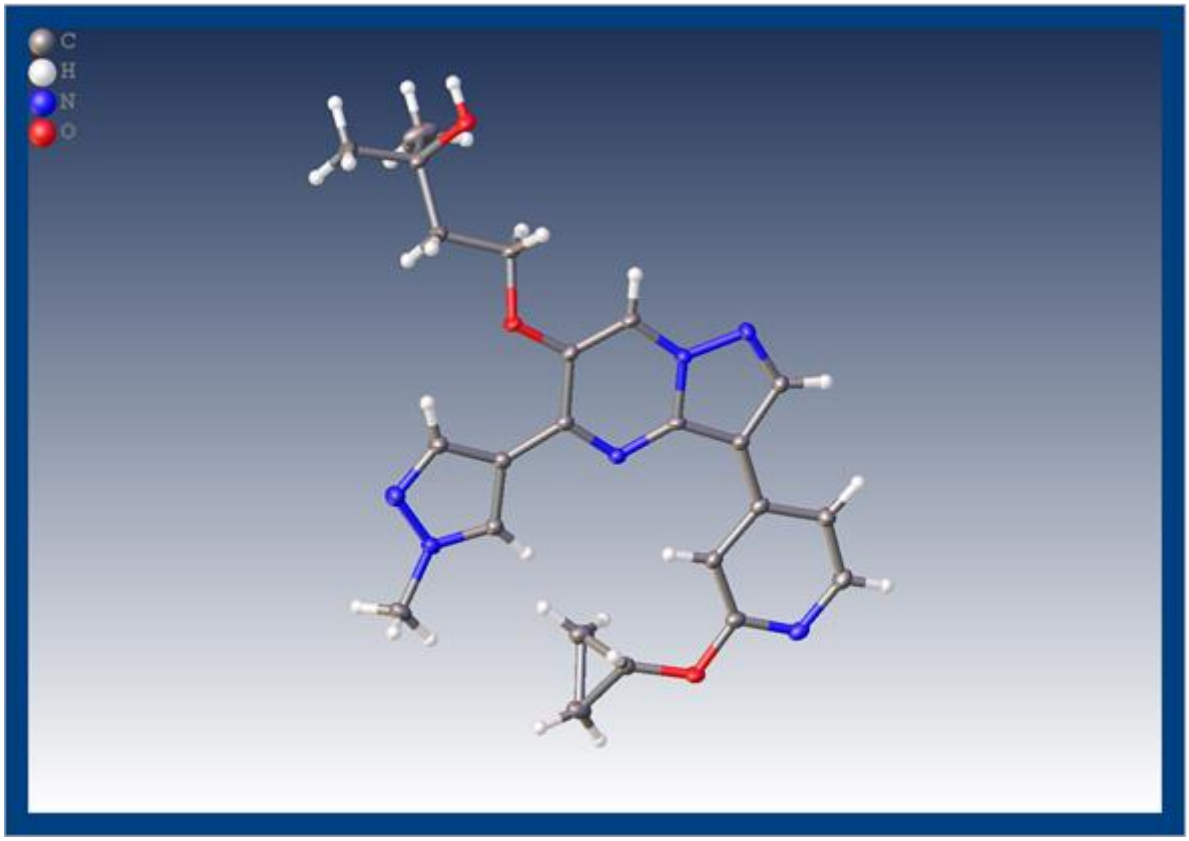


Figure S2. X-ray crystal structure of compound 29.

Table S2. Crystal data and structure refinement for compound 29.

Compound Name	**4-((3-(2-cyclopropoxypyridin-4-yl)-5-(1-methyl-1*H*-pyrazol-4-yl)pyrazolo[1,5-*a*]pyrimidin-6-yl)oxy)-2-methylbutan-2-ol**
Deposition Number	**1989276**
Data Block Name	**data_exp_268**
Unit Cell Parameters	**a 24.4253(4) b 6.41790(10) c 29.7759(5) C2/c**
Empirical formula	**$C_{23}H_{26}N_6O_3$**
Formula weight	**434.50**
Temperature/K	**100(1)**
Crystal system	**monoclinic**
Space group	**C2/c**
a/Å	**24.4253(4)**
b/Å	**6.41790(10)**
c/Å	**29.7759(5)**
α/°	**90**
β/°	**110.634(2)**
γ/°	**90**

