Supporting Information

Development of 2-Aminooxazoline 3-Azaxanthene β-Amyloid Cleaving Enzyme (BACE) Inhibitors with Improved Selectivity Against Cathepsin D

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Description of in vitro Assays and in vivo Studies

BACE1 Enzymatic Assay. BACE1 enzymatic activity was determined by the enhancement of fluorescence intensity upon enzymatic cleavage of the fluorescence resonance energy transfer substrate. The BACE1 recognition and cleavage sequence of the substrate is derived from the reported literature,[i] and the fluorophore and quencher dyes are attached to side chain of Lys residues at the termini of the substrate peptide. The human recombinant BACE1[ii] assay was performed in 50 mM acetate, pH 4.5/8% DMSO/100 μM Genepol/0.002% Brij-35. In doseresponse IC_{50} assays, 10 point 1:3 serial dilutions of compound in DMSO were pre-incubated with the enzyme for 60 min at room temperature. Subsequently, the substrate was added to initiate the reaction. After 60 min at room temperature, the reaction was stopped by addition of 0.1 M Tris base to raise the pH above the enzyme active range, and the increase of fluorescence intensity was measured on Safire II microplate reader (Tecan, Mannedorf, Switzerland). IC_{50} values were averaged values determined by at least two independent experiments. The standard deviation and the number of experiments for each compounds are listed in Table S1.

Cat D Enzymatic Assay. The enzymatic activity of Cat D is determined by the enhancement of fluorescence intensity upon enzymatic cleavage of the FRET (fluorescence resonance energy transfer) substrate. The cleavage sequence of the substrate is derived from the literature,[iii] and a fluorophor and a quencher dye are attached to the Lys side chain at the termini of the substrate. The human recombinant Cat D assay was performed in 50 mM Citrate, pH 3.5 / 8% DMSO / 5 mM Chaps / 0.002% Brij-35 in a Costar 96-well black polypropylene plate. All compounds were initially

tested in a dose-response IC_{50} assay with an end-point read at 400 μM top concentration. Any compounds of interest showing no inhibitory effect up to 400 μM were subsequently tested in a dose-response IC_{50} assay with kinetic reads at 4 mM top concentration. In all dose-response IC_{50} assays, 10 various concentrations of each compound that are made at 1:3 serial dilutions in DMSO were pre-incubated with the enzyme for 60 min at room temperature. Afterwards, the FRET substrate was added to initiate the reaction. For the end-point read assay, the reaction was stopped by the addition of un-titrated 0.1 M Tris Base to raise the pH above the enzyme active range after 60 min at room temperature. The fluorescence intensity of each well was measured on Safire II microplate reader (Tecan, Männedorf, Switzerland). However, for the kinetic-read assay, the fluorescence intensity of each well was measured immediately after the addition of substrate for 60 minutes in 20 kinetic cycles. The IC_{50}s were calculated by fitting normalized activity data with a 4-parameter non-linear regression equation via Screener software (Genedata AG, Basel, Switzerland). The standard deviation and the number of experiments for each compounds are listed in Table S1.

BACE1 Cell-based Assay. Human embryonic kidney cells (HEK293) stably expressing APPSW were plated at a density of 100K cells/well in 96 well plates (Costar). The cells were cultivated for 6 h at 37 °C and 5% CO_2 in DMEM supplemented with 10% FBS. Cells were incubated overnight with test compounds at concentrations ranging from 0.0005 to 10 μM. Following incubation with the test compounds the conditioned media was collected and the $A\beta_{40}$ levels were determined using a sandwich ELISA. The IC_{50} was calculated from the percent of control $A\beta_{40}$ as a function of the concentration of the test compound. The sandwich ELISA to detect $A\beta_{40}$ was performed in 96 well microtiter

plates, which were pre-coated with goat anti-rabbit IgG (Pierce). The capture and detection antibody pair that was used to detect $A\beta_{40}$ from cell supernatants consists of affinity purified $pA\beta_{40}$ (Invitrogen) and biotinylated 6E10 (Covance), respectively. Conditioned media was incubated with capture antibody overnight at 4 °C, followed by washing. The detecting antibody incubation was for 3 h at 4 °C, again followed by the wash steps as described previously. The plate was developed using Delfia reagents (Streptavidin-Europium and Enhancement solution (Perkin Elmer) and time-resolved fluorescence was measured on an EnVision multilabel plate reader (Perkin Elmer). The standard deviation and the number of experiments for each compounds are listed in Table S1.

Table S1. Standard Deviation of BACE1 Enzymatic Assay, CatD Enzymatic Assay and BACE1 Cell-based Assay

Cmpd #	BACE1 Enzyme IC50 (μM)	CatD IC50 (μM)	BACE1 Cell HEK293T IC50 (μM)
1	0.00092 +/- .00015 (n=25)	0.66 +/- .17 (n=16)	0.021 +/- .01 (n=8)
6	0.072 +/- .020 (n=10)	15 +/- 7.7 (n=8)	0.83 +/- .21 (n=3)
7	0.32 +/- .11 (n=4)	130 +/- 12 (n=2)	4.7 (n=1)
9	0.013 +/- .0088 (n=4)	72 +/- 3.1 (n=2)	0.12 (n=1)
15	0.00043 +/- .000065 (n=5)	4.5 +/- 2.0 (n=4)	0.0041 +/- .0016 (n=2)
14	0.00046 +/- .000058 (n=4)	2.7 +/- .96 (n=4)	0.0022 +/- .0016 (n=2)
13	0.00031 +/- .000092 (n=5)	5.9 +/- 2.8 (n=4)	0.0012 +/- .00072 (n=2)
16	0.0018 +/- .00039 (n=4)	3.6 +/- 1.7 (n=4)	0.087 (n=1)
17	0.00058 +/- .00023 (n=4)	4.4 +/- 1.9 (n=4)	0.0024 +/- .00018 (n=2)
18	0.0055 +/- .0036 (n=4)	12 +/- 5.2 (n=4)	0.10 (n=1)
21	0.021 +/- .012 (n=5)	1100 (n=1)	0.19 +/- .069 (n=2)
32	0.0075 +/- .0027 (n=4)	1200 +/- 162 (n=2)	0.052 (n=1)
27	0.031 +/- .011 (n=4)	640 +/- 309 (n=4)	0.054 +/- .014 (n=2)
28	0.0036 +/- .0014 (n=9)	310 +/- 156 (n=6)	0.013 +/- .0061 (n=3)
29	0.00057 +/- .00014 (n=6)	1000 +/- 230 (n=2)	0.0025 +/- .0022 (n=3)
30	0.00062 +/- .0002 (n=10)	480 +/- 77 (n=2)	0.0014 +/- .00025 (n=4)

Table S2. Calculation of Selectivity (CatD/BACE1):

Cmpd #	BACE1 IC50 (μM)	Standard Deviation: BACE1 IC50 (μM)	CatD IC50 (μM)	Standard Deviation: CatD IC50 (μM)	CatD Potency Max	CatD Potency Min	Selectivity: [CatD IC50 /BACE1 IC50]	Selectivity Max	Selectivity Min
1	0.00092	0.00015	0.66	0.17	0.83	0.48	660	910	530
6	0.072	0.020	15	7.7	23	7.4	210	320	100
7	0.32	0.11	130	12	140	120	400	430	360
9	0.013	0.0088	72	3.1	75	69	5,600	5,900	5,400
15	0.00043	0.000065	4.5	2.0	6.5	2.4	10,000	15,000	5,600
14	0.00046	0.000058	2.7	0.96	3.7	1.8	5,900	8,000	3,900
13	0.00031	0.000092	5.9	2.8	8.8	3.1	19,000	28,000	10,000
16	0.0018	0.00039	3.6	1.7	5.3	1.9	2,100	3,000	1,100
17	0.00058	0.00023	4.4	1.9	6.3	2.5	7,500	11,000	4,300
18	0.0055	0.0036	12	5.2	17	7.0	2,200	3,200	1,300
21	0.021	0.012	1100	-	-	-	53,000	-	-
32	0.0075	0.0027	1200	162	1300	1020	160,000	180,000	135,000
27	0.031	0.011	640	309	950	330	20,000	30,000	11,000
28	0.0036	0.0014	310	156	460	150	85,000	130,000	42,000
29	0.00057	0.00014	1000	230	1250	790	1,800,000	2,200,000	1,400,000
30	0.00062	0.0002	480	77	550	400	770,000	900,000	660,000

