

Supporting Information

Discovery of GBT440, an Orally Bioavailable R State Stabilizer of Sickle Cell Hemoglobin

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EXPERIMENTAL SECTION

Blood source. SCD blood was obtained from homozygous sickle cell patients through the Hemoglobinopathy Center at Children's Hospital Oakland Research Institute [CHRCO, Oakland, CA (IRB #2013-006)]. Apheresis bags in acid-citrate- dextrose anticoagulant (ACD) from transfused sickle cell donors (CHRCO) routinely contained a mix of ~30% HbS/~70% HbA.

Hemoglobin Purification. A mixture of HbS/HbA as well as HbS alone were purified from the apheresis bags from transfused sickle cell donor RBCs by gel filtration and DE-52 anion exchange chromatography. Samples of the eluted fractions were run on a Tris-glycine 12% acrylamide (non-SDS containing) gel where they were separated according to their isoelectric point (pI) allowing for their identification. The fractions were then pooled and used for different experiments. The mixture of purified HbS/HbA was used for determination of oxygen equilibrium curves (OECs) and purified HbS was used for polymerization assays.¹

Hematocrit determination. The hematocrit (Hct) was determined using an uncoated capillary tube and a HemataSTAT-II instrument. When necessary, autologous plasma was added or removed to adjust the Hct to the values indicated in the experiment.

Hemoximetry.¹ Hemoximetry was used to assess oxygen saturation in purified HbS/HbA or whole blood by generating oxygen equilibrium curves (OECs) which relate the extent of Hb-O₂ saturation to the partial pressure of O₂ (pO₂) and measure the binding affinity of O₂ to Hb. OECs were determined with a Hemox Analyzer (TCS Scientific Company) that determines the oxygen partial pressure and degree of HbS saturation with O₂ by means of spectrophotometry and a Clark oxygen electrode, respectively.

Purified Hb (25 μM, tetrameric) samples were incubated in TES/saline buffer (30 mM TES, 130 mM NaCl, 5 mM KCl, pH 7.4 at 25 °C) for 45 min at 37 °C in the absence or presence of test compound. In vitro blood samples (100 μl, 20% Hct, 1 mM Hb,) from transfused sickle cell donors were incubated for 1 hour at 37 °C. In vitro blood samples were then diluted 50-fold, in TES/saline buffer and transferred to the TCS Hemox Analyzer chamber where they were oxygenated with compressed air for 10 min (for purified Hb) or 20 min (for whole blood) and then deoxygenated with compressed nitrogen (N₂).¹

During deoxygenation, the p50 values were determined automatically by the Hemox Analytical Software (HAS) [TCS Scientific Company].

Time dependence of hemoglobin-oxygen affinity. The time dependence of hemoglobin-oxygen affinity was measured using hemoximetry. Purified Hb (25 μM, tetrameric) samples were incubated in hemox buffer (30 mM TES, 130 mM NaCl, 5 mM KCl, pH 7.4 at 25 °C) for 0-130 min at 37 °C in the presence of Compound **1** (5 mM, tested for 0-113 minutes), Compound **4** (100 μM) or Compound **5** (100 μM). Samples were then transferred to the TCS Hemox Analyzer chamber where they were oxygenated with compressed air for 10 min (for purified Hb) and then deoxygenated with compressed nitrogen (N₂). During deoxygenation (10 min), the p50 values were determined automatically by the Hemox Analytical Software (HAS) [TCS Scientific Company]. e.

Polymerization assay HbS polymerization assays were performed as previously described¹ with minor modifications. For all polymerization experiments, 50 μM of HbS in 1.8 M KH₂PO₄ at pH 7.4 was used. To evaluate the ability of the test compound to delay polymerization, HbS was first incubated with the test compound (prepared from a 100% DMSO stock to the indicated concentrations) for 1 hr at 37°C. The final DMSO concentration was 0.3%. The Hb solution was deoxygenated in a hypoxic chamber (99.5% N₂/0.5% O₂) for 90 min at 4°C and polymerization was initiated by increasing the temperature from 4°C to 37°C. Polymerization was measured by the increase in absorbance at 700 nm

for 50 minutes. A plot of the optical density (at 700 nm) against time was analyzed using the Boltzman sigmoidal fit and the delay time, defined as the time at half Vmax, was determined from the fitted curve.

Table 1

Cmpd	6	7	8	9	10	11	12
$\Delta p50^a$	44.4 ± 4.7	11.7 ± 2.2	42.2 ± 7.8	37.0 ± 2.7	32.7 ± 5.4	3.5 ± 8.8	37.9 ± 1.0
N (number of tests)	12	2	2	2	4	2	2
Cmpd	13	14	15	16	17	18	19
$\Delta p50^a$	16.4 ± 0.9	23.1 ± 6.2	40.1 ± 8.9	43.2 ± 2.9	13.7 ± 9.1	4.0 ± 1.6	36.0 ± 1.4
N (number of tests)	2	2	6	4	4	2	2

^aData expressed as $\Delta p50\%$: the % change from baseline of pO_2 at which HbS (at 25 μM) is 50% saturated with O_2 in the presence of test compound at 30 μM ; data format: data ± SD dev.

Table 2

Cmpd	20	21	22	23	24
$\Delta p50^a$	28.2 ± 3.5	21.1 ± 2.5	2.8 ± 6.7	29.1 ± 0.1	14.2 ± 7.2
N (number of tests)	2	2	2	2	2

^aData expressed as $\Delta p50\%$: the % change from baseline of pO_2 at which HbS (at 25 μM) is 50% saturated with O_2 in the presence of test compound at 30 μM ; data format: data ± SD dev.

Table 3

Cmpd	25	26	27	28	29	30	31	32	33	34	35
$\Delta p50^a$ (Purified Hb)	40.2 ± 2.5	9.0 ± 2.8	29.4 ± 1.3	11.2 ± 5.9	51.7 ± 7.0	27.4 ± 2.1	66.1 ± 4.5	17.1 ± 10.0	21.9 ± 0.7	31.8 ± 0.9	32.7 ± 5.3
N (number of tests)	6	4	2	2	17	2	83	2	2	2	2
$\Delta p50^b$ (Whole Blood)	19.2	ND ^d	ND	ND	43.1 ± 16.0	ND	66.9 ± 6.4	ND	ND	ND	64.7 ± 0.1
N (number of tests)	1	ND	ND	ND	22	ND	58	ND	ND	ND	2
$\Delta T\%^c$ (Polymerization)	ND	ND	ND	ND	64.5	64.5	115.2 ± 22.9	ND	ND	ND	105.2
N (number of tests)	NA ^e	NA	NA	NA	1	1	26	NA	NA	NA	1

^aData expressed as $\Delta p50\%$: the % change from baseline of pO_2 at which HbS (at 25 μM) is 50% saturated with O_2 in the presence of test compound at 30 μM ; ^bData presented as Value ± SD Dev; ^cN: number of tests; ^dData expressed as $\Delta p50\%$: the % change from baseline of pO_2 at which whole blood (at 20% hematocrit, 1 mM) is 50% saturated with O_2 in the presence of test compound at 1 mM. ^eData

expressed as $\Delta T\%$: the % change from baseline of delay time of HbS at 50 μM in the presence of test compound at 75 μM ; ^dNot determined; data format: data \pm SD dev.

Table 4

Cmpd	31	36	37	38	39	40	41	42	43	44	45	46
$\Delta p50^a$ (Purified Hb)	66.1 \pm 4.5	72.1 \pm 6.2	59.6 \pm 2.7	43.1 \pm 4.4	55.9 \pm 1.8	53.3 \pm 8.7	61.7 \pm 3.6	70.5 \pm 5.2	54.5 \pm 0.4	ND ^d	67.8 \pm 2.2	2.6 ^e
N (number of tests)	83	42	2	2	4	2	2	6	2	NA ^f	2	NA
$\Delta p50^b$ (Whole Blood)	66.9 \pm 6.4	79.9 \pm 3.6	62.5 \pm 2.0	49.7 \pm 7.2	68.9 \pm 0.6	56.4 \pm 3.3	65.4 \pm 1.4	72.5 \pm 0.8	55.0 \pm 1.3	80.6 \pm 3.1	72.8 \pm 1.3	ND
N (number of tests)	58	133	2	2	2	2	2	2	2	2	2	NA
$\Delta T\%^c$ (Polymerization)	115.2 \pm 22.9	206.1 \pm 46.4	128.2 \pm 2.0	73.1	77.8	70.3	62.6	167.1	130.6	135.9	103.9	ND
N (number of tests)	26	8	2	1	1	1	1	1	1	1	1	NA

^aData expressed as $\Delta p50\%$: the % change from baseline of pO_2 at which HbS (at 25 μM) is 50% saturated with O_2 in the presence of test compound at 30 μM ; ^bData presented as Value \pm SD Dev; ^cN: number of tests; ^dData expressed as $\Delta p50\%$: the % change from baseline of pO_2 at which whole blood (at 20% hematocrit, 1 mM) is 50% saturated with O_2 in the presence of test compound at 1 mM. ^eData expressed as $\Delta T\%$: the % change from baseline of delay time of HbS at 50 μM in the presence of test compound at 75 μM ; ^dNot determined; ^eTested at 500 μM ; ^fNot applicable; data format: data \pm SD dev.

***In-vitro* P-450 Inhibition Studies.** Pooled human liver microsomes were obtained from BD Gentest and were used for in vitro assessment of compound inhibitory potential of six major drug metabolizing human cytochrome P450 (CYP) enzymes (CYP1A2, CYP2C8, CYP2C19, CYP2C9, CYP2D6 and CYP3A4). The selective drug substrates used were phenacetin (CYP1A2), paclitaxel (CYP2C8), tolbutamide (CYP2C9), s-mephenytoin (CYP2C19), bufuralol (CYP2D6), midazolam (CYP3A4), and testosterone (CYP3A4). Assay conditions were optimized for each human cytochrome P450 substrate. Effect of GBT440 (**36**) on formation of the respective metabolites (acetaminophen, 6 α -hydroxypaclitaxel, 4-hydroxytolbutamide, 4'-hydroxymephenytoin, 1'-hydroxybufuralol, 1'-hydroxymidazolam, and 6 β -hydroxtestosterone) was determined. The assays were performed in duplicate in 96-well plates at 37°C for 5-15 minutes. The reaction mixtures (200 μL) contained a final concentration of 0.1-0.2 mg/mL pooled human liver microsomes, and 1 mM NADPH in 100 mM potassium phosphate, pH 7.4 buffer with 3 mM MgCl_2 . Human cytochrome specific inhibitors were included as reference compounds.

***In-vivo* Studies.** The vivo work was a non-GLP exploratory study that was not subjected to U.S. Food and Drug Administration (FDA) or international Good Laboratory Practice (GLP) regulations. The

execution of this study followed the Institutional Animal Care and Use Committee (IACUC) requirements. Pharmacokinetics was evaluated in Sprague-Dawley rats following an oral administration at 7.2 to 120 mg/kg. Blood samples were collected serially up to 96 hrs post-dose. Both blood and plasma samples were extracted and the concentration of each compound was determined by high pressure liquid chromatography/tandem mass spectrometry (LC-MS/MS). Relevant pharmacokinetic parameters were derived by noncompartmental analysis (Phoenix WinNonlin version 6.4).

Crystallography/HbS crystallization. Hemoglobin S (HbS) was purified and saturated with carbon monoxide (CO) gas. Carbon-monoxo HbS (CO-HbS) was diluted to 30 mg/mL in 20 mM Hepes buffer pH 7.4. CO-HbS protein was mixed with a 2.5-fold molar excess of compound 6 or compound 31 and incubated at room temperature for 30 minutes prior to adding an equal volume of the crystallization condition. Compound 6 was co-crystallized with HbS using a condition 100 mM Hepes pH 7.4, 40 mM NaCl and 29% PEG 3350 and compound 31 was co-crystallized with HbS using a condition of 100 mM Hepes pH 7.4, 20 mM NaCl and 23% PEG 3350. Diffracting crystals grew within 7-10 days using the hanging drop vapor diffusion method at 23 °C. Crystals were cryoprotected by adding glycerol (10-12% (v/v) final concentration) to the crystallization condition before flash-freezing in liquid nitrogen. X-ray diffraction data was collected at beamline 8.3.1. at the Advanced Light Source (ALS), Berkeley CA, using a wavelength of 1.11 Å. CO-HbS was co-crystallized with either compound **6** or compound **31** in the P2₁2₁2₁ spacegroup with a single HbS tetramer per asymmetric unit. Data reduction was carried out using MOSFLM.² All models were built using COOT³ and further refinement was carried out using the latest builds of the PHENIX suite.⁴ The coordinates have been deposited to the Protein Data Bank (PDB codes 5UFJ and 5U3I). Figures were made using PyMOL version 1.6 (Schrodinger, 2016).

Table 1. Crystallization Data

Data Set (PDB CODE)	Compound 6 (5UFJ)	Compound 31 (5U3I)
Data Collection		
Wavelength	1.11	1.11
Space group	P2 ₁ 2 ₁ 2 ₁	P2 ₁ 2 ₁ 2 ₁
Cell dimensions:		
a,b,c (Å)	56.4, 58.9, 174.4	57.3, 59.0, 172.6
Resolution (Å)	30 - 2.05 (2.12 - 2.05)	30 - 1.95 (2.02-1.95)
R _{sym} ^a	0.08 (1.3)	0.06 (1.7)
R _{p.i.m.} ^b	0.023 (0.371)	0.018 (0.503)
I/σ	14.3 (2.1)	17.2 (1.7)
CC _{1/2}	(0.678)	(0.77)
CC*	(0.899)	(0.933)
Completeness	100 (100)	100 (100)
Redundancy	12.8 (13.0)	12.8 (13.4)
Wilson B factor (Å ²)	47.25	48.57
Refinement		
Resolution (Å)	29.8 – 2.05	29.8-1.95
Reflections	37,327	43,683
Nonhydrogen Atoms	4,649	4,642
Water Molecules	30	39
R _{work} ^c	21.39	20.77
R _{free} ^d	25.57	25.43
R.m.s. deviations		
Bond lengths (Å)	0.009	0.009
Bond angles (°)	1.102	0.978
B factors (Å ²)		
Protein	76.6	76.5
Water	60.8	60.5
Coordinate error (Å)	0.27	0.33
Ramachandran plot ^d		
Most favored (%)	97	97
Allowed (%)	3	3
Disallowed (%)	0	0

Highest resolution shell is shown in parenthesis.

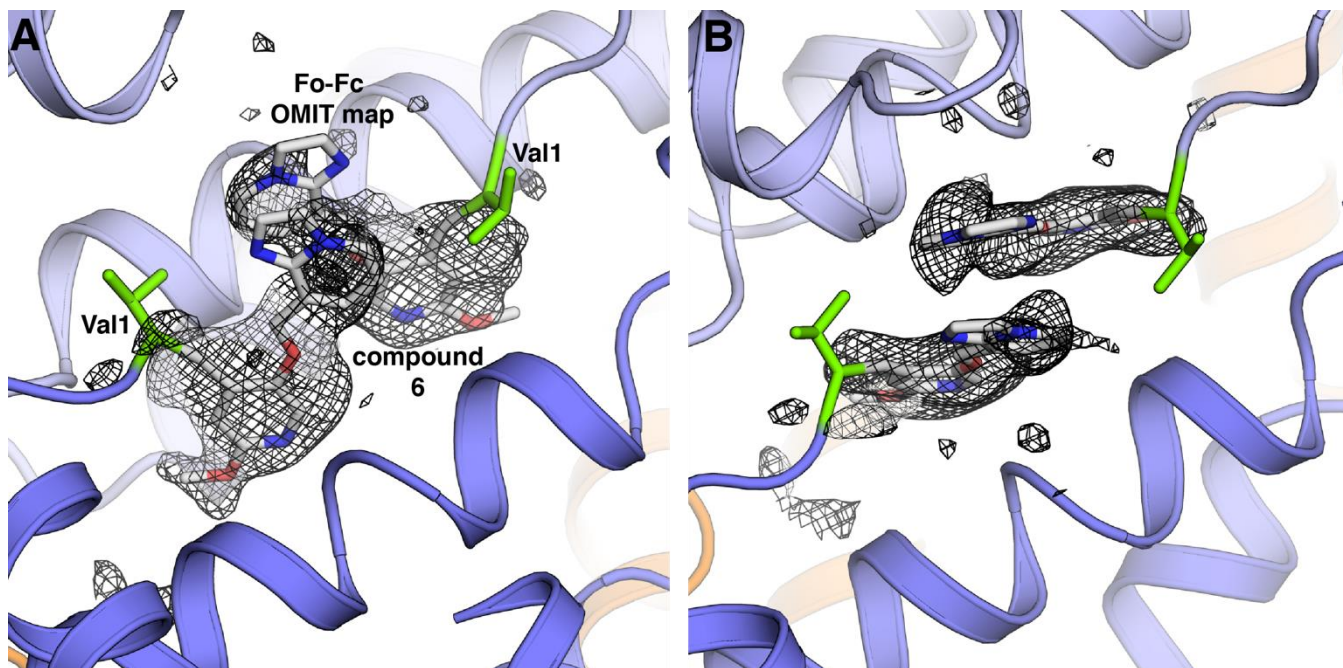
^a $R_{sym} = \sum |I_i - \langle I_i \rangle| / \sum I_i$, where I_i is the intensity of the i th observation and $\langle I_i \rangle$ is the mean intensity of the reflection.

^b $R_{p.i.m.} = \sum_{hkl} [I/(N-1)]^{1/2} \sum_i |I_i(hkl) - \langle I(hkl) \rangle| / \sum_{hkl} \sum_i I_i(hkl)$, where $I_i(hkl)$ is the observed intensity and $\langle I(hkl) \rangle$ is the average intensity of multiple observations of symmetry-related reflections.