Volume/Å^3	**4368.22(13)**
Z	**8**
ρ_{calc}g/cm^3	**1.321**
μ/mm^{-1}	**0.739**
F(000)	**1840.0**
Crystal size/mm^3	**0.25 × 0.05 × 0.05**
Radiation	**CuKα (λ = 1.54184)**
2Θ range for data collection/°	**6.344 to 147.908**
Index ranges	**-25 ≤ h ≤ 30, -7 ≤ k ≤ 7, -37 ≤ l ≤ 32**
Reflections collected	**11909**
Independent reflections	**4285 [R_{int} = 0.0297, R_{sigma} = 0.0308]**
Data/restraints/parameters	**4285/0/293**
Goodness-of-fit on F^2	**1.062**
Final R indexes [I>=2σ (I)]	**R_1 = 0.0369, wR_2 = 0.0965**
Final R indexes [all data]	**R_1 = 0.0398, wR_2 = 0.0987**
Largest diff. peak/hole / e Å^{-3}	**0.29/-0.29**

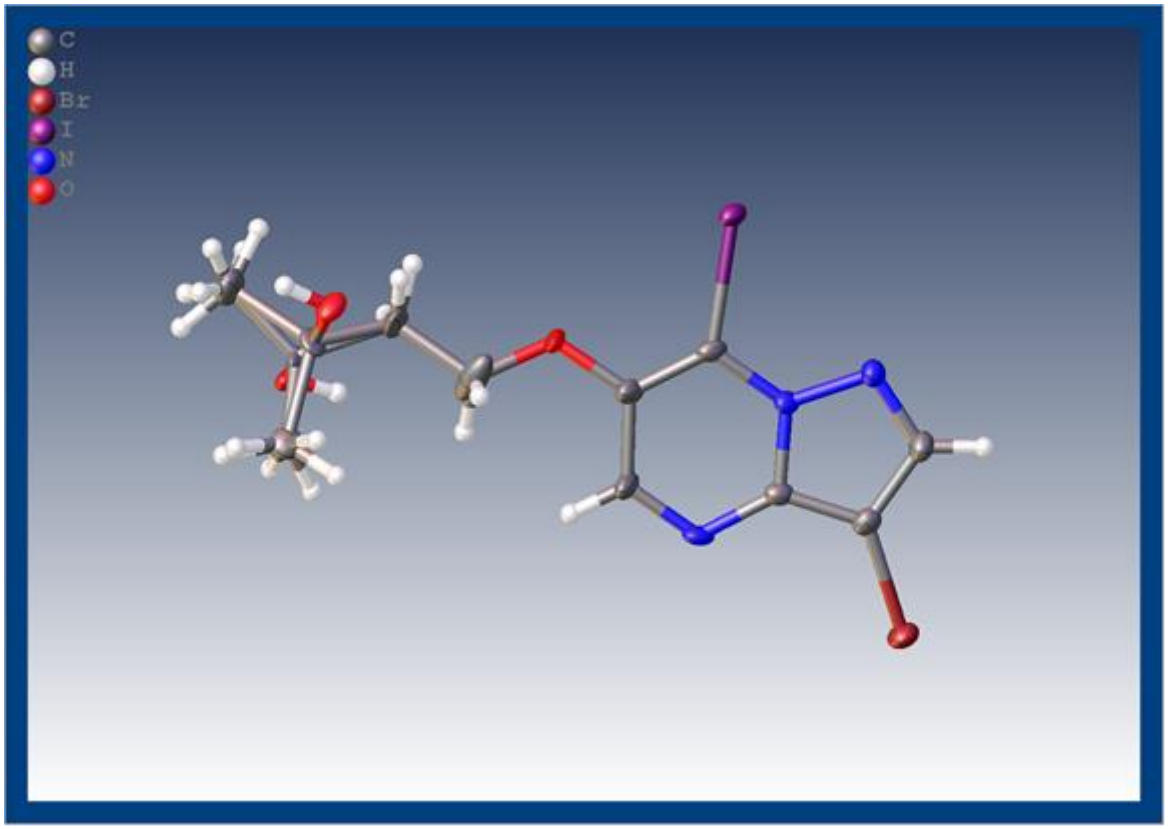


Figure S3. X-ray crystal structure of SI-69.

Table S3. Crystal data and structure refinement for SI-69.