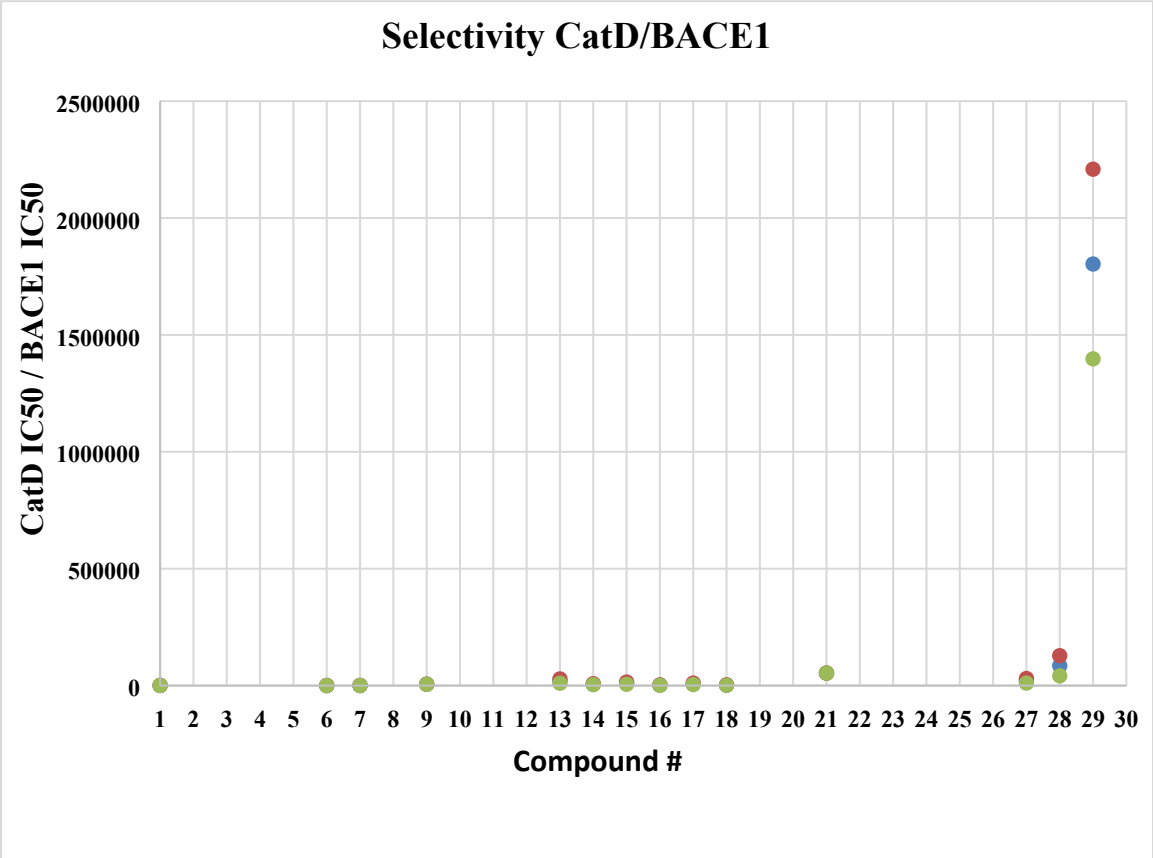
CatD Potency Max = CatD IC_{50} + Standard Deviation CatD IC_{50}

Selectivity Max = CatD Potency Max / BACE1 IC_{50}

CatD Potency Min = CatD IC_{50} - Standard Deviation CatD IC_{50}

Selectivity Min = CatD Potency Min / BACE1 IC_{50}

Graph S1: CatD/BACE1 Selectivity Plot



Permeability assay. The wild type cell line LLC-PK1 (porcine renal epithelial cells, WTLLC-PK1) was purchased from American Type Culture Collection (ATCC, Manassass, VA). Transfections of WT-LLC-PK1 cells with human MDR1 gene (hMDR1-LLC-PK1) and rat mdr1a gene (rMdr1a-LLC-PK1) were generated. Cells were grown in Medium 199 supplemented with 10% fetal bovine serum.[iv] Cells were seeded onto matrigel-coated transwell filter membranes at a density of 90,000 cells/well. Media change was performed on day 3. Compound incubations were performed 5-6 days post seeding. All cultures were incubated at 37°C in a humidified (95% relative humidity) atmosphere of 5% CO_2 / 95% air. Prior to the transport experiment,[v] culture medium was aspirated from both apical and basolateral wells, cells were rinsed with warmed (37 °C) Hank's balanced salt solution supplemented with 10 mM Hepes at pH 7.4 (HHBSS, Invitrogen, Grand Island, NY). HHBSS was removed from wells prior to dosing with test drugs at 5 μM in transport buffer (HHBSS containing 0.1% bovine serum albumin). One hundred-fifty microliters of transport buffer were added to receiver chambers prior to dosing in triplicate to apical or basolateral chambers. The dosed transwell plates containing the cell monolayers were incubated for two hours at 37 °C on a shaking platform. At the end of the incubation period, 100 μL samples were collected from receiver reservoirs, and analyzed by LC-MS/MS on an API4000 (Applied Biosystem, Foster City, CA) triple quadruple mass spectrometer interfaced with turbo IonSpray operated in positive

mode using Analyst 1.4.2 software. The apparent permeability coefficient (Papp) of all tested agents was estimated from the slope of a plot of cumulative amount of the agent versus time based on the following equation:

Papp = (dQ / dt) / (A * C0)

where

dQ / dt = penetration rate of the agent (ng/s)

A = surface area of the cell layer on the Transwell (0.11 cm^2)

C0 = initial concentration of the test compound (ng/ml).

Efflux ratio (ER) was calculated from the basolateral-to-apical permeability divided by the apical-to-basolateral permeability: ER = Papp B>A/ Papp A>B.

Microsomal Stability Assay. Compounds (1 μM) were incubated with liver microsomes (0.25 mg/mL in 67 mM phosphate buffer, pH 7.4) from human and rat at 37 °C for 30 min with or without 1 mM NADPH in a total volume of 0.2 mL. The final concentration of DMSO in the incubation was <0.1%. Incubations were stopped by addition of 200 μL of ice-cold acetonitrile containing 0.5% formic acid and an internal standard (500 ng/mL) followed by centrifugation at 3100 rpm for 20 min. The supernatants were analyzed directly (without any further sample cleanup) by high performance liquidchromatography (HPLC) and mass spectrometric detection.

hERG Functional Assay. A stable HEK293 cell line expressing the hERG channel was procured from Cytomyx (Cambridge, UK). Standard electrophysiological assay conditions for recording hERG have been described elsewhere,[vi] but are summarized here in brief. Extracellular buffer consisted of (135 mM NaCl, 5 mM KCl, 1.8 mM $CaCl_2$, 10 mM HEPES, 5 mM glucose, pH adjusted to 7.4), intracellular buffer consisted of (130 mM KF, 2 mM $MgCl_2$, 10 mM EGTA, 10 mM HEPES, pH adjusted to 7.25). Once solubilized as a 10 mM stock in DMSO, compounds were diluted serially in DMSO with

a final transfer into extracellular buffer so as to maintain a consistent vehicle concentration of 0.3% DMSO (vol/vol). After establishing the whole-cell configuration with a holding potential of -80 mV, hERG tail currents were elicited every 10 s by a step depolarization to +30 mV for 2 s followed immediately by a repolarization to -50 mV. Compounds were applied as cumulative ascending concentrations. Peak tail currents were normalized to each cell's baseline, plotted against compound concentration, and fit with a Hill equation to yield a potency estimate (IC_{50}). All experiments were performed on the PatchXpress® 7000A automated electrophysiology platform (Molecular Devices, Sunnyvale, CA).