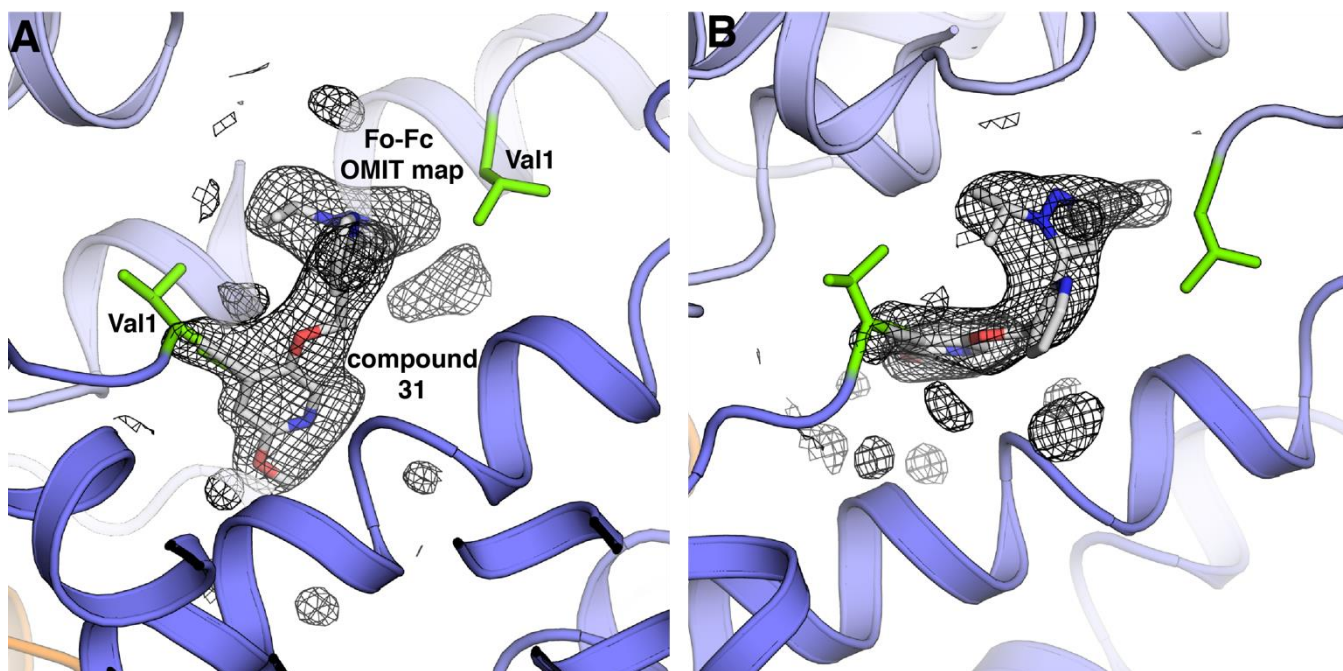
^c $R_{work} = \sum (||F_{obs}| - |F_{calc}|| / \sum |F_{obs}|)$

^d R_{free} = R value for a randomly selected subset (5%) of the data that were not used for minimization of the crystallographic residual.

^d Calculated with the program PROCHECK.



Supplemental Figure 1. A) Fo-Fc omit map within 5 angstroms of compound 6 with HbS. The Fo-Fc map is scaled to 2.5 sigma. B) Top view of the same map and compound bound to HbS. This view more clearly shows that two compounds are bound to a single tetramer.

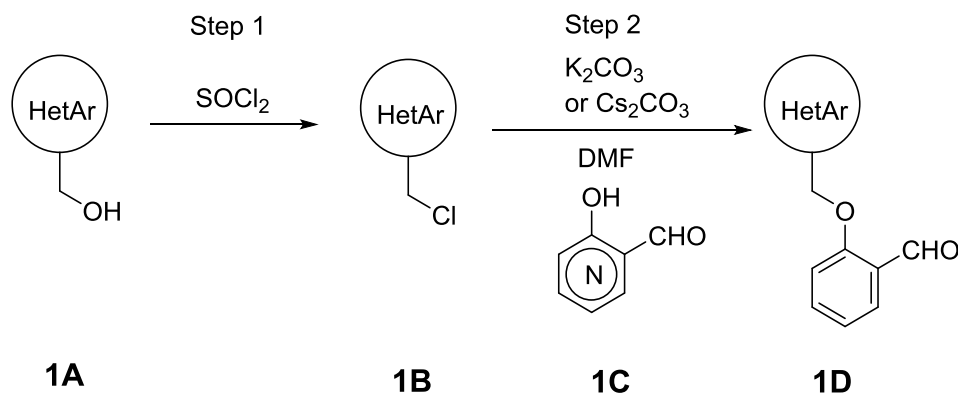


Supplemental Figure 2. A) Fo-Fc omit map within 5 angstroms of compound 31 with HbS. The Fo-Fc map is scaled to 2.5 sigma. B) Top view of the same map and compound bound to HbS. This view more clearly shows density for a single compound bound to a single tetramer.

Chemistry/Compound Characterization: Reagents and solvents were purchased from Aldrich Chemical, Acros Organics, Alfa Aesar, AK Scientific, TCI America, Shanghai BePharm Ltd, J&K Scientific Ltd and used as received unless otherwise indicated. Air- and/or moisture-sensitive reactions were carried out under a nitrogen or argon atmosphere in oven-dried glassware using anhydrous solvents from Aldrich. Air- and/or moisture-sensitive reagents were transferred via syringe or cannula, and introduced into reaction vessels through rubber septa. Solvent removal was accomplished with a rotary evaporator at ~10–50 Torr. Microwave irradiation was carried out with a Biotage initiator system. Automated silica gel column chromatography was carried out using a Biotage Isolera system and silica gel cartridges from Biotage. Analytical TLC plates from Merk (Silica Gel 60 F₂₅₄) were employed for TLC analyses. ¹H NMR spectra were recorded on an Agilent 400 MR NMR spectrometer or Bruker Avance III 300 MHz. Chemical shifts are reported in δ units (ppm) relative to TMS as an internal standard. Coupling constants (*J*) are reported in hertz (Hz). Characterization data are reported as follows: chemical shift, multiplicity (s=singlet, d=doublet, t=triplet, q=quartet, br=broad, m=multiplet), coupling constants, number of protons, mass to charge ratio.

All analogs submitted for testing were judged to be of $\geq 95\%$ purity based on analytical LC/MS analysis performed on a Waters ACQUITY UPLC and SQ detector 2 MS system or Shimadzu LCMS-2020 Series equipped with a BEH C₁₈ column (50 x 2.1 mm, 1.7 μ m) at 25 °C using a mobile phase of water-acetonitrile containing 0.1% trifluoroacetic acid with a flow rate of 1.2 mL/min. Gradient elution was employed wherein the acetonitrile: water ratio was increased linearly from 5% to 95% acetonitrile over 1.5 min, then maintained at 95% acetonitrile for 0.1 min, and then decreased to 5% acetonitrile over 0.02 min, and maintained at 5% acetonitrile for 0.38 min. Compound purity was determined by integrating peak areas of the liquid chromatogram, monitored at 254 nm.

Scheme 1. Synthetic route to HbS modulator **1D**.

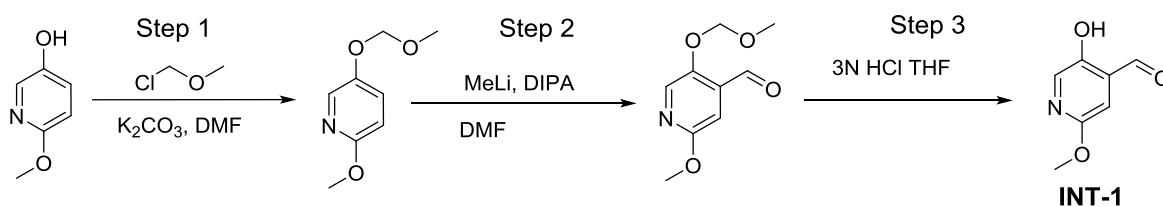


General method step 1 (Scheme 1) for preparing substituted methylene chloride (1B). To a solution of substituted methylene alcohol (**1A**) (0.1 to 2 mmol) in DCM (1-10 mL) was added SOCl_2 dropwise (2 to 5 equiv) at 0 °C or rt. The reaction mixture was stirred at rt for 10min to 6 h, or until reaction is judged complete (LC/MS). The reaction mixture is concentrated to dryness over a rotavap. The crude chloride residue was suspended in toluene, sonicated and concentrated to dryness. The process was repeated three times and dried under vacuum to give the substituted methylene chloride (**1B**), usually as an off-white solid, which was used for next step without further purification. Alternatively, a solution of aqueous 1N Na_2CO_3 is then added to produce a solution of pH~ 8. the mixture was extracted with DCM (3 x10-50mL), dried over sodium sulfate, and concentrated to the crude substituted methylene chloride (**1B**), which is then purified by column chromatography on silica gel (0-100% ethyl acetate-hexanes).

General method step 2 (Scheme 1) for preparing aryloxy/heteroarylether analogs (1D) from substituted methylene alcohol (1B) and hydroxyl (hetero)aryl aldehyde derivatives (1C). A hydroxyl (hetero)arylaldehyde derivatives (**1C**) (0.1-2 mmol) mixture with substituted methylene alcohol (**2**) (0.8 to 1.2equiv) and (polymer-supported)/ PPh_3 (1-1.5equiv) in anhydrous THF (1-10mL) was stirred under nitrogen until complete dissolution. The solution was cooled to 0 °C on ice bath and DIAD or DEAD (1.1 equiv) in THF or toluene was added drop wise over a 1-20 min period. The ice cooling bath was allowed to expire over 90 min and the mixture was stirred at rt for 2-48 hours. The mixture was stirred for 10 min, then filtered through a pad of silica. The silica was washed with ethyl acetate 2-20mL. The combined filtrates were evaporated and the residue was dried on highvac. The residue was purified by preparative HPLC or flash silica gel chromatography.

General method step 2 (Scheme 1) for preparing aryloxy/heteroarylether analogs (1D) from substituted methylene halide (1B) and hydroxyl (hetero)aryl aldehyde derivatives (1C). A mixture of hydroxyl (hetero)arylaldehyde derivatives (**1C**) (0.1-2 mmol, 1-4 equiv), substituted methylene chloride or bromide (**1B**) (1equiv), and K_2CO_3 (2-5 equiv) (catalytic amount of NaI or Bu_4NI may also be added) in DMF, acetonitrile, NMP or DMSO (1 to 10 mL) was stirred at rt or heating up to 120 °C for 1-24 h under nitrogen atmosphere. In workup A, water was added to the reaction mixture, the precipitated product was collected, washed with water, and then subjected to preparative HPLC or flash silica gel chromatography purification. In workup B (for products that did not precipitate), diluted HCl or aqueous NH_4Cl was added at 0 °C to adjusted the pH to ~7, the reaction mixture was partitioned between ethyl acetate or dichloromethane and aqueous sodium chloride and the organic layer separated, dried, and solvent removed under vacuum to afford crude product which was purified by automated silica gel column chromatography using appropriate solvents mixture (e.g., ethyl acetate/hexanes).

5-hydroxy-2-methoxyisonicotinaldehyde (INT-1).

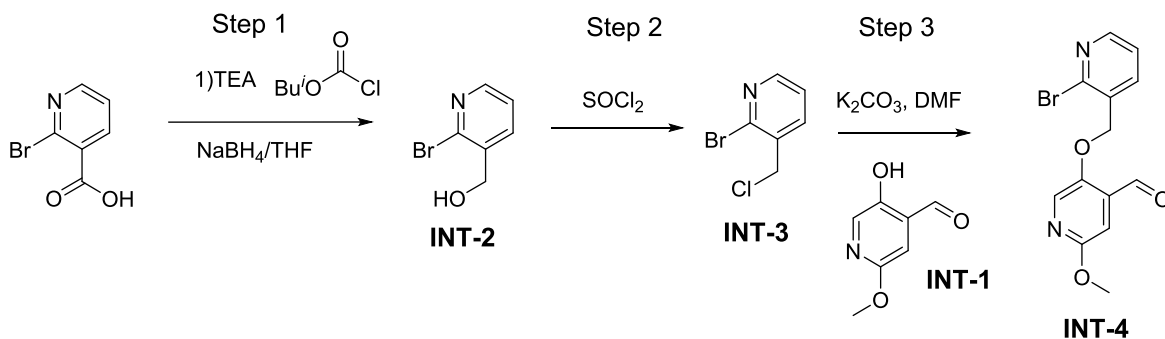


Step 1. To a mixture of 6-methoxy-3-hydroxypyridine (25 g, 0.2 mol) and K₂CO₃ (82.8 g, 0.6 mol) in DMF (250 mL) was added bromomethyl methyl ether (30 g, 0.24 mol) slowly at rt for a period of 1h. The reaction mixture was filtered and the filtrate was concentrated. The residue was purified on silica gel with 25% EtOAc/hexanes eluent to give 2-methoxy-5-(methoxymethoxy)pyridine (20 g, 59%) as a colorless oil. MS (ESI) *m/z* 170.1 [M+H]⁺.

Step 2: To a solution of 2-methoxy-5-(methoxymethoxy)pyridine (20 g, 0.12 mol) in THF was added diisopropylamine (0.24 g, 2.4 mmol). The solution was cooled to -40 °C followed by addition of MeLi (3M/THF, 72mL, 0.216 mol) slowly. The resulting mixture was warmed to 0 °C, stirred at 0 °C for 3 h, cooled back down to -40 °C and added N-formylpiperidine (24mL, 0.216mol). After stirring at -40 °C for 2 h, the mixture was quenched with a mixed solution of HCl (37%, 120 mL) and THF (250 mL). The temperature was then raised to rt and diluted with water (200 mL) and EtOAc (200 mL). The pH of the mixture was adjusted to 8-9 with solid K₂CO₃ and extracted with EtOAc (300 mL) twice. The organic layer was combined, dried over Na₂SO₄, and concentrated. The residue was purified on silica gel with 25%EtOAc/hexanes as eluent to give 2-methoxy-5-(methoxymethoxy)isonicotinaldehyde (10 g, 42%) as a pale yellow oil. MS (ESI) *m/z* 198.1 [M+H]⁺; ¹H NMR (400 MHz; CD₃OD) δ 7.90 (s, 1 H), 6.92 (s, 1 H), 5.64 (s, 1 H), 5.20 (s, 2 H), 3.84 (s, 3 H), 3.48 (s, 3 H).

Step 3: To a solution of 2-methoxy-5-(methoxymethoxy)isonicotinaldehyde (10 g, 0.05 mol) in THF (100 mL) was added 3 N HCl (150 mL). The reaction was stirred at 50 °C for 30 min, cooled to rt, and diluted with water (100 mL). The mixture was neutralized to pH 7-8 and extracted with EtOAc (200 mL) three times. The organic layer was dried over Na₂SO₄ and concentrated to give 5-hydroxy-2-methoxyisonicotinaldehyde (4.2 g, 55%) as a yellow solid. MS (ESI) *m/z* 154.1 [M+H]⁺; ¹H NMR (400 MHz; DMSO) δ = 10.31(s, 1 H), 8.03 (s, 1 H), 6.89 (s, 1 H), 3.80 (s, 3 H).

(2-Bromopyridin-3-yl)methanol (INT-2), 2-bromo-3-(chloromethyl)pyridine (INT-3) and 5-((2-bromopyridin-3-yl)methoxy)-2-methoxyisonicotinaldehyde (INT-4).

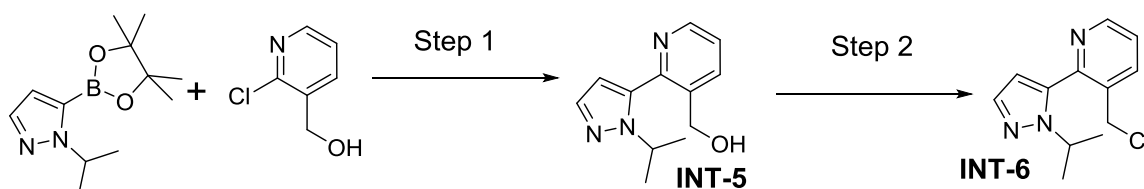


Step1. To a solution of 2-bromonicotinic acid (4.0 g, 20 mmol) and triethylamine (3.34 mL, 24 mmol, 1.2 equiv) in THF (100 mL) was added *i*-butyl chloroformate (3.12 mL, 24 mmol, 1.2 eq.) at 0 °C. The mixture was stirred at 0 °C for 10 min and filtered. To this filtrate was added a suspension of NaBH₄ (1.52 g, 40 mmol, 2 equiv) in water (1.0 mL) at 0 °C. The mixture was stirred for 30 min, added water (3 mL), continued to stir for 2 h, and concentrated to dryness. The crude was purified on silica gel using a mixture of ethylacetate and hexanes as eluent to give (2-bromopyridin-3-yl)methanol (**INT-2**) (3.4 g, 90%) as a white solid. MS (ESI) m/z 188.0 [M+H]⁺.

Step2. To (2-bromopyridin-3-yl)methanol (380 mg, 2 mmol) in DCM (5 mL) was added SOCl₂ (1 mL) at rt. The reaction mixture was stirred at rt for 4 h and concentrated to dryness. The crude solid was suspended in toluene and concentrated to dryness. The process was repeated three times and dried under vacuum to give an off-white solid (**INT-3**) (480 mg), which was used for next step without further purification.

Step 3. A mixture of 5-hydroxy-2-methoxyisonicotinaldehyde (306 mg, 2 mmol, 1 equiv), 2-bromo-3-(chloromethyl)pyridine (crude above, 2 mmol), and K₂CO₃ (828 mg, 6 mmol, 3 equiv) in DMF (1.0 mL) was heated at 50 °C for 2 h. The mixture was cooled and added to water (50 mL) dropwise. The precipitate was filtered, washed with water, dried under high *vacuo* to give 5-((2-bromopyridin-3-yl)methoxy)-2-methoxyisonicotinaldehyde (350 mg, 85%) as an yellow solid (**INT-4**). MS (ESI) m/z 323.0 [M+H]⁺; ¹H NMR (400 MHz; CDCl₃) δ 10.51 (s, 1 H), 8.42 (dd, 1 H), 8.09 (s, 1 H) 7.91 (d, 1 H), 7.40 (dd, 1 H), 7.15 (s, 1 H), 5.27 (s, 2 H), 3.95 (s, 3 H).

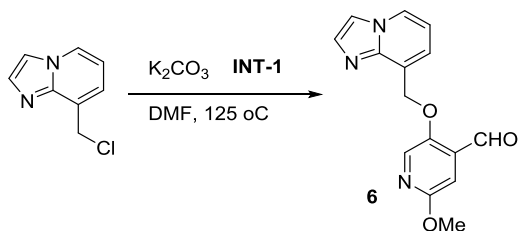
(2-(1-isopropyl-1H-pyrazol-5-yl)pyridin-3-yl)methanol (INT-5) and 3-(chloromethyl)-2-(1-isopropyl-1H-pyrazol-5-yl)pyridine (INT-6).



