Supporting Information

Discovery of Triazole CYP11B2 Inhibitors with In vivo Activity in Rhesus Monkeys

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List of abbreviations. In the following procedures and schemes, abbreviations are used with the following meanings unless otherwise indicated: Ac = acetate; ACTH = adrenocorticotropic hormone; aq, aq. = aqueous; Ar = aryl; BOC, Boc = *t*-butyloxycarbonyl; Bn = benzyl; BSA = bovine serum albumin; Bu = butyl, *t*-Bu = *tert*-butyl; BuLi, *n*-BuLi = *n*-butyllithium; CBZ, Cbz = Benzyloxycarbonyl; conc, conc. = concentrated; *c*-Bu = cyclobutyl; *c*-Pr = cyclopropyl; DAST = (diethylamino)sulfur trifluoride; dba = dibenzylideneacetone; DCM = dichloromethane; DIAD = diisopropylazodicarboxylate; DIBAL, DIBAL-H = diisobutylaluminum hydride; DIEA = diisopropylethylamine; DMAC, DMA = dimethylacetamide; DME = 1,2-dimethoxyethane; DMEM = Dulbecco's modified eagle medium; DMAP = 4-

dimethylaminopyridine; DMF = *N,N*-dimethylformamide; DMSO = dimethylsulfoxide; eq. = equivalent(s); EDC = N-[3-(dimethylamino)propyl]-N-ethylcarbodiimide; EDTA = ethylenediaminetetraacetic acid; ESI = electrospray ionization; Et = ethyl; EtOAc = ethyl acetate; EtOH = ethanol; FBS = Fetal Bovine Serum; h, hr = hour; HATU = N-[(dimethylamino)-1H-1,2,3-triazolo[4,5-b]pyridin-1-ylmethylene]-N-methylmethanaminium hexafluorophosphate N-oxide; HOAc = acetic acid; HOAt = 3H-[1,2,3]-triazolo[4,5-b]pyridin-3-ol; HOBt = 1H-benzotriazol-1-ol; HPLC = High pressure liquid chromatography; HTRF = homogenous time resolved fluorescence; IPA, i-PrOH = isopropanol; iPr = isopropyl; LAH = lithium aluminum hydride; LCMS = liquid chromatography - mass spectroscopy; LHMDS = lithium bis(trimethylsilyl)amide; Me = methyl; MeOH = methanol; min, min. = minute; μW = microwave; NMP = N-methylpyrrolidinone; NMR = nuclear magnetic resonance; OMs, mesyl = methanesulfonyl; Oxone, OXONE = potassium peroxymonosulfate; PBS = phosphate buffered saline; Pd_2dba_3 = *tris*(dibenzylidineacetone)dipalladium; Pd/C = palladium on activated carbon; Ph = phenyl; Pr = propyl; Py = pyridyl; RT, rt = room temperature; sat. = saturated; TBAF = tetrabutylammonium fluoride; TBAI = tetrabutylammonium iodide; *t*-Bu = tert-butyl; TFA = trifluoroacetic acid; THF = tetrahydrofuran; TLC = thin layer chromatography; prep TLC = preparative thin layer chromatography; Tosyl = toluenesulfonyl; triflate, OTf = trifluoromethanesulfonate; triflic = trifluoromethanesulfonic; Xantphos = 4,5-bis(diphenylphosphino)-9,9-dimethylxanthene.

Human CYP11B2 and CYP11B1 Assays. V79 cell lines stably expressing the either the human CYP11B2 or the human CYP11B1 enzyme were generated using a standard transfection protocol. V79 cells were transfected with plasmids pTriEx3-Hygro-hCYP11B2 or pTriEx3-Hygro-hCYP11B1 using Lipofectamine2000 reagent. V79 cells that stably express the human CYP11B2 or human CYP11B1 enzyme were selected for and maintained in DMEM supplemented with 10% FBS and 400 μg/mL hygromycin for ~2 weeks. Single cell clones were generated by infinite dilution in DMEM supplemented with 10% FBS and 400 μg/mL hygromycin until single colonies were obtained. Clones V79-hCYP11B2-CLE9 and V79-hCYP11B1-8C7 were determined to produce the most aldosterone and cortisol, respectively, and were selected for inhibitor screening. For

testing of inhibitors, cells were harvested at 80% confluency with 0.5% Trypsan-EDTA, washed once in PBS, and reconstituted in DMEM + 0.1% BSA media at a cell concentration of 400,000 cells / mL. 25 µL of cells were added to a 384-well tissue culture treated plate and mixed with 0.25 µL of inhibitor or DMSO (1% final DMSO concentration) for 1 hour at 37 °C, 5% CO_2. After pre-incubation with inhibitor, the reaction was initiated by adding 5 µL of substrate (final concentration of 125 nM 11-deoxycorticosterone for the CYP11B2 assay or 250 nM 11-deoxycortisol for the CYP11B1 assay). The reaction was carried out for 3 hours at 37 °C, 5% CO_2 and was stopped by harvesting the supernatants. The amount of product in the supernatant (aldosterone for CYP11B2 assay and cortisol for the CYP11B1 assay) was measured using HTRF-based assay kit (Aldosterone HTRF-CisBio #64ALDPEB, Cortisol HTRF-CisBio #63IDC002-CORT). IC_{50}'s for the inhibitor were determined by plotting the amount of product formed against the concentration of inhibitor using sigmoidal dose-response curve (variable slope) fit in GraphPad.

Rhesus and Rat CYP11B2 Assays: V79 cell lines stably expressing either rhesus or rat CYP11B2 enzyme were generated using a standard transfection protocol. V79 cells were transfected with plasmids pTriEx3-Hygro-rhesusCyp11B2 or pTriEx3-Hygro-ratCyp11B2 using Lipofectamine2000 reagent. V79 cells that stably express the rhesus or rat Cyp11B2 enzyme were selected for and maintained in DMEM supplemented with 10% FBS and 400 µg/mL hygromycin for ~2 weeks. Single cell clones were generated by infinite dilution in DMEM supplemented with 10% FBS and 400 µg/mL hygromycin until single colonies were obtained. Clones V79-rhesusCyp11B2-1F4 and V79-ratCyp11B1-5C3 were determined to produce the most aldosterone, and were selected for inhibitor screening. For testing of inhibitors, cells were harvested at 80% confluency with 0.5% Trypsan-EDTA, washed once in PBS, and reconstituted in DMEM + 0.1% BSA media at a cell concentration of 400,000 cells / mL. 25 µL of cells were added to a 384 well tissue culture treated plate and mixed with 0.25 µL of inhibitor or DMSO (1% final DMSO concentration) for 1 hour at 37 °C, 5% CO_2. After pre-incubation with inhibitor, the reaction was initiated by adding 5 µL of substrate (final concentration of

125 nM 11-deoxycorticosterone for the rhesus CYP11B2 assay or 500 nM 11-deoxycorticosterone for the rat CYP11B2 assay). The reaction was carried out for 3 hours at 37 °C, 5% CO_2 and was stopped by harvesting the supernatants. The amount of aldosterone product in the supernatant was measured using HTRF-based assay kit (Aldosterone HTRF-CisBio#64ALDPEB). IC_{50}'s for the inhibitor were determined by plotting the amount of product formed against the concentration of inhibitor using sigmoidal dose-response curve(variable slope) fit in GraphPad.

Human CYP17 Assay: COS were maintained in DMEM supplemented with 10% fetal calf serum. For transfection of COS-7, cells were grown to 70% confluency and transfected with pTrieX3-Hygro-Human CYP17 using Lipofectamine 2000 according to the manufacturer's instructions. For testing of inhibitors, cells were harvested at 48 h post transfection with 0.5% Trypsan-EDTA, washed once in PBS, and reconstituted in DMEM + 0.1% BSA media at a cell concentration of 800,000 cells / mL. 100 µL of cells were added to a 96 well tissue culture treated plate and mixed with 1.0 µL of inhibitor or DMSO (1% final DMSO concentration) for 1 hour at 37 °C, 5% CO_2. After pre-incubation with inhibitor, the reaction was initiated by adding 5 µL of substrate (final concentration 360 nM 17-hydroxypregnenolone). The reaction was carried out for 3 hours at 37 °C, 5% CO_2 and was stopped by harvesting the supernatants. The amount of dehydroepiandrosterone (DHEA) product in the supernatant was measured using EIA-based assay kit (DHEA EIA kit (Enzo Life Sciences, cat no. 901-093). IC_{50}'s for the inhibitor were determined by plotting the amount of product formed against the concentration of inhibitor using sigmoidal dose-response curve(variable slope) fit in GraphPad.