Compound Name	**4-((3-bromo-7-iodopyrazolo[1,5-*a*]pyrimidin-6-yl)oxy)-2-methylbutan-2-ol**
Deposition Number	**1989277**
Data Block Name	**data_exp_279**
Unit Cell Parameters	**a 5.5503(3) b 20.0305(11) c 12.2541(8) P21/c**
Empirical formula	**$C_{11}H_{12}BrIN_3O_2$**
Formula weight	**425.05**
Temperature/K	**100.0(2)**
Crystal system	**monoclinic**
Space group	**$P2_1/c$**
a/Å	**5.5503(3)**
b/Å	**20.0305(11)**
c/Å	**12.2541(8)**
α/°	**90**
β/°	**94.438(5)**
γ/°	**90**
Volume/Å^3	**1358.27(14)**

Z	**4**
ρ_{calc}g/cm^3	**2.079**
μ/mm^{-1}	**21.976**
F(000)	**812.0**
Crystal size/mm^3	**0.05 × 0.05 × 0.02**
Radiation	**CuKα (λ = 1.54184)**
2Θ range for data collection/°	**8.478 to 148.202**
Index ranges	**-6 ≤ h ≤ 6, -24 ≤ k ≤ 23, -15 ≤ l ≤ 15**
Reflections collected	**6792**
Independent reflections	**2659 [R_{int} = 0.0466, R_{sigma} = 0.0551]**
Data/restraints/parameters	**2659/96/201**
Goodness-of-fit on F^2	**1.095**
Final R indexes [I>=2σ (I)]	**R_1 = 0.0449, wR_2 = 0.1197**
Final R indexes [all data]	**R_1 = 0.0485, wR_2 = 0.1220**
Largest diff. peak/hole / e Å^{-3}	**1.62/-1.27**