Step1. To a 500-mL flask containing the pyrazole boronate (9.0g, 38.1mmol), 2-chloropyridine (5.47g, 38.1mmol), Pd(dppf)Cl₂ ([1,1-bis(diphenylphosphino)ferrocene]dichloropalladium) (1.39g, 1.91mmol, 5% mol), and sodium bicarbonate (9.61g, 114.4mmol, 3 equiv) was added 100 mL of dioxane and 30 mL of water. The mixture was heated under nitrogen at 100 °C for 12 hrs. Then solvents were removed on a rotavap at 40 °C under vacuum. The resulting brown residue was suspended in 20%EtOAc/DCM (60mL), filtered through a pad of silica gel (15g); washed with 20%EtOAc/DCM (4x20mL). The combined filtrate were concentrated to afford a brown oil (13 g). The residue was dissolved 10% EtOAc/hexanes (20mL) and loaded on a Biotage 100g snap SiO₂ column and eluted with 0-50% EtOAc. (2-(1-isopropyl-1H-pyrazol-5-yl)pyridin-3-yl)methanol (**INT-5**) was obtained as a light brown oil (3.32 g, 40%). MS (ESI) *m/z* 218.1 [M+H]⁺. ¹H NMR (400 MHz, CDCl₃) δ 8.67 (dd, *J* = 4.7, 1.5 Hz, 1H), 8.0 (d, *J* = 7.8 Hz, 1H), 7.61 (d, *J* = 1.8 Hz, 1H), 7.39 (dd, *J* = 7.8, 4.8 Hz, 1H), 6.37 (d, *J* = 1.8 Hz, 1H), 4.67 (s, 2H), 4.55 (sep, *J* = 6.6 Hz 1H), 1.98-2.05 (br, 1H), 1.47 (d, *J* = 6.6 Hz, 6H).

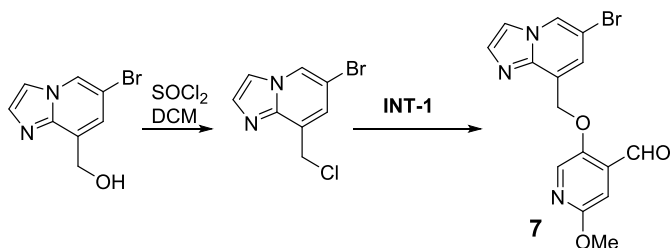
Step2. To a solution of (2-(1-isopropyl-1H-pyrazol-5-yl)pyridin-3-yl)methanol (440mg, 2.02mmol) in DCM (4 mL) was added SOCl₂ (2equiv) at 0 °C. The reaction mixture was stirred at rt for 15mins and concentrated to dryness. The crude solid was suspended in toluene and concentrated to dryness. The process was repeated three times and dried under vacuum to give 3-(chloromethyl)-2-(1-isopropyl-1H-pyrazol-5-yl)pyridine hydrochloride (**INT-6**) (432 mg) as an off-white solid, which was used for next step without further purification. MS (ESI) *m/z* 236.5 [M+H]⁺. ¹H NMR (400 MHz, DMSO-*d*₆) δ 8.70 (dd, *J* = 4.8, 1.7 Hz, 1H), 8.09 (dt, *J* = 7.9, 2.0 Hz, 1H), 7.60 – 7.49 (m, 2H), 6.57 – 6.50 (m, 1H), 4.72 (s, 2H), 4.49 – 4.37 (m, 1H), 1.33 (d, *J* = 6.6 Hz, 6H).

5-(Imidazo[1,2-a]pyridin-8-ylmethoxy)-2-methoxyisonicotinaldehyde (**6**).



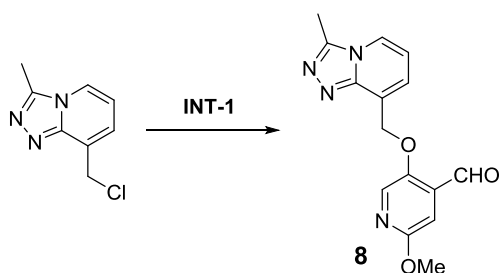
The title compound was prepared from INT-1 and 8-(chloromethyl)imidazo[1,2-a]pyridine according to the general procedure step 2 to give the product (cmpd **6**) as a yellow solid in 32% yield; MS (ESI) *m/z* 284.0 [M+H]⁺; ¹H NMR (400 MHz, DMSO-*d*₆) δ 10.37 (s, 1H), 8.57 (dd, *J* = 6.8, 1.1 Hz, 1H), 8.39 (s, 1H), 8.02 (s, 1H), 7.61 (d, *J* = 1.2 Hz, 1H), 7.45 (dd, *J* = 6.9, 1.2 Hz, 1H), 7.00 – 6.89 (m, 2H), 5.61 (s, 2H), 3.84 (s, 3H).

5-((6-Bromoimidazo[1,2-a]pyridin-8-yl)methoxy)-2-methoxyisonicotinaldehyde (**7**).



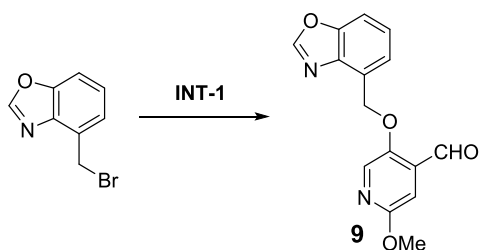
The title compound was prepared from INT-1 and (6-bromoimidazo[1,2-a]pyridin-8-yl)methanol according to the general procedure steps 1&2 to give the product as a light yellow solid in 8% overall yield. MS (ESI) m/z 362.0, 364.0 $[M+H]^+$; 1H NMR (400 MHz, Chloroform-*d*) δ 10.40 (s, 1H), 8.17 (dt, $J = 1.6, 0.8$ Hz, 1H), 7.99 (d, $J = 0.6$ Hz, 1H), 7.51 (dd, $J = 12.5, 1.3$ Hz, 2H), 7.29 (q, $J = 1.3$ Hz, 1H), 6.98 (d, $J = 0.6$ Hz, 1H), 5.49 (t, $J = 1.0$ Hz, 2H), 3.78 (s, 3H).

2-methoxy-5-((3-methyl-[1,2,4]triazolo[4,3-a]pyridin-8-yl)methoxy)isonicotinaldehyde (8).



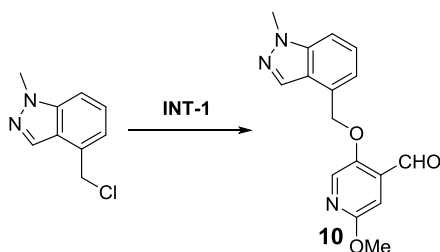
The title compound was prepared from INT-1 and 8-(chloromethyl)-3-methyl-[1,2,4]triazolo[4,3-a]pyridine according to the general procedure step 2 to give the product as a light yellow solid in 41% yield. MS (ESI) m/z 299.1 $[M+H]^+$; 1H NMR (400 MHz, CDCl₃) δ 10.53 (s, 1H), 8.14 (s, 1H), 7.89 (d, $J = 6.9$ Hz, 1H), 7.44 (dd, $J = 6.8, 1.1$ Hz, 1H), 7.11 (s, 1H), 6.94 (t, $J = 6.9$ Hz, 1H), 5.67 (s, 2H), 3.92 (s, 3H), 2.80 (s, 3H).

5-(Benzo[d]oxazol-4-ylmethoxy)-2-methoxyisonicotinaldehyde (9).



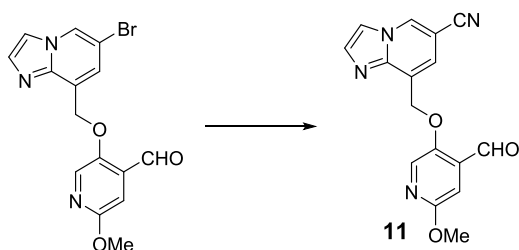
The title compound was prepared from INT-1 and 4-(bromomethyl)benzo[d]oxazole according to the general procedure step 2 to give the product as a white solid in 29% yield. MS (ESI) m/z 285.1 $[M+H]^+$; 1H NMR (400 MHz, CDCl₃) δ 10.51 (s, 1H), 8.18 (d, $J = 7.3$ Hz, 2H), 7.63 (dd, $J = 8.1, 1.0$ Hz, 1H), 7.52 (d, $J = 7.1$ Hz, 1H), 7.46 (t, $J = 7.8$ Hz, 1H), 7.11 (d, $J = 0.5$ Hz, 1H), 5.65 (s, 2H), 3.92 (s, 3H).

3-(5-(3-(((4-Formyl-6-methoxypyridin-3-yl)oxy)methyl)pyridin-2-yl)-1H-pyrazol-1-yl)propanoic acid (10).



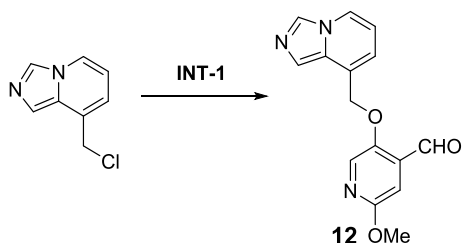
The title compound was prepared from INT-1 and 4-(chloromethyl)-1-methyl-1H-indazole according to the general procedure step 2 to give the product as a light yellow solid in 54% yield. MS (ESI) m/z 298.1 $[M+H]^+$; **[0001]** ^1H NMR (400 MHz, CDCl_3) δ 10.33 (s, 1H), 8.66 (dd, $J = 4.8, 1.6$ Hz, 1H), 7.96 (dd, $J = 7.9, 1.5$ Hz, 1H), 7.83 (s, 1H), 7.53 (d, $J = 1.9$ Hz, 1H), 7.37 (dd, $J = 7.9, 4.8$ Hz, 1H), 7.02 (s, 1H), 6.33 (d, $J = 1.9$ Hz, 1H), 5.13 (s, 2H), 4.49 (t, $J = 6.5$ Hz, 2H), 3.81 (s, 3H), 2.94 (t, $J = 6.5$ Hz, 2H).

8-(((4-Formyl-6-methoxypyridin-3-yl)oxy)methyl)imidazo[1,2-a]pyridine-6-carbonitrile (11).



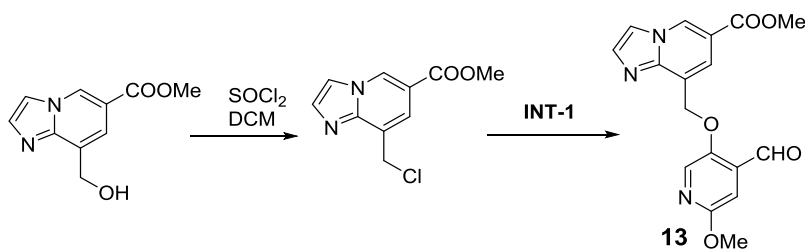
A mixture of 5-((6-bromoimidazo[1,2-a]pyridin-8-yl)methoxy)-2-methoxyisonicotinaldehyde (60.0 mg, 0.166 mmol, 1 equiv) and $\text{Zn}(\text{CN})_2$ (38.0 mg, 0.332 mmol, 2.0 equiv) in DMF (4 mL) was purged with N_2 for 5 min and added $\text{Pd}(\text{PPh}_3)_4$ (38.0 mg, 0.032 mmol, 0.2 equiv). The mixture was heated at 125 °C for 12 min under N_2 , cooled, filtered, concentrated, and purified on silica gel using a mixture of EtOAc and hexanes as eluent to give the title compound (5.3 mg, 20%) as a white solid. MS (ESI) m/z 309.1 $[M+H]^+$; ^1H NMR (400 MHz, $\text{DMSO}-d_6$) δ 10.42 (s, 1H), 9.39 (d, $J = 1.6$ Hz, 1H), 8.37 (s, 1H), 8.13 (d, $J = 1.4$ Hz, 1H), 7.79 (dd, $J = 4.7, 1.4$ Hz, 2H), 7.01 (d, $J = 0.6$ Hz, 1H), 5.64 (s, 2H), 3.86 (s, 3H).

5-(Imidazo[1,5-a]pyridin-8-ylmethoxy)-2-methoxyisonicotinaldehyde (12).



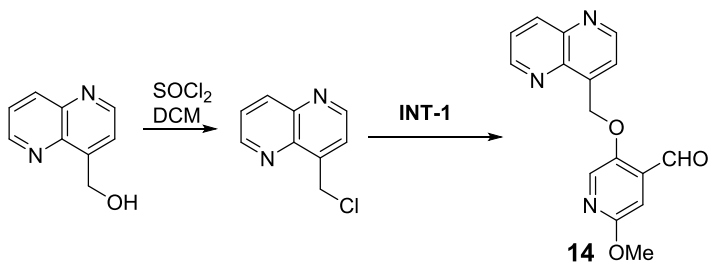
The title compound was prepared from INT-1 and 8-(chloromethyl)imidazo[1,5-a]pyridine according to the general procedure step 2 to give the product as an off-white solid in 20% yield. MS (ESI) m/z 284.0 $[M+H]^+$; 1H NMR (400 MHz, $CDCl_3$) δ 10.47 (s, 1H), 8.21 (s, 1H), 8.11 (s, 1H), 7.97 (d, $J = 7.0$ Hz, 1H), 7.52 (s, 1H), 7.12 (s, 1H), 6.87 (t, $J = 8.1$ Hz, 1H), 6.62 (t, $J = 6.8$ Hz, 1H), 5.37 (s, 2H), 3.92 (s, 3H).

Methyl 8-(((4-formyl-6-methoxypyridin-3-yl)oxy)methyl)imidazo[1,2-a]pyridine-6-carboxylate (13).



The title compound was prepared from INT-1 and methyl 8-(hydroxymethyl)imidazo[1,2-a]pyridine-6-carboxylate according to the general procedure steps 1&2 to give the product as an off-white solid in 85% yield. MS (ESI) m/z 342.1 $[M+H]^+$; 1H NMR (400 MHz, $DMSO-d_6$) δ 10.35 (s, 1H), 9.38 (d, $J = 1.7$ Hz, 1H), 8.42 (s, 1H), 8.19 (d, $J = 1.2$ Hz, 1H), 7.86 (d, $J = 1.6$ Hz, 1H), 7.73 (d, $J = 1.2$ Hz, 1H), 6.99 (s, 1H), 5.64 (s, 2H), 3.90 (s, 3H), 3.85 (s, 3H).

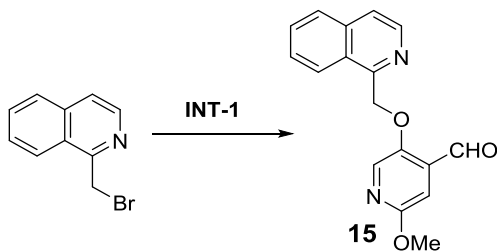
5-((1,5-Naphthyridin-4-yl)methoxy)-2-methoxyisonicotinaldehyde (14).



The title compound was prepared from INT-1 and (1,5-naphthyridin-4-yl)methanol according to the general procedure steps 1& 2 to give the product as an off-white solid in 26% yield. MS (ESI) m/z 296.0 $[M+H]^+$; 1H NMR (400 MHz, $Chloroform-d$) δ 10.53 (s, 1H), 9.11 (d, $J = 2.1$ Hz, 1H), 9.06 (dd, $J = 4.2$,

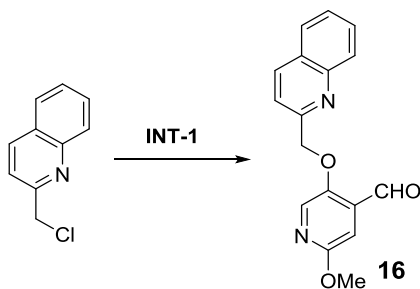
1.7 Hz, 1H), 8.53 – 8.44 (m, 2H), 8.15 (d, $J = 0.7$ Hz, 1H), 7.72 (dd, $J = 8.5, 4.2$ Hz, 1H), 7.16 (d, $J = 0.6$ Hz, 1H), 5.50 (d, $J = 0.8$ Hz, 2H), 3.94 (s, 3H).

5-(Isoquinolin-1-ylmethoxy)-2-methoxyisonicotinaldehyde (15).



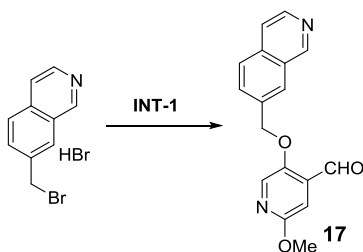
The title compound was prepared from INT-1 and 1-(bromomethyl)isoquinoline according to the general procedure step 2 to give the product as a yellow solid in 41% yield. MS (ESI) m/z 295.1 $[M+H]^+$; 1H NMR (400 MHz, $CDCl_3$) δ 10.40 (s, 1H), 8.54 (d, $J = 5.7$ Hz, 1H), 8.30 (s, 1H), 8.31 (d, $J = 8.6$ Hz, 1H), 7.90 (d, $J = 8.4$ Hz, 1H), 7.78 – 7.63 (m, 3H), 7.07 (d, $J = 0.5$ Hz, 1H), 5.82 (s, 2H), 3.91 (s, 3H).

2-Methoxy-5-(quinolin-2-ylmethoxy)isonicotinaldehyde (16).



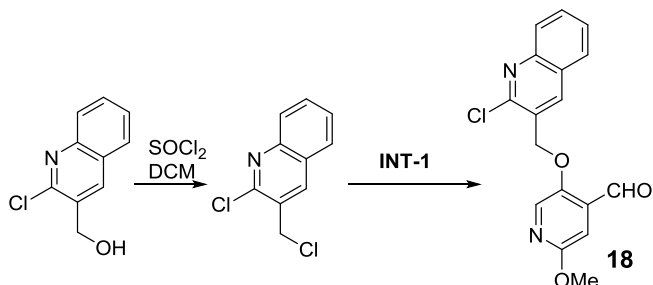
The title compound was prepared from INT-1 and 2-(chloromethyl)quinoline according to the general procedure step 2 to give the product as a yellow solid in 36% yield. MS (ESI) m/z 295.1 $[M+H]^+$; 1H NMR (400 MHz, $CDCl_3$) δ 10.61 (s, 1H), 8.26 (d, $J = 8.5$ Hz, 1H), 8.12 (s, 1H), 8.10 (d, $J = 8.5$ Hz, 1H), 7.87 (dd, $J = 8.2, 1.1$ Hz, 1H), 7.78 (ddd, $J = 8.4, 6.9, 1.4$ Hz, 1H), 7.67 (d, $J = 8.5$ Hz, 1H), 7.60 (ddd, $J = 8.1, 7.0, 1.1$ Hz, 1H), 7.13 (s, 1H), 5.52 (s, 2H), 3.91 (s, 3H).