CYP19 Assay: CYP19 assay was performed using the CYP19/MFC High Throughput Inhibitor Screening Kit according to manufacturer's instructions. Briefly, inhibitor was first disolved in 100% acetonitirile, then 6 µL of inhibitor was mixed with 144 µL of NADPH-cofactor mix (16.25 µM NADP, 825 µM MgCl2, 825 µM Glucose-6-Phosphate, 0.4 Units/mL Glucose-6-Phosphate Dehydrogenase). The inhibitor was furthered titratrated against the same NADPH-cofactor mix. 100 µL of inhibitor / NADPH-cofactor

mix was added to 96 well plate and incubated at 37 °C for 10 minutes. The assay was initiated by adding100 µL of enzyme and substrate mix (15 nM CYP19 and 50 µM MFC in 0.1 M Phosphate buffer, pH 7.4). The reaction was carried out for 30 minutes at 37 °C and was stopped by adding 75 µL stop solution (0.5 M Tris Base). The amount of product formed was detected by measuring the HFC metabolite at 409 nm excitation / 530 nm emission wavelengths.

Human and Rhesus In Vitro CYP11B2 and CYP11B1 Data for Compounds 1 – 22

Cpd	hCYP11B2 (IC_{50}, nM)	n	SEM	hCYP11B1 (IC_{50}, nM)	n	SEM	rhCYP11B2	n	SEM
1	300	2	64.5	>10000	2				
2	423	2	131	>10000	2				
3	487	2	92.4	>10000	2				
4	506	2	187	>8333	2				
5	2400	3	470	>8333	3				
6	103	2	15.4	1357	2	288			
7	558	2	18.8	>10000	2				
8	486	2	11.6	>10000	2				
9	58	2	7.9	2198	2	378			
10	>8333	3		>8333	3				
11	>8333	3		>8333	3				
12	>8333	3		>8333	3				
13	40	3	4.2	1501	3	498			
14	283	3	43.1	>8333	3		34	6	5.68
15	19	2	3.2	219	2	83.2	3	6	0.35
16	19	2	5.1	317	2	119			
17	5	2	1.1	407	2	52.9			
18	526	1	126	1473	1	261			
19	64	8	7.9	4402	8	530	12	6	1.62
20	109	2	37.5	>8333	3				
21	461	2	87.9	887	2	187			
22	476	3	25.8	>8333	3				

Determination of Intrinsic Clearance. Reactions were carried out in a 96-well plate (Thermo Fisher Scientific Inc., Waltham, MA). The reaction mixture (450 µL), containing 100 mM potassium phosphate buffer, pH 7.4, the appropriate liver microsomes (0.25 mg/mL), and the test compound (0.3 mM), was preincubated at 37 °C for 10 minutes. Reactions were initiated by the addition of NADPH (50 µL, 10 mM) to the incubation mixture. At time points 5, 15, 30, and 45 min, 50-µL aliquots of the reaction mixture were quenched with 200 µL of acetonitrile with 0.1% v/v formic acid and an internal standard cocktail. The samples were centrifuged at 4 °C for 10 min at 3,000 rpm. The supernatant was transferred to a 96-well plate for analysis using a generic

LC/MS method. The unscaled microsomal intrinsic clearance was estimated using the equation $Cl_{int,\ unscaled} = K_e \times$ (volume of incubation/mg microsomal protein), where K_e is the first-order rate constant describing the disappearance of parent compound in the incubation and can be obtained from regressing the initial slope of the natural log of the analyte area/internal standard area (designated as C at an appointed time t) versus time (min) profile.

Determination of Plasma Protein Binding. In vitro plasma protein binding (PPB) was estimated in rat and monkey plasma by the equilibrium dialysis method. Equilibrium dialysis was performed in a HT dialysis plate (model HTD96b, HTDialysis LLC, Gales Ferry, CT) with chambers separated by a dialysis membrane (mol. wt. cutoff, 12-14 kDa). One side of the chamber was filled with 120 mL of plasma containing test compound at a concentration of 2.5 mM, and the other was filled with 120 mL of phosphate-buffered saline. Dialysis was performed in a humidified incubator with a 5% CO_2 environment at 37 °C for 4 h. After dialysis, 50 mL of plasma and buffer samples from each well were extracted with 250 mL of acetonitrile solution containing internal standard, vertexed for 4 minutes and then centrifuged at 3,000 rpm for 10 minutes. Supernatant was transferred to a 96-well injection plate for LC/MS analysis. The fraction unbound (f_u) is the ratio of free drug peak area ratio of analyte/internal standard in buffer to that of peak area ratio of analyte/internal standard in plasma.

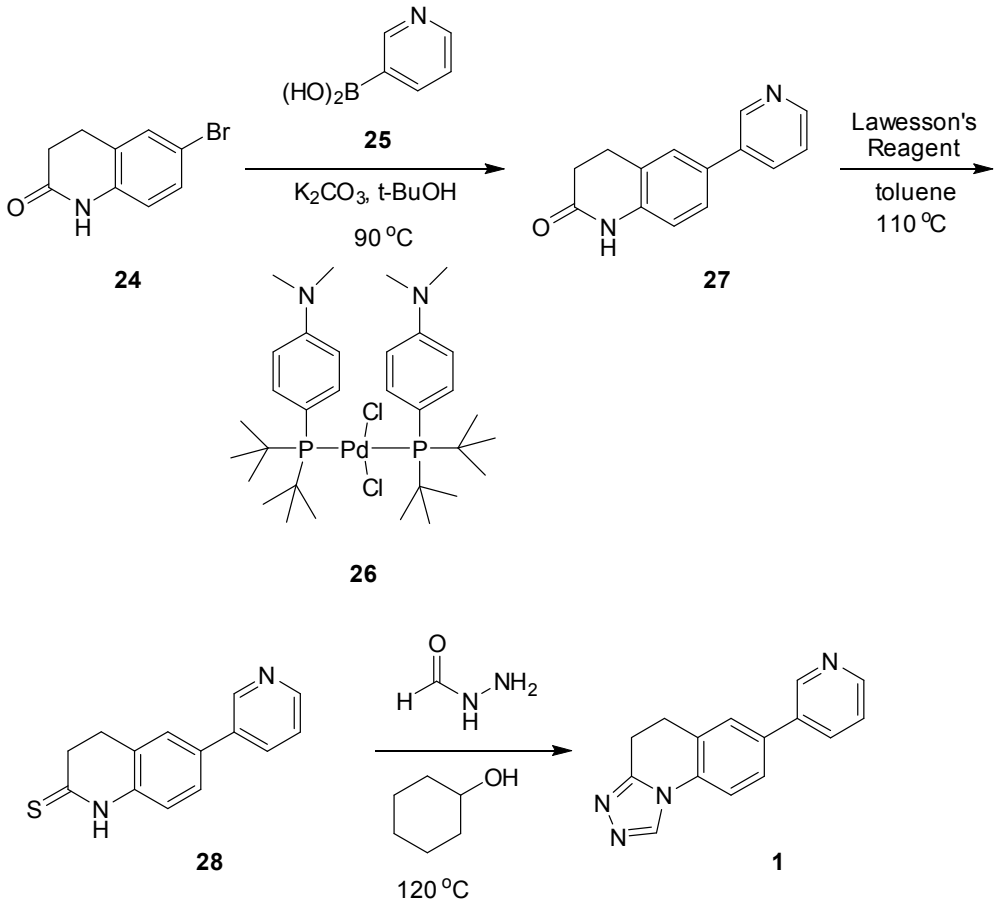
Rat and Rhesus Monkey Pharmacokinetic Assays. Rat and rhesus monkey pharmacokinetic experiments were conducted as follows: test compounds were typically formulated as 1.0 mg/mL solutions in mixtures of ethanol/PEG200/water (10:40:50, v/v/v). Fasted male Sprague-Dawley rats and fasted male adult rhesus monkeys were given either a 1.0 mg/kg i.v. (n = 2) dose of test compound solution via a cannula implanted in the femoral vein (or saphenous vein in the case of monkeys) or a 2.0 mg/kg p.o. dose by gavage (n = 3). Serial blood samples were collected at 5 (i.v. only), 15, and 30 minutes, and at 1, 2, 4, 6, and 8 hours post dose. Plasma was collected by centrifugation, and plasma concentrations of test compound were determined by LC-MS/MS following protein precipitation with acetonitrile.