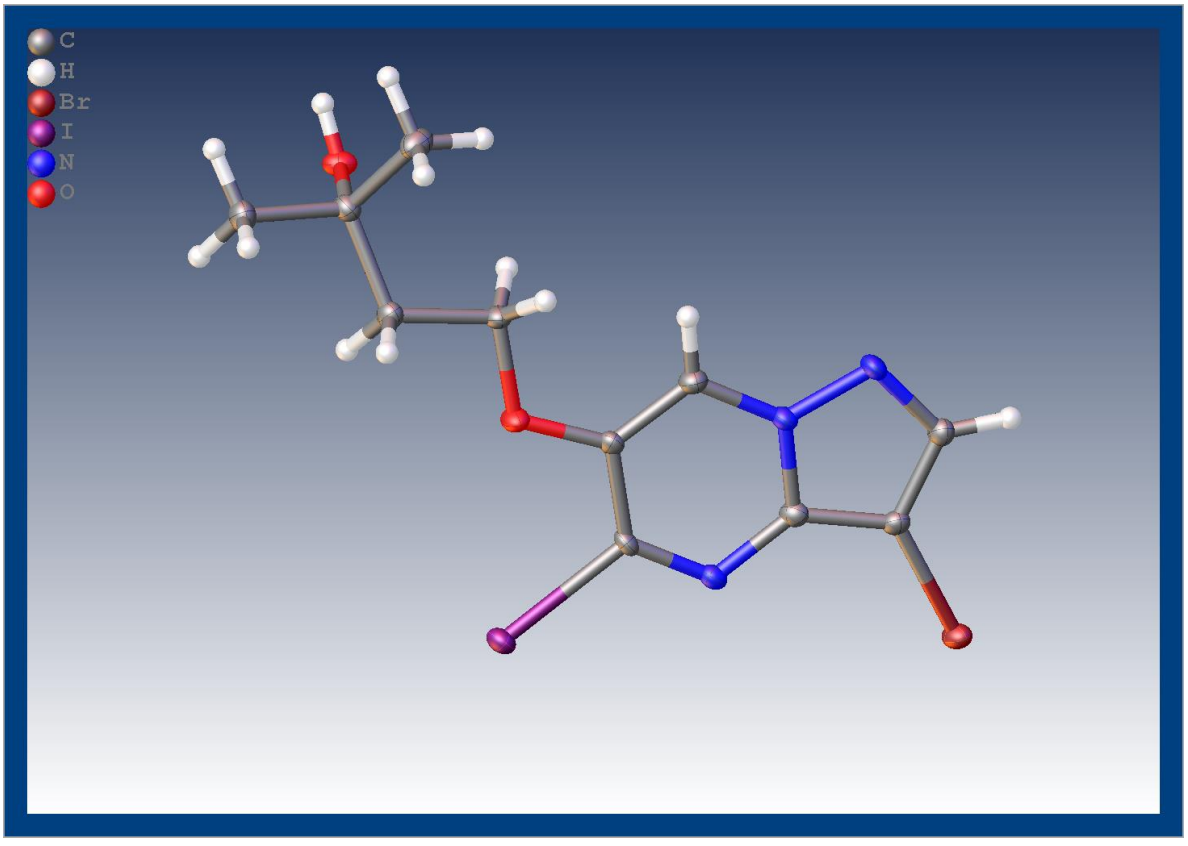


Figure S4. X-ray crystal structure of SI-70.

Table S4. Crystal data and structure refinement for SI-70.

Compound Name	**4-((3-bromo-5-iodopyrazolo[1,5-*a*]pyrimidin-6-yl)oxy)-2-methylbutan-2-ol**
Empirical formula	**$C_{11}H_{13}BrIN_3O_2$**
Deposition Number	**1989278**
Data Block Name	**data_exp_274**
Unit Cell Parameters	**a 5.4582(2) b 10.1221(7) c 12.4533(9) P-1**
Formula weight	**426.05**
Temperature/K	**100.0(3)**
Crystal system	**triclinic**
Space group	**P-1**
a/Å	**5.4582(2)**
b/Å	**10.1221(7)**
c/Å	**12.4533(9)**
α/°	**87.736(6)**
β/°	**81.643(5)**
γ/°	**83.202(5)**
Volume/$Å^3$	**675.76(7)**
Z	**2**
ρ_{calc}g/cm^3	**2.094**
μ/mm^{-1}	**22.086**
F(000)	**408.0**
Crystal size/mm^3	**0.1 × 0.05 × 0.05**
Radiation	**CuKα (λ = 1.54184)**
2Θ range for data collection/°	**7.176 to 147.284**
Index ranges	**$-6 \le h \le 6$, $-12 \le k \le 12$, $-13 \le l \le 15$**
Reflections collected	**6230**
Independent reflections	**2621 [R_{int} = 0.0439, R_{sigma} = 0.0433]**
Data/restraints/parameters	**2621/0/166**
Goodness-of-fit on F^2	**1.044**
Final R indexes [I>=2σ (I)]	**R_1 = 0.0303, wR_2 = 0.0817**
Final R indexes [all data]	**R_1 = 0.0306, wR_2 = 0.0822**
Largest diff. peak/hole / e $Å^{-3}$	**0.91/-1.27**

6. Kinase panel

Kinase selectivity of compound **29** was concisely assessed using KINOMEscan® (DiscoverX co.), by picking one

or two representative kinases out of the eight branches (TK, TKL, STE, CK1, AGC, CAMK, CMGC, Others) of the Kinome. Values are percentage of inhibition by treatment of 0.1 μM of compound **29**.

Table S5. Selectivity profiling of compound 29.

Kinase	% inhibition
ASK1	0
CSNK1D	26
DAPK3	18
DYRK2	75
FGFR1	1
JAK2	36
MARK2	8
NEK3	4
PAK4	3
PCTK1	51
PLK2	2
PRKCH	0
RSK1	19
TGFBR1	54
TGFBR2	95.7