5-(Isoquinolin-7-ylmethoxy)-2-methoxyisonicotinaldehyde (17).



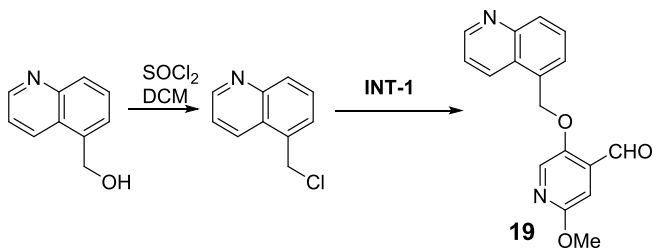
The title compound was prepared from INT-1 and 7-(bromomethyl)isoquinoline HBr salt according to the general procedure step 2 to give the product as a white solid in 20% yield. MS (ESI) m/z 295.1 $[M+H]^+$; 1H NMR (400 MHz, Chloroform- d) δ 10.53 (s, 1H), 9.32 (s, 1H), 8.59 (d, J = 5.4 Hz, 1H), 8.12 (d, J = 0.7 Hz, 1H), 8.07 (dt, J = 1.7, 0.8 Hz, 1H), 7.93 (d, J = 8.5 Hz, 1H), 7.81 (dd, J = 8.5, 1.7 Hz, 1H), 7.73 (d, J = 5.7 Hz, 1H), 7.14 (d, J = 0.6 Hz, 1H), 5.42 (d, J = 0.8 Hz, 2H), 3.93 (s, 3H).

5-((2-Chloroquinolin-3-yl)methoxy)-2-methoxyisonicotinaldehyde (18).



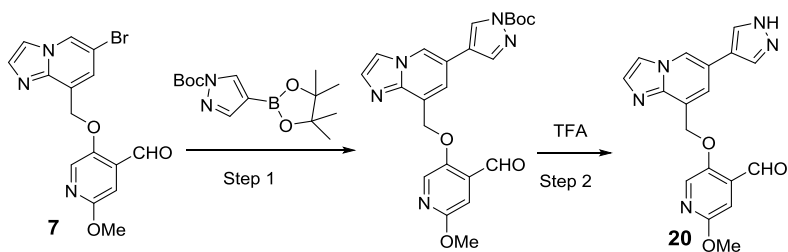
The title compound was prepared from INT-1 and (2-chloroquinolin-3-yl)methanol according to the general procedure steps 1&2 to give the product as an off-white solid in 77% yield. MS (ESI) m/z 329.0, 331.0 $[M+H]^+$; 1H NMR (400 MHz, Chloroform- d) δ 10.57 (s, 1H), 8.37 (s, 1H), 8.16 (s, 1H), 8.12 – 8.05 (m, 1H), 7.90 (d, J = 8.1 Hz, 1H), 7.80 (ddd, J = 8.5, 6.9, 1.4 Hz, 1H), 7.64 (dd, J = 8.2, 7.0 Hz, 1H), 7.28 (s, 1H), 7.17 (s, 1H), 5.44 (d, J = 1.1 Hz, 2H), 3.95 (d, J = 0.7 Hz, 3H).

2-Methoxy-5-(quinolin-5-ylmethoxy)isonicotinaldehyde (19).



The title compound was prepared from INT-1 and quinolin-5-ylmethanol according to the general procedure steps 1&2 to give the product as a yellow solid in 22% yield. MS (ESI) m/z 295.1 $[M+H]^+$; 1H NMR (400 MHz, CDCl $_3$) δ 10.23 (s, 1H), 8.94 (dd, J = 4.3, 1.5 Hz, 1H), 8.43 (d, J = 8.5 Hz, 1H), 8.16 (d, J = 14.1 Hz, 1H), 8.13 (s, 2H), 7.68 (dd, J = 8.3, 7.2 Hz, 1H), 7.61 (d, J = 6.7 Hz, 1H), 7.47 (dd, J = 8.6, 4.3 Hz, 1H), 7.02 (s, 1H), 5.56 (s, 2H), 3.84 (s, 3H).

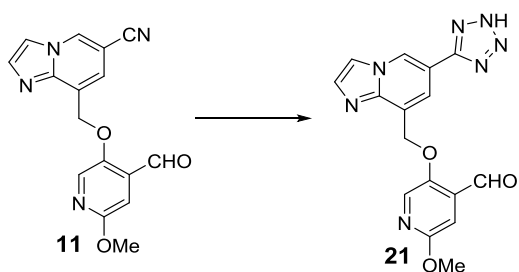
5-((6-(1H-pyrazol-4-yl)imidazo[1,2-a]pyridin-8-yl)methoxy)-2-methoxyisonicotinaldehyde (20).



Step 1. To a mixture of 5-((6-bromoimidazo[1,2-a]pyridin-8-yl)methoxy)-2-methoxyisonicotinaldehyde (100 mg, 0.28 mmol, 1 equiv), tert-butyl 4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1H-pyrazole-1-carboxylate (87 mg, 0.36 mmol, 1.5 equiv), Pd(PPh₃)₄ (65 mg, 0.056 mmol, 0.2 equiv), K₂CO₃ (155 mg, 1.12 mmol, 4 equiv) in a 5 mL microwave tube was added DMF (2 mL). The mixture was heated 30 min at 125 °C in a microwave reactor. The solid was filtered off and the filtrate was concentrated to dryness. The crude was purified on silica gel using a mixture of EtOAc and hexanes as eluent to give tert-butyl 4-(8-(((4-formyl-6-methoxy-pyridin-3-yl)oxy)methyl)imidazo[1,2-a]pyridin-6-yl)-1H-pyrazole-1-carboxylate (84 mg, 67%) as a colorless oil which was used directly for the next step without further purification.

Step 2. The Boc-intermediate (45 mg, 0.1 mmol) was dissolved in DCM (3 mL), to this was added TFA (1 mL). The mixture was allowed to stir at rt for 2h and then concentrated. The crude product was purified on silica gel using a mixture of EtOAc and hexanes as eluent to give **20** (5.0 mg, 14%) as a white solid. MS (ESI) *m/z* 350.1 [M+H]⁺; ¹H NMR (400 MHz, Acetonitrile-*d*₃) δ 10.48 (s, 1H), 8.58 (d, *J* = 1.7 Hz, 1H), 8.33 (s, 1H), 7.94 (s, 2H), 7.81 (d, *J* = 1.2 Hz, 1H), 7.67 (d, *J* = 1.5 Hz, 1H), 7.60 (d, *J* = 1.2 Hz, 1H), 7.05 (d, *J* = 0.7 Hz, 1H), 5.65 (d, *J* = 0.8 Hz, 2H), 3.90 (s, 3H).

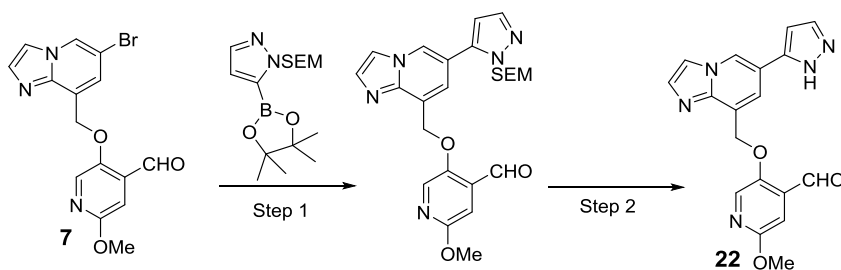
5-((6-(2H-tetrazol-5-yl)imidazo[1,2-a]pyridin-8-yl)methoxy)-2-methoxyisonicotinaldehyde (**21**).



A mixture of 8-(((4-formyl-6-methoxy-pyridin-3-yl)oxy)methyl)imidazo[1,2-a]pyridine-6-carbonitrile (**11**) (51mg, 0.166 mmol), trimethylamine HCl salt (91 mg, 0.66 mmol), and sodium azide (36 mg, 0.66 mmol) in chlorobenzene (5.0 mL) was heated to 110 °C for 2 h under N₂. It was then cooled, filtered, concentrated, and purified on silica gel using a mixture of EtOAc and hexanes as eluent to give the product as a light yellow solid in 12% yield. MS (ESI) *m/z* 352.1 [M+H]⁺; ¹H NMR (400 MHz, DMSO-

d_6) δ 10.41 (s, 1H), 9.12 (d, $J = 1.6$ Hz, 1H), 8.44 (s, 1H), 8.33 (s, 1H), 8.12 (d, $J = 1.2$ Hz, 1H), 8.04 (d, $J = 1.5$ Hz, 1H), 7.60 (d, $J = 1.2$ Hz, 1H), 6.97 (s, 1H), 5.67 (s, 2H), 3.84 (s, 3H).

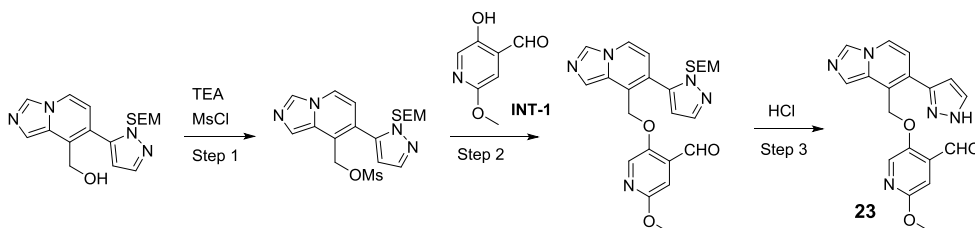
5-((6-(1H-pyrazol-5-yl)imidazo[1,2-a]pyridin-8-yl)methoxy)-2-methoxyisonicotinaldehyde (22).



Step 1. To a mixture of 5-((6-bromoimidazo[1,2-a]pyridin-8-yl)methoxy)-2-methoxyisonicotinaldehyde (100 mg, 0.28 mmol, 1 equiv), 5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1-((2-(trimethylsilyl)ethoxy)methyl)-1H-pyrazole (87 mg, 0.36 mmol, 1.5 equiv), Pd(PPh₃)₄ (65 mg, 0.056 mmol, 0.2 equiv), K₂CO₃ (155 mg, 1.12 mmol, 4 equiv) in a 5 mL microwave tube was added DMF (2 mL). The mixture was heated 30 min at 125 °C in a microwave reactor. The solid was filtered off and the filtrate was concentrated to dryness. The crude was purified on silica gel using a mixture of EtOAc and hexanes as eluent to give 2-methoxy-5-((6-(1-((2-(trimethylsilyl)ethoxy)methyl)-1H-pyrazol-5-yl)imidazo[1,2-a]pyridin-8-yl)methoxy)isonicotinaldehyde (84 mg, 63%) as a colorless oil.

Step 2. To the SEM protected intermediate (82 mg, 0.17 mmol) in EtOH (4.0 mL) was aqueous 3N HCl (0.6 mL). The mixture was stirred at rt overnight. It was basified with 3 N NaOH to pH around 8, extracted with EtOAc. The crude product was purified on silica gel using a mixture of EtOAc and hexanes as eluent to give the title compound as a white solid in 49% yield. MS (ESI) m/z 350.3 [M+H]⁺; ¹H NMR (400 MHz, DMSO- d_6) δ 13.02 (s, 1H), 10.39 (s, 1H), 9.04 (d, $J = 1.7$ Hz, 1H), 8.44 (s, 1H), 8.03 (d, $J = 1.2$ Hz, 1H), 7.96 (d, $J = 1.6$ Hz, 1H), 7.85 (dd, $J = 2.4, 1.5$ Hz, 1H), 7.61 (d, $J = 1.3$ Hz, 1H), 6.99 (s, 1H), 6.76 (t, $J = 2.1$ Hz, 1H), 5.66 (s, 2H), 3.85 (s, 3H).

5-((6-(1H-pyrazol-5-yl)imidazo[1,2-a]pyridin-2-yl)methoxy)-2-methoxyisonicotinaldehyde (23).

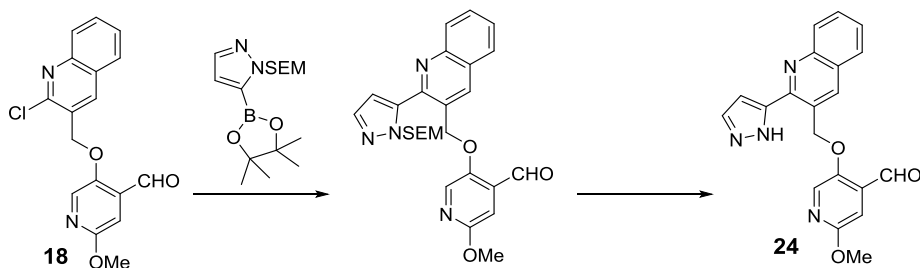


Step 1. To a solution of (7-(1-((2-(trimethylsilyl)ethoxy)methyl)-1H-pyrazol-5-yl)imidazo[1,5-a]pyridin-8-yl)methanol (46 mg, 0.133 mmol) in THF (10 mL) was added TEA (117 μ L, 0.6 mmol) and MsCl (37 μ L, 0.48 mmol). The mixture was stirred at ambient temperature until completion. It was diluted with water and extracted with EtOAc. The crude product was used directly for Step 2 without purification.

Step 2. To a solution of above crude product in DMF (5 mL) was added **INT-1** (60 mg, 0.4 mmol) and K_2CO_3 (73 mg, 0.53 mmol). The mixture was heated at 70 $^{\circ}C$ for 2 h. It was filtered and the filtrate was concentrated. The crude product was purified on silica gel using a mixture of EtOAc and hexanes as eluent to give 2-methoxy-5-((7-(1-((2-(trimethylsilyl)ethoxy)methyl)-1H-pyrazol-5-yl)imidazo[1,5-a]pyridin-8-yl)methoxy)isonicotinaldehyde (21 mg, 33% yield).

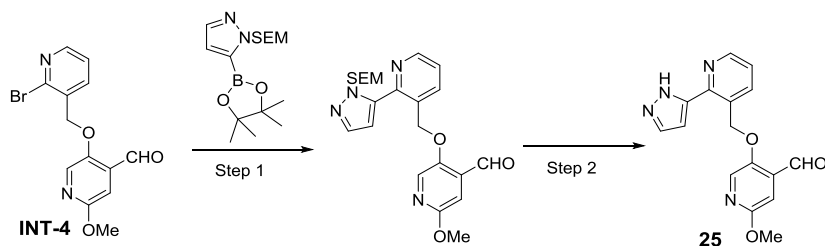
Step 3. To a solution of SEM protected intermediate (21 mg, 0.044 mmol) in EtOH (2 mL) was added HCl (3N solution, 0.16 mL, 0.44 mmol). The mixture was stirred at ambient temperature for 15 h. It was basified to pH = 9 and extracted with EtOAc. The crude product was purified on silica gel using a mixture of EtOAc and hexanes as eluent to give 5-((6-(1H-pyrazol-5-yl)imidazo[1,2-a]pyridin-2-yl)methoxy)-2-methoxyisonicotinaldehyde (2.8 mg, 14% yield). MS (ESI) m/z 350.1[M+H]⁺; ¹H NMR (400 MHz, Chloroform-*d*) δ 10.24 (s, 1H), 8.09 (d, J = 0.8 Hz, 1H), 8.01 (s, 1H), 7.92 (d, J = 7.3 Hz, 1H), 7.61 (d, J = 2.4 Hz, 1H), 7.53 (t, J = 0.9 Hz, 1H), 6.99 (d, J = 0.6 Hz, 1H), 6.82 (d, J = 7.3 Hz, 1H), 6.49 (d, J = 2.4 Hz, 1H), 5.57 (s, 2H), 3.83 (s, 3H), 1.21 (s, 1H).

5-((2-(1H-pyrazol-5-yl)quinolin-4-yl)methoxy)-2-methoxyisonicotinaldehyde (**24**).



The title compound was prepared using similar procedures as these for **22**, starting from 5-((2-chloroquinolin-3-yl)methoxy)-2-methoxyisonicotinaldehyde (**18**) and 5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1-((2-(trimethylsilyl)ethoxy)methyl)-1H-pyrazole, the product was obtained as a white solid in 33% yield over two steps. MS (ESI) m/z 361.3 [M+H]⁺; ¹H NMR (400 MHz, Chloroform-*d*) δ 10.38 (s, 1H), 8.35 (s, 1H), 8.12-8.03 (m, 2H), 7.84 – 7.76 (m, 1H), 7.70 (ddd, J = 8.5, 6.9, 1.5 Hz, 1H), 7.64 (d, J = 2.3 Hz, 1H), 7.52 (ddd, J = 8.1, 6.9, 1.2 Hz, 1H), 7.06 (d, J = 0.6 Hz, 1H), 5.66 (s, 2H), 3.85 (s, 3H).

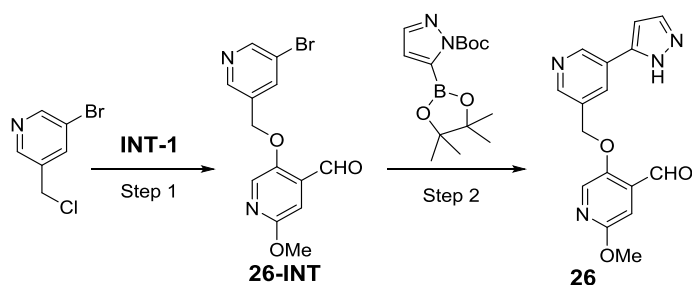
5-((2-(1H-pyrazol-5-yl)pyridin-3-yl)methoxy)-2-methoxyisonicotinaldehyde (25).