Rhesus Pharmacodynamic Assay. In vivo effects of aldosterone synthase (CYP11B2) inhibition on circulating levels of adrenal steroids were investigated in male rhesus macaques using methods that have recently been described in detail.[1] Briefly, animals on low sodium diet were anesthetized. Vehicle (saline, 0.3 mL/kg, IV) or increasing doses of test compound were administered. 60 minutes post compound or vehicle treatment, adrenocorticotropic hormone (ACTH) at 0.3 mg/kg was administered. Blood samples were collected at 0 (right before ACTH administration) and 30, 60, 90, 120, 150 and 180 minutes post ACTH administration. Plasma samples were prepared and concentrations of adrenal steroid including aldosterone and 11-deoxycortisol (RSS) were measured via an ultra performance liquid chromatography (Waters Acquity UPLC) coupled with tandem mass spectrometry (Applied Biosystem Sciex API 5000 MS).

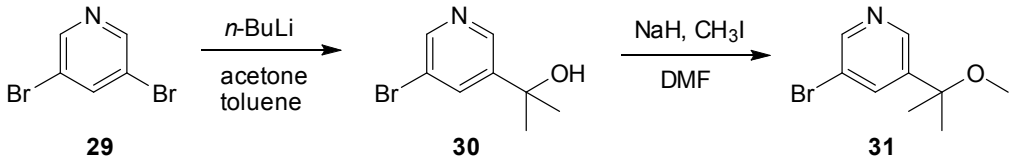
General Synthetic Procedures. Unless otherwise noted, commercially available materials were used without further purification. Air or moisture sensitive reactions were carried out under a nitrogen or argon atmosphere. Anhydrous solvents were obtained from Sigma-Aldrich and used as received. Flash chromatography was performed using pre-packed silica gel cartridges using Biotage Horizon or Biotage SP-1 instruments equipped with UV detectors. Preparative HPLC was performed using a SunFire Prep C18 OBD column (5 μM, 19 x 100 mm i.d.) on Gilson instruments equipped with UV detectors. Chemical reactions were monitored by LCMS, and the purity and identity of the reaction products were assayed by LCMS (electrospray ionization) and NMR. LCMS spectra were recorded on an Agilent 1100 series instrument equipped with an Xterra MS C18 column (3.5 μM, 3.0 x 50 mm i.d.) and UV detector. ^{1}H NMR spectra were recorded on a Varian 500 HHz spectrometer, and are internally referenced to residual protio solvent signals. Data for ^{1}H NMR are reported with chemical shift (δ ppm), multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, br s = broad singlet, br m = broad multiplet), coupling constant (Hz), and integration. Unless otherwise noted, all LCMS ions listed are [M + H]. All temperatures are degrees Celsius unless otherwise noted. All final compounds with reported biological data were determined to be >95% purity based on LC-MS and NMR data unless otherwise noted.

Synthesis Schemes. Detailed procedures for the synthesis of these compounds have been reported previously.[2] Compounds **1** – **6** were prepared using general synthesis A and the appropriate commercially available starting materials. Compounds **7** – **18** were synthesized using general synthesis B and bromide **30**, **31** or **40**, or the appropriate commercially available pyridyl halide. Compounds **19** – **22** were synthesized using general synthesis C, and either bromide **48** or **49**.

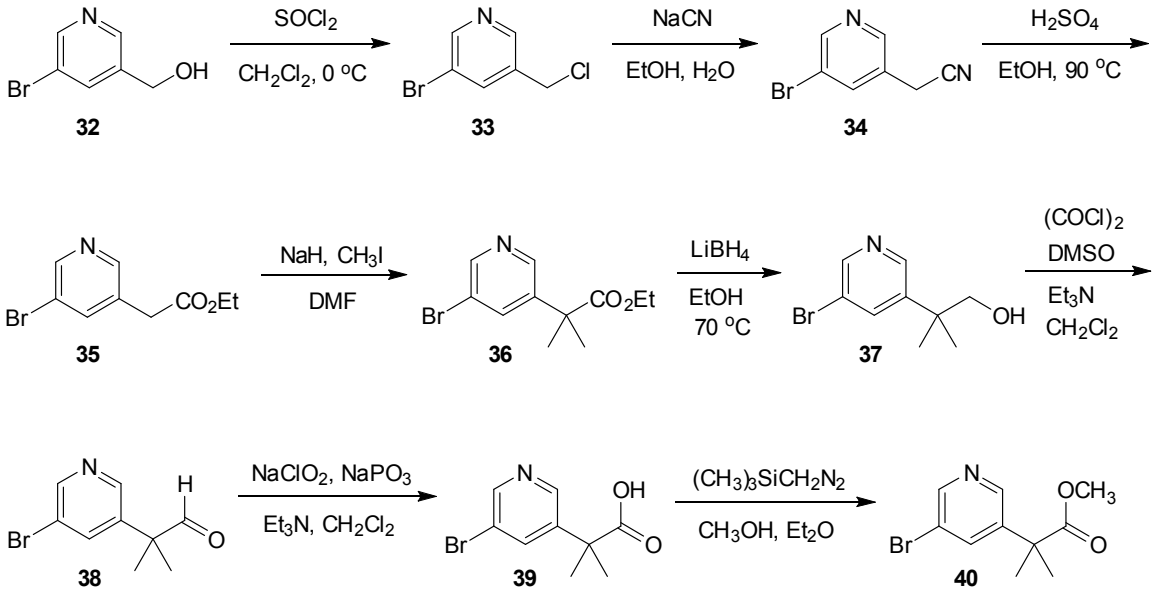
Scheme 1. General synthesis A of triazole compounds **1** – **6**.



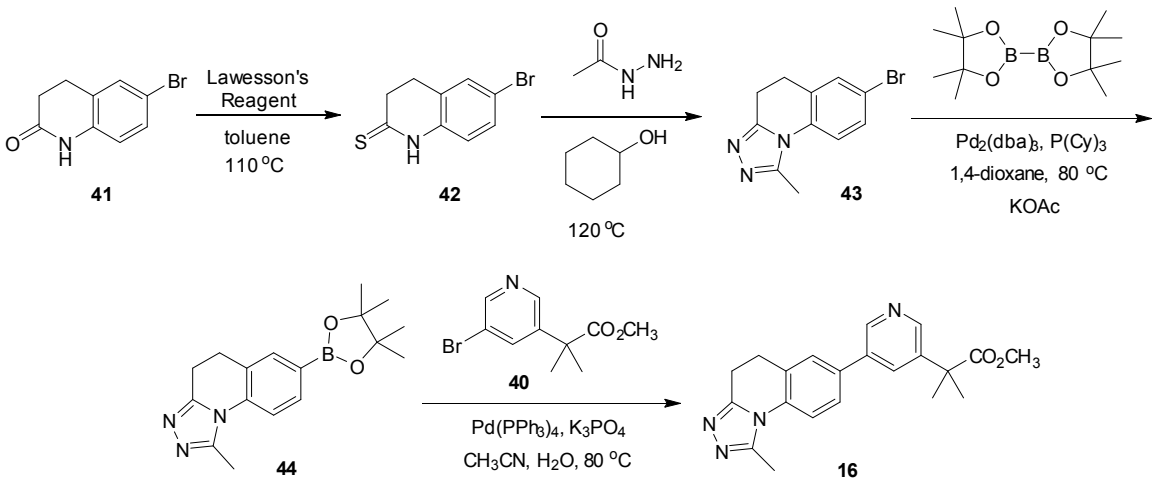
Scheme 2. Preparation of 2-(5-bromo-3-pyridinyl)-2-propanol and 3-bromo-5-(2-methoxypropan-2-yl)pyridine.