7. Biological assay

Kinase assay

Kinase assays using TGF-βRII, ALK5, and ACVR2A enzyme reactions were carried out as previously described[13] with some modifications. Briefly, TGF-βRII enzyme reaction was performed with 2 nmol/L of TGF-βRII (Promega), 50 μmol/L biotinylated substrate (Biotin-TTLKDLIYDMTTSGSGSGLPLLVQRTIART), 0.3 μmol/L ATP, and 1 μCi/reaction [γ-^{33}P] ATP(PerkinElmer) in assay buffer (40mmol/L Tris-HCl pH7.5, 20mmol/L $MgCl_2$, 0.1% BSA, 50μM DTT) at room temperature for 15 min. For 0.1 nmol/L TGF-βRII, the enzyme was incubated for 50 min. ALK5 kinase assay was performed with 10 nmol/L of ALK5 enzyme (Promega), 100 μmol/L biotinylated substrate (Biotin-KKKVLTQMGSPSIRCSpSVS), 11 μmol/L ATP, and 1 μCi/reaction [γ-^{33}P]ATP in assay buffer (40 mmol/L Tris-HCl pH 7.5, 20 mmol/L $MgCl_2$, 0.1% BSA, 50 μM DTT) at room temperature for 40 min. ACVR2A reaction was performed with 20 nmol/L of the ACVR2A enzyme,(Carna biosciense), 50 μmol/L biotinylated substrate (Biotin-KTLQDLVYDLSTSGSGSGLPLFVQRTVART), 9.5 μmol/L ATP, and 1 μCi/reaction [γ-^{33}P]ATP in kinase buffer(20 mmol/L Tris-HCl pH7.5, 10mmol/L $MnCl_2$, 1mmol/L EGTA, 5 mmol/L β-Glycerophosphate, 0.02 mmol/L Triton X-100, 0.1% BSA, 1 mmol/L DTT) at room temperature for 20 min. Enzyme reaction was performed with 2 nmol/L of TGF-βRII for Table 1 and 2. Enzyme reaction was performed with 0.1 nmol/L of TGF-βRII for

Table 3. Mean IC_{50} values (± SD) for reference compound **3** are shown in Table S6.

Table S6. Mean IC_{50} values (± SD) for reference compound 3

	Enzyme IC_{50} (μM)		
Compd	TGF-βRII	ACVR2A	ALK5
3	0.075 (± 0.010), n = 183	1.3 (± 0.20), n = 183	4.4 (± 1.4), n = 59

Phosphorylation of Smad3 in Expi293F cells

Expi293F cells purchased from (Thermo Fisher Scientific) were cultured in Expi293 Expression Medium. These cells were then inoculated into 384-well plates at 5.0×10^3 cells/well with test compounds. After pre-incubation with test compounds for 10 min at room temperature, the cells were stimulated by 1 μmol/L TGF-β1 (R&D) or 4 μmol/L Activin A(R&D) for 120 min at room temperature. Phosphorylated Smad3 signaling in Expi293F cells were detected by AlphaLISA SureFire Ultra p-SMAD3 (Ser423/425) Assay Kit (PerkinElmer Inc.). Mean IC_{50} values (± SD) for reference compound SB431542[14] are shown in Table S7. In this assay, there was no sign of cytotoxicity observed for any of the compounds including compound **29** at concentrations up to 10 μM.

Table S7. Mean IC_{50} values (± SD) for reference compound SB431542

	Cell IC_{50} (μM)	
Compd	TGF-β	activin
SB431542	0.19 (± 0.060), n = 190	0.19 (± 0.030), n = 177

In vivo assay

All procedures related to the use of animals in this manuscript were reviewed and approved by the Institutional Animal Care and Use Committee of Japan Tobacco Inc., Central pharmaceutical Research Institute. Male C57BL/6J mice were obtained from Charles River Laboratories Japan (Yokohama, Japan). 10μL of 30ng/μL mouse recombinant TGF-β1 (Cell signalling technology) were injected intradermally in an ear once daily for 3 days. The test compounds with 30.0% PEG-60 Hydrogenated Castor Oil, 33.8% Polyethylene Glycol #400 and 36.2% Propylene carbonate were administered orally twice daily. 24hr after the last TGF-β1 injection, the ear was collected under anesthesia. Total RNA was extracted from each ear with TRIzol Reagent (Thermo Fisher Scientific) and RNeasy mini kit (Qiagen). Reverse-transcribed using a High Capacity cDNA Reverse Transcription Kit (Thermo Fisher Scientific). qRT-PCR was carried out using TaqMan(R) Gene Expression Assays with QuantStudio 7 Flex(Thermo Fisher Scientific). The relative Col1A1 mRNA(Assay ID: Mm99999915 g1) expression was normalized measuring the amount of GAPDH mRNA(Assay ID: Mm00801666 g1) in each sample. The results of cycle threshold values (Ct values) were calculated by the $\Delta\Delta C_T$ method to obtain the fold differences.