Rat Pharmacodynamic assay. Male Sprague-Dawley rats (175-200 g) were purchased from Harlan and were maintained on a 12 h light/dark cycle with unrestricted access to food and water until use. Rats were administered compound by oral gavage at the appropriate dose. Rats were euthanized with CO_2 inhalation for 2 min and cisterna magna was quickly exposed by removing the skin and muscle above it. CSF (50-100 µl) was collected with a 30 gauge needle through the dura membrane covering the cisterna magna. Blood was withdrawn by cardiac puncture and plasma obtained by centrifugation for drug exposures. Brains were removed and, along with the CSF, immediately frozen on dry ice and stored at -80 °C until use. The frozen brains were subsequently homogenized in 10 volumes of (w/v) of 0.5% Triton X-100 in TBS with protease inhibitors. The homogenates were centrifuged at 100,000 rpm for 30 min at 4 °C. The supernatants were analyzed for Aβ levels by immunoassay as follows: Meso Scale 96-well avidin plates were coated with Biotin-4G8 (Covance) and detected with Ruthenium-labeled Fab specific for $A\beta_{40}$. Plates were read in MSD Sector6000 imager according to

manufacturer's recommended protocol (Meso Scale Discovery, Inc.). $A\beta_{40}$ concentrations were plotted using Graphpad Prism and analyzed by one-way ANOVA followed by Dunnett's multiple comparison analysis to compare drug-treated animals to vehicle-treated controls. Five rats were tested per group, the mean was used to calculate the percentage of Aβ lowering and EC_{50}. The conversion percentage is ranged 5-20%.

Determination of in vivo plasma IC_{50},unbound. The IC_{50} estimation used an indirect effect model for modeling brain and CSF Aβ, similar to Model 1 (section 3.3) as described by Femlee et al.[vii]

$$\frac{dR}{dt} = k_{\text{in}} \left(1 - \frac{I_{\max} \times C_p}{\text{IC}_{50} + C_p}\right) - k_{\text{out}} \times R.$$

where:

kin = the zero order formation rate constant of Aβ

kout = first order elimination rate constant for Aβ

R = Aβ concentration

Cp = concentration of drug in plasma

Imax = 1.

Dog Cardiovascular Safety Studies.

i) Animal Preparation. Male beagle dogs (10−12 kg, Marshall Farms) were anesthetized with 2 mg/kg morphine, dosed subcutaneously, followed by a 110 mg/kg intravenous bolus of α-chloralose. After induction, animals were intubated and ventilated at an

appropriate rate and volume with ambient air. Maintenance on surgical plane was achieved with a constant infusion of 45 mg/kg/h of α-chloralose. Animals were kept on a thermal blanket and heat lamp to maintain normal body temperature and monitored throughout the study by veterinary staff. The study was conducted with approval of the Institutional Animal Care and Use Committee.

ii) Cardiovascular Monitoring. Arterial pressure and heart rate were measured directly from a femoral artery catheter and catheters were placed in both femoral veins for infusion of drug and blood sampling, using standard surgical techniques. ECG signals (l II and V) were captured simultaneously from subcutaneous limb and precordial needle electrodes, respectively. After surgery, the animals were allowed to stabilize for a minimum of 45 min prior to collecting pretreatment (baseline) data. After baseline values were collected, the test article was administered in a rising-dose paradigm in which vehicle and escalating doses were infused intravenously. Each dose (vehicle or test article) was given over 30 min. Animals were euthanized at the conclusion of the study.

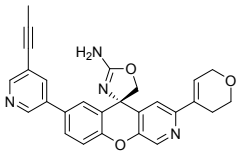
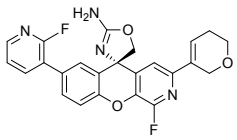
iii) Data Acquisition and Analysis. A computerized data acquisition system (CARecorder; DISS, Inc.) was used to collect all raw input signals. Arterial pressure, heart rate, and cardiac intervals were recorded continuously throughout the study and averaged at 30 s intervals. All raw data were converted with EMKA ecg-auto (version 2.5.1.30; Paris, France) into *.d01 files for analysis purposes. In the tables, values were compared to end of vehicle infusion. Data were analyzed using ecg-auto v2.5.1.30 (EMKA Technologies).

Description of X-ray Crystallography

Co-crystallization of BACE1 Complex.[viii] The catalytic domain of human BACE1 was expressed as inclusion bodies, purified, and crystallized as described previously (Patel, 2004). The cocrystal structure of BACE1 with compound **13** was obtained by soaking apo crystals in a modified mother liquor solution (25 % PEG 5000 MME, 0.1 M sodium citrate, pH 6.6, 0.2 M ammonium iodide, 3% (v/v) dimethylsulfoxide) containing 1.0 mM compound for 5.5 hours at room temperature. The crystals were then transferred briefly to the same solution supplemented with 20% (v/v) glycerol prior to flash-cooling for data collection. Diffraction data were collected on a Rigaku FR-E Superbright rotating anode X-ray source equipped with an R-Axis IV++ image plate detector. Data were reduced with HKL2000 (Otwinowski, 1997) and the structures were refined using REFMAC (Murshudov, 1997). Model building was performed with COOT (Emsley, 2004). Data collection and refinement statistics appear in Table S2 of the Supporting Information.

Co-crystallization of CatD Complex.[ix] Rat Cathepsin D was concentrated to 12 mg/ml in a buffer containing 40 mM Tris and 20 mM Na Citrate pH 6.3. Co-crystals of rat Cathepsin D with compound **4** [(S)-3-(5,6-dihydro-2H-pyran-3-yl)-1-fluoro-7-(3-fluoropyridin-2-yl)-5'Hspiro[chromeno[2,3-c]pyridine-5,4'-oxazol]-2'-amine][x] was grown at 20°C using the hanging drop method/vapor diffusion method, the drops contained 1.0 ul Cathepsin D solution and 1.0 ul reservoir solution. The reservoir solution consisted of 20% PEG 3350, 2% glycerol, 200 mM Lithium chloride. Multiple hits were obtained from Qiagen Peg Suite 1 screen that also diffracted well including calcium acetate, sodium nitrate, or potassium sulfate. Cathepsin D crystals were soaked overnight in a 5.0 mM compound solution and briefly transferred to 20% glycerol, 20% PEG 3350, 200 mM lithium chloride prior to flash freezing in liquid nitrogen. Data was collected at Advanced Light Source Beamline 5.0.2 (Lawrence Berkeley National Laboratory, Berkeley, CA) at 100 K. Data was processed and scaled using HKL2000 (1). The crystals belong to the space group C2 with approximate unit cell dimensions of a = 136.5 b = 66.1 Å, c = 99.5 and Å β=99.5o. Molecular replacement was performed using Phaser (2) with human Cathepsin D (PDB entry 1LYA) as a search model. The structure was refined using Phenix (3), and the model with ligand was built with Coot (4). Validation was performed using tools in Coot and Phenix. Ramachandran contained 97% in favored, 2.8% in allowed and none in outliers as determined by Phenix. Data collection and refinement statistics appear in Table S3 of the Supporting Information.