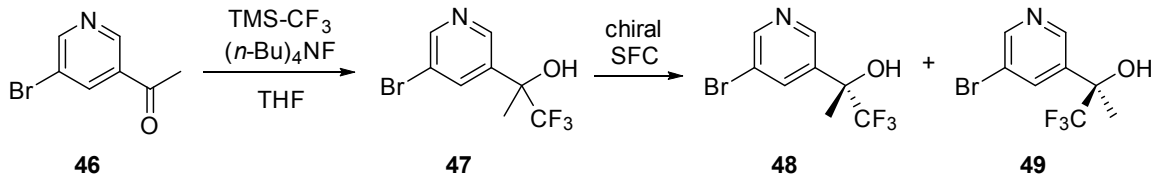
Scheme 3. Preparation of methyl 2-(5-bromopyridin-3-yl)-2-methylpropanoate.



Scheme 4. General synthesis B of triazole compounds **7** – **18**.



Scheme 5. Preparation of (*R*)- and (*S*)- 2-(5-bromopyridin-3-yl)-1,1,1-trifluoropropan-2-ol.



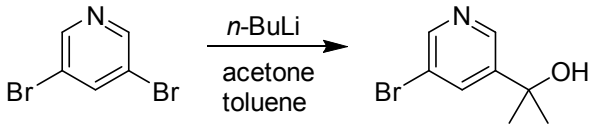
46 47 48 49

Scheme 6. General synthesis C of triazole compounds **19** – **22**.

Compound Preparation.

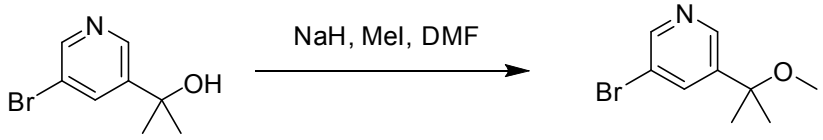
Preparation of 3-bromo-5-(2-methoxypropan-2-yl)pyridine

Step A. 2-(5-bromo-3-pyridinyl)-2-propanol



To a 5000-mL 4-necked round-bottom flask purged and maintained with an inert atmosphere of nitrogen was added a solution of 3,5-dibromopyridine (264 g, 1.12 mol) in toluene (3000 mL). The solution was cooled to -78 ^{o}C, and a solution of *n*-butyllithium in hexanes (2.6 M, 475 mL, 1.24 mol) was then added, giving a solution that was stirred for 2 hours at -78 ^{o}C. Acetone (108 g, 1.86 mol) was then added. After 1 hour, the reaction mixture was quenched by addition of 350 mL saturated aqueous ammonium chloride solution. The resulting solution was extracted with ethyl acetate. The organic extracts were combined, dried over anhydrous sodium sulfate and concentrated under

reduced pressure. Purification by flash chromatography on silica gel (ethyl acetate/petroleum ether 1:10 – 1:5) provided the title compound: ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.63 (s, 1 H), 8.56 (s, 1 H), 8.01 (s, 1 H), 1.61 (s, 6 H).



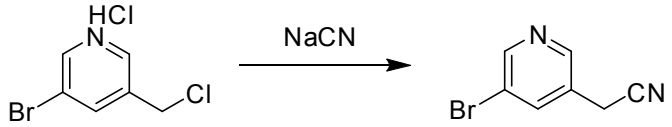
Step B. 3-bromo-5-(2-methoxypropan-2-yl)pyridine

To a solution of sodium hydride (60% dispersion in mineral oil, 46 mg, 1.2 mmol) in *N,N*-dimethylformamide (4.6 mL) at 0 °C was added a solution of the title compound from Step A (100 mg, 0.463 mmol) in *N,N*-dimethylformamide (4.6 mL). After warming to room temperature and stirring for 1 hour, the solution was cooled back to 0 °C and iodomethane (35 µl, 0.56 mmol) was added. After stirring overnight, the reaction was poured into water and extracted with ethyl acetate. The organic extracts were combined, washed with saturated aqueous sodium chloride solution, dried over sodium sulfate and concentrated under reduced pressure to afford the title compound: LCMS m/z 232.97 $[M + H]^+$.

Preparation of methyl 2-(5-bromopyridin-3-yl)-2-methylpropanoate

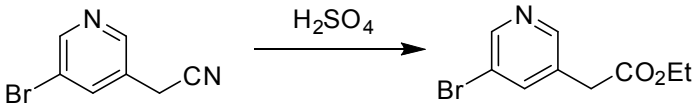
Step A. 3-bromo-5-(chloromethyl)pyridine

To a cooled (0 ^{o}C) solution of (5-bromopyridin-3-yl)methanol (38.8 g, 0.206 mol) in dichloromethane (0.6 L) was added a solution of thionyl chloride (149 mL, 2.05 mol) in dichloromethane (200 mL). The resulting mixture was stirred at reflux overnight. The reaction was then concentrated under reduced pressure, and the resulting residue was recrystallized from diethyl ether to provide the title compound: LCMS m/z 206 $[M + H]^+$; ^{1}H NMR (300 MHz, d6-DMSO) δ 12.66 (s, 1 H), 8.69 – 8.74 (m, 2 H), 8.25 (s, 1 H), 4.83 (s, 2 H).



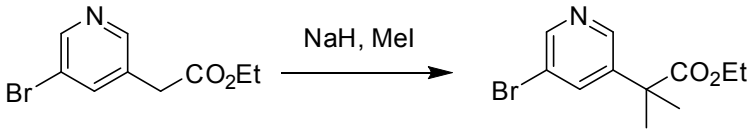
Step B. (5-bromo-3-pyridinyl)acetonitrile

A solution of the title compound from Step A dissolved in 500 mL of ethyl acetate was made basic with saturated aqueous sodium bicarbonate solution. The organic layer was washed with saturated aqueous sodium chloride solution and concentrated. The residue was dissolved in 400 mL of ethanol at room temperature and was added to a solution of sodium cyanide in 90 mL of water. Then the mixture was stirred at reflux overnight. The mixture was cooled to room temperature and poured into water. It was extracted with dichloromethane, the organic layer was dried and concentrated to give the crude product which was purified by flash chromatograph on silica gel (petroleum ether : ethyl acetate = 20:1 – 5:1) to give the title compound: LCMS m/z 197 $[M + H]^+$; ^1H NMR (300 MHz, $CDCl_3$) δ 8.67 (s, 1 H), 8.51 (s, 1 H), 7.89 (s, 1 H), 3.78 (s, 2 H).



Step C. ethyl (5-bromo-3-pyridinyl)acetate

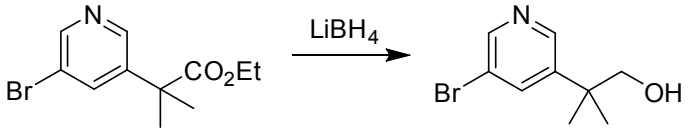
To a mixture of ethanol (10 mL) and *conc.* H_2SO_4 (4 mL) was added the title compound from Step B (0.50 g, 2.5 mmol) in 3 mL of ethanol at room temperature. The mixture was stirred at 90 ^oC overnight. The reaction was poured into ice and made basic with saturated aqueous sodium bicarbonate solution. It was then extracted with ethyl acetate (2 x 30 mL), the organic layer was dried, concentrated and purified by flash chromatography on silica gel (petroleum ether:ethyl acetate = 10:1) to give the title compound: LCMS m/z 244 $[M + H]^+$; ^1H NMR (300 MHz, $CDCl_3$) δ 8.60 (s, 1 H), 8.44 (s, 1 H), 7.82 (s, 1 H), 4.17 – 4.20 (m, 2 H), 3.61 (s, 2 H) , 1.27 – 1.30 (m, 3 H).



