Supporting Information

Discovery of Benzimidazole 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; 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 either the human CYP11B2 or 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 CYP21 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 CYP21 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. 25 µL of cells were added to a 96 well tissue culture treated

plate and mixed with 0.25µL 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 1.0 µM 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 11-deoxycortisol product in the supernatant was measured using the HTRF-based assay kit (Cortisol HTRF-CisBio-62CO2PEB). 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.

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) in versus time (min) profile.

Determination of Plasma Protein Binding. In vitro plasma protein binding (PPB) was estimated in rat, dog, monkey and human 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 Pharmacokinetic Assay. Rat 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 were given either a 1.0 mg/kg i.v. dose of test compound solution via a cannula implanted in the femoral vein (n = 2) 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 Pharmacokinetic Assay. Pharmacokinetics for rhesus were typically determined using an intravenous solution formulation at 1.0 mg/mL in ethanol/PEG400/water (10:40:50, v/v/v) and an oral suspension formulation at 2.0 mg/mL in 0.5% methyl cellulose. Fasted rhesus monkeys were given either a 0.5 mg/kg i.v. dose of test compound solution via a saphenous vein catheter (n = 2) or a 1.0 mg/kg p.o. dose by gavage (n = 2). Serial blood samples were collected at 5 (i.v. only), 15, and 30 minutes, and at 1, 2, 4, 6, 8, 24 and 48 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.

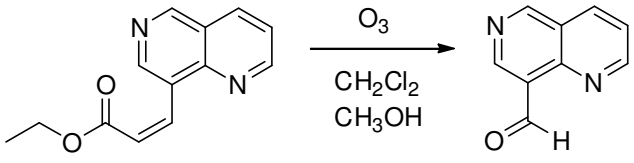
Rhesus Pharmacodynamic Assay. In vivo effects of aldosterone synthase (CYP11B2) inhibition on circulating levels of adrenal steroids were investigated in male rhesus macaques using methods that have recently been described in detail.[1] Briefly, animals on low sodium diet were anesthetized. Vehicle (saline, 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.

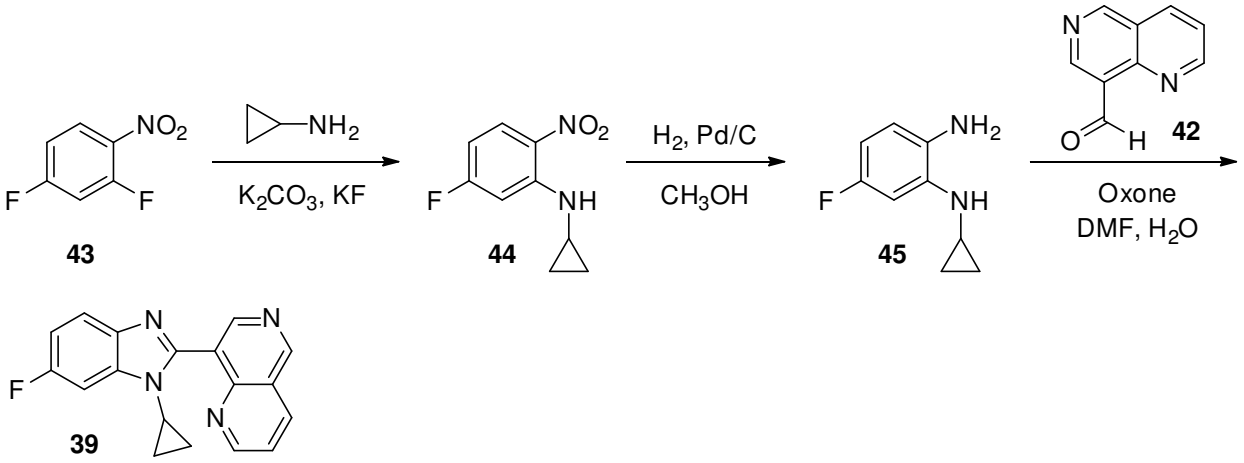
Synthesis Schemes. Detailed procedures for the synthesis of these compounds have been reported previously.[2]

Scheme 1. Preparation of 1,6-napthyridine-8-carbaldehyde.