8. ADMET assays

Rat PK (IV, PO)

Male CD(SD) rats (Charles River Laboratories (Japan)) were intravenously or orally administered a single dose of compound **29** at 0.3 mg/kg (dimethyl sulfoxide solution) or 1 mg/kg (propylene glycol solution), respectively. After the administration, the plasma samples were collected over a period of 25 h. The time-course of the plasma concentrations of compound **29** was analyzed by non-compartmental analysis and the pharmacokinetic parameters were calculated. A control experiment applying the same amount of DMSO was conducted and that quantity of DMSO caused no interference.

Metabolic stability in liver microsomes

Test compounds (1.0 μM) were incubated with rat or human liver microsomes (Xenotech, US) for up to 60 min and the remaining ratios were determined.

Solubility study

Test compound solutions were placed in 96 well plates, and DMSO was removed with a centrifugal evaporator for 2 h. FaSSIF solvent was added to each well. The plates were mixed at 2500 rpm at room temperature for 4 h. Incubation samples were filtered twice with 96 well filtration plates. An aliquot of the second filtrate and acetonitrile were mixed, and injected into the LC/MS to quantify the amount of each compound in the filtrates.

***In vitro* Caco-2 Permeability study**

A sample of the test compound (final concentrations: 25 μmol/L) was added to the apical side of Caco-2 cell monolayers, and incubated at 37 °C for 2 h. After incubation, the transported amounts of test compound were measured by liquid chromatography/tandem mass spectrometry (LC/MS/MS). Apparent permeability coefficients (Papp) were calculated from the transported amounts.

Protein Binding

Mouse plasma protein binding was determined using an equilibrium dialysis method[15] with some modifications and the percentage of test compound bound to plasma protein (% Bound) was calculated according to the following equation: % Bound = $[(C_{tot}-C_{buf})/C_{tot}] \times 100$ where C_{tot} is the concentration of the incubated standard plasma sample at the time of the measurement and C_{buf} is the compound concentration in the buffer sample at the time of the measurement.

CYP inhibition assay

A cocktail of typical substrates of each CYP isozyme was incubated with human liver microsomes (Xenotech, US) in the presence or absence of the test compounds and the inhibitory effects of the test compounds were evaluated according to the published method[16,17] with some modifications.

hERG inhibition assay

The hERG current was measured by the whole cell patch clamp method. hERG-transfected HEK293 cells were cultured in MEM solution containing 10% fetal bovine serum, 1 mmol/L MEM sodium pyruvate solution,

0.1 mmol/L MEM non-essential amino acid solution, 100 U/mL penicillin, 100 μg/mL streptomycin, and 400 μg/mL geneticin. Cells on the cover slip were set in the measurement chamber and the chamber was superfused with the external solution containing (in mM): 137 NaCl, 4 KCl, 1 $MgCl_2 \cdot 6H_2O$, 1.8 $CaCl_2 \cdot 2H_2O$, 10 HEPES and 10 glucose (pH 7.4), maintained at 24 ± 2 °C. The hERG current was measured with a glass electrode (resistance: 2 to 6 MΩ) filled with the internal solution containing (in mM): 130 KCl, 1 $MgCl_2 \cdot 6H_2O$, 5 EGTA, 10 HEPES and 5 MgATP (pH 7.2), through a patch clamp amplifier (EPC-10, HEKA Elektronik). The cell membrane voltage was held at –80 mV by the patch clamp software (PULSE, HEKA Elektronik) with the amplifier. A test pulse consisting of +20 mV for 1.5 seconds and –40 mV for 1.5 seconds was applied with intervals of 15 seconds. The currents before and 11 minutes after initiation of the treatment with the vehicle and test article were analyzed.

9. References

(1) Munchhof, M. J. Pyrazine compounds as transforming growth factor (TGF) inhibitors. WO/2004/080982 A1; International Application No. PCT/IB2004/000581; September 23, 2004.

(2) Munchhof, M. J. Isothiazole derivatives. WO/2004/067530 A1; International Application No. PCT/IB2004/000122; August 12, 2004.

(3) Tigno-Aranjuez, J. T.; Benderitter, P.; Rombouts, F.; Deroose, F.; Bai, X.; Mattioli, B.; Cominelli, F.; Pizarro, T. T.; Hoflack, J.; Abbott, D. W. In vivo inhibition of RIPK2 kinase alleviates inflammatory disease. *J. Biol. Chem.* **2014**, *289*, 29651−29664.