Step D. ethyl 2-(5-bromo-3-pyridinyl)-2-methylpropanoate

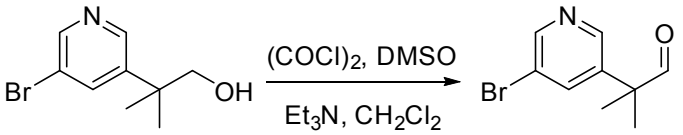
To a suspension of sodium hydride (251 mg, 5.33 mmol) in 10 mL of *N,N*-dimethylformamide at 0 ^oC was added a solution of the title compound from Step C (520

mg, 2.13 mmol) in *N,N*-dimethylformamide (2 mL). After 1 hour, iodomethane (0.29 mL, 4.67 mmol) in 2 mL of *N,N*-dimethylformamide was added. The resulting mixture was then allowed to warm to room temperature over 2 hours. The reaction was then quenched by addition of water, extracted and purified by flash chromatograph on silica gel (petroleum ether:ethyl acetate = 40:1-20:1) to give the title compound: LCMS m/z 272 $[M + H]^+$; ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.53 – 8.56 (m, 2 H), 7.80 (s, 1 H), 4.10 – 4.18 (m, 2 H), 1.60 (s, 2 H), 1.18 – 1.22 (m, 3 H).



Step E. 2-(5-bromo-3-pyridinyl)-2-methyl-1-propanol

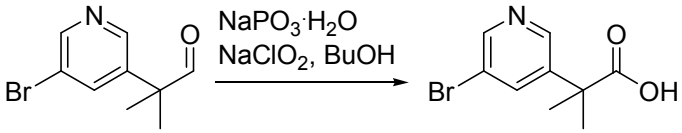
To a solution of the title compound from Step D (93 g, 0.34 mol) in ethanol (1.2 L) was added lithium borohydride (16.6 g, 0.75 mol). The resulting mixture was heated to reflux and stirred overnight. The reaction was then cooled to room temperature and poured onto ice. The mixture was extracted with ethyl acetate, and the organic layer washed with saturated aqueous sodium chloride solution, dried over magnesium sulfate, filtered and concentrated under reduced pressure. Purification by flash chromatograph on silica gel (petroleum ether/ethyl acetate = 40/1-8/1) provided the title compound: LCMS m/z 230 $[M + H]^+$; ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.58 (s, 1 H), 8.52 (s, 1 H), 7.97 (s, 1 H), 4.82 – 4.86 (m, 1 H), 3.44 – 3.46 (m, 2 H), 1.24 (s, 6 H).



Step F. 2-(5-bromopyridin-3-yl)-2-methylpropanal

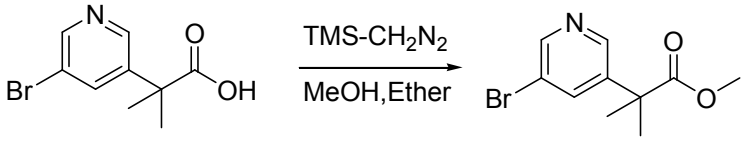
A solution of oxalyl chloride in dichloromethane (2.0 M, 0.869 mL, 1.74 mmol) was diluted with dichloromethane (2 mL) and then a solution of dimethylsulfoxide (0.250 mL, 3.50 mmol) in dichloromethane (1 mL) was added at -78 °C. After stirring for 15 minutes, a solution of the title compound from Step E (0.200 g, 0.869 mmol) in dichloromethane (3 mL) was added. The resulting reaction stirred for 30 minutes before triethylamine (0.600 mL, 4.35 mmol) was added. The reaction warmed to room temperature slowly and stirred overnight. It was quenched with the addition of water and

then extracted with dichloromethane. The combined organics were washed with saturated aqueous sodium chloride solution, dried over sodium sulfate, filtered and concentrated under reduced pressure. Purification by flash chromatography on silica gel (25 – 100% ethyl acetate in hexanes) provided the title compound: LCMS m/z 229.87 [M + 2 + H]$^+$; ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.53 (s, 1 H), 8.61 (s, 1 H), 8.47 (s, 1 H), 7.73 (s, 1 H), 1.51 (s, 6 H).



Step G. 2-(5-bromopyridin-3-yl)-2-methylpropanoic acid

To a cooled (0 °C) solution of the title compound from Step F (0.100 g, 0.438 mmol) in tert-butanol (2.2 mL) was added an aqueous solution of sodium-m-phosphate monohydrate (2.0 M, 0.658 mL, 1.32 mmol). After several minutes, an aqueous solution of sodium chlorite (2.0M, 0.77 ml, 1.54 mmol) was added. The resulting reaction was allowed to warm to room temperature where it stirred until complete. The reaction was concentrated under reduced pressure, then diluted with aqueous 0.1% trifluoroacetic acid solution and passed through a syringe filter. Purification by HPLC (C18 column, 10 to 100% acetonitrile/water, both 0.1% v/v trifluoroacetic acid) provided the title compound: LCMS m/z 245.97 [M + 2 + H]$^+$; ^{1}H NMR (500 MHz, CD_3OD) δ 8.64 (s, 1 H), 8.62 (s, 1 H), 8.19 (s, 1 H), 1.62 (s, 6 H).

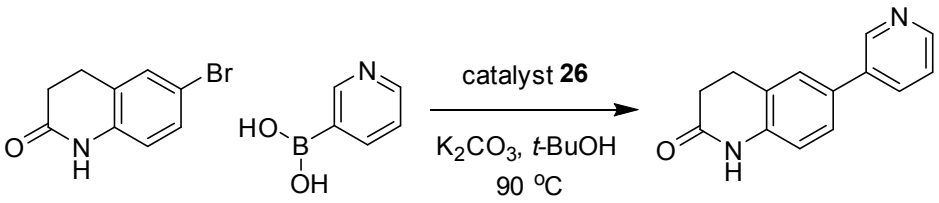


Step H. methyl 2-(5-bromopyridin-3-yl)-2-methylpropanoate

To a solution of the title compound from Step G (0.350 g, 1.43 mmol) in diethyl ether (8.6 mL) and methanol (5.7 mL) was added a solution of (trimethylsilyl)diazomethane in diethyl ether (2.0 M, 1.08 mL, 2.16 mmol). The reaction stirred for 1 hour before being quenched with acetic acid. The reaction solution was then concentrated under reduced pressure and purified by flash chromatography on silica gel (0 – 80% ethyl acetate in hexanes) to provide the title compound: LCMS m/z 259.83 [M

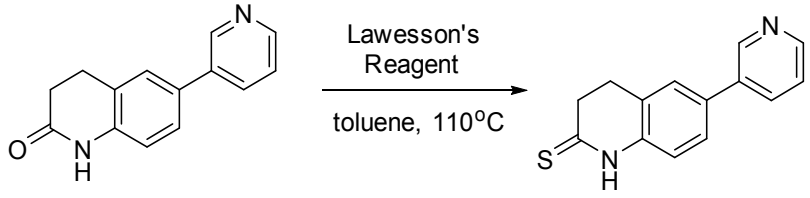
+ 2 + H]$^+$; ^{1}H NMR (500 MHz, DMSO) δ 8.54 (s, 1 H), 8.50 (s, 1 H), 7.99 (s, 1 H), 3.68 (s, 3 H), 1.60 (s, 6 H).

Example 1: Exemplification of General Synthesis A: Preparation of Compound **1**.