Step 1: 5-((2-bromopyridin-3-yl)methoxy)-2-methoxyisonicotinaldehyde (INT-4) (258 mg, 0.8 mmol, 1 equiv), 1-((2-(trimethylsilyl)ethoxy)methyl)-1H-pyrazol-5-ylboronic acid (233 mg, 0.96 mmol, 1.2 equiv), Pd(PPh₃)₄ (92 mg, 0.08 mmol, 0.1 equiv), K₂CO₃ (331 mg, 2.4 mmol, 3 equiv) in a round bottom flask were added dioxane (8 mL) and water (2 mL). The mixture was heated 2 h at 90 °C, cooled, filtered, and concentrated. The crude was purified on silica gel using a mixture of EtOAc and hexanes as eluent to give 2-methoxy-5-((2-(1-((2-(trimethylsilyl)ethoxy)methyl)-1H-pyrazol-5-yl)pyridin-3-yl)methoxy)isonicotinaldehyde (208 mg, 79%) as a white solid. MS (ESI) *m/z* 441.2 [M+H]⁺; ¹H NMR (400 MHz; CDCl₃) δ 10.54 (s, 1 H), 8.85 (d, 1 H), 8.18 (d, 1 H) 8.03 (s, 1 H), 7.73 (d, 1 H), 7.56 (dd, 1 H), 7.21 (s, 1 H), 6.60 (d, 1H), 5.79 (s, 2 H), 5.27 (s, 2 H), 4.01 (s, 3 H), 3.65 (t, 2 H), 0.88 (t, 2 H), 0.05 (s, 9 H).

Step 2: To 2-methoxy-5-((2-(1-((2-(trimethylsilyl)ethoxy)methyl)-1H-pyrazol-5-yl)pyridin-3-yl)methoxy)isonicotinaldehyde (120 mg, 0.27 mmol, 1 equiv) suspended in EtOH (1 mL) was added HCl (1.0 mL, 3 N). The solution turned homogeneous and the mixture was stirred at rt overnight. The EtOH was partially removed by blowing in N₂ gas and the precipitate was collected. The solid was washed with acetonitrile and EtOAc and dried under high *vacuo* to give 5-((2-(1H-pyrazol-5-yl)pyridin-3-yl)methoxy)-2-methoxyisonicotinaldehyde dihydrochloride (100 mg, 96%) as a white solid. MS (ESI) *m/z* 311.1 [M+H]⁺; ¹H NMR (400 MHz; DMSO, 80 °C) δ 10.27 (s, 1 H), 8.68 (br, 1 H), 8.32 (br, 1 H), 8.22 (s, 1 H), 7.82 (br, 1 H), 7.57 (br, 1 H), 7.00 (br, 2 H), 5.75 (s, 2 H), 3.89 (s, 3 H).

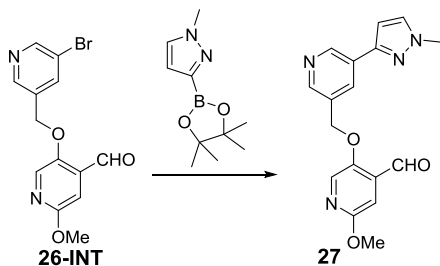
5-((5-(1H-pyrazol-5-yl)pyridin-3-yl)methoxy)-2-methoxyisonicotinaldehyde (26).



Step 1. 005-59 was prepared from INT-1 and 3-bromo-5-(chloromethyl)pyridine according to the general procedure step 2 to give the product as an off-white solid in 63% yield. MS (ESI) m/z 323.0, 325.0 $[M+H]^+$; 1H NMR (400 MHz, Chloroform- d) δ 10.47 (d, $J = 0.9$ Hz, 1H), 8.73 (d, $J = 2.1$ Hz, 1H), 8.65 (d, $J = 1.7$ Hz, 1H), 8.07 (d, $J = 0.9$ Hz, 1H), 7.99 (q, $J = 2.2, 1.6$ Hz, 1H), 7.15 (s, 1H), 5.22 (s, 2H), 3.95 (d, $J = 0.9$ Hz, 3H).

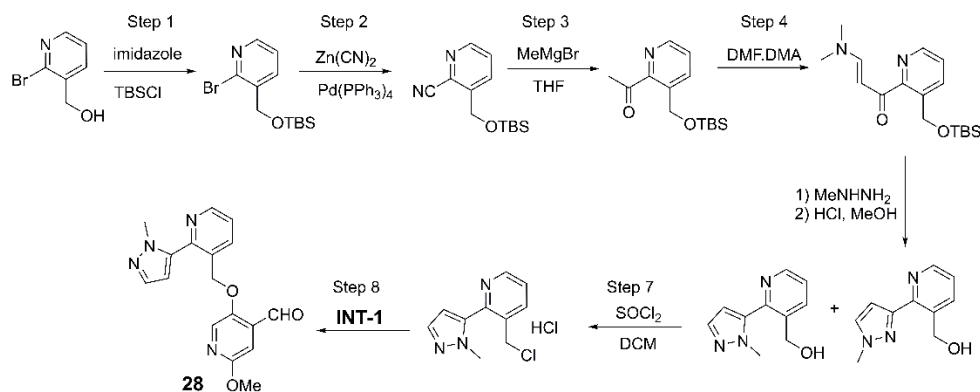
Step 2. The title compound was prepared using similar procedures as these for **20**, starting from 5-((2-bromopyridin-3-yl)methoxy)-2-methoxyisonicotinaldehyde and tert-butyl 5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1H-pyrazole-1-carboxylate, the product was obtained as a white solid in 30% yield over two steps. MS (ESI) m/z 311.1 $[M+H]^+$; 1H NMR (400 MHz, Chloroform- d) δ 10.50 (d, $J = 0.5$ Hz, 1H), 9.07 (s, 1H), 8.68 (s, 1H), 8.27 (s, 1H), 8.12 (s, 1H), 7.71 (d, $J = 2.5$ Hz, 1H), 7.14 (s, 1H), 6.76 (d, $J = 2.3$ Hz, 1H), 5.30 (s, 2H), 3.94 (d, $J = 0.5$ Hz, 3H), 3.52 (d, $J = 0.5$ Hz, 1H).

2-methoxy-5-((5-(1-methyl-1H-pyrazol-3-yl)pyridin-3-yl)methoxy)isonicotinaldehyde (**27**).



The title compound was prepared using similar procedures as these for **20**, step1 (Suzuki coupling), starting from 5-((2-bromopyridin-3-yl)methoxy)-2-methoxyisonicotinaldehyde (**26-INT**) and 1-methyl-3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1H-pyrazole, the product was obtained as an off-white solid in 56% yield. MS (ESI) m/z 325.1 $[M+H]^+$; 1H NMR (400 MHz, Chloroform- d) δ 10.45 (s, 1H), 8.77 (d, $J = 2.1$ Hz, 1H), 8.74 (d, $J = 2.2$ Hz, 1H), 8.14 – 8.06 (m, 1H), 7.89 (t, $J = 2.1$ Hz, 1H), 7.59 (d, $J = 1.9$ Hz, 1H), 7.14 (d, $J = 0.6$ Hz, 1H), 6.43 (d, $J = 1.9$ Hz, 1H), 5.31 (s, 2H), 3.96 (s, 3H), 3.95 (s, 3H).

2-methoxy-5-((2-(1-methyl-1H-pyrazol-5-yl)pyridin-3-yl)methoxy)isonicotinaldehyde (28).



Step 1. To a mixture of (2-bromopyridin-3-yl)methanol (20.0 g, 106.4 mmol, 1 equiv; refer to example 14) and imidazole (14.5 g, 212.8 mmol, 2 equiv) in DMF (50.0 mL) was added TBSCl (19.2 g, 150.7 mmol, 1.2 eq.) at rt. The mixture was stirred at rt for 1 h and diluted with a mixture of water (100 mL) and EtOAc (300 mL). The organic layer was washed with $\text{NH}_4\text{Cl}_{(\text{sat.})}$ solution and brine, dried over Na_2SO_4 , concentrated, and purified on silica gel using 10% EtOAc/hexanes as eluent to give 2-bromo-3-((tert-butyldimethylsilyloxy)methyl)pyridine (30.1 g, 94%) as a colorless oil. MS (ESI) m/z 302.0 $[\text{M}+\text{H}]^+$.

Step 2. A mixture of 2-bromo-3-((tert-butyldimethylsilyloxy)methyl)pyridine (30.1 g, 100.0 mmol, 1 eq.) and $\text{Zn}(\text{CN})_2$ (23.5 g, 200.0 mmol, 2.0 equiv) in DMF (100.0 mL) was purged with N_2 for 5 min and added $\text{Pd}(\text{PPh}_3)_4$ (5.78 g, 5.0 mmol, 0.05 equiv). The mixture was heated at 120 °C for 2 h under N_2 , cooled, filtered, concentrated, and purified on silica gel using a mixture of EtOAc and hexanes as eluent to give 3-((tert-butyldimethylsilyloxy)methyl)picolinonitrile (20.4 g, 82%) as a colorless oil. MS (ESI) m/z 249.1 $[\text{M}+\text{H}]^+$.

Step 3. Methylmagnesium bromide (3M/ether, 41.0 mL, 123.4 mmol) was added to a stirred solution of 3-((tert-butyldimethylsilyloxy)methyl)picolinonitrile (20.4 g, 82.25 mmol) in THF (100.0 mL) at -78 °C. The reaction mixture was warm to rt, quenched with aqueous citric acid solution, and extracted with EtOAc (50 mL) twice. The combined organic layers were washed with $\text{NaHCO}_3_{(\text{sat.})}$ solution and brine, dried over Na_2SO_4 , concentrated, and purified on silica gel using a mixture of EtOAc/hexanes as eluent to give 1-(3-((tert-butyldimethylsilyloxy)methyl)pyridin-2-yl)ethanone (12.9 g, 59%) as a colorless oil. MS (ESI) m/z 266.2 $[\text{M}+\text{H}]^+$.

Step 4. 1-(3-((tert-butyldimethylsilyloxy)methyl)pyridin-2-yl)ethanone (10.8 g, 40.75 mmol) in dimethoxy-N,N-dimethylmethanamine (15.0 mL) was heated to reflux for 3 days. The mixture was concentrated and used for next step without further purification. MS (ESI) m/z 321.1 $[\text{M}+\text{H}]^+$.

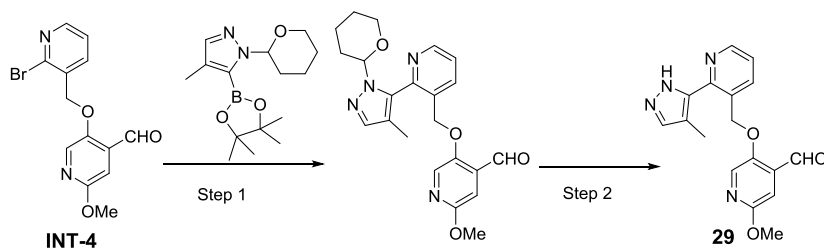
Step 5. To (*E*)-1-(3-((tert-butyldimethylsilyloxy)methyl)pyridin-2-yl)-3-(dimethylamino)prop-2-en-1-one (crude above, 966.4 mg, 3.02 mmol, 1 eq.) in EtOH (10 mL) was added methylhydrazine (1.0 mL) at rt. The mixture was heated at 80 °C for 2 h, concentrated, and purified on silica gel using a mixture of EtOAc and hexanes as eluent to give a mixture of regio-isomers (420 mg; 46% for 2 steps). MS (ESI) m/z 304.2 $[M+H]^+$.

Step 6. To a mixture of 3-((tert-butyldimethylsilyloxy)methyl)-2-(1-methyl-1H-pyrazol-5-yl)pyridine and 3-((tert-butyldimethylsilyloxy)methyl)-2-(1-methyl-1H-pyrazol-3-yl)pyridine (420 mg, 1.38 mmol) in MeOH (20 mL) was added HCl (4 N, 2.0 mL). The mixture was stirred at rt for 1 h, concentrated, and diluted with EtOAc (50 mL) and NaHCO₃ (sat) solution (10 mL). The layers were separated and aqueous layer was extracted with EtOAc three times. The combined organic layers were dried over Na₂SO₄, concentrated, and purified on silica gel using EtOAc as eluent to give (2-(1-methyl-1H-pyrazol-5-yl)pyridin-3-yl)methanol (187 mg, 72%) and (2-(1-methyl-1H-pyrazol-3-yl)pyridin-5-yl)methanol (55 mg, 21%) as white solids. Data for 2-(1-methyl-1H-pyrazol-5-yl)pyridin-3-yl)methanol. MS (ESI) m/z 190.1 $[M+H]^+$. ¹H NMR (400 MHz; CDCl₃) 8.58 (d, 1 H), 7.91 (d, 1 H), 7.46 (s, 1H), 7.30 (dd, 1 H), 6.36 (s, 1 H), 4.62 (d, 2 H), 3.83 (s, 3 H), 2.1 (t, 1 H). MS (M+H⁺) m/z 190.1; data for (2-(1-methyl-1H-pyrazol-3-yl)pyridin-5-yl)methanol: ¹H NMR (400 MHz; CDCl₃) 8.60 (d, 1 H), 7.70 (d, 1 H), 7.47 (s, 1H), 7.22 (dd, 1 H), 6.99 (s, 1 H), 5.91 (t, 1 H), 4.68 (d, 2 H), 4.01 (s, 3 H).

Step 7. To (2-(1-methyl-1H-pyrazol-5-yl)pyridin-3-yl)methanol (182 mg, 0.96 mmol) in DCM (5 mL) was added SOCl₂ (1.5 mL) at rt. The reaction mixture was stirred at rt for 4 h and concentrated to dryness. The crude solid was suspended in toluene and concentrated to dryness. The process was repeated three times and dried under vacuum to give 3-(chloromethyl)-2-(1-methyl-1H-pyrazol-5-yl)pyridine (236 mg) as an off-white solid, which was used for next step without further purification. MS (ESI) m/z 208.0, 210.0 $[M+H]^+$; ¹H NMR (400 MHz, DMSO-*d*₆) δ 8.69 (dd, *J* = 4.8, 1.7 Hz, 1H), 8.09 (dd, *J* = 7.9, 1.7 Hz, 1H), 7.57 – 7.48 (m, 2H), 6.61 (d, *J* = 1.9 Hz, 1H), 4.73 (s, 2H), 3.75 (s, 3H).

Step 8. A mixture of 5-hydroxy-2-methoxyisonicotinaldehyde (147 mg, 0.96 mmol, 1 eq.), 3-(chloromethyl)-2-(1-methyl-1H-pyrazol-5-yl)pyridine hydrochloride (236 mg, 0.96 mmol, 1 equiv), and K₂CO₃ (532 mg, 3.85 mmol, 3 equiv) in DMF (3.0 mL) was heated at 70 °C for 2 h. The mixture was cooled, filtered, concentrated, and purified on silica gel using a mixture of EtOAc and hexanes as eluent to give 2-methoxy-5-((2-(1-methyl-1H-pyrazol-5-yl)pyridin-3-yl)methoxy)isonicotinaldehyde (232.5 mg, 75%) as an off-white solid. MS (ESI) m/z 325.1 $[M+H]^+$; ¹H NMR (400 MHz, CDCl₃) δ 10.40 (s, 1H), 8.77 (dd, *J* = 4.7, 1.7 Hz, 1H), 8.03 (dd, *J* = 7.9, 1.7 Hz, 1H), 7.93 (s, 1H), 7.55 (d, *J* = 1.9 Hz, 1H), 7.44 (dd, *J* = 7.9, 4.8 Hz, 1H), 7.11 (d, *J* = 0.4 Hz, 1H), 6.43 (d, *J* = 1.9 Hz, 1H), 5.20 (s, 2H), 3.97 (s, 3H), 3.92 (s, 3H).

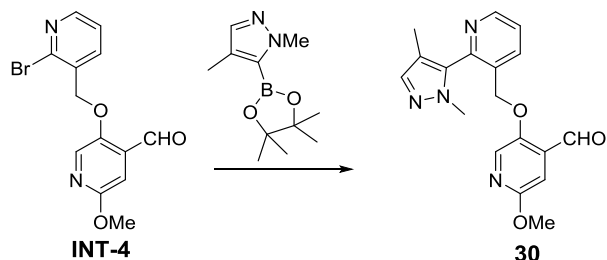
2-methoxy-5-((2-(4-methyl-1H-pyrazol-5-yl)pyridin-3-yl)methoxy)isonicotinaldehyde (**29**).



Step 1. To a mixture of 5-((2-bromopyridin-3-yl)methoxy)-2-methoxyisonicotinaldehyde (100 mg, 0.31 mmol, 1 equiv), 4-methyl-1-(tetrahydro-2H-pyran-2-yl)-1H-pyrazol-5-ylboronic acid (98 mg, 0.47 mmol, 1.5 equiv), Pd(PPh₃)₄ (70 mg, 0.06 mmol, 0.2 equiv), K₂CO₃ (171 mg, 1.24 mmol, 4 equiv) in a 5 mL microwave tube was added DMF (2 mL). The mixture was heated 30 min at 125 °C in a microwave reactor. The solid was filtered off and the filtrate was concentrated to dryness. The crude was purified on silica gel using a mixture of EtOAc and hexanes as eluent to give 2-methoxy-5-((2-(4-methyl-1-(tetrahydro-2H-pyran-2-yl)-1H-pyrazol-5-yl)pyridin-3-yl)methoxy)isonicotinaldehyde (110 mg, 87%) as a colorless oil. MS (ESI) *m/z* 409.2 [M+H]⁺.

Step 2. To 2-methoxy-5-((2-(4-methyl-1-(tetrahydro-2H-pyran-2-yl)-1H-pyrazol-5-yl)pyridin-3-yl)methoxy)isonicotinaldehyde (110 mg, 0.27 mmol, 1 equiv) suspended in EtOH (1 mL) was added HCl (1.0 mL, 3 N). The solution turned homogeneous and the mixture was stirred at rt overnight. The EtOH was partially removed by blowing in N₂ gas and basified to pH 9. The aqueous solution was extracted with EtOAc three times. The organic layer was dried over Na₂SO₄ and concentrated. The crude was purified on silica gel using a mixture of MeOH and DCM as eluent to give 2-methoxy-5-((2-(4-methyl-1H-pyrazol-5-yl)pyridin-3-yl)methoxy)isonicotinaldehyde (40 mg, 46%) as a white solid. MS (ESI) *m/z* 325.1 [M+H]⁺; ¹H NMR (400 MHz; CDCl₃) δ 10.45 (s, 1 H), 8.76 (d, 1 H), 8.07 (br, 1 H), 8.05 (s, 1H), 7.53 (s, 1 H), 7.40 (dd, 1 H), 7.13 (s, 1 H), 5.52 (br, 2 H), 3.98 (s, 3 H).