(4) Hoflack, J. M. C. J.; Berthet, C.; Benderitter, P. A. R.; Blom, P. M. F.; Gomez, S.; Daoubi, M. Macrocyclic TGF-βRII kinase inhibitors. WO/2015/136073 A1; International Application No. PCT/EP2015/055294; September 17, 2015.

(5) Tebben, A. J.; Ruzanov, M.; Gao, M.; Xie, D.; Kiefer, S. E.; Yan, C.; Newitt, J. A.; Zhang, L.; Kim, K.; Lu, H.; Kopcho, L. M.; Sheriff, S. Crystal structures of apo and inhibitor-bound TGFβR2 kinase domain: insights into TGFβR isoform selectivity. *Acta Crystallogr. Sect. D: Biol. Crystallogr.* **2016**, *72*, 658–674.

(6) Kabsch, W. XDS. *Acta Crystallogr. Sect. D: Biol. Crystallogr* **2010**, *66*, 125–132.

(7) Evans, P. R.; Murshudov, G. N. How good are my data and what is the resolution? *Acta Crystallogr. Sect. D: Biol. Crystallogr.* **2013**, *69*, 1204–1214.

(8) Vagin, A.; Teplyakov, A. MOLREP: an automated program for molecular replacement. *J. Appl. Crystallogr.* **1997**, *30*, 1022–1025.

(9) Winn, M. D.; Ballard, C. C.; Cowtan, K. D.; Dodson, E. J.; Emsley, P.; Evans, P. R.; Keegan, R. M.; Krissinel, E. B.; Leslie, A. G. W.; McCoy, A.; McNicholas, S. J.; Murshudov, G. N.; Pannu, N. S.; Potterton, E. A.; Powell, H. R.; Read, R. J.; Vagin, A.; Wilson, K. S. Overview of the CCP4 suite and current developments. *Acta Crystallogr. Sect. D: Biol. Crystallogr.* **2011**, *67*, 235–242.

(10) Emsley, P.; Cowtan, K. *Coot*: Model-building tools for molecular graphics. *Acta Crystallogr. Sect. D: Biol. Crystallogr.* **2004**, *60*, 2126–2132.

(11) Murshudov, G. N.; Skubák, P.; Lebedev, A. A.; Pannu, N. S.; Steiner, R. A.; Nicholls, R. A.; Winn, M. D.; Long, F.; Vagin, A. A. *REFMAC5* for the Refinement of macromolecular crystal structures. *Acta Crystallogr. Sect. D: Biol. Crystallogr.* **2011**, *67*, 355–367.

(12) The PyMOL Molecular Graphics System, Shrödinger, LLC; http://www.pymol.org

(13) Dinh, M.; Grunberger, D.; Ho, H.; Tsing, S. Y.; Shaw, D.; Lee, S.; Barnett, J.; Hill, R. J.; Swinney, D. C.; Bradshaw, J. M. Activation mechanism and steady state kinetics of Bruton's tyrosine kinase. *J. Biol. Chem.* **2007**, *282*, 8768–8776.

(14) Inman, G. J.; Nicolás, F. J.; Callahan, J. F.; Harling, J. D.; Gaster, L. M.; Reith, A. D.; Laping, N. J.; Hill, C. S. SB-431542 is a potent and specific inhibitor of transforming growth factor-β superfamily type I activin receptor-like kinase (ALK) Receptors ALK4, ALK5, and ALK7. *Mol. Pharmacol.* **2002**, *62*, 65–74.

(15) Lin, Z. J.; Desai-Krieger, D.; Shum, L. Simultaneous determination of glipizide and rosiglitazone unbound drug concentrations in plasma by equilibrium dialysis and liquid chromatography- tandem mass spectrometry. *J. Chromatogr. B.* **2004**, *801*, 265–272.

(16) Dierks, E. A.; Stams, K. R.; Lim, H.-K.; Cornelius, G.; Zhang, H.; Ball, S. E. A method for the simultaneous evaluation of the activities of seven major human drug-metabolizing cytochrome P450s using an in vitro cocktail of probe substrates and fast gradient liquid chromatography tandem mass spectrometry. *Drug Metab. Dispos.* **2001**, *29*, 23–29.

(17) Polasek, T. M.; Miners, J. O. Time-dependent inhibition of human drug metabolizing cytochromes P450 by tricyclic antidepressants. *Br. J. Clin. Pharmacol.* **2008**, *65*, 87–97.