Step A. 6-(pyridin-3-yl)-3,4-dihydroquinolin-2(1H)-one

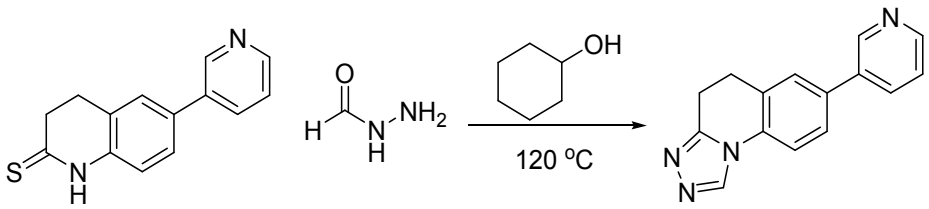
A sealable tube containing 6-bromo-3,4-dihydroquinolin-2(1H)-one (0.10 g, 0.44 mmol), 3-pyridylboronic acid (0.56 g, 4.6 mmol), bis(di-tert-butyl(4-dimethylaminophenyl)phosphine)dichloropalladium(II) (6.3 mg, 8.9 µmol), and potassium carbonate (0.18 g, 1.3 mmol) was flushed with nitrogen before tert-butanol (4.9 mL) and water (0.6 mL) were added. The tube was flushed again with nitrogen, sealed tightly and heated to 100 °C overnight. The reaction was then cooled to room temperature, poured into saturated aqueous sodium chloride solution and extracted with ethyl acetate. The organic extracts were combined, washed with water, dried over sodium sulfate, filtered and concentrated. Purification by flash chromatography on silica gel (0 – 15% methanol in ethyl acetate) provided the title compound: LCMS m/z 225.27 [M + H]$^+$; ^{1}H NMR (500 MHz, CD_3OD) δ 8.77 (s, 1 H), 8.47 (d, J = 4.8 Hz, 1 H), 8.06 (ddd, J = 1.7, 2.0, 8.1 Hz, 1 H), 7.52 (s, 1 H), 7.50 – 7.47 (m, 2 H), 6.99 (d, J = 8.1 Hz, 1 H), 3.05 (t, J = 7.5, 7.7 Hz, 2 H), 2.61 (t, J = 7.5, 7.7 Hz, 2 H.



Step B. 6-(pyridin-3-yl)-3,4-dihydroquinoline-2(1H)-thione

To the title compound from Example 1 Step A (200 mg, 0.892 mmol) and 2,4-bis(4-methoxyphenyl)-1,3,2,4-dithiadiphosphetane-2,4-disulfide (180 mg, 0.446 mmol)

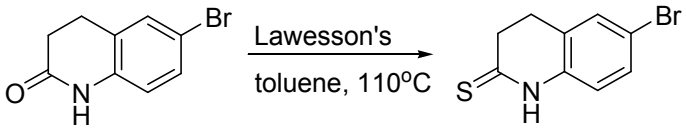
was added toluene (1.8 mL). The suspension was heated to reflux for 45 minutes. The reaction was then cooled to room temperature and concentrated under reduced pressure. Purification by flash chromatography on silica gel (0 – 15% methanol in ethyl acetate) provided the title compound: LCMS m/z 241.22 $[M + H]^+$; ^{1}H NMR (500 MHz, CD_3OD) δ 8.86 (d, *J* = 2.0 Hz, 1 H), 8.55 (dd, *J* = 1.5, 4.8 Hz, 1 H), 8.11 (ddd, *J* = 1.9, 2.2, 8.2 Hz, 1 H), 7.61 – 7.58 (m, 2 H), 7.53 (dd, *J* = 4.9, 8.0 Hz, 1 H), 7.20 (d, *J* = 8.1 Hz, 1 H), 3.09 - 3.06 (m, 2 H), 2.99 – 2.96 (m, 2 H).



Step C. 7-(pyridin-3-yl)-4,5-dihydro[1,2,4]triazolo[4,3-a]quinoline

A flask containing the title compound from Example 1 Step B (20 mg, 0.08 mmol), formic hydrazide (6.0 mg, 0.10 mmol) and cyclohexanol (0.50 ml, 0.08 mmol) was heated to reflux for 6 hours. The reaction was cooled to room temperature, diluted with dimethylsulfoxide, acidified with trifluoroacetic acid and passed through a syringe filter. Purification by reverse phase HPLC (C18 column, 10 to 100% acetonitrile/water, both 0.1% v/v trifluoroacetic acid) provided the title compound: LCMS m/z 249.16 $[M + H]^+$; ^{1}H NMR (500 MHz, CD_3OD) δ 9.90 (s, 1 H), 9.30 (d, *J* = 2.0 Hz, 1 H), 9.01 (ddd, *J* = 1.5, 1.9, 8.2 Hz, 1 H), 8.93 (d, *J* = 5.6 Hz, 1 H), 8.23 (dd, *J* = 5.8, 8.2 Hz, 1 H), 8.10 – 8.07 (m, 2 H), 8.02 (dd, *J* = 1.9, 8.4Hz, 1 H), 3.46 – 3.43 (m, 2 H), 3.38 – 3.35 (m, 2 H).

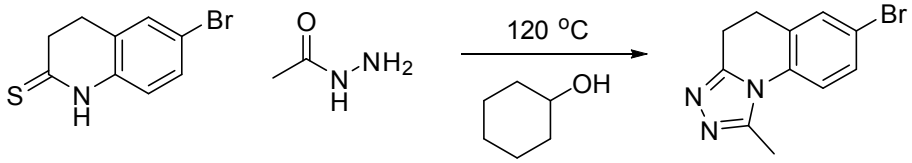
Example 2: Exemplification of General Synthesis B: Preparation of Compound **16**.



Step A. 6-bromo-3,4-dihydroquinoline-2(1H)-thione

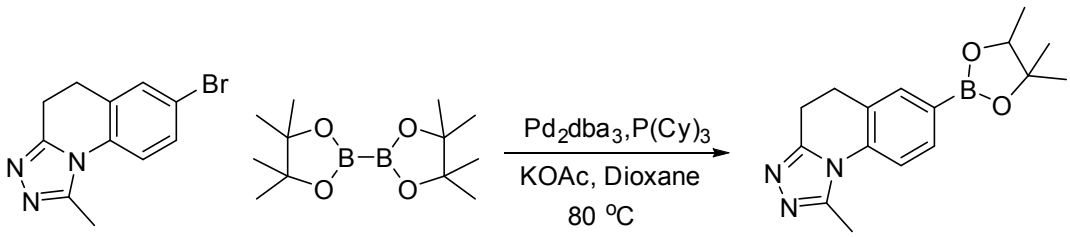
6-bromo-3,4-dihydroquinolin-2-(1H)-one (1.00 g, 4.42 mmol) and 2,4-bis(4-methoxyphenyl)-1,3,2,4-dithiadiphosphetane2,4-disulfide (0.895 g, 2.21 mmol) were

suspended in toluene (110 mL) and then heated to 110 °C overnight. The reaction was then cooled to room temperature and concentrated under reduced pressure. Dichloromethane was added, giving a mixture that was filtered to provide the title compound: LCMS m/z 243.95 $[M + 2 + H]^+$; ^{1}H NMR (500 MHz, DMSO) δ 7.44 (s, 1 H), 7.39 (d, *J* = 8.4 Hz, 1 H), 7.01 (d, *J* = 8.4 Hz, 1 H), 2.92 – 2.89 (m, 2 H), 2.81 – 2.78 (m, 2 H).



Step B. 7-bromo-1-methyl-4,5-dihydro[1,2,4]triazolo[4,3-a]quinoline

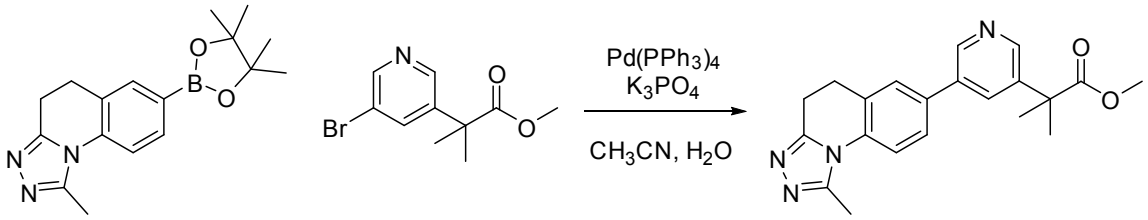
A flask containing the title compound from Example 2 Step A (100 mg, 0.413 mmol), acetic hydrazide (40.8 mg, 0.496 mmol) and cyclohexanol (2.00 ml, 0.413 mmol) was heated at reflux for 2 days. The reaction was then poured into water and extracted with ethyl acetate. The organic extracts were combined, washed with brine, dried over sodium sulfate, filtered and concentrated under reduced pressure. Purification by flash chromatography on silica gel (0 – 15% methanol in ethyl acetate) provided the title compound: LCMS m/z 265.99 $[M + 2 + H]^+$; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.53 (br s, 1 H), 7.50 (d, *J* = 8.57 Hz, 1 H), 7.35 (d, *J* = 8.57 Hz, 1 H), 3.13 – 3.11 (m, 2 H), 3.00 - 2.98 (m, 2 H), 2.74 (s, 3 H).