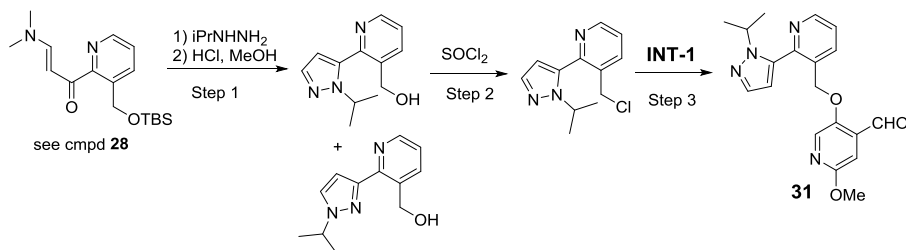
5-((2-(1,4-dimethyl-1H-pyrazol-5-yl)pyridin-3-yl)methoxy)-2-methoxyisonicotinaldehyde (**30**).



The title compound was prepared using similar procedures as these for **20**, step1 (Suzuki coupling), starting from 5-((2-bromopyridin-3-yl)methoxy)-2-methoxyisonicotinaldehyde (INT4) and 1,4-dimethyl-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1H-pyrazole, the product was obtained as a

light yellow oil in 82% yield. MS (ESI) m/z 339.2 $[M+H]^+$; 1H NMR (400 MHz, Chloroform- d) δ 10.38 (s, 1H), 8.78 (dd, $J = 4.8, 1.7$ Hz, 1H), 8.08 (dt, $J = 8.0, 1.1$ Hz, 1H), 7.82 (s, 1H), 7.49 (dd, $J = 7.9, 4.8$ Hz, 1H), 7.44 – 7.38 (m, 1H), 7.08 (d, $J = 0.6$ Hz, 1H), 5.04 (d, $J = 13.7$ Hz, 2H), 3.90 (s, 3H), 3.72 (s, 3H), 1.96 (s, 3H).

5-((2-(1-isopropyl-1H-pyrazol-5-yl)pyridin-3-yl)methoxy)-2-methoxyisonicotinaldehyde (31).

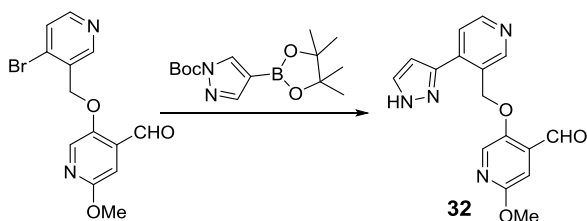


Step 1. To (*E*)-1-(3-((tert-butyldimethylsilyloxy)methyl)pyridin-2-yl)-3-(dimethylamino)prop-2-en-1-one (crude, 1.03 g, 3.22 mmol, 1 eq.; refer to Example 28, step 4) in EtOH (10 mL) was added isopropylhydrazine hydrochloride (430 mg, 3.86 mmol, 1.2 eq.). The mixture was heated at 80 °C for 2 h, cooled, added HCl (6 N, 0.5 mL), and stirred overnight. The mixture was concentrated and diluted with EtOAc (80 mL) and NaHCO₃(sat) (10 mL) solution. The layers were separated and the aqueous layer was extracted with EtOAc three times. The combined organic layers were dried over Na₂SO₄, concentrated, and purified on silica gel using EtOAc as eluent to give (2-(1-isopropyl-1H-pyrazol-3-yl)pyridin-3-yl)methanol (500 mg, 71%) and (2-(1-isopropyl-1H-pyrazol-5-yl)pyridin-3-yl)methanol (55 mg, 25%) as pale yellow oils. Data for 2-(1-isopropyl-1H-pyrazol-5-yl)pyridin-3-yl)methanol. MS (ESI) m/z 218.1 $[M+H]^+$; 1H NMR (400 MHz, CDCl₃) δ 8.67 (dd, $J = 4.7, 1.5$ Hz, 1H), 8.0 (d, $J = 7.8$ Hz, 1H), 7.61 (d, $J = 1.8$ Hz, 1H), 7.39 (dd, $J = 7.8, 4.8$ Hz, 1H), 6.37 (d, $J = 1.8$ Hz, 1H), 4.67 (s, 2H), 4.55 (sep, $J = 6.6$ Hz 1H), 1.98-2.05 (br, 1H), 1.47 (d, $J = 6.6$ Hz, 6H); Data for (2-(1-isopropyl-1H-pyrazol-3-yl)pyridin-5-yl)methanol: MS ($M+H^+$) m/z 218.1; 1H NMR (400 MHz, CDCl₃) δ 8.62 (dd, $J = 4.8, 1.6$ Hz, 1H), 7.72 (d, $J = 7.6$ Hz, 1H), 7.55 (d, $J = 2.4$ Hz, 1H), 7.23 (dd, $J = 7.6, 4.8$ Hz, 1H), 6.99 (dd, $J = 8.0, 6.5$ Hz, 1H), 6.07 (t, $J = 7.6$ Hz, 1H), 4.67 (d, $J = 7.6$ Hz, 2H), 4.58 (sep, $J = 6.7$ Hz, 1H), 1.60 (d, $J = 6.7$ Hz, 1H).

Step 2. To (2-(1-isopropyl-1H-pyrazol-5-yl)pyridin-3-yl)methanol (560 mg, 2.58 mmol) in DCM (10 mL) was added SOCl₂ (3.0 mL) at rt. The reaction mixture was stirred at rt for 4 h and concentrated to dryness. The crude solid was suspended in toluene and concentrated to dryness. The process was repeated three times and dried under vacuum to give 3-(chloromethyl)-2-(1-isopropyl-1H-pyrazol-5-yl)pyridine hydrochloride (700 mg) as an off-white solid, which was used for next step without further purification.

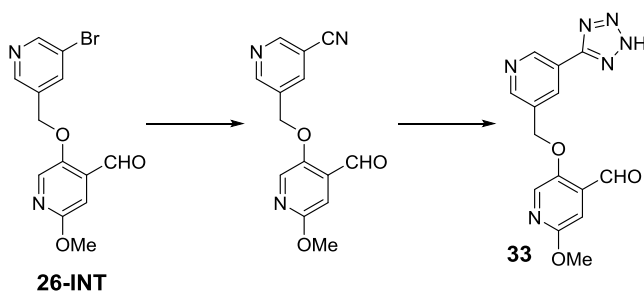
Step 3. A mixture of 5-hydroxy-2-methoxyisonicotinaldehyde (395 mg, 2.58 mmol, 1 eq.), 3-(chloromethyl)-2-(1-isopropyl-1H-pyrazol-5-yl)pyridine hydrochloride (700 mg, 2.58 mmol, 1 equiv), and K_2CO_3 (1.4 g, 10.32 mmol, 4 equiv) in DMF (10.0 mL) was heated at 70 °C for 2 h. The mixture was cooled, filtered, concentrated, and purified on silica gel using a mixture of EtOAc and hexanes as eluent to give 5-((2-(1-isopropyl-1H-pyrazol-5-yl)pyridin-3-yl)methoxy)-2-methoxyisonicotinaldehyde (**31**) (590 mg, 65%) as an off-white solid. HRMS calcd for $C_{19}H_{21}N_4O_3$ ($M+H^+$) 353.1608, found 353.1606; MS (ESI) m/z 353.1 [$M+H^+$]⁺; ¹H NMR (400 MHz, $CDCl_3$) δ 10.41 (s, 1H), 8.76 (dd, $J = 4.7$, 1.6 Hz, 1H), 8.04 (dd, $J = 7.9$, 1.6 Hz, 1H), 7.90 (s, 1H), 7.61 (d, $J = 1.8$ Hz, 1H), 7.44 (dd, $J = 7.9$, 4.8 Hz, 1H), 7.10 (s, 1H), 6.37 (d, $J = 1.8$ Hz, 1H), 5.14 (s, 2H), 4.65 (sep, $J = 6.6$ Hz, 1H), 3.91 (s, 3H), 1.49 (d, $J = 6.6$ Hz, 6H); ¹³C NMR (101 MHz, Chloroform-*d*) δ 188.68, 159.71, 149.69, 149.49, 149.26, 138.37, 137.85, 136.87, 133.85, 133.03, 131.26, 123.55, 108.80, 107.12, 68.68, 54.12, 51.01, 22.97.

5-((5-(1H-pyrazol-4-yl)pyridin-3-yl)methoxy)-2-methoxyisonicotinaldehyde (**32**).



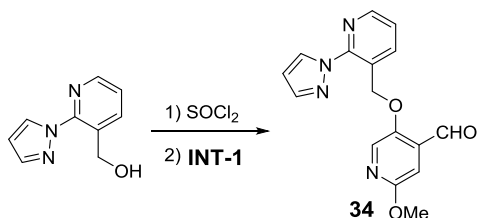
The title compound was prepared using similar procedures as these for **20**, starting from 5-((4-bromophenyl)methoxy)-2-methoxyisonicotinaldehyde and tert-butyl 4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1H-pyrazole-1-carboxylate, the product was obtained as a white solid in 63% yield over two steps. MS (ESI) m/z 311.1 [$M+H^+$]⁺; ¹H NMR (400 MHz, $DMSO-d_6$) δ 13.01 (s, 1H), 10.17 (s, 1H), 8.76 (t, $J = 2.9$ Hz, 1H), 8.57 (dt, $J = 5.2$, 2.8 Hz, 1H), 8.33 (d, $J = 4.0$ Hz, 1H), 8.09 (s, 1H), 7.98 (s, 1H), 7.56 (s, 1H), 7.01 (d, $J = 2.8$ Hz, 1H), 5.46 (d, $J = 2.8$ Hz, 2H), 3.93 (t, $J = 2.3$ Hz, 3H).

5-((5-(2H-tetrazol-5-yl)pyridin-3-yl)methoxy)-2-methoxyisonicotinaldehyde (**33**).



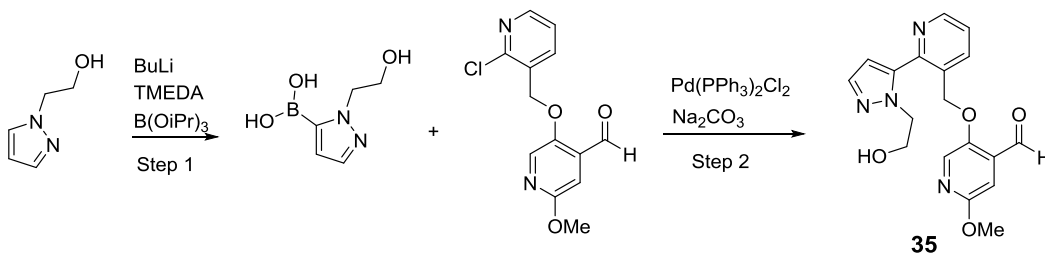
The title compound was prepared using similar procedures as these for **21**, starting from 5-((5-bromopyridin-3-yl)methoxy)-2-methoxyisonicotinaldehyde, the product was obtained as a white solid in 13% yield over two steps. MS (ESI) m/z 313.2 $[M+H]^+$; 1H NMR (400 MHz, DMSO- d_6) δ 10.40 (s, 1H), 9.20 (d, $J = 2.1$ Hz, 1H), 8.90 (d, $J = 2.1$ Hz, 1H), 8.55 (t, $J = 2.1$ Hz, 1H), 8.38 (s, 1H), 7.02 (s, 1H), 5.49 (s, 2H), 3.86 (s, 3H).

5-((2-(1H-pyrazol-1-yl)pyridin-3-yl)methoxy)-2-methoxyisonicotinaldehyde (34).



The title compound was prepared from INT-1 and (2-(1H-pyrazol-1-yl)pyridin-3-yl)methanol according to the general procedure steps 1&2 to give the product as a white solid in 6% yield over two steps. MS (ESI) m/z 311.2 $[M+H]^+$; 1H NMR (400 MHz, CDCl₃) δ 10.47 (s, 1H), 8.53 (d, $J = 2.1$ Hz, 1H), 8.48 (d, $J = 4.7$ Hz, 1H), 8.19 (d, $J = 7.8$ Hz, 1H), 8.10 (s, 1H), 7.75 (s, 1H), 7.35 (dd, $J = 7.7, 4.7$ Hz, 1H), 7.12 (s, 1H), 6.51 (dd, $J = 2.5, 1.8$ Hz, 1H), 5.75 (s, 2H), 3.93 (s, 3H).

5-((2-(1-(2-hydroxyethyl)-1H-pyrazol-5-yl)pyridin-3-yl)methoxy)-2-methoxyisonicotinaldehyde (35).

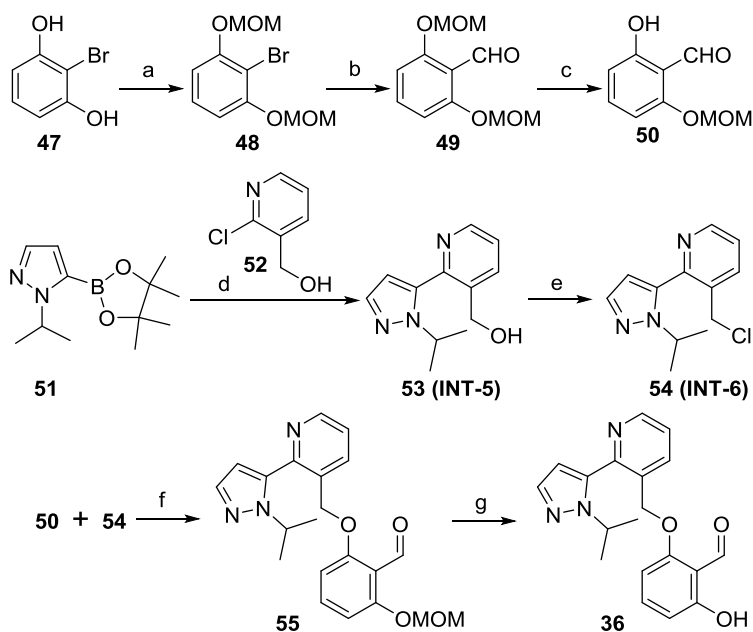


Step 1. To a solution of 2-(1H-pyrazol-1-yl)ethan-1-ol (250 mg, 2.23 mmol) in THF (7.5 mL) at 0 °C was added TMEDA (670 μ L, 4.46 mmol) and BuLi (2.5 M, 2 mL, 5 mmol) dropwise. The mixture was stirred at 0 °C for 30 min. The mixture was added triisopropylborate (620 μ L, 2.68 mmol). Ice bath was removed and the reaction mixture was warmed up to ambient temperature. After stirred for additional 3 h, the reaction mixture was quenched with MeOH. After removal of organic solvents, the crude product was used directly for Step 2 without purification.

Step 2. To a solution of above crude product in dioxane (6 mL) was added 5-((2-chloropyridin-3-yl)methoxy)-2-methoxyisonicotinaldehyde (145 mg, 0.52 mmol) followed by [1,1'-Bis(diphenylphosphino)ferrocene]palladium(II) dichloride (140 mg, 0.19 mmol) and a solution of

Na₂CO₃ (200 mg, 1.89 mmol) in water (2 mL). The reaction was heated at 100 °C for 1 h. The mixture was filtered and the filtrate was concentrated. The crude product was purified on silica gel using a mixture of EtOAc and hexanes as eluent to give 5-((2-(1-(2-hydroxyethyl)-1H-pyrazol-5-yl)pyridin-3-yl)methoxy)-2-methoxyisonicotinaldehyde (28 mg, 15% yield). MS (ESI) *m/z* 355.2[M+H]⁺; ¹H NMR (400 MHz, Chloroform-*d*) δ 10.38 (s, 1H), 8.70 (dd, *J* = 4.8, 1.8 Hz, 1H), 8.09 (dd, *J* = 7.9, 1.7 Hz, 1H), 7.92 (s, 1H), 7.62 (d, *J* = 2.0 Hz, 1H), 7.47 (dd, *J* = 7.9, 4.9 Hz, 1H), 7.10 (d, *J* = 0.7 Hz, 1H), 6.46 (d, *J* = 2.0 Hz, 1H), 5.48 (s, 1H), 5.22 (d, *J* = 16.9 Hz, 2H), 4.43 – 4.35 (m, 2H), 4.14 – 4.06 (m, 2H), 3.91 (s, 3H).

2-Hydroxy-6-((2-(1-isopropyl-1H-pyrazol-5-yl)pyridin-3-yl)methoxy)benzaldehyde (36).



Step a. To a solution of 2-bromobenzene-1,3-diol (**47**) (5 g, 26.45 mmol) in DCM (50 ml) at 0 °C was added DIPEA (11.54 mL, 66.13 mmol) and MOMCl (4.42 mL, 58.19 mmol). The mixture was stirred at 0 °C for 1.5 h, and then warmed to room temperature. The solution was diluted with DCM, washed with sat. NaHCO₃, brine, dried and concentrated to give crude product, which was purified by column (hexanes/EtOAc=4:1) to give desired product 15.58 g (90%). MS (ESI) *m/z* 244.8 [M-OCH₃]⁺. ¹H-NMR (400 MHz, Chloroform-*d*) δ 7.18 (ddd, *J* = 8.6, 8.1, 0.5 Hz, 1H), 6.84 (d, *J* = 8.2 Hz, 2H), 5.25 (d, *J* = 0.4 Hz, 4H), 3.52 (d, *J* = 0.4 Hz, 6H).

Step b. To a solution of 2-bromo-1,3-bis(methoxymethoxy)benzene (**48**) (19.9g, 71.8 mmol) in THF (150 mL) at -78 °C was added BuLi (2.5 M, 31.6 mL, 79.0 mmol) dropwise. The solution was stirred at -78 °C for 25 min (resulting white cloudy mixture), then it was warmed to 0 °C and stirred for 25 min.