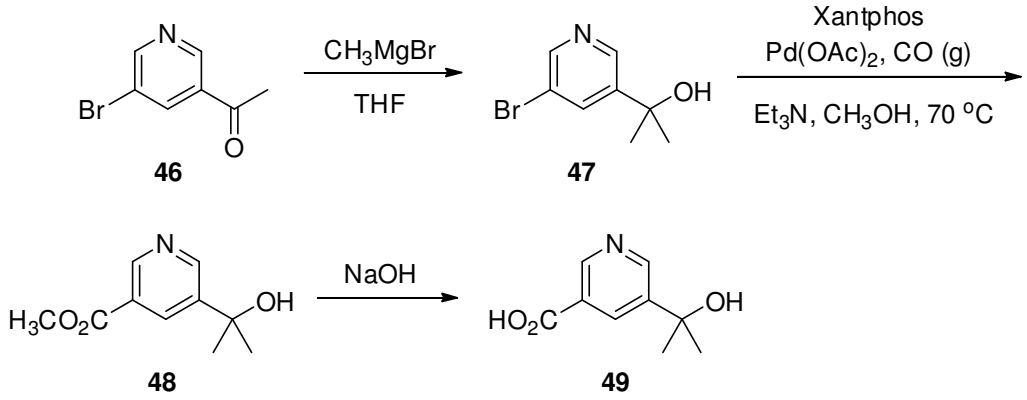


41 **42**

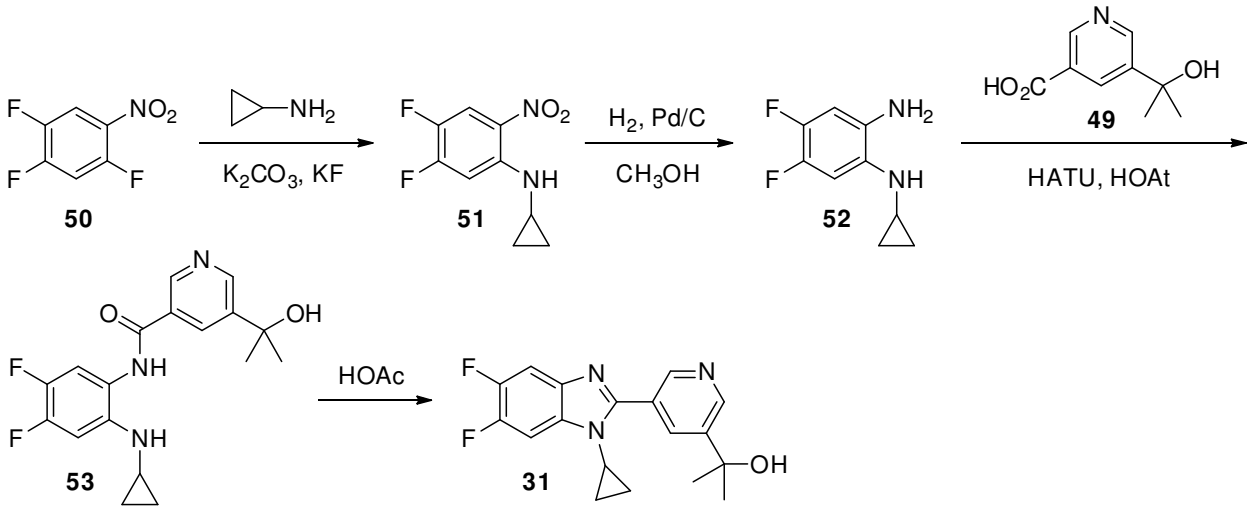
Scheme 2. General synthesis A of benzimidazole target compounds 1 – 30, 32, 34, and 36 – 40.



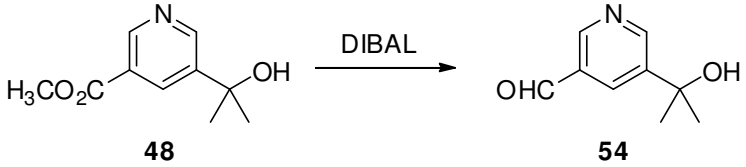
Scheme 3. Preparation of 5-(2-hydroxypropan-2-yl)nicotinic acid.



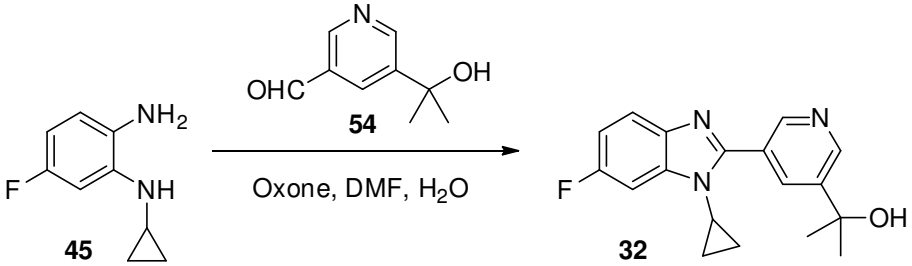
Scheme 4. General synthesis B of target compound 31.



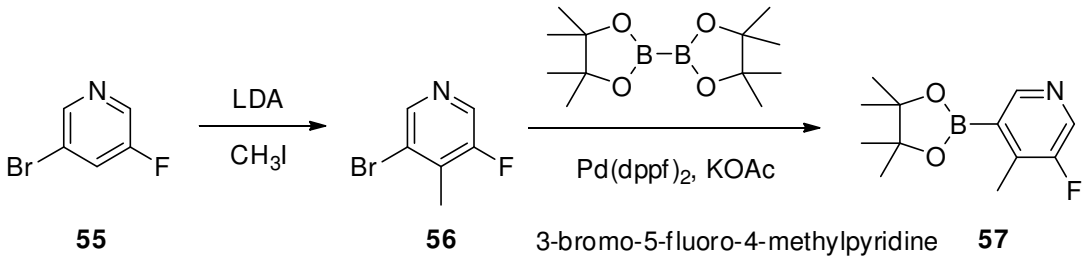
Scheme 5. Preparation of 5-(2-hydroxypropan-2-yl)nicotinaldehyde.