Step C. 1-methyl-7-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-4,5-dihydro[1,2,4]triazolo[4,3-a]quinoline

To a vial containing the title compound from Example 2 Step B (0.100 g, 0.379 mmol), bis(pinacolato)diboron (0.115 g, 0.454 mmol), potassium acetate (0.111 g, 1.14 mmol), tris(dibenzylideneacetone)dipalladium (0) (0.035 g, 0.038 mmol) and

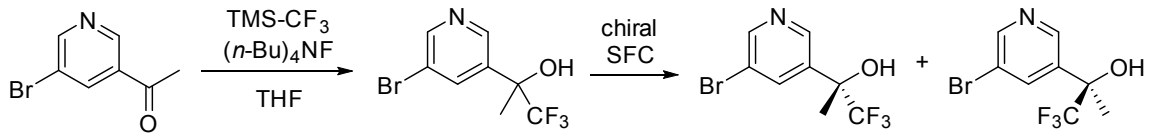
tricyclohexylphosphine (0.021 g, 0.076 mmol) was added 1,4-dioxane (3.8 mL). The vial was flushed with nitrogen, sealed tightly and heated to 80 °C overnight. The reaction was poured into saturated aqueous sodium chloride solution and extracted with ethyl acetate. The combined organic extracts were washed with water, dried over sodium sulfate, filtered and concentrated. Purification by flash chromatography on silica gel (0 – 12% methanol in ethyl acetate) provided the title compound: LCMS m/z 311.98 $[M + H]^+$; ^{1}H NMR (500 MHz, CD_3OD) δ (s, 1 H), 8.50 (1 H), 7.99 (s, 1 H), 3.68 (s, 3 H), 1.60 (s, 6 H).



Step D. methyl 2-methyl-2-[5-(1-methyl-4,5-dihydro[1,2,4]triazolo[4,3-a]quinolin-7-yl)pyridin-3-yl]propanoate

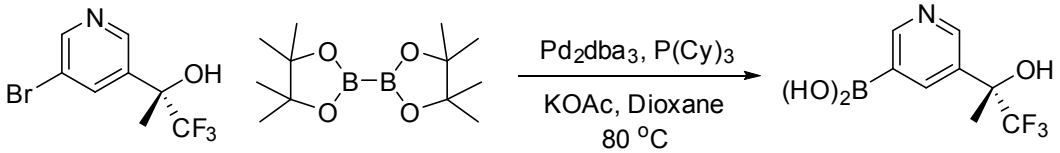
To a vial containing the title compound from Example 2 Step C (12.5 mg, 0.040 mmol), methyl 2-(5-bromopyridin-3-yl)-2-methylpropanoate (8.0 mg, 0.03 mmol) and tetrakis(triphenylphosphine)palladium (0) (7.2 mg, 6.2 µmol) was added acetonitrile (0.34 mL). The vial was flushed with nitrogen prior to the addition of potassium phosphate tribasic (19.7 mg, 0.093 mmol) in water (0.04 mL). The vial was capped tightly and heated to 100 °C overnight. The reaction was then poured into saturated aqueous sodium chloride solution and extracted with ethyl acetate. The combined organic extracts were washed with water, dried over sodium sulfate, filtered and concentrated under reduced pressure. The resulting residue was diluted with dimethylsulfoxide, passed through a syringe filter and purified by HPLC (C18 column, 10 to 100% acetonitrile/water, both 0.1% v/v trifluoroacetic acid) to provide the title compound: LCMS m/z 363.11 $[M + 2 + H]^+$; ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.73 (s, 1 H), 8.64 (s, 1 H), 7.82 (m, 1 H), 7.61-7.57 (m, 3 H), 3.70 (s, 3 H), 3.20 – 3.17 (m, 2 H), 3.12 – 3.09 (m, 2 H), 2.81 (s, 3 H), 1.69 (s, 6H).

Example 3: Exemplification of General Synthesis C: Preparation of Compound **19**.



Step A. (*R*)- and (*S*)-2-(5-bromopyridin-3-yl)-1,1,1-trifluoropropan-2-ol

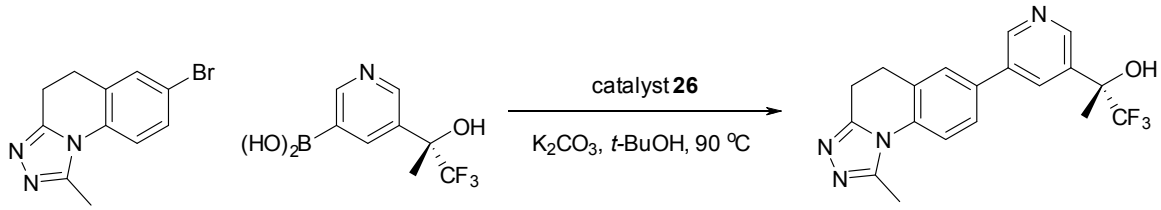
To a flask containing 3-acetyl-5-bromopyridine (2.27 g, 11.4 mmol) was added a solution of (trifluoromethyl)trimethylsilane in tetrahydrofuran (0.5 M, 40 mL, 20 mmol) at 0 °C. A solution of tetrabutylammonium fluoride in tetrahydrofuran (1.0 M, 11.4 mL, 11.4 mmol) was then added, and the reaction stirred at room temperature until the reaction was complete. The reaction was then concentrated under reduced pressure, diluted with ethyl acetate, and washed with water and saturated aqueous sodium bicarbonate solution. The organic layer was separated, dried over sodium sulfate, filtered and concentrated to give a residue that was purified by flash chromatography on silica gel (10% to 50% ethyl acetate / hexanes linear gradient) to provide the racemic title compound: LCMS m/z 269.85 $[M + 2 + H]^+$; ^{1}H NMR (500 MHz, CD_3OD) δ 8.70 (s, 1 H), 8.65 (1 H), 8.13 (s, 1 H), 1.81 (s, 3 H). The racemic title compound was resolved by supercritical fluid chromatography on a chiral AD column, eluting with 10% ethanol:CO_2. Data for the fast-eluting (*R*)-enantiomer: LCMS m/z 271.85 $[M + H]^+$; ^{1}H NMR (500 MHz, $CDCl_3$) 8.71 (s, 1 H), 8.68 (d, *J* = 2.0 Hz, 1 H), 8.10 (s, 1 H), 1.82 (s, 3 H). Data for the slower-eluting (*S*)-enantiomer: LCMS m/z 271.83 $[M + H]^+$; ^{1}H NMR (500 MHz, $CDCl_3$) 8.71 (s, 1 H), 8.68 (s, 1 H), 8.10 (s, 1 H), 1.81 (s, 3 H).



Step B. (*S*)-[5-(1,1,1-trifluoro-2-hydroxypropan-2-yl)pyridin-3-yl]boronic acid

A vial containing the title compound from Example 4 Step A [(*S*)-enantiomer, (0.647 g, 2.40 mmol)], bis(pinacolato)diboron (1.22 g, 4.79 mmol), tris(dibenzylideneacetone)dipalladium (0) (0.439 g, 0.479 mmol), tricyclohexylphosphine (0.269 g, 0.958 mmol) and potassium acetate (0.705 g, 7.19 mmol) in 1,4-dioxane (12

mL) was flushed with nitrogen, sealed tightly and heated to 80 °C overnight. The reaction was then passed through a syringe filter and and concentrated under reduced pressure to provide the title compound: LCMS m/z 235.95 $[M + H]^+$.