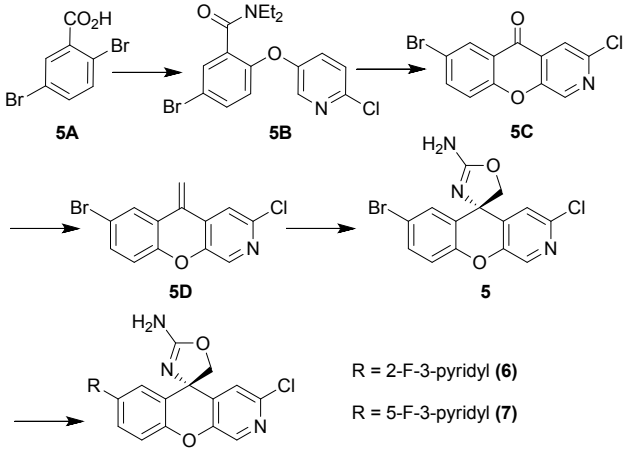
Table S3 – Crystallographic Data Collection & Refinement Statistics

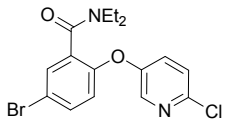
	BACE1 + **13**	CatD + **4**
		
Data Collection		
Space group	P6122	C2
Cell dimensions		
a, b, c (Å)	102.3, 102.3, 170.4	136.5, 66.1, 99.5
α, β, γ (°)	90, 90, 120	90, 99.5, 90
Resolution (Å)	50-1.90 (1.97-1.90)	30 – 2.80 (2.90 – 2.80)
Total reflections	287633	449341
Unique reflections	41991	21617
Completeness (%)[†]	99.4 (95.0)	99.4 (96.7)
R_{merge}[†]	0.071 (0.453)	0.09 (0.39)
$I/\sigma(I)$[†]	17.2 (2.4)	9.4 (2.8)
Refinement		
Reflections used	39815	21441
R_{work}/R_{free}	0.23/0.26	0.19/0.24
Average B-value (Å^2)	28.0	46.8
Number of atoms		
Protein	2914	5244
Ligand	34	136
Solvent	357	25
R.m.s. deviations		
Bond lengths (Å)	0.008	0.003
Bond angles (°)	1.26	0.64
PDB ID code	5UYU	5UX4

[†]Values in parentheses are for the highest resolution shell.

Synthesis of Compounds.

Unless otherwise noted, all materials were obtained from commercial suppliers and used without further purification. Anhydrous solvents were obtained from Aldrich or EM Science and used directly. All reactions involving air- or moisture-sensitive reagents were performed under a nitrogen or Argon atmosphere. All microwave assisted reactions were conducted with a Smith synthesizer from Personal Chemistry, Uppsala, Sweden. Silica gel chromatography was performed using either glass columns packed with silica gel (230-400 mesh, EMD Chemicals, Gibbstown, NJ) or prepacked silica gel cartridges (Biotage or ISCO). ^{1}H NMR spectra were recorded on a Bruker 300 MHz or Varian 400 MHz spectrometer at ambient temperature. Chemical shifts are reported in parts per million (ppm, δ units) downfield from tetramethylsilane. Data are reported as follows: chemical shift, multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, br = broad, m = multiplet), coupling constants, and number of protons. Purity for final compounds was greater than 95% unless otherwise noted and was measured using Agilent 1100 series high performance liquid chromatography (HPLC) systems with UV detection at 254 nm (system A, Agilent Zorbax Eclipse XDB-C8 4.6 mm × 150 mm, 5 µm, 5 – 100% CH3CN in H_2O with 0.1% TFA for 15 min at 1.5 mL/min; system B, Waters Xterra 4.6mm × 150 mm, 3.5 µm, 5 – 95% CH_3CN in H_2O with 0.1% TFA for 15 min at 1.0 mL/min).

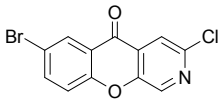




5-Bromo-2-((6-chloropyridin-3-yl)oxy)-N,N-diethylbenzamide **(5B)**

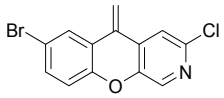
To a 5 L three neck flask was added 2,5- dibromobenzoic acid (**5A**, 200 g, 0.72 mol), 5-hydroxyl-2-chloropyridine (111 g, 0.86 mol) and cesium carbonate (469 g, 1.4 mol). The flask was purged with nitrogen for 20 minutes then copper (I) trifloromethanesulfonate toluene complex (9.62 g, 18.6 mmol) was added followed by toluene (2.5 L). The resulting suspension was heated

to 105°C and stirred for 1.5 hours. The liquid layer was decanted, and water (1.5 L) and ethyl acetate (500 mL) were added to the remaining residue. The reaction was stirred until all of the solids had dissolved. The organic phase was separated and the aqueous phase was neutralized with 6N HCl to pH 2. The acidic aqueous phase was extracted with ethyl acetate (800 mL x3) and the organic phases were combined, dried over sodium sulfate, filtered, and concentrated to afford crude 5-bromo-2-(6-chloropyridin-3-yloxy)benzoic acid. The crude material was dissolved in DCM (2.5 L). TBTU (230 g, 0.72 mol) was added followed by slow addition of diethylamine (148.6 mL, 1.43 mol). The resulting solution was stirred at ambient temperature for 16 hours. To the reaction was added saturated ammonium chloride (500 mL) and water (500 mL) and the reaction was stirred for 15 minutes. The organic phase was separated, washed with saturated sodium carbonate (1 L), dried over sodium sulfate, and filtered. Purification via silica gel flash chromatography eluting with a gradient of 5% to 20% ethyl acetate in hexanes afforded 5-bromo-2-((6-chloropyridin-3-yl)oxy)-N,N-diethylbenzamide **(5B)** (145 g, 0.38 mol, 53% yield). *m/z* calculated for $C_{16}H_{16}BrClN_2O_2$: 382.01, found 382.9 (M^+)



7-Bromo-3-chloro-5H-chromeno[2,3-c]pyridin-5-one **(5C)**

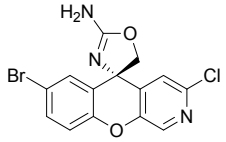
5-Bromo-2-((6-chloropyridin-3-yl)oxy)-N,N-diethylbenzamide **(5B)** (290 g, 0.76 mol) was dissolved in THF (2 L) and cooled to -78 °C. To this solution was added a solution of LDA (prepared by the reaction of diisopropylamine (393 mL, 2.8 mol) with *n*BuLi (2.5 M in hexane, 1060 mL) in THF (700 mL) under typical conditions). Upon complete addition of the LDA solution, the reaction was stirred for 30 minutes at -78 °C. The reaction was gradually warmed to ambient temperature and quenched by the slow addition saturated ammonium chloride (2 L). Ethyl acetate (500 mL) was added and the phases were mixed. The organic phase was separated and the aqueous layer was extracted with ethyl acetate (2 x 500 mL) The organic phases were combined, dried over sodium sulfate, and passed through a plug of silica gel to afford, after concentration, 7-bromo-3-chloro-5H-chromeno[2,3-c]pyridin-5-one **(5C)** as a light yellow solid (141 g, 0.45 mol, 60% yield). *m/z* calculated for $C_{12}H_5BrClNO_2$: 308.92, found 309.8 (M^+)



7-Bromo-3-chloro-5-methylene-5H-chromeno[2,3-c]pyridine (**5D)**

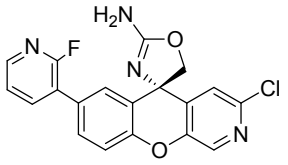
A solution of 7-bromo-3-chloro-5H-chromeno[2,3-c]pyridin-5-one **(5C)** (400 g, 1.3 mol) in dry THF (5 L) was cooled to 0 °C. Methylmagnesium chloride (2 M in THF, 1.5 L) was added slowly. Upon complete addition the reaction was gradually warmed to ambient temperature and stirred for 16 hours. The reaction was cooled to 0 °C and quenched with saturated ammonium chloride (3 L). Ethyl acetate (2 L) was added . The phases were mixed and the organic phase was separated. The aqueous phase was extracted with ethyl acetate (1 L) and the organic phases

were combined and passed through a plug of silica gel to provide, after concentration, the intermediate alcohol which was used directly in the following step. The alcohol was taken up in dichloromethane (5.5 L) and PPTS (18 g, 71.6 mmol) was added. The resulting solution was heated to 70 °C for 6 hours then cooled to ambient temperature. The reaction was concentrated to 10% of the original volume then purified by silica gel chromatography eluting with DCM to afford 7-bromo-3-chloro-5-methylene-5H-chromeno[2,3-c]pyridine (**5D)** (183 g, 0.59 mol, 46% overall yield). *m/z* calculated for $C_{13}H_7BrClNO$: 306.94, found 308.0 (M^+)