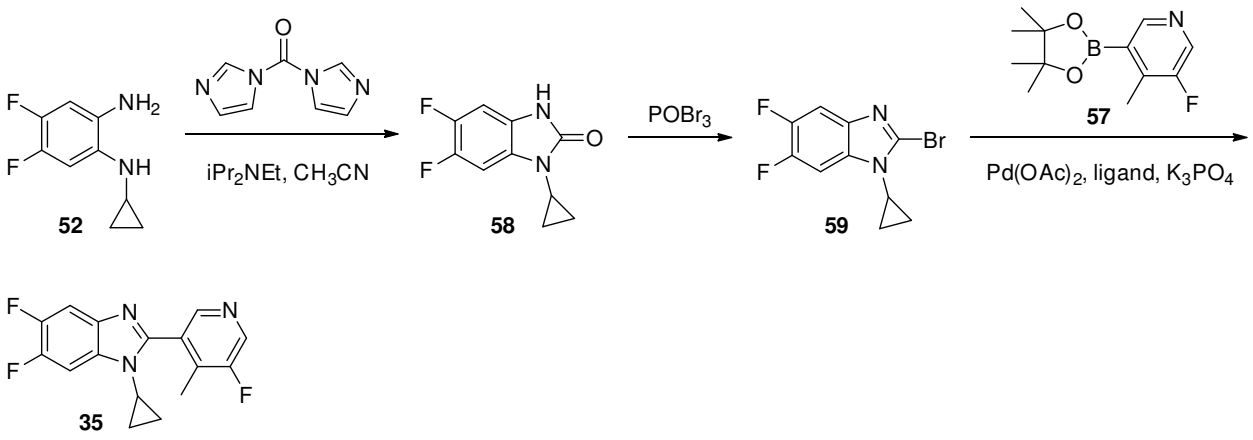
Scheme 6. Preparation of compound **32**.



Scheme 7. Preparation of 3-fluoro-4-methyl-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)pyridine.

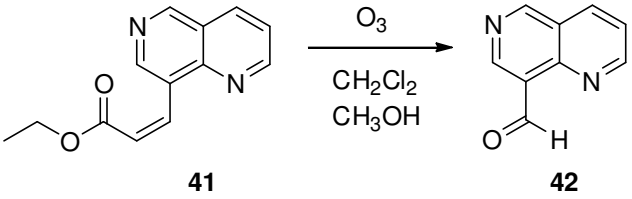


Scheme 8. General synthesis C of final targets 33 and 35.



Compound Preparation.

Preparation of 1,6-napthyridine-8-carbaldehyde.

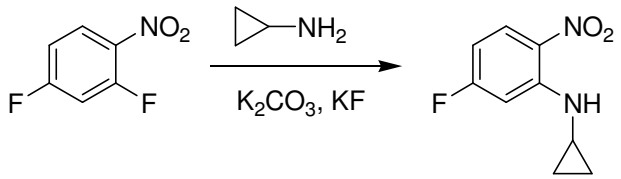


A solution of ethyl (2Z)-3-(1,6-naphthyridin-8-yl)prop-2-enoate (30 g) in dichloromethane (500 mL) and methanol (125 mL) was cooled to -78 °C and reacted with ozone until the color of the reaction mixture became light blue (~3 h). The reaction was purged with nitrogen to remove the residual ozone. Methyl sulfide (60 mL) was then added, and the reaction was allowed to warm to room temperature and then concentrated. The resulting residue was dissolved in dichloromethane (300 mL), washed with water (100 mL) and saturated aqueous sodium bicarbonate (100 mL) and dried over sodium sulfate. The solvent was removed and product was slurried in heptane/ethyl acetate mixture (5:1). The solid was then filtered and dried under reduced pressure to provide the title compound: LCMS m/z 158.97 $[M + H]^+$; ^{1}H NMR (500 MHz, $CDCl_3$) δ 11.39 (s, 1 H), 9.09 (s, 1 H), 9.13 (m, 1 H), 9.07 (s, 1 H), 8.43 (d, J = 8.3

Hz, 1 H), 7.78 (dd, J = 8.7, 4.8 Hz, 1 H), 7.63 (dd, J = 8.2, 4.2 Hz, 1 H), 7.34 (dd, J = 8.7, 2.2 Hz, 1 H), 7.07 (m, 1 H), 3.55 – 3.53 (m, 1 H), 0.62 – 0.61 (m, 4 H).

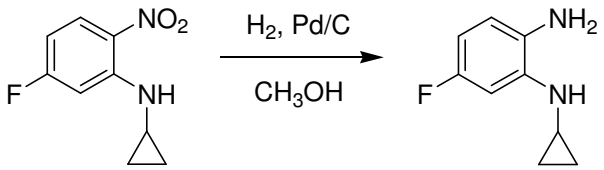
Example 1: Exemplification of General Synthesis A: Preparation of Compound **39**.

Step A. N-cyclopropyl-5-fluoro-2-nitroaniline



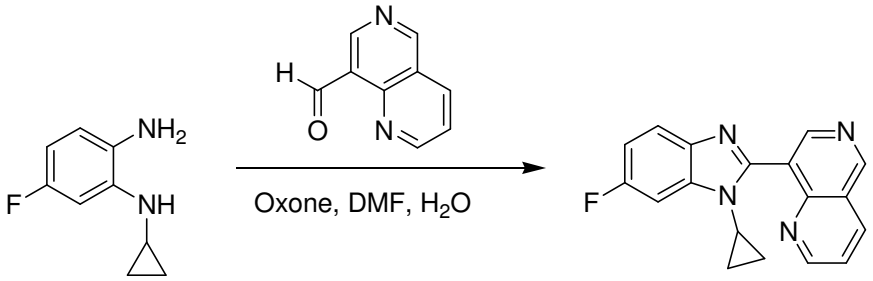
2,4-Difluoronitrobenzene (1.00 g, 6.29 mmol), potassium fluoride (0.365 g, 6.29 mmol), potassium carbonate (0.869 g, 6.29 mmol), and cyclopropylamine (0.52 mL, 7.54 mmol) were added to a vial and heated in a microwave at 90 °C for 10 min. The reaction was then diluted with dichloromethane, washed with water and brine, dried over magnesium sulfate, and concentrated to provide the title compound: LCMS m/z 196.95 $[M + H]^+$; ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.20 (dd, J = 9.5, 6.0 Hz, 2 H), 6.95 (dd, J = 11.4, 2.6 Hz, 1 H), 6.43 – 6.39 (m, 1 H), 2.58 – 2.53 (m, 1 H), 0.96 – 0.92 (m, 2 H), 0.69 – 0.66 (m, 2 H).

Step B. N^2-cyclopropyl-4-fluorobenzene-1,2-diamine



To the title compound from Example 1, Step A (1.23 g, 6.27 mmol) were added 10% palladium on carbon (0.123 g, 1.16 mmol) and methanol (31.3 mL). The resulting mixture was stirred under an atmosphere of hydrogen at room temperature overnight. The reaction mixture was then filtered through Celite using dichloromethane and concentrated under reduced pressure. Purification by flash chromatography on silica gel (0 to 30% ethyl acetate in hexanes) provided the title compound: ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.76 (dd, J = 10.9, 2.8 Hz, 1 H), 6.60 (dd, J = 8.4, 5.6 Hz, 1 H), 6.34 (ddd, J = 11.2, 8.4, 2.8 Hz, 1 H), 4.17 (br s, 1 H), 3.01 (br s, 2 H), 2.42 – 2.38 (m, 1 H), 0.77 – 0.72 (m, 2 H), 0.54 – 0.51 (m, 2 H).

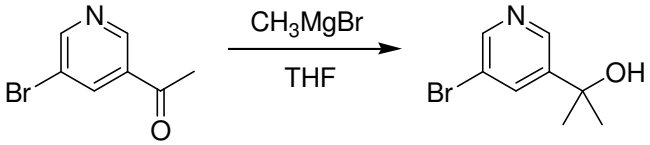
Step C. 8-(1-cyclopropyl-6-fluoro-1*H*-benzimidazol-2-yl)-1,6-naphthyridine



To a solution of the title compound from Example 1, Step B (0.080 g, 0.48 mmol) in DMF (1.17 mL) and water (0.04 mL) were added 1,6-napthyridine-8-carbaldehyde (0.084 g, 0.53 mmol) and OXONE® (0.192 g, 0.313 mmol). The resulting mixture was stirred at room temperature for 1 hour, then diluted with ethyl acetate and water. Solid potassium carbonate was added until the aqueous layer was basic (pH ~ 9). Ethyl acetate was added, and the organic layer was separated, washed with water and brine, dried over magnesium sulfate, filtered and concentrated. Purification by flash chromatography on silica gel (50% ethyl acetate in hexanes, then 100 % ethyl acetate) provided the title compound: LCMS m/z 304.99 $[M + H]^+$; ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.09 (s, 1 H), 9.13 (m, 1 H), 9.07 (s, 1 H), 8.43 (d, *J* = 8.3 Hz, 1 H), 7.78 (dd, *J* = 8.7, 4.8 Hz, 1 H), 7.63 (dd, *J* = 8.2, 4.2 Hz, 1 H), 7.34 (dd, *J* = 8.7, 2.2 Hz, 1 H), 7.07 (m, 1 H), 3.55 – 3.53 (m, 1 H), 0.62 – 0.61 (m, 4 H).

Preparation of 5-(2-hydroxypropan-2-yl)nicotinic acid.

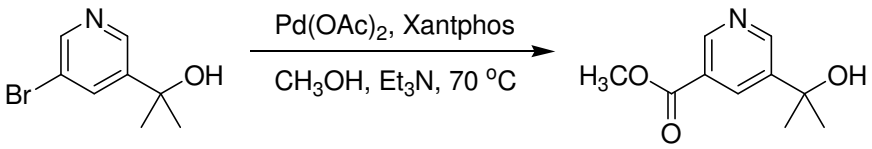
Step A. 2-(5-Bromopyridin-3-yl)propan-2-ol



To a cooled (-78 °C) solution of 3-acetyl-5-bromo pyridine (1.98 g, 9.90 mmol) in tetrahydrofuran (33 mL) was added dropwise a solution of methyl magnesium bromide in diethyl ether (3.0 M, 6.60 mL, 19.8 mmol). The reaction was warmed to room temperature, and the resulting mixture was stirred at room temperature overnight. The reaction was then quenched with saturated aqueous ammonium chloride solution and extracted with diethyl ether. The organic extracts were combined, washed with brine, dried over magnesium sulfate, filtered and

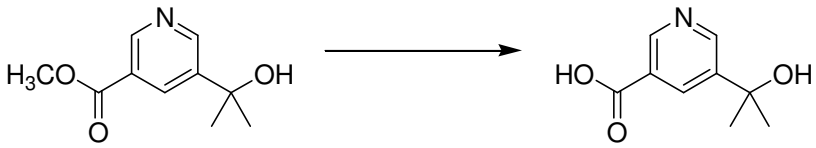
concentrated. Purification by flash chromatography on silica gel (0 – 60% ethyl acetate in hexanes) provided the title compound: LCMS m/z 217.83 $[M + H]^+$; ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.61 (s, 1 H), 8.53 (s, 1 H), 8.01 (d, *J* = 1.9 Hz, 1 H), 2.38 (br s, 1 H), 1.61 (6 H).

Step B. Methyl 5-(2-hydroxypropan-2-yl)pyridine-3-carboxylate



A mixture of 2-(5-bromopyridin-3-yl)propan-2-ol (1.99 g, 9.21 mmol), palladium acetate (0.041 g, 0.041 mmol), Xantphos (0.213 g, 0.368 mmol), methanol (3.73 mL, 92.1 mmol), and triethylamine (18.33 mL, 132.0 mmol) was stirred under an atmosphere of carbon monoxide at 70 °C overnight. The reaction was then cooled to room temperature, diluted with ethyl acetate, filtered through Celite and concentrated under reduced pressure. Purification by flash chromatography on silica gel (30% ethyl acetate in hexanes) provided the title compound: LCMS m/z 195.99 $[M + H]^+$; ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.09 (d, *J* = 1.8 Hz, 1 H), 8.92 (d, *J* = 2.2 Hz, 1 H), 8.41 (t, *J* = 2.0 Hz, 1 H), 3.96 (s, 3 H), 2.27 (br s, 1 H), 1.64 (s, 6 H).

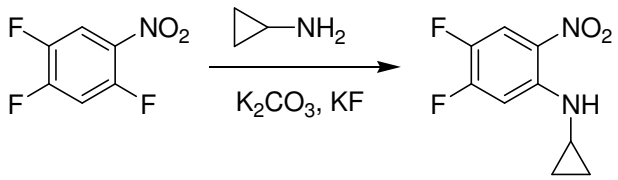
Step C. 5-(2-Hydroxypropan-2-yl)pyridine-3-carboxylic acid



To a solution of methyl 5-(2-hydroxypropan-2-yl)pyridine-3-carboxylate (0.179 g, 0.915 mmol) in methanol (4.57 mL) was added 1 N aqueous sodium hydroxide solution (2.74 mL, 2.74 mmol). The reaction was stirred at room temperature for 30 min and then concentrated under reduced pressure. The resulting residue was acidified via the addition of 1 N aqueous hydrogen chloride solution until the pH was ~2. Ethyl acetate was then added, and the organic extracts were dried over magnesium sulfate, filtered and concentrated to provide the title compound: LCMS m/z 182.01 $[M + H]^+$; ^{1}H NMR (500 MHz, CD_3OD) δ 8.91 (s, 1 H), 8.79 (d, *J* = 1.8 Hz, 1 H), 8.43 (d, *J* = 1.9 Hz, 1 H), 1.51 (s, 6 H).

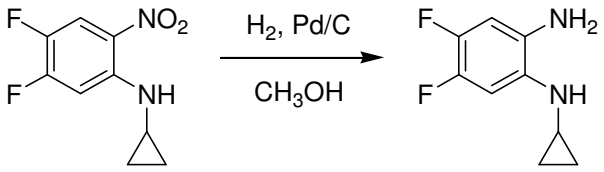
Example 2: Exemplification of General Synthesis B: Preparation of Compound **31**.

Step A. N-cyclopropyl-4,5-difluoro-2-nitroaniline



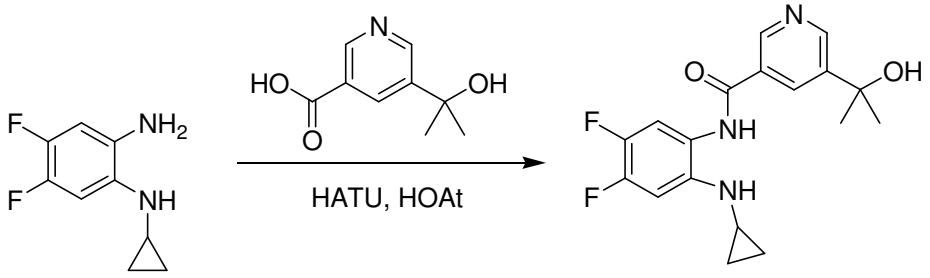
2,4,5-Trifluoronitrobenzene (2.00 g, 11.3 mmol), potassium fluoride (0.656 g, 11.3 mmol), potassium carbonate (1.561 g, 11.29 mmol), and cyclopropylamine (0.93 ml, 13.55 mmol) were added to a vial and heated in a microwave at 90 °C for 10 min. The reaction was then diluted with dichloromethane, washed with water and brine, dried over magnesium sulfate, and concentrated under reduced pressure. Purification by flash chromatography on silica gel (0 – 15% ethyl acetate in hexanes) provided the title compound: ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.04 (dd, *J* = 10.4, 8.5 Hz, 1 H), 7.09 (dd, *J* = 12.6, 7.0 Hz, 1 H), 2.55 – 2.54 (m, 1 H), 0.97 – 0.93 (m, 2 H), 0.69 – 0.66 (m, 2 H).

Step B. N-cyclopropyl-4,5-difluorobenzene-1,2-diamine



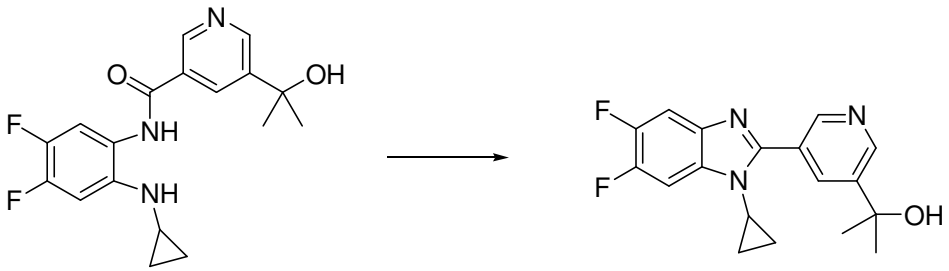
To the title compound from Example 2 Step A (1.22 g, 5.69 mmol) were added 10% palladium on carbon (0.122 g, 1.15 mmol) and methanol (28.5 mL). The resulting mixture was stirred under an atmosphere of hydrogen at room temperature overnight. The mixture was then filtered through Celite using dichloromethane, and the filtrate was concentrated. Purification by flash chromatography on silica gel (0 – 30% ethyl acetate in hexanes) provided the title compound: ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.83 (dd, *J* = 12.4, 7.9 Hz, 1 H), 6.51 (dd, *J* = 11.3, 7.8 Hz, 1 H), 3.82 (br s, 1 H), 3.15 (br s, 2 H), 2.40 – 2.37 (m, 1 H), 0.77 – 0.73 (m, 2 H), 0.52 – 0.50 (m, 2 H).

Step C. N-[2-(cyclopropylamino)-4,5-difluorophenyl]-5-(2-hydroxypropan-2-yl)pyridine-3-carboxamide



To a solution of the title compound from Example 2 Step B (0.074 g, 0.40 mmol) in dichloromethane (2.1 mL) were added 5-(2-hydroxypropan-2-yl)pyridine-3-carboxylic acid (0.080 g, 0.44 mmol), HATU (0.168 g, 0.442 mmol), HOAt (0.060 g, 0.44 mmol) and diisopropylethylamine (0.285 mL, 1.61 mmol). The reaction was stirred at room temperature for 1 hour, then diluted with dichloromethane, washed sequentially with water and brine, dried over magnesium sulfate, filtered and concentrated under reduced pressure. Purification by flash chromatography on silica gel (50 – 100% ethyl acetate in hexanes) provided the title compound: LCMS m/z 330.04 $[M + H]^+$; ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.96 (s, 1 H), 8.90 (d, J = 7.2 Hz, 1 H), 8.35 (s, 1 H), 7.66 (br s, 1 H), 7.31 (d, J = 10.7 Hz, 1 H), 7.04 – 7.00 (m, 1 H), 4.31 (br s, 1 H), 2.80 (br s, 1 H), 2.44 (t, J = 6.1, 3.1 Hz, 1 H), 1.66 (s, 6 H), 0.78 – 0.77 (m, 2 H), 0.53 – 0.51 (m, 2 H).

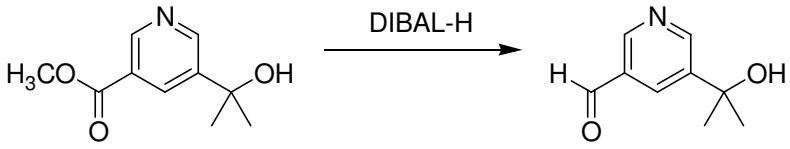
Step D. 2-[5-(1-Cyclopropyl-5,6-difluoro-1*H*-benzimidazol-2-yl)pyridin-3-yl]propan-2-ol



A solution of the title compound from Example 2 Step C (0.070 g, 0.20 mmol) in acetic acid (1.0 mL) was heated at 100 °C for 1 hour, and then cooled to room temperature. The resulting mixture was concentrated under reduced pressure, diluted with ethyl acetate, washed sequentially with aqueous 1 N sodium hydroxide solution and brine, dried over magnesium

sulfate, filtered and concentrated. Purification by flash chromatography on silica gel (50 – 80% ethyl acetate in hexanes) provided the title compound: LCMS m/z 330.04 $[M + H]^+$; ^{1}H NMR (500MHz, $CDCl_3$) δ 9.09 (s, 1 H), 8.87 (s, 1 H), 8.44 (d, *J* = 1.8 Hz, 1 H), 7.58 (dd, *J* = 10.3, 7.3 Hz, 1 H), 7.40 (dd, *J* = 9.7, 7.0 Hz, 1 H), 3.59 – 3.58 (m, 1 H), 2.89 (br s, 1 H), 1.69 (s, 6 H), 1.22 – 1.19 (m, 2 H), 0.79 – 0.78 (m, 2 H).

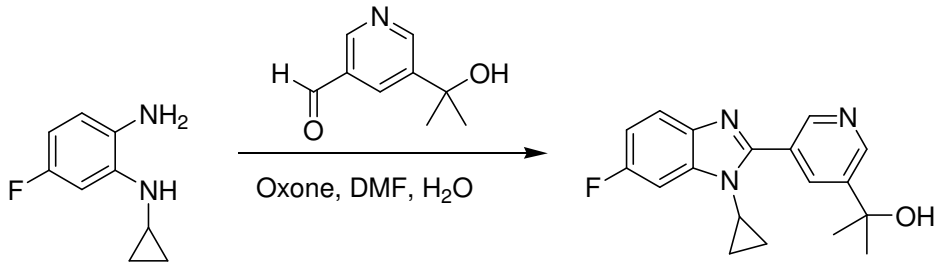
Preparation of 5-(2-Hydroxypropan-2-yl)pyridine-3-carbaldehyde.



To a cooled (-78 °C) solution of methyl 5-(2-hydroxypropan-2-yl)pyridine-3-carboxylate (0.50 g, 2.6 mmol) in toluene (15 mL) and dichloromethane (15 mL) was added a solution of diisobutyl aluminum hydride in toluene (1.0 M, 6.0 mL, 6.0 mmol). After 10 min, the reaction was quenched with water (5 mL), allowed to warm to room temperature and dried with magnesium sulfate. Concentration of the filtrate under reduced pressure provided the title compound: ^{1}H NMR (500 MHz, $CDCl_3$) δ 10.10 (s, 1 H), 8.99 (d, *J* = 2.3 Hz, 1 H), 8.93 (s, 1 H), 8.30 (s, 1 H), 2.22 (br s, 1 H), 1.67 (s, 6 H).