Step C. (*S*)-1,1,1-trifluoro-2-[5-(1-methyl-4,5-dihydro[1,2,4]triazolo[4,3-a]quinolin-7-yl)pyridin-3-yl]propan-2-ol

To a vial containing the title compound from Example 4 Step B (0.58 g, 2.2 mmol), the title compound from Example 2 Step B (0.563 g, 2.34 mmol), bis(di-tert-butyl(4-dimethylaminophenyl)phosphine) dichloropalladium(II) (0.031 g, 0.044 mmol), and potassium carbonate (0.912 g, 6.60 mmol) was added tert-butanol (24 mL) and water (3.0 mL). The vial was flushed with nitrogen, sealed tightly and heated to 80 °C overnight. The reaction was then cooled to room temperature, concentrated under reduced pressure and purified by HPLC (C18 column, 10 to 100% acetonitrile/water, both 0.1% v/v trifluoroacetic acid). Fractions containing product were combined, aqueous 1 M hydrochloric acid solution was added, and the solution concentrated under reduced pressure to provide the title compound: HRMS (Positive ESI) m/z 375.1447 (375.1433 calcd for C19H17F3N4OH, M+H); $[\alpha]_D$ +4.2, (*c* 0.66, CH_3OH); ^{1}H NMR (500 MHz, CD_3OD) δ 9.31 (s, 1 H), 9.13 (1 H), 9.08 (s, 1 H), 8.09 (s, 1 H), 8.03 (s, 2 H), 3.34 (m, 4 H), 3.06 (s, 3 H), 1.96 (s, 3 H). ^{13}C NMR (125 MHz, CD_3OD) δ 152.7, 149.2, 146.7, 146.2, 136.5, 135.8, 135.2, 133.1, 130.6, 127.9, 126.8, 126.5, 124.5, 118.4, 72.8 (q, J = 30 Hz), 26.3, 21.7, 20.5, 12.0.

Example 4: Preparation of Compound **14**.

Compound **14** was prepared from 3-bromo-5-(2-methoxypropan-2-yl)pyridine (synthesis described above) and the title compound from Example 2 step B using the coupling method described in Example 3 step C: HRMS (Positive ESI) m/z 321.1717 (321.1715 calcd for C19H20N4OH, M+H); ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.73 (s, 2 H),

8.06 (s, 1 H), 7.62 – 7.58 (m, 3 H), 3.19 – 3.16 (m, 2 H), 3.11 – 3.08 (m, 2 H), 2.80 (s, 3 H), 1.69 (s, 6 H).

Example 5: Preparation of Compound **18**.

Compound **18** was prepared from 3-bromo-5-(2-methoxypropan-2-yl)pyridine (synthesis described above) using a procedure analogous to the one described in Example 2: HRMS (Positive ESI) m/z 375.1437 (375.1433 calcd for C19H17N4OH, M+H); ^{1}H NMR (500 MHz, CD_3OD) δ 9.06 (s, 1 H), 8.95 (s, 1 H), 8.83 (s, 1H), 7.99 (s, 1 H), 7.91 (d, 1 H, J = 2.78 Hz), 7.81 (d, 1 H, , J = 2.79 Hz), 3.28 – 3.20 (m, 4 H), 1.67 (s, 6 H). ^{13}C NMR (125 MHz, d6-DMSO) δ 156.3, 148.1, 142.7, 140.6 (q, J = 39 Hz), 135.8, 135.3, 134.8, 132.3, 131.6, 128.8, 127.3, 120.4, 119.4, 118.2, 70.4, 31.9 (2 C), 25.8, 21.0.

X-ray Crystallographic Analysis of Bromide 48. The absolute configurations of bromides **48** and **49**, and of the final compounds **20** – **23** prepared from **48** and **49**, were established by X-ray crystallographic analysis of bromide **48**. An ORTEP representation and associated data are shown below.

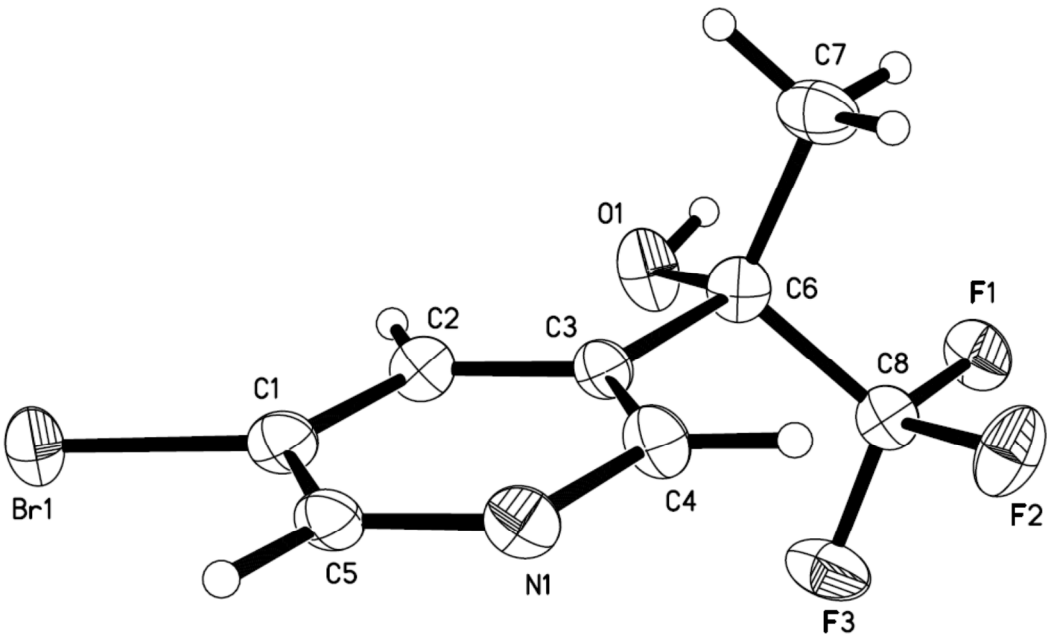


Figure 1: ORTEP represenation of Compound 48. Thermal ellipsoids are set at the 50%

probability level.

Table 1. Crystal data and structure refinement for Compound 48.

Identification code	MDF078	
Empirical formula	C8 H7 Br F3 N O	
Formula weight	270.047	
Temperature	101K	
Wavelength	1.5418 Å	
Crystal system	Orthorhombic	
Space group	$P2_1\ 2_1\ 2_1$	
Unit cell dimensions	a = 6.9491(2) Å	α= 90°
	b = 7.4935(2) Å	β= 90°
	c = 17.5809(10) Å	γ = 90°
Volume	915.22(4) $Å^3$	
Z	4	
Density (calculated)	1.960 Mg/m^3	
Absorption coefficient	6.3350 mm^{-1}	
F(000)	528	
Crystal size	0.04 x 0.18 x 0.19mm^3	
Theta range for data collection	5.03 to 66.82°	
Index ranges	-8<=h<=8, -8 <=k<=8, -20<=l<=20	
Reflections collected	7971	
Independent reflections	1626 [R(int) = 0.0563]	
Completeness to theta = . °	100 %	
Absorption correction	Analytical numeric	
Max. and min. transmission	0.8364 and 0.4592	
Refinement method	Full-matrix least-squares on F^2	
Data / restraints / parameters	1626 / 0 / 129	
Goodness-of-fit on F^2	1.12	
Final R indices [I>2sigma(I)]	R1 = 0.038, wR2 = 0.106	
R indices (all data)	R1 = 0.039, wR2 = 0.107	
Absolute structure parameter	-0.06(4)	
Extinction coefficient	None	
Largest diff. peak and hole	0.552and -0.934 (e.$Å^{-3}$	

References

1. Cai, T.-Q.; Stribling, S.; Tong, X.; Xu, L.; Wisniewski, T.; Fontenot, J. A.; Struthers, M.; Akinsanya, K. O. Rhesus monkey model for concurrent analyses of pharmacodynamics, pharmacokinetics and in vivo selectivity of aldosterone synthase inhibitors. *J. Pharmacol. Toxicol. Methods* **2015,** *71*, 137 – 146.
2. Hoyt, S. B.; Petrilli, W. L.; London, C.; Xiong, Y.; Taylor, J. A.; Ali, A.; Lo, M.; Henderson, T. J.; Hu, Q.; Hartmann, R.; Yin, L.; Heim, R.; Bey, E.; Saxena, R.; Samanta, S. K.; Kulkarni, B. A. Aldosterone Synthase Inhibitors. WO 2012148808, 11/01/2012.