(S)-7-bromo-3-chloro-5'H-spiro[chromeno[2,3-c]pyridine-5,4'-oxazol]-2'-amine **(5)**

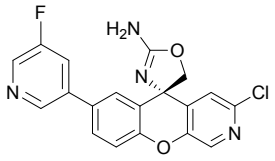
To a solution of iodine (181.2g, 0.714 mol) in THF (5 L) at -20 °C was added silver cyanate (291.5 g, 1.95 mol). The resulting slurry was stirred at this temperature for 1 hour. A solution of 7-bromo-3-chloro-5- methylene-5H-chromeno[2,3-c]pyridine **(5D)** (200 g, 0.65 mol) in THF (1 L) was added and the reaction was stirred at 0 °C for 3 hours. The mixture was filtered through a pad of celite, washing with THF. The filtrate was cooled to 10 °C and treated with a solution of ammonia (2 M in *i*PrOH, 972 mL). The resulting dark solution was stirred at ambient temperature overnight at which point saturated sodium thiosulfate (1 L) and saturated sodium bicarbonate (1.5 L) were added. The mixture was stirred 1 hour then the organic phase was separated and the aqueous phase was extracted with ethyl acetate (1 L). The combined organic phases were dried over sodium sulfate, filtered and concentrated. The obtained solid was triturated with DCM (1 L) and water (1 L) before being filtered to provide 7-bromo- 3-chloro-5'H-spiro[chromeno[2,3-c]pyridine-5,4'-oxazol]-2'-amine as yellow solid. Chiral separation provided (S)-7-bromo-3-chloro-5'H-spiro[chromeno[2,3- c]pyridine-5 ,4'-oxazol]-2'-amine **(5)** (59.4 g, 0.16 mol, 25% yield). *m/z* calculated for $C_{14}H_9BrClN3O_2$: 364.96, found 365.9 (M^+)



(S)-3-Chloro-7-(2-fluoropyridin-3-yl)-5'H-spiro[chromeno[2,3-c]pyridine-5,4'-oxazol]-2'-amine **(6)**

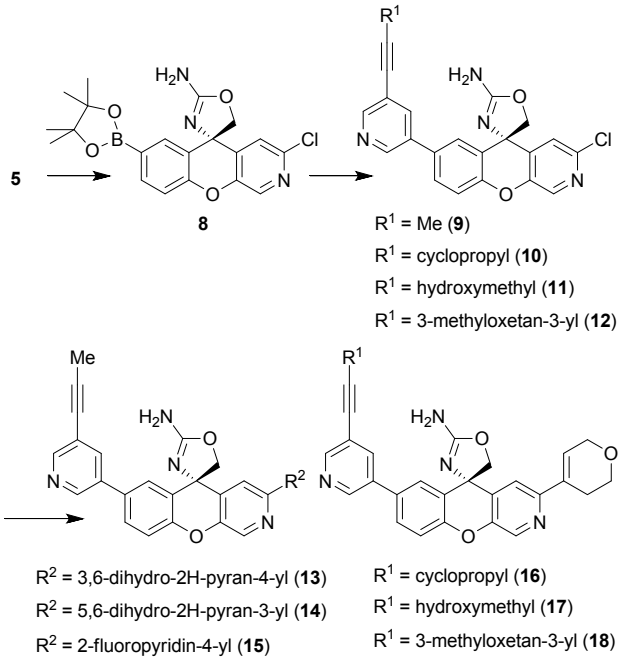
A 20 mL glass microwave reaction vessel was charged with (S)-7-bromo-3-chloro-5'H-spiro[chromeno[2,3-c]pyridine-5,4'-oxazol]-2'-amine **(5)** (0.5 g, 1.4 mmol), potassium phosphate (0.87 g, 4.1 mmol), 2-fluoropyridin-3-ylboronic acid (0.48 g, 3.4 mmol) and bis(di-tert-butyl(4-dimethylaminophenyl)phosphine)dichloropalladium (II) (0.097 g, 0.14 mmol). The vial was sealed and evacuated / backfilled with nitrogen 3x. Dioxane (4.8 mL) and water (1.6 mL) were added. The reaction mixture was heated to 100 °C for 30 minutes in a Biotage microwave reactor.

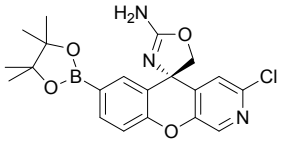
The mixture was diluted with EtOAc and water. The organic layer was washed with saturated sodium carbonate (2x) then dried over sodium sulfate and concentrated under reduced pressure. Silica gel chromatography using a gradient of 2-10% MeOH in DCM afforded (S)-3-chloro-7-(2-fluoropyridin-3-yl)-5'H-spiro[chromeno[2,3-c]pyridine-5,4'-oxazol]-2'-amine (0.5 g, 1.3 mmol, 96% yield). *m/z* calculated for $C_{19}H_{12}ClFN_4O_2$: 382.78, found 383.0 (M^+); δH (400 MHz; $CDCl_3$; Me_4Si) 4.29 - 4.41 (2 H, m) 4.73 (2 H, br s) 7.26 - 7.31 (2 H, m) 7.39 (1 H, s) 7.52 - 7.57 (1 H, m) 7.62 (1 H, s) 7.87 (1 H, ddd, *J*=9.73, 7.58, 1.86 Hz) 8.20 (1 H, d, *J*=4.70 Hz) 8.33 (1 H, s)



(S)-3-chloro-7-(5-fluoropyridin-3-yl)-5'H-spiro[chromeno[2,3-c]pyridine-5,4'-oxazol]-2'-amine **(7)**

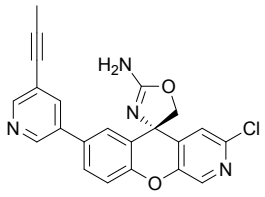
The title compound was prepared using the same procedure as described for compound **(6)** but with 5-fluoropyridin-3-ylboronic acid (58% yield). *m/z* calculated for $C_{19}H_{12}ClFN_4O_2$: 382.78, found 383.0 (M^+); δH NMR (400 MHz, DMSO-d_6; ; Me_4Si) 4.30 - 4.36 (1 H, m) 4.37 - 4.41 (1 H, m) 6.65 (2 H, s) 7.30 (1 H, s) 7.37 (1 H, d, *J*=8.61 Hz) 7.71 (1 H, d, *J*=2.15 Hz) 7.80 (1 H, dd, *J*=8.61, 2.35 Hz) 8.07 (1 H, dt, *J*=10.37, 2.25 Hz) 8.42 (1 H, s) 8.59 (1 H, d, *J*=2.74 Hz) 8.76 (1 H, t, *J*=1.66 Hz)





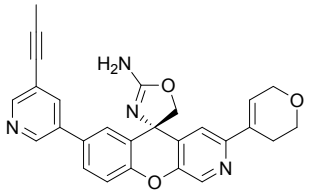
(S)-3-Chloro-7-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-5'H-spiro[chromeno[2,3-c]pyridine-5,4'-oxazol]-2'-amine B **(8)**

A flask was charged with (S)-7-bromo-3-chloro-5'H-spiro[chromeno[2,3-c]pyridine-5,4'-oxazol]-2'-amine **(5)** (3.1 g, 8.3 mmol), 4,4,4',4',5,5,5',5'-octamethyl-2,2'-bi(1,3,2-dioxaborolane) (4.2 g, 16.7 mmol) , dichloro(1,1-bis(diphenylphosphinoferrocene))palladium(II) complex with dichloromethane (0.68 g, 0.8 mmol), and potassium acetate (2.45 g, 25.0 mmol). Dioxane (39 mL) was added and the flask was purged with Argon. The reaction was heated at 100 °C for 4 hours. The reaction was cooled then filtered through celite, rinsing with methanol. The filtrate was concentrated under reduced pressure and the residue was diluted with water and extracted with EtOAc (2X). The organic phase was dried over sodium sulfate, and concentrated. Purification via silica gel chromatography using a gradient of 2-10% MeOH in DCM afforded the title compound (3.1 g, 7.6 mmol, 91% yield). *m/z* calculated for $C_{20}H_{21}BClN_3O_4$: 413.13, found 414.0 (M^+)



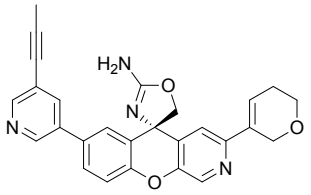
(S)-3-chloro-7-(5-(prop-1-ynyl)pyridin-3-yl)-5'H-spiro[chromeno[2,3-c]pyridine-5,4'-oxazol]-2'-amine (**9**) **GENERAL METHOD A**

A flask was charged with 3-bromo-5-(prop-1-ynyl)pyridine (0.31 g, 1.6 mmol), (S)-3-chloro-7-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-5'H-spiro[chromeno[2,3-c]pyridine-5,4'-oxazol]-2'-amine **(8)** (0.64 g, 1.6 mmol), 1,1-bis[(di-t-butyl-p-methylaminophenyl]palladium(II) chloride (0.060 g, 0.085 mmol), and potassium phosphate, tribasic (0.850 g, 4.00 mmol). Dioxane (10 mL) and water (3.5 mL) were added and the reaction was flushed with Argon. The reaction was heated to 100 °C for 1 hour. The reaction was cooled to room temperature and diluted with dichloromethane (75 mL) and water (35 mL). The phases were mixed and the organic layer was separated, dried over sodium sulfate and concentrated under reduced pressure. Purification via silica gel chromatography using a gradient of 0-10% MeOH in DCM afforded the title compound (0.29 g, 0.72 mmol, 45 % yield). *m/z* calculated for $C_{22}H_{15}ClN_4O_2$: 402.83, found 403.1 (M^+); δH (400 MHz; DMSO-d_6; Me_4Si) 2.12 (3 H, s) 4.32 - 4.43 (2 H, m) 6.65 (2 H, br s) 7.31 (1 H, s) 7.34 (1 H, d, *J*=8.61 Hz) 7.66 (1 H, d, *J*=1.96 Hz) 7.77 (1 H, dd, *J*=8.61, 2.35 Hz) 8.07 (1 H, t, *J*=2.05 Hz) 8.42 (1 H, s) 8.58 (1 H, d, *J*=1.76 Hz) 8.80 (1 H, d, *J*=2.15 Hz)



(S)-3-(3,6-Dihydro-2H-pyran-4-yl)-7-(5-(prop-1-ynyl)pyridin-3-yl)-5'H-spiro[chromeno[2,3-c]pyridine-5,4'-oxazol]-2'-amine **(13) General Method B**

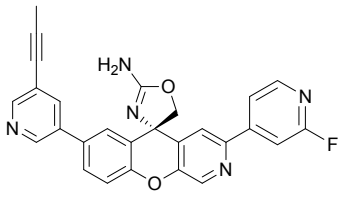
A microwave vial was charged with (S)-3-chloro-7-(5-(prop-1-ynyl)pyridin-3-yl)-5'H-spiro[chromeno[2,3-c]pyridine-5,4'-oxazol]-2'-amine **(9)** (0.23 g, 0.57 mmol), 2-(3,6-dihydro-2H-pyran-4-yl)-4,4,5,5-tetramethyl-1,3,2-dioxaborolane (0.25 g, 1.18 mmol), tetrakis(triphenylphosphine)palladium(0) (0.036 g, 0.031 mmol), and potassium carbonate (0.27 g, 1.9 mmol). Dioxane (3 mL) and water (1 mL) were added and the reaction was flushed with Argon. The reaction was heated to 120 °C in a Biotage microwave reactor for 30 minutes. The reaction vial was cooled to room temperature. The reaction was diluted with dichloromethane (35 mL) and water (10 mL). The phases were mixed and the organic layer was separated, dried over sodium sulfate and concentrated under reduced pressure the organic layer was separated. The organic layer was dried over sodium sulfate and concentrated under reduced pressure. Purification via silica gel chromatography using a gradient of 0-10% MeOH in DCM afforded the title compound (0.090 g, 0.200 mmol, 35 % yield). *m/z* calculated $C_{27}H_{22}N_4O_3$: 450.17, found 451.1 (M^+); δH (400 MHz; DMSO-d_6; Me_4Si) 2.12 (3 H, s) 2.51 - 2.57 (1 H, m) 3.84 (2 H, t, *J*=5.48 Hz) 4.25 - 4.30 (2 H, m) 4.30 - 4.39 (2 H, m) 6.56 (2 H, s) 6.65 (1 H, br s) 7.33 (1 H, d, *J*=8.41 Hz) 7.41 (1 H, s) 7.64 (1 H, d, *J*=2.15 Hz) 7.75 (1 H, dd, *J*=8.51, 2.25 Hz) 8.05 (1 H, t, *J*=1.96 Hz) 8.50 (1 H, s) 8.57 (1 H, d, *J*=1.76 Hz) 8.79 (1 H, d, *J*=2.15 Hz)



(S)-3-(5,6-Dihydro-2H-pyran-3-yl)-7-(5-(prop-1-yn-1-yl)pyridin-3-yl)-5'H-spiro[chromeno[2,3-c]pyridine-5,4'-oxazol]-2'-amine **(14) General Method C**

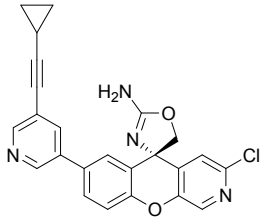
A microwave vial was charged with (S)-3-chloro-7-(5-(prop-1-ynyl)pyridin-3-yl)-5'H-spiro[chromeno[2,3-c]pyridine-5,4'-oxazol]-2'-amine **(9)** (0.23 g, 0.57 mmol), 2-(5,6-dihydro-2H-pyran-3-yl)-4,4,5,5-tetramethyl-1,3,2-dioxaborolane (0.32 g, 1.52 mmol), 1,1-bis[(di-t-butyl-p-methylaminophenyl]palladium(II) chloride (0.020 g, 0.028 mmol), and potassium phosphate, tribasic (0.400 g, 1.88 mmol). Dioxane (3 mL) and water (1 mL) were added and the reaction was flushed with Argon. The reaction was heated to 100 °C in a Biotage microwave reactor for 25 minutes. The reaction vial was cooled to room temperature and diluted with dichloromethane

(35 mL) and water (10 mL). the phases were mixed and the organic layer was separated, dried over sodium sulfate and concentrated under reduced pressure. Purification via silica gel chromatography using a gradient of 0-10% MeOH in DCM afforded the title compound (0.11 g, 0.24 mmol, 42 % yield). *m/z* calculated for $C_{27}H_{22}N_4O_3$: 450.17, found 451.1 (M^+); δH (400 MHz, DMSO-d_6; Me_4Si) 2.12 (3 H, s) 2.31 (2 H, m) 3.74 (2 H, t, *J*=5.48 Hz) 4.34 (2 H, q, *J*=8.61 Hz) 4.46 - 4.60 (2 H, m) 6.56 (2 H, s) 6.63 (1 H, br s) 7.32 (1 H, d, *J*=8.61 Hz) 7.43 (1 H, s) 7.63 (1 H, d, *J*=2.15 Hz) 7.75 (1 H, dd, *J*=8.61, 2.15 Hz) 8.05 (1 H, t, *J*=1.86 Hz) 8.46 (1 H, s) 8.57 (1 H, d, *J*=1.76 Hz) 8.79 (1 H, d, *J*=2.15 Hz)



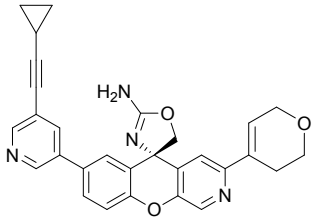
(S)-3-(2-Fluoropyridin-4-yl)-7-(5-(prop-1-ynyl)pyridin-3-yl)-5'H-spiro[chromeno[2,3-c]pyridine-5,4'-oxazol]-2'-amine **(15) General Method B**

The title compound was prepared as described for compound (**13)** but using 2-fluoropyridine-4-boronic acid (12 % yield). *m/z* calculated $C_{27}H_{18}FN_5O_{23}$: 463.14, found 464.1 (M^+); δH (400 MHz, DMSO-d_6; Me_4Si) 2.13 (3 H, s) 4.45 - 4.53 (2 H, m) 6.56 (2 H, s) 7.36 - 7.40 (1 H, m) 7.64 (1 H, d, *J*=2.35 Hz) 7.77 - 7.81 (2 H, m) 7.96 - 8.00 (1 H, m) 8.05 - 8.08 (2 H, m) 8.37 (1 H, d, *J*=5.28 Hz) 8.59 (1 H, d, *J*=1.76 Hz) 8.71 (1 H, s) 8.80 (1 H, d, *J*=2.15 Hz)



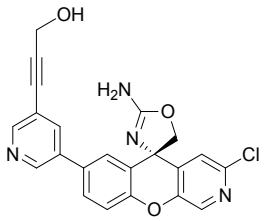
(S)-3-chloro-7-(5-(cyclopropylethynyl)pyridin-3-yl)-5'H-spiro[chromeno[2,3-c]pyridine-5,4'-oxazol]-2'-amine **(10) General Method A**

The title compound was prepared as described for compound **(9)** but using 3-bromo-5-(cyclopropylethynyl)pyridine (28 % yield). *m/z* calculated $C_{24}H_{17}ClN_4O_2$: 428.10, found 429.0 (M^+)



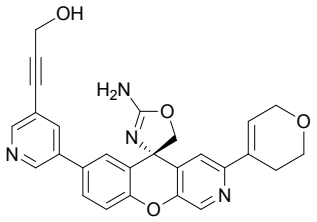
(S)-7-(5-(Cyclopropylethynyl)pyridin-3-yl)-3-(3,6-dihydro-2H-pyran-4-yl)-5'H-spiro[chromeno[2,3-c]pyridine-5,4'-oxazol]-2'-amine **(16) GENERAL METHOD C**

The title compound was prepared as described for compound **(14)** but using (S)-3-chloro-7-(5-(cyclopropylethynyl)pyridin-3-yl)-5'H-spiro[chromeno[2,3-c]pyridine-5,4'-oxazol]-2'-amine **(10)** and 2-(3,6-dihydro-2H-pyran-4-yl)-4,4,5,5-tetramethyl-1,3,2-dioxaborolane (28 % yield). *m/z* calculated $C_{29}H_{24}N_4O_3$: 476.18, found 477.2 (M^+); δH (400 MHz; DMSO-d_6; Me_4Si) 0.55 - 0.62 (2 H, m) 0.69 - 0.75 (2 H, m) 1.34 - 1.45 (1 H, m) 2.29 - 2.36 (2 H, m) 3.61 (2 H, t, *J*=5.48 Hz) 4.05 (2 H, br d, *J*=2.54 Hz) 4.07 - 4.18 (2 H, m) 6.33 (2 H, s) 6.39 - 6.46 (1 H, m) 7.10 (1 H, d, *J*=8.41 Hz) 7.18 (1 H, s) 7.41 (1 H, d, *J*=2.35 Hz) 7.52 (1 H, dd, *J*=8.51, 2.25 Hz) 7.80 (1 H, t, *J*=2.05 Hz) 8.28 (1 H, s) 8.32 (1 H, d, *J*=1.96 Hz) 8.55 (1 H, d, *J*=2.15 Hz)



(S)-3-(5-(2'-Amino-3-chloro-5'H-spiro[chromeno[2,3-c]pyridine-5,4'-oxazole]-7-yl)pyridin-3-yl)prop-2-yn-1-ol **(11) GENERAL METHOD A**

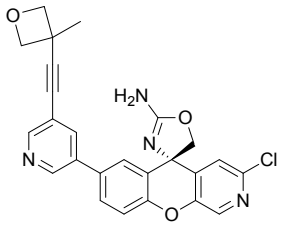
The title compound was prepared as described for compound **(9)** but using 3-(5-bromopyridin-3-yl)prop-2-yn-1-ol (26 % yield). *m/z* calculated $C_{22}H_{15}ClN_4O_3$: 418.08, found 419.0 (M^+)



(S)-3-(5-(2'-Amino-3-(3,6-dihydro-2H-pyran-4-yl)-5'H-spiro[chromeno[2,3-c]pyridine-5,4'-oxazole]-7-yl)pyridin-3-yl)prop-2-yn-1-ol **(17) GENERAL METHOD C**

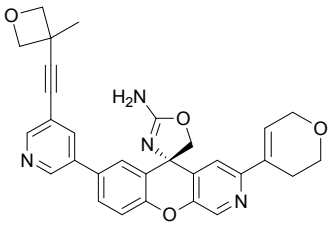
The title compound was prepared as described for compound **(14)** but using (S)-3-(5-(2'-amino-3-chloro-5'H-spiro[chromeno[2,3-c]pyridine-5,4'-oxazole]-7-yl)pyridin-3-yl)prop-2-yn-1-ol **(11)** and 2-(3,6-dihydro-2H-pyran-4-yl)-4,4,5,5-tetramethyl-1,3,2-dioxaborolane (32 % yield). *m/z*

calculated $C_{27}H_{22}N_4O_4$: 466.16, found 467.1 (M^+); δH (400 MHz; DMSO-d_6; Me_4Si) 2.52 - 2.62 (2 H, m) 3.84 (2 H, t, *J*=5.48 Hz) 4.26 - 4.31 (2 H, m) 4.31 - 4.35 (1 H, m) 4.36 - 4.41 (3 H, m) 5.40 - 5.50 (1 H, m) 6.58 (2 H, s) 6.63 - 6.69 (1 H, m) 7.35 (1 H, d, *J*=8.41 Hz) 7.41 (1 H, s) 7.65 (1 H, d, *J*=2.15 Hz) 7.78 (1 H, dd, *J*=8.51, 2.25 Hz) 8.10 (1 H, t, *J*=1.96 Hz) 8.51 (1 H, s) 8.62 (1 H, d, *J*=1.76 Hz) 8.85 (1 H, d, *J*=2.15 Hz)



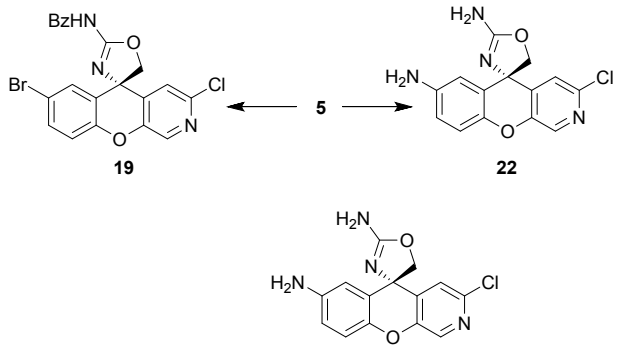
(S)-3-Chloro-7-(5-((3-methyloxetan-3-yl)ethynyl)pyridin-3-yl)-5'H-spiro[chromeno[2,3-c]pyridine-5,4'-oxazol]-2'-amine **(12) GENERAL METHOD A**

The title compound was prepared as described for compound **(9)** but using 3-bromo-5-((3-methyloxetan-3-yl)ethynyl)pyridine (58 % yield). *m/z* calculated $C_{25}H_{19}ClN_4O_3$: 458.11, found 459.0 (M^+)



(S)-3-(3,6-dihydro-2H-pyran-4-yl)-7-(5-((3-methyloxetan-3-yl)ethynyl)pyridin-3-yl)-5'H-spiro[chromeno[2,3-c]pyridine-5,4'-oxazol]-2'-amine **(18) GENERAL METHOD C**

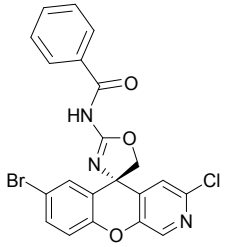
The title compound was prepared as described for compound **(14)** but using (S)-3-chloro-7-(5-((3-methyloxetan-3-yl)ethynyl)pyridin-3-yl)-5'H-spiro[chromeno[2,3-c]pyridine-5,4'-oxazol]-2'-amine **(12)** and 2-(3,6-dihydro-2H-pyran-4-yl)-4,4,5,5-tetramethyl-1,3,2-dioxaborolane (45 % yield)**.** *m/z* calculated $C_{30}H_{26}N_4O_4$: 506.2, found 507.2 (M^+); δH (400 MHz; DMSO-d_6; ; Me_4Si) 1.68 (3 H, s) 2.53 (2 H, br d, *J*=2.74 Hz) 3.83 (2 H, t, *J*=5.48 Hz) 4.27 (2 H, br d, *J*=2.54 Hz) 4.35 (2 H, q, *J*=8.48 Hz) 4.47 (2 H, d, *J*=5.48 Hz) 4.80 (2 H, d, *J*=5.48 Hz) 6.56 (2 H, s) 6.60 - 6.68 (1 H, m) 7.33 (1 H, d, *J*=8.61 Hz) 7.40 (1 H, s) 7.65 (1 H, d, *J*=2.15 Hz) 7.77 (1 H, dd, *J*=8.61, 2.15 Hz) 8.11 (1 H, t, *J*=2.05 Hz) 8.50 (1 H, s) 8.62 (1 H, d, *J*=1.96 Hz) 8.82 (1 H, d, *J*=2.15 Hz)



(S)-3-Chloro-5'H-spiro[chromeno[2,3-c]pyridine-5,4'-oxazole]-2',7-diamine **(22)**

Step 1: A resealable vial was charged with (S)-7-bromo-3-chloro-5'H-spiro[chromeno[2,3-c]pyridine-5,4'-oxazol]-2'-amine **(5)** (1.0 g, 2.73 mmol), sodium azide (0.53 g, 8.2 mmol), sodium L-ascorbate (0.054 g, 0.273 mmol), and copper(I) iodide (0.104 g, 0.546 mmol). The vial was sealed and evacuated / backfilled with nitrogen three times. EtOH (7 mL) was added followed by water (3 mL) and trans-N,N'-dimethyl-1,2-cyclohexanediamine (0.13 mL, 0.82 mmol). The reaction was stirred in a pre-heated 90 °C oil bath for 2 hours. The reaction was poured into water (150 mL) and a tan precipitate formed. The solid was collected by vacuum filtration, dissolved in EtOAc and washed with water and brine. The organic phase was dried over magnesium sulfate and concentrated under reduced pressure to afford the intermediate azide (S)-7-azido-3-chloro-5'H-spiro[chromeno[2,3-c]pyridine-5,4'-oxazol]-2'-amine (0.804 g, 2.45 mmol, 90 % yield).

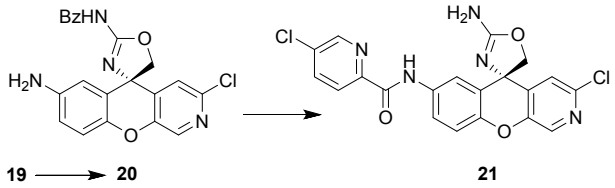
Step 2: To a 50 mL round bottom flask was added (S)-7-azido-3-chloro-5'H-spiro[chromeno[2,3-c]pyridine-5,4'-oxazol]-2'-amine (0.55 g, 1.67 mmol) followed by MeOH (11 mL). The solution was cooled to 0 °C before adding sodium borohydride (0.32 g, 8.37 mmol) in portions. The reaction was stirred for 25 minutes then quenched by the careful addition of water. The reaction was diluted with DCM and the phases were mixed. The aqueous phase was extracted with additional DCM. The combined organic phases were washed with brine, dried over magnesium sulfate and concentrated under reduced pressure to afford the title compound (406 mg, 1.34 mmol, 80 % yield). *m/z* calculated for $C_{14}H_{11}ClN_4O_2$: 302.06, found 303.0 (M^+)

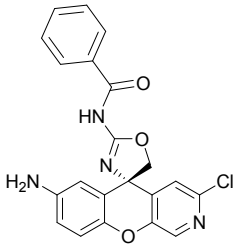


(S)-N-(7-Bromo-3-chloro-5'H-spiro[chromeno[2,3-c]pyridine-5,4'-oxazol]-2'-yl)benzamide **(19)**

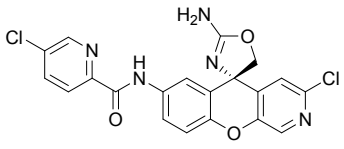
STo a solution of (S)-7-bromo-3-chloro-5'H-spiro[chromeno[2,3-c]pyridine-5,4'-oxazol]-2'-amine **(5)** (5.0 g, 13.6 mmol) in DMF (40 mL) was added benzoic acid anhydride (3.4 g, 15 mmol) and the resulting mixture was stirred at room temperature for 17 h. The mixture was poured into

water (200 mL) and extracted with EtOAc (2x). The combined organics were washed sequentially with saturated sodium bicarbonate and brine then dried over magnesium sulfate. Purification via silica gel chromatography using a gradient of 0-50% EtOAc in hexanes afforded (S)-N-(7-bromo-3-chloro-5'H-spiro[chromeno[2,3-c]pyridine-5,4'-oxazol]-2'-yl)benzamide (5.77 g, 12.3 mmol, 90 % yield).



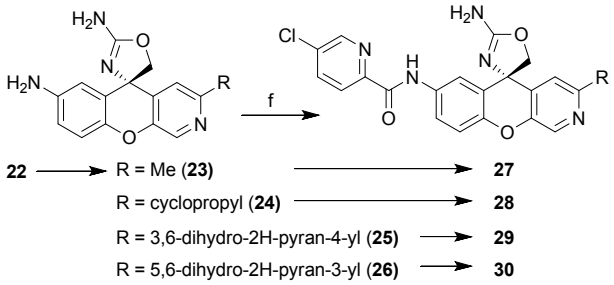


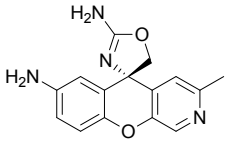
(S)-N-(7-Amino-3-chloro-5'H-spiro[chromeno[2,3-c]pyridine-5,4'-oxazol]-2'-yl)benzamide **(20)**
A resealable vial was charged with (S)-N-(7-bromo-3-chloro-5'H-spiro[chromeno[2,3-c]pyridine-5,4'-oxazol]-2'-yl)benzamide **(19)** (0.78 g, 1.65 mmol), tris(dibenzylideneacetone)dipalladium (0) (0.053 g, 0.058 mmol), and (2-biphenyl)dicyclohexylphosphine (0.040 g, 0.115 mmol). The vial was sealed and evacuated / backfilled with nitrogen 3x. A solution of Lithium bis(trimethylsilyl)amide (1 M in THF, 4.6 mL, 4.6 mmol) was added. The reaction was stirred in a pre-heated 70 °C oil bath for 16 hours. The reaction was quenched with saturated ammonium chloride and diluted with water and EtOAc. The organic layer was separated, washed with brine, dried over magnesium sulfate, and concentrated under reduced pressure. Purification via silica gel chromatography using a gradient of 20-70% EtOAc in hexanes afforded (S)-N-(7-amino-3-chloro-5'H-spiro[chromeno[2,3-c]pyridine-5,4'-oxazol]-2'-yl)benzamide (0.46 g, 1.13 mmol, 69 % yield) as an orange solid. *m/z* calculated for $C_{21}H_{15}ClN_4O_3$: 406.09, found 407.0 (M^+)



(S)-N-(2'-amino-3-chloro-5'H-spiro[chromeno[2,3-c]pyridine-5,4'-oxazol]