The reaction mixture slowly turns homogenous. To the solution was added DMF at 0 °C. After 25 min, HPLC showed reaction completed. The mixture was quenched with sat. NH₄Cl (150 mL), diluted with ether (300 mL). The organic layer was separated, aq layer was further extracted with ether (2X200 mL), and organic layer was combined, washed with brine, dried and concentrated to give crude product, which was triturated to give 14.6 g desired product. The filtrate was then concentrated and purified by column to give additional 0.7 g, total mass is 15.3 g. MS (ESI) *m/z* 249.3 [M+Na]⁺. ¹H-NMR (400 MHz, CDCl₃) δ 3.51 (s, 6H, 2 OCH₃), 5.28 (s, 4H, 2 OCH₂O), 6.84 (d, 2H, J = 8.40 Hz, H-3, H-5), 7.41 (t, 1H, J = 8.40 Hz, H-4), 10.55 (s, 1H, CHO).

Step c. To a solution of 2,6-bis(methoxymethoxy)benzaldehyde (**49**) (15.3 g, 67.6 mmol) in THF (105 mL) (solvent was purged with N₂) was added conc. HCl (12N, 7 mL) under N₂, then it was further stirred under N₂ for 1.5 h. To the solution was added brine (100 mL) and ether (150 mL). The organic layer was separated and the aqueous layer was further extracted with ether (2x200 mL). The organic layer was combined, washed with brine, dried and concentrated to give crude product, which was purified by column (300g, hexanes/EtOAc=85:15) to give desired product **50** (9.9 g) as yellow liquid. MS (ESI) *m/z* 139.1 [M-CH₂OCH₃]⁺. ¹H-NMR (400 MHz, Chloroform-*d*) δ 7.40 (td, *J* = 8.4, 0.4 Hz, 1H), 7.26 (s, 1H), 7.30 – 7.19 (m, 1H), 6.64 – 6.54 (m, 2H), 5.27 (s, 2H), 3.52 (s, 3H).

Step d. To a 500-mL flask containing the pyrazole boronate (9.0g, 38.1mmol), 2-chloropyridine (5.47g, 38.1mmol), Pd(dppf)Cl₂ ([1,1-bis(diphenylphosphino)ferrocene]dichloropalladium) (1.39g, 1.91mmol, 5%mol), and sodium bicarbonate (9.61g, 114.4mmol, 3 equiv) was added 100 mL of dioxane and 30 mL of water. The mixture was heated under nitrogen at 100 °C for 12 hrs. Then solvents were removed on a rotavap at 40 °C under vacuum. The resulting brown residue was suspended in 20%EtOAc/DCM (60mL), filtered through a pad of silica gel (15g); washed with 20%EtOAc/DCM (4x20mL). The combined filtrate were concentrated to afford a brown oil (13 g). The residue was dissolved 10% EtOAc/hexanes (20mL) and loaded on a Biotage 100g snap SiO₂ column and eluted with 0-50% EtOAc. (2-(1-isopropyl-1H-pyrazol-5-yl)pyridin-3-yl)methanol (**53**, **INT-5**) was obtained as a light brown oil (3.32 g, 40%). MS (ESI) *m/z* 218.1 [M+H]⁺; ¹H-NMR (400 MHz, CDCl₃) δ 8.67 (dd, *J* = 4.7, 1.5 Hz, 1H), 8.0 (d, *J* = 7.8 Hz, 1H), 7.61 (d, *J* = 1.8 Hz, 1H), 7.39 (dd, *J* = 7.8, 4.8 Hz, 1H), 6.37 (d, *J* = 1.8 Hz, 1H), 4.67 (s, 2H), 4.55 (sep, *J* = 6.6 Hz 1H), 1.98-2.05 (br, 1H), 1.47 (d, *J* = 6.6 Hz, 6H).

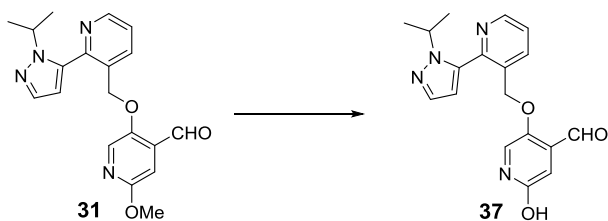
Step e. To a solution of (2-(1-isopropyl-1H-pyrazol-5-yl)pyridin-3-yl)methanol (**53**, **INT-5**) (440mg, 2.02mmol) in DCM (4 mL) was added SOCl₂ (2eq) at 0 °C. The reaction mixture was stirred at rt for 15mins and concentrated to dryness. The crude solid was suspended in toluene and concentrated to dryness. The process was repeated three times and dried under vacuum to give 3-(chloromethyl)-2-(1-

isopropyl-1H-pyrazol-5-yl)pyridine hydrochloride (**54**, **INT-6**, 432 mg) as an off-white solid, which was used for next step without further purification. MS (ESI) m/z 236.5 $[M+H]^+$; 1H NMR (400 MHz, DMSO- d_6) δ 8.70 (dd, $J = 4.8, 1.7$ Hz, 1H), 8.09 (dt, $J = 7.9, 2.0$ Hz, 1H), 7.60 – 7.49 (m, 2H), 6.57 – 6.50 (m, 1H), 4.72 (s, 2H), 4.49 – 4.37 (m, 1H), 1.33 (d, $J = 6.6$ Hz, 6H).

Step f. To a solution of 2-hydroxy-6-(methoxymethoxy)benzaldehyde (**50**) (10.88 g, 59.72 mmol) in DMF (120 mL) (DMF solution was purged with N_2 for 10 min) was added K_2CO_3 (32.05 g, 231.92 mmol) and 3-(chloromethyl)-2-(1-isopropyl-1H-pyrazol-5-yl)pyridine hydrochloride (**54**, **INT-6**) (15.78 g, 57.98 mmol). The mixture was heated at 65 °C for 1.5 h, cooled to rt, poured into ice water (800 mL). The precipitated solids were isolated by filtration, dried and concentrated to give desired product (**56**, 18 g). MS (ESI) m/z 382.5 $[M+H]^+$; 1H NMR (400 MHz, Chloroform- d) δ 8.69 (ddt, $J = 4.8, 1.8, 0.5$ Hz, 1H), 8.32 (ddt, $J = 7.9, 1.7, 0.8$ Hz, 1H), 7.61 (dd, $J = 1.8, 0.5$ Hz, 1H), 7.45 (dd, $J = 7.9, 4.7$ Hz, 1H), 7.39 (t, $J = 8.5$ Hz, 1H), 6.83 (dd, $J = 8.6, 0.7$ Hz, 1H), 6.49 (dt, $J = 8.5, 0.7$ Hz, 1H), 6.36 (d, $J = 1.9$ Hz, 1H), 5.28 (s, 2H), 5.05 (s, 2H), 4.66 – 4.54 (m, 1H), 3.51 (s, 3H), 1.46 (d, $J = 6.7$ Hz, 7H).

Step g. To a solution of 2-((2-(1-isopropyl-1H-pyrazol-5-yl)pyridin-3-yl)methoxy)-6-(methoxymethoxy)benzaldehyde (**55**) (18 g, 47.19 mmol) in THF (135 mL, solution was purged with N_2) was added conc. HCl (12N, 20 mL). The solution was stirred at rt for 3 h when HPLC showed the reaction complete. The mixture was added to a solution of $NaHCO_3$ (15 g) in water (1.2 L), and the resulting precipitate was collected by filtration, dried to give crude solid, which was further purified by column (DCM/EtOAc=60:40) to give GBT440 (**36**) (15.3 g). HRMS calcd for $C_{19}H_{20}N_3O_3$ ($M+H^+$) 338.1499, found 338.1497; MS (ESI) m/z 338.4 $[M+H]^+$; 1H NMR (400 MHz, Chloroform- d) δ 11.94 (s, 1H), 10.37 (d, $J = 0.6$ Hz, 1H), 8.75 (dd, $J = 4.8, 1.7$ Hz, 1H), 7.97 (dd, $J = 7.8, 1.6$ Hz, 1H), 7.63 – 7.57 (m, 1H), 7.46 – 7.33 (m, 2H), 6.57 (dt, $J = 8.6, 0.7$ Hz, 1H), 6.34 (d, $J = 1.9$ Hz, 1H), 6.27 (dt, $J = 8.3, 1.0$ Hz, 1H), 5.07 (s, 2H), 4.65 (hept, $J = 6.6$ Hz, 1H), 1.47 (d, $J = 6.6$ Hz, 7H); ^{13}C NMR (101 MHz, DMSO- d_6) δ 194.0, 162.9, 161.1, 149.6, 149.1, 139.1, 138.2, 138.2, 138.0, 131.6, 124.0, 111.1, 110.2, 107.4, 103.5, 67.8, 50.5, 23.1.

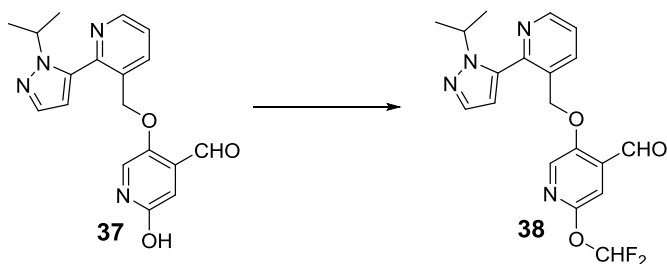
5-((2-(1-isopropyl-1H-pyrazol-5-yl)pyridin-3-yl)methoxy)-2-oxo-1,2-dihydropyridine-4-carbaldehyde (**37**).



To 5-((2-(1-isopropyl-1H-pyrazol-5-yl)pyridin-3-yl)methoxy)-2-methoxyisonicotinaldehyde (**31**) (400 mg, 1.135 mmol) in a vial was added HCl (12 N, 1 mL). The reaction was heated in a microwave reactor at 125 °C for 45 min. The mixture was concentrated and dried, and dissolved in NaOH (3 N, 5 mL), filtered, and washed with EtOAc twice. The pH of the aqueous layer was adjusted to pH 6-7, filtered, and purified by RP-HPLC (Gemini 21.2 x 150 mm) with a mixture of CH₃CN and water (0.1% HCOOH) as eluent to give the product (100 mg, 26%) as a yellow solid. HRMS calcd for C₁₈H₁₉N₄O₃ (M+H⁺) 339.1435, found 339.1452; MS (ESI) *m/z* 339.4 [M+H]⁺; ¹H NMR (400 MHz, Chloroform-*d*) δ 10.3 (s, 1H), 8.6 (d, 1H), 8.1 (d, 1H), 7.2 (s, 1H), 7.4 (dd, 1H), 7.1 (s, 1H), 7.0 (s, 1H), 6.3 (s, 1H), 4.9 (s, 2H), 4.6 (m, 1H), 1.5 (d, 6H).

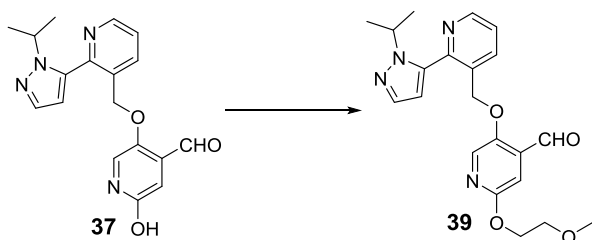
2-(difluoromethoxy)-5-((2-(1-isopropyl-1H-pyrazol-5-yl)pyridin-3-yl)methoxy)isonicotinaldehyde (38**).**

The title compound was prepared according to the procedure in Example 62.



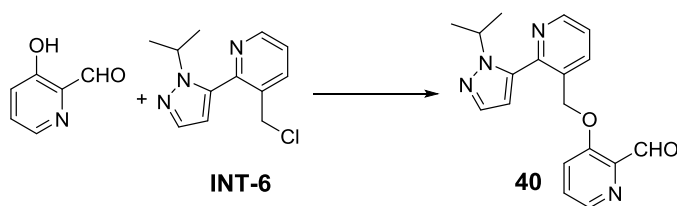
To 5-(imidazo[1,2-*a*]pyridin-8-ylmethoxy)-2-oxo-1,2-dihydropyridine-4-carbaldehyde (**37**) (100 mg, 0.295 mmol, 1 equiv) in CH₃CN (10 mL) was added sodium 2-chloro-2,2-difluoroacetate (84.5 mg, 0.56 mmol, 1.5 eq.). The mixture was stirred at rt overnight and concentrated. The crude was purified on silica gel using 10% MeOH/DCM as eluent to give 2-(difluoromethoxy)-5-(imidazo[1,2-*a*]pyridin-8-ylmethoxy)isonicotinaldehyde (6.0 mg, 5%) as a yellow solid. MS (ESI) *m/z* 389.1 [M+H]⁺; ¹H NMR (400 MHz, CDCl₃) δ 10.44 (s, 1H), 8.80 (d, *J* = 3.7 Hz, 1H), 8.03 (d, *J* = 8.0 Hz, 1H), 7.95 (s, 1H), 7.63 (d, *J* = 1.6 Hz, 1H), 7.47 (dd, *J* = 7.9, 4.8 Hz, 1H), 7.33 (t, *J* = 72.8 Hz, 1H), 6.37 (d, *J* = 1.7 Hz, 1H), 5.21 (s, 2H), 4.67 (sep, *J* = 6.6 Hz, 1H), 1.50 (d, *J* = 6.6 Hz, 6H).

5-((2-(1-isopropyl-1H-pyrazol-5-yl)pyridin-3-yl)methoxy)-2-(2-methoxyethoxy)isonicotinaldehyde (39**).**



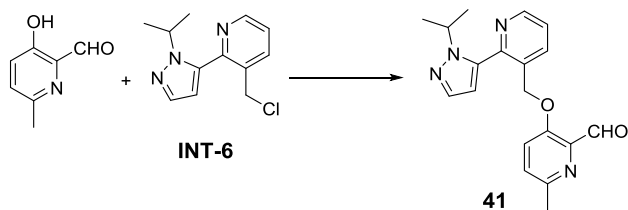
To a mixture of 5-(imidazo[1,2-a]pyridin-8-ylmethoxy)-2-oxo-1,2-dihydropyridine-4-carbaldehyde (100 mg, 0.295 mmol, 1 equiv) and K_2CO_3 (153.2 mg, 1.11, 3.0 eq.) in DMF (5 mL) was added 1-bromo-2-methoxyethane (154.3 mg, 1.11 mmol, 3.0 eq.). The mixture was stirred at rt overnight, filtered, concentrated, and purified on silica gel using 10% MeOH/DCM as eluent to give 5-(imidazo[1,2-a]pyridin-8-ylmethoxy)-2-(2-methoxyethoxy)isonicotinaldehyde (**39**) (6.0 mg, 5%) as a yellow solid. MS (ESI) m/z 397.1 $[M+H]^+$; 1H NMR (400 MHz, $CDCl_3$) δ 10.40 (s, 1H), 8.76 (dd, $J = 4.7, 1.5$ Hz, 1H), 8.04 (dd, $J = 7.9, 1.2$ Hz, 1H), 7.87 (s, 1H), 7.61 (d, $J = 1.8$ Hz, 1H), 7.44 (dd, $J = 7.9, 4.8$ Hz, 1H), 7.16 (s, 1H), 6.37 (d, $J = 1.8$ Hz, 1H), 5.14 (s, 2H), 4.65 (sep, $J = 6.6$ Hz, 1H), 4.42 (t, $J = 4.8$ Hz, 2H), 3.74 (t, $J = 4.8$ Hz, 2H), 3.44 (s, 3H), 1.49 (d, $J = 6.6$ Hz, 6H).

3-((2-(1-isopropyl-1H-pyrazol-5-yl)pyridin-3-yl)methoxy)picolinaldehyde (**40**).



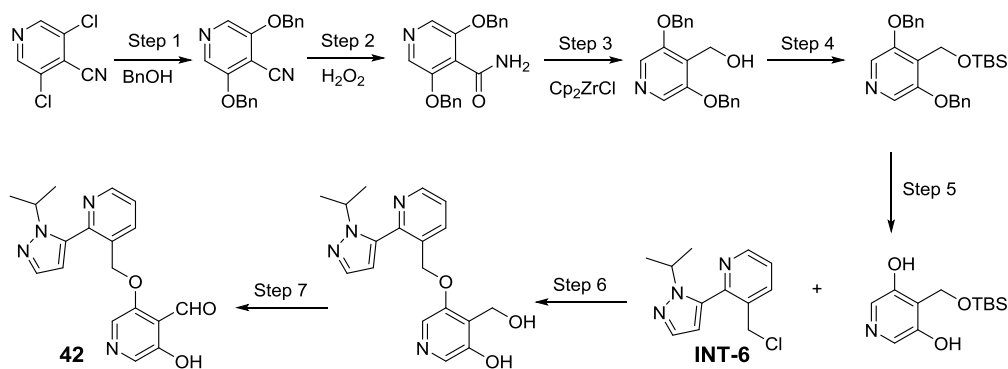
The title compound was prepared from 3-(chloromethyl)-2-(1-isopropyl-1H-pyrazol-5-yl)pyridine (INT-6) and 3-hydroxypicolinaldehyde using general procedure step 2 to give the product as a light yellow solid in 88% yield. MS (ESI) m/z 323.2 $[M+H]^+$; 1H NMR (400 MHz, $CDCl_3$) δ 10.34 (s, 1H), 8.76 (dd, $J = 4.7, 1.6$ Hz, 1H), 8.47 (dd, $J = 4.4, 1.0$ Hz, 1H), 8.32 (dd, $J = 7.9, 1.5$ Hz, 1H), 7.64 (d, $J = 1.8$ Hz, 1H), 7.49 (td, $J = 8.3, 4.6$ Hz, 2H), 7.31 (d, $J = 8.5$ Hz, 1H), 6.39 (d, $J = 1.8$ Hz, 1H), 5.15 (s, 2H), 4.65 (sep, $J = 6.6$ Hz, 1H), 1.49 (d, $J = 6.6$ Hz, 6H).

3-((2-(1-isopropyl-1H-pyrazol-5-yl)pyridin-3-yl)methoxy)-6-methylpicolinaldehyde (**41**).