Example 3: Preparation of Compound **32.**

Step A. 2-[5-(1-cyclopropyl-6-fluoro-1*H*-benzimidazol-2-yl)pyridin-3-yl]propan-2-ol

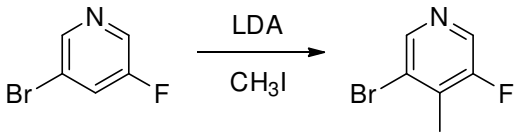


To a solution of the title compound from Example 1, Step B (0.479 g, 2.88 mmol) in DMF (6.97 mL) and water (0.23 mL) were added 5-(2-hydroxypropan-2-yl)pyridine-3-carbaldehyde (0.302 g, 1.83 mmol) and OXONE® (0.729 g, 1.19 mmol). The resulting mixture was stirred at room temperature for 1 hour, then diluted with ethyl acetate and water. Solid

potassium carbonate was added until the aqueous layer was basic (pH ~ 9). Ethyl acetate was added, and the organic layer was separated, washed with water and brine, dried over magnesium sulfate, filtered and concentrated. Purification by flash chromatography on silica gel (100% ethyl acetate) provided a product that was further purified by reverse phase HPLC (C18 OBD column, 20 to 90% acetonitrile/water, both 0.1% v/v trifluoroacetitic acid). Fractions containing the desired product were combined, washed sequentially with aqueous 1 N sodium hydroxide solution and brine, dried over magnesium sulfate, filtered and concentrated to provide the title compound: LCMS m/z 312.01 $[M + H]^+$; ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.09 (d, *J* = 1.7 Hz, 1 H), 9.85 (d, *J* = 1.9 Hz, 1 H), 8.40 (s, 1 H), 7.73 (dd, *J* = 8.8, 4.8 Hz, 1 H), 7.29 (dd, *J* = 8.7, 2.2 Hz, 1 H), 7.08 – 7.04 (m, 1 H), 3.57 – 3.55 (m, 1 H), 2.21 (br s, 1 H), 1.69 (s, 6 H), 1.20 – 1.16 (m, 2 H), 0.79 – 0.75 (m, 2 H).

Preparation of 3-fluoro-4-methyl-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)pyridine.

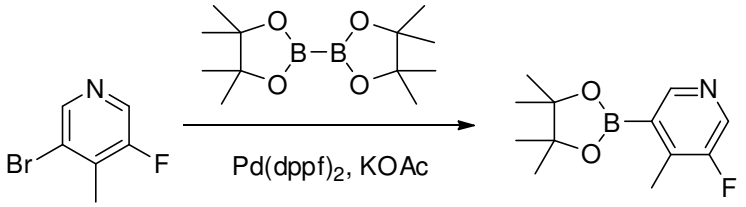
Step A. 3-bromo-5-fluoro-4-methylpyridine



An oven-dried 3-necked flask was equipped with a dropping funnel and stirbar and flushed with nitrogen. Tetrahydrofuran (120 mL) and diisopropylamine (19.11 mL, 13.80 g, 136.0 mmol) were added, giving a colorless solution that was cooled to -78 °C. A solution of N-butyllithium in hexanes (2.5 M, 50.0 mL, 125 mmol) was then added dropwise, and the resulting solution was stirred for 30 minutes at -78 °C. A solution of 3-bromo-5-fluoropyridine (20.0 g, 114 mmol) in tetrahydrofuran (40.0 mL) was added, and stirring was continued for an additional 30 minutes. Iodomethane (8.49 mL, 19.4 g, 136 mmol) was then added, and the resulting mixture was allowed to slowly warm to room temperature. After 18 hours, the reaction was poured into saturated aqueous sodium bicarbonate solution (100 mL) and water (100 mL) and extracted with ethyl acetate (3 x 200 mL). The organic extracts were combined, washed with saturated aqueous sodium chloride solution (100 mL), dried over sodium sulfate, filtered, and concentrated under reduced pressure. Purification by flash chromatography on silica gel (0 –

15% ethyl acetate in heptane) provided the title compound: ^{1}H NMR (500MHz, $CDCl_3$) δ 8.49 (s, 1 H), 8.31 (s, 1 H), 2.37 (s, 3 H).

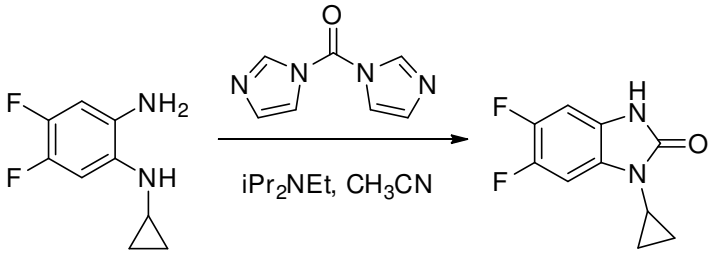
Step B. 3-fluoro-4-methyl-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)pyridine



A mixture of the title compound from Step A (2.93 g, 9.25 mmol), bis(pinacolato)diboron (2.82 g, 11.1 mmol), 1,1' bis(diphenylphosphino)ferrocene dichloropalladium(II) (0.335 g, 0.462 mmol) and potassium acetate (1.82 g, 18.5 mmol) in 1,4-dioxane was heated under a nitrogen atmosphere to 80 °C. After 24 hours, the reaction was cooled to room temperature and diluted with ethyl acetate (400 mL). The resulting mixture was filtered through celite and washed sequentially with 1:1 saturated aqueous sodium bicaronate solution:water (2 x 200 mL) and saturated aqueous sodium chloride solution (100 mL). The organic layer was separated, dried over sodium sulfate, filtered and concentrated under reduced pressure. Purification by flash chromatography on silica gel (25 – 50% ethyl acetate in heptane) provided the title compound: ^{1}H NMR (500MHz, $CDCl_3$) δ 8.68 (s, 1 H), 8.39 (s, 1 H), 2.48 (s, 3 H), 1.38 (s, 12 H).

Example 4: Exemplification of General Synthesis C: Preparation of Compound **35**.

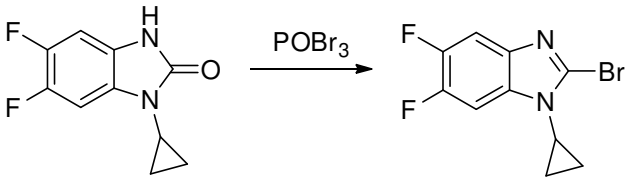
Step A. 1-cyclopropyl-5,6-difluoro-1H-benzo[d]imidazol-2(3H)-one



Carbonyldiimidazole (4.3 g) was added to a solution of N-cyclopropyl-4,5-difluorobenzene-1,2-diamine (3.2 g) in THF (30 mL). The mixture was stirred at room temperature for 20 hours, then diluted with ethyl acetate (200 mL) and washed with an aqueous

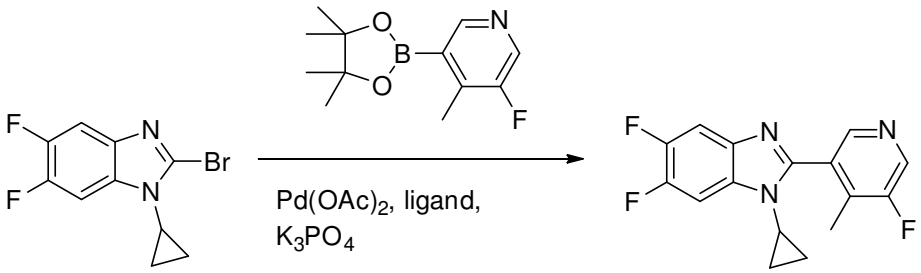
solution of hydrochloric acid (1 M, 200 mL). The organic layer was dried over sodium sulfate, filtered, and concentrated under reduced pressure to afford the title compound: ^{1}H NMR (CD_3OD) δ 7.20 (dd, 1 H), 6.95 (dd, 1 H), 2.86 (m, 1 H), 1.11 (m, 2 H), 0.94 (m, 2 H).

Step B. 2-bromo-1-cyclopropyl-5,6-difluoro-1H-benzo[d]imidazole



To a solution of the title compound from Example 4 Step A (2.50 g) in toluene (150 mL) was added phosporyl tribromide (13.64 g). The reaction mixture was then warmed and heated at reflux for 18 hours. The reaction was then cooled to room temperature, diluted with dichloromethane (150 mL) and slowly added to stirring 40 °C water, giving a mixture that was stirred for 30 minutes. Solid sodium carbonate was then added until the pH of the mixture was ~10. The mixture was then separated, and the organic layer washed with water, dried over sodium sulfate and concentrated under reduced pressure to give the title compound: ^{1}H NMR ($CDCl_3$) δ 7.45 (dd, 1 H), 7.30 (dd, 1 H), 3.20 (m, 1 H), 1.30 (m, 2 H), 1.20 (m, 2 H).

Step C. 1-cyclopropyl-5,6-difluoro-2-(5-fluoro-4-methylpyridin-3-yl)-1H-benzo[d]imidazole



To a microwave vial were added the title compound from Example 4, Step B (0.150 g, 0.549 mmol), 3-fluoro-4-methyl-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)pyridine (0.128 g, 0.824 mmol), palladium (II) acetate (0.0062 g, 0.027 mmol), 2-dicyclohexylphosphino-2',6'-dimethoxybiphenyl (0.023 g, 0.055 mmol), potassium phosphate, tribasic (0.233 g, 1.10 mmol), 1,4-dioxane (2.0 mL) and water (0.80 mL). The resulting mixture was then heated in a microwave to 180 °C for 1 hour. The reaction was then diluted with ethyl acetate (20 mL),

filtered through celite and washed with saturated aqueous sodium bicarbonate solution (20 mL). The organic layer was dried over sodium sulfate, filtered and concentrated under reduced pressure. Purification by reverse phase HPLC (C18 OBD column) provided the title compound: LCMS m/z 304.0 $[M + H]^+$; ^1H NMR (500 MHz, $CDCl_3$) δ 8.53 (s, 1 H), 8.52 (s, 1 H), 7.58 (t, 1 H), 7.41 (t, 1 H), 3.35 (m, 1 H), 2.35 (s, 3 H), 1.01 (m, 2 H), 0.67 (m, 2 H).

References

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2. Hoyt, S. B.; Park, M. K.; London, C.; Xiong, Y.; Bennett, J. D.; Cai, J.; Ratcliffe, P.; Cooke, A.; Carswell, E.; MacLean, J. WO 012478, 2012.