The title compound was prepared from 3-(chloromethyl)-2-(1-isopropyl-1H-pyrazol-5-yl)pyridine and 3-hydroxy-6-methylpicolinaldehyde using general procedure step 2 to give the product as a white solid in 77% yield. MS (ESI) m/z 337.4 $[M+H]^+$; 1H NMR (400 MHz, $CDCl_3$) δ 10.31 (s, 1H), 8.75 (dd, $J = 4.7, 1.3$ Hz, 1H), 8.29 (d, $J = 7.9$ Hz, 1H), 7.64 (d, $J = 1.7$ Hz, 1H), 7.48 (dd, $J = 7.9, 4.8$ Hz, 1H), 7.31 (d, $J = 8.6$ Hz, 1H), 7.20 (d, $J = 8.6$ Hz, 1H), 6.38 (d, $J = 1.7$ Hz, 1H), 5.11 (s, 2H), 4.64 (sep, $J = 6.6$ Hz, 1H), 2.61 (s, 3H), 1.49 (d, $J = 6.6$ Hz, 6H).

3-hydroxy-5-((2-(1-isopropyl-1H-pyrazol-5-yl)pyridin-3-yl)methoxy)isonicotinaldehyde (42).



Step 1. To a mixture of NaH (60% in mineral oil) (2.77 g, 69.25 mmol, 2.5 equiv) in DMF (40.0 mL) was added benzyl alcohol (6.6 g, 61.0 mmol, 2.2 equiv) at 0 °C. The mixture was stirred at 0 °C for 10 min, added 3,5-dichloroisonicotinonitrile (4.8 g, 27.7 mmol, 1 equiv), continued to stir at 0 °C for 30 min, gradually warm to rt, stirred at rt overnight, and quenched with NH₄Cl (sat.) solution. The aqueous layer was extracted with EtOAc three times. The combined organic layers were washed with brine, dried over Na₂SO₄, concentrated, and purified on silica gel using a mixture of EtOAc and hexanes as eluent to give 3,5-bis(benzyloxy)isonicotinonitrile (4.94 g, 56%) as an off-white solid. MS (ESI) *m/z* 317.1 [M+H]⁺; ¹H NMR (400 MHz, CDCl₃) δ 8.14 (s, 2H), 7.58-7.30 (m, 10H), 5.33 (s, 4H).

Step 2. To a mixture of 3,5-bis(benzyloxy)isonicotinonitrile (2.5 g, 7.9 mmol, 1 equiv) and K₂CO₃ (4.37 g, 31.6 mmol, 4 equiv) in DMSO (10 mL) was added H₂O₂ (30% in water, 2.0 mL) at rt. The mixture was stirred at rt overnight and added water (50 mL). The solid was collected and dried to give 3,5-bis(benzyloxy)isonicotinamide (2.2 g, 83%) as a white solid. MS (ESI) *m/z* 335.1 [M+H]⁺; ¹H NMR (400 MHz, CDCl₃) δ 8.13 (s, 2H), 7.59 – 7.33 (m, 10H), 5.83 (s, 2H), 5.25 (s, 4H), 4.81 (s, 2 H).

Step 3. To 3,5-bis(benzyloxy)isonicotinamide (1.6 g, 4.79 mmol) in THF (30 mL) was added Cp₂ZrCl (3.7 g, 14.4 mmol, 3 equiv) at rt. The mixture was stirred at rt for 2 h, concentrated, and purified on silica gel using a mixture of EtOAc and hexanes as eluent to give 3,5-bis(benzyloxy)isonicotinaldehyde (580 mg, 38%) and (3,5-bis(benzyloxy)pyridin-4-yl)methanol (710 mg, 46%) as white solids. Data for aldehyde MS (ESI) *m/z* 322.1 [M+H]⁺; ¹H NMR (400 MHz, CDCl₃) δ 10.53 (s, 1H), 8.13 (s, 2H), 7.51 – 7.22 (m, 10H), 5.21 (s, 4H); LRMS (M+H⁺) *m/z* 320.1. Data for alcohol ¹H NMR (400 MHz, CDCl₃) δ 8.12 (s, 2H), 7.58 – 7.34 (m, 10H), 5.22 (s, 4H), 4.87 (s, 1H).

Step 4. To a solution of (3,5-bis(benzyloxy)pyridin-4-yl)methanol (910 mg, 2.83 mmol) and imidazole (385 mg, 5.66 mmol) in DMF (10.0 mL) was added TBSCl (513 mg, 3.4 mmol) at rt. The mixture was stirred at rt for 1 h and diluted with a mixture of water (10 mL) and EtOAc (40 mL). The organic layer was washed with NH₄Cl_(sat.) solution and brine, dried over Na₂SO₄, concentrated, and purified on silica

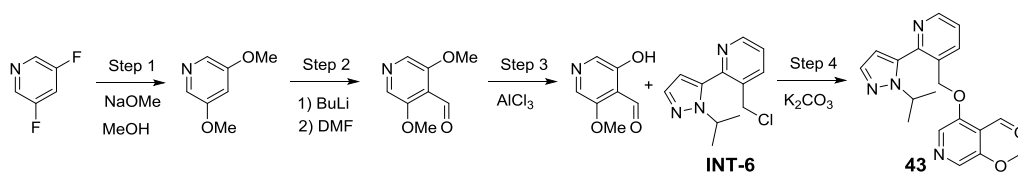
gel using 10% EtOAc/hexanes as eluent to give 3,5-bis(benzyloxy)-4-((tert-butyltrimethylsilyloxy)methyl)pyridine (728 mg, 59%) as an off-white solid. MS (ESI) m/z 436.3 $[M+H]^+$.

Step 5. To 3,5-bis(benzyloxy)-4-((tert-butyltrimethylsilyloxy)methyl)pyridine (720 mg, 1.66 mmol, 1 equiv) in a mixture of EtOAc/EtOH (5/2, 28 mL) was added Pd/C (400.0 mg). The mixture was charged with H₂ (60 psi), stirred at rt for 2 h, filtered, and concentrated to give 4-((tert-butyltrimethylsilyloxy)methyl)pyridine-3,5-diol as a yellow solid. MS (ESI) m/z 256.1 $[M+H]^+$; ¹H NMR (400 MHz, CDCl₃) δ 7.54 (s, 2H), 4.91 (s, 2H), 0.73 (s, 9H), -0.00 (s, 6H).

Step 6. A mixture of 4-((tert-butyltrimethylsilyloxy)methyl)pyridine-3,5-diol (100 mg, 0.39 mmol, 2 equiv) and Cs₂CO₃ (381 mg, 1.17 mmol, 3 equiv) in DMF (15 mL) was stirred at rt for 30 min. To this mixture was added 3-(chloromethyl)-2-(1-isopropyl-1H-pyrazol-5-yl)pyridine hydrochloride (INT-6) (53 mg, 0.39 mmol, 1 equiv) at rt. The mixture was continued to stir at rt overnight, filtered, concentrated, and purified on silica gel using a mixture of EtOAc and hexanes as eluent to give 4-(hydroxymethyl)-5-((2-(1-isopropyl-1H-pyrazol-5-yl)pyridin-3-yl)methoxy)pyridin-3-ol (36 mg, 27%) as a pale yellow oil. MS (ESI) m/z 341.1 $[M+H]^+$; ¹H NMR (400 MHz, CDCl₃) δ 8.51 (dt, $J = 33.0, 16.5$ Hz, 1H), 7.72 (d, $J = 1.6$ Hz, 1H), 7.69 (s, 1H), 7.47 (s, 1H), 7.33 (s, 1H), 7.21 (dd, $J = 7.8, 4.8$ Hz, 1H), 6.10 (d, $J = 1.8$ Hz, 1H), 4.84 (s, 2H), 4.68 (s, 1H), 4.44 (sep, 6.6 Hz, 1H), 1.24 (d, $J = 6.6$ Hz, 6H).

Step 7. To 4-(hydroxymethyl)-5-((2-(1-isopropyl-1H-pyrazol-5-yl)pyridin-3-yl)methoxy)pyridin-3-ol (26 mg, 0.076 mmol, 1 equiv) in CH₃CN (10 mL) was added MnO₂ (66 mg, 0.76 mmol, 10equiv). The mixture was heated to 46 °C with stirring overnight, cooled to rt, filtered, and concentrated to give 3-hydroxy-5-((2-(1-isopropyl-1H-pyrazol-5-yl)pyridin-3-yl)methoxy)isonicotinaldehyde (**42**) as a pale yellow oil. HRMS calcd for C₁₈H₁₉N₄O₃ ($M+H^+$) 339.1435, found 339.1452; MS (ESI) m/z 339.1 $[M+H]^+$; ¹H NMR (400 MHz, CDCl₃) δ 11.06 (s, 1H), 10.35 (s, 1H), 8.70 (dd, $J = 4.7, 1.5$ Hz, 1H), 8.11 (s, 1H), 7.89 (dd, $J = 7.9, 1.1$ Hz, 1H), 7.80 (s, 1H), 7.53 (d, $J = 1.8$ Hz, 1H), 7.36 (dd, $J = 7.9, 4.8$ Hz, 1H), 6.27 (d, $J = 1.8$ Hz, 1H), 5.14 (s, 2H), 4.61 (sep, $J = 6.6$ Hz, 1H), 1.41 (d, $J = 6.6$ Hz, 6H).

3-((2-(1-isopropyl-1H-pyrazol-5-yl)pyridin-3-yl)methoxy)-5-methoxyisonicotinaldehyde (**43**).



Step 1. To a solution of 3,5-difluoropyridine (5.4 g, 46.8 mmol, 1 equiv) in MeOH (45 mL) was added NaOMe (7.5 g, 140.4 mmol). The mixture was divided into three microwave tubes and individually

heated at 135 °C for 1 h in a microwave reactor. The three tubes were combined, concentrated, and diluted with a mixture EtOAc (100 mL) and brine (30 mL). The organic layer was dried over Na₂SO₄ and concentrated. The crude was re-dissolved in MeOH (45 mL) and added NaOMe (7.5 g, 140.4 mmol). The mixture was again divided into three microwave tubes and individually heated at 135 °C for 1 h in a microwave reactor. The three tubes were combined and concentrated. The crude was dissolved in a mixture of EtOAc (200 mL) and brine (30 mL). The organic layer was dried over Na₂SO₄, concentrated, and purified on silica gel using a mixture of EtOAc and hexanes as eluent to give 3,5-dimethoxypyridine (3.73 g, 57%) as an off-white solid. HRMS calcd for C₁₉H₂₁N₄O₃ (M+H⁺) 353.1608, found 353.1593; MS (ESI) *m/z* 140.1 [M+H]⁺; ¹H NMR (400 MHz, CDCl₃) δ 7.98 (d, *J* = 2.4 Hz, 2H), 6.76 (t, *J* = 2.4 Hz, 1H), 3.88 (s, 6H).

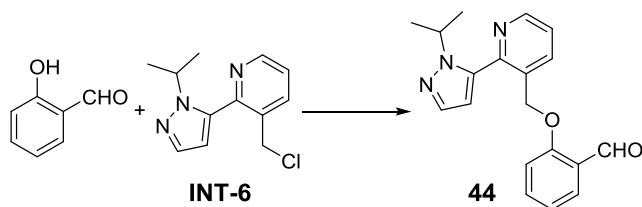
Step 2. To a solution of 3,5-dimethoxypyridine (3.6 g, 25.90 mmol, 1 equiv) in THF (80 mL) was added BuLi (3M/hexanes, 13.0 mL, 38.85 mmol, 1.5 equiv) at -20 °C. The mixture was warmed to 0 °C, stirred at 0 °C for 30 min, cooled back down to -78 °C, and added DMF (3.8 g, 51.8 mmol, 2 equiv). The mixture was gradually warmed to 0 °C, quenched with NH₄Cl(sat.) solution, and diluted with EtOAc. The aqueous layer was extracted with EtOAc twice. The combined organic layers were washed with brine, dried over Na₂SO₄, concentrated, and purified on silica gel using a mixture of EtOAc and hexanes as eluent to give 3,5-dimethoxyisonicotinaldehyde (2.7 g, 62%) as a yellow solid. MS (ESI) *m/z* 168.1 [M+H]⁺.

Step 3. To a solution of 3,5-dimethoxyisonicotinaldehyde (2.7 g, 16.16 mmol, 1 equiv) in DCM (100 mL) was added AlCl₃ (4.31 g, 32.32 mmol, 2.0 equiv) at rt. The mixture was reflux overnight, cooled to rt, and added into ice (200 g). The aqueous layer was extracted with DCM three times. The combined organic layers were dried over Na₂SO₄, concentrated, and purified on silica gel using a mixture of EtOAc and hexanes as eluent to give 3-hydroxy-5-methoxyisonicotinaldehyde (420 mg, 17%) as an off-white solid. MS (ESI) *m/z* 154.1 [M+H]⁺; ¹H NMR (400 MHz, CDCl₃) δ 10.96 (s, 1H), 10.26 (s, 1H), 7.96 (s, 1H), 7.80 (s, 1H), 3.84 (s, 3H).

Step 4. A mixture of 3-hydroxy-5-methoxyisonicotinaldehyde (30 mg, 0.20 mmol, 1 equiv), 3-(chloromethyl)-2-(1-isopropyl-1H-pyrazol-5-yl)pyridine hydrochloride (54 mg, 0.20 mmol, 1 equiv), and K₂CO₃ (110 mg, 0.80 mmol, 4 equiv) in DMF (2.0 mL) was heated at 70 °C for 2 h. The mixture was cooled, filtered, concentrated, and purified on silica gel using a mixture of EtOAc and hexanes to give 3-((2-(1-isopropyl-1H-pyrazol-5-yl)pyridin-3-yl)methoxy)-5-methoxyisonicotinaldehyde (**43**) (30 mg, 43%) as an off-white solid. MS (ESI) *m/z* 353.1 [M+H]⁺; ¹H NMR (400 MHz, CDCl₃) δ 10.46 (s, 1H), 8.65 (dd, *J* = 4.7, 1.1 Hz, 1H), 8.13 (s, 1H), 8.11 (dd, *J* = 7.9, 1.1 Hz, 1H), 7.96 (s, 1H), 7.54 (d, *J* =

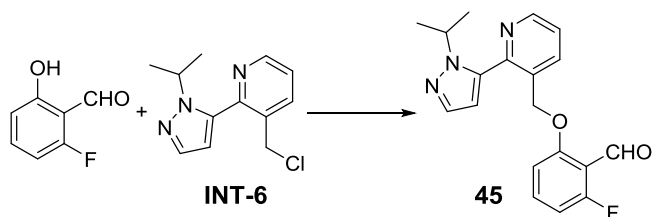
1.7 Hz, 1H), 7.37 (dd, $J = 7.9, 4.8$ Hz, 1H), 6.29 (d, $J = 1.7$ Hz, 1H), 5.11 (s, 2H), 4.55 (sep, $J = 6.6$ Hz, 1H), 3.95 (s, 3H), 1.40 (d, $J = 6.6$ Hz, 6H).

2-((2-(1-Isopropyl-1H-pyrazol-5-yl)pyridin-3-yl)methoxy)benzaldehyde (44).



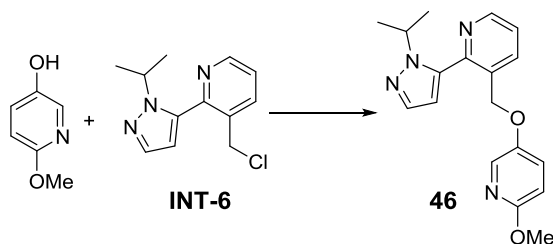
The compound was prepared by O-alkylation of 2-hydroxybenzaldehyde with 3-(chloromethyl)-2-(1-isopropyl-1H-pyrazol-5-yl)pyridine (INT-6) according to general procedure step 2. The product of TFA salt as white solid was obtained after HPLC purification in 87% yield. HRMS calcd for $C_{19}H_{20}N_3O_2$ ($M+H^+$) 322.155, found 322.1538; MS (ESI) m/z 322 [$M+H$] $^+$; 1H NMR (300MHz, $CDCl_3$) δ 10.49(s, 1H), 8.78(m, 1H), 8.16(m, 1H), 7.88(m, 1H), 7.69(d, $J=6.0$ Hz, 1H), 7.54(m, 2H), 7.13(m, 1H), 6.90(d, $J=8.4$ Hz, 1H), 6.41(d, $J=1.8$ Hz, 1H), 5.11 (s, 2H), 4.62 (m, 1H).

2-fluoro-6-((2-(1-isopropyl-1H-pyrazol-5-yl)pyridin-3-yl)methoxy)benzaldehyde (45).



The title compound was prepared from INT-6 and 2-fluoro-6-hydroxybenzaldehyde according to the general procedure step 2 to give the product as white solid in 99% yield. HRMS calcd for $C_{19}H_{19}FN_3O_2$ ($M+H^+$) 340.1456, found 340.1451; MS (ESI) m/z 340.1 [$M+H$] $^+$; 1H NMR (400 MHz, $CDCl_3$) δ 10.41 (s, 1H), 8.66 (dd, $J = 4.7, 1.6$ Hz, 1H), 8.13 (dd, $J = 7.9, 1.4$ Hz, 1H), 7.55 (d, $J = 1.8$ Hz, 1H), 7.46 – 7.29 (m, 2H), 6.72 (dd, $J = 10.0, 8.7$ Hz, 1H), 6.59 (d, $J = 8.5$ Hz, 1H), 6.29 (d, $J = 1.8$ Hz, 1H), 5.03 (s, 2H), 4.56 (sep, $J = 6.7$ Hz, 1H), 1.40 (d, $J = 6.6$ Hz, 6H).

2-(1-isopropyl-1H-pyrazol-5-yl)-3-(((6-methoxypyridin-3-yl)oxy)methyl)pyridine (46).



The title compound was prepared from INT-6 and 6-methoxypyridin-3-ol according to the general procedure step 2 to give the product as a white solid in 45% yield. MS (ESI) m/z 325.2 $[M+H]^+$; 1H NMR (400 MHz, Methanol- d_4) δ 8.71 (dd, $J = 4.8, 1.7$ Hz, 1H), 8.15 (dd, $J = 7.9, 1.7$ Hz, 1H), 7.73 (d, $J = 3.0$ Hz, 1H), 7.63 – 7.54 (m, 2H), 7.32 (dd, $J = 9.0, 3.1$ Hz, 1H), 6.74 (d, $J = 9.0$ Hz, 1H), 6.50 (d, $J = 2.0$ Hz, 1H), 5.00 (s, 2H), 4.52 (p, $J = 6.7$ Hz, 1H), 3.85 (s, 3H), 1.44 (d, $J = 6.7$ Hz, 6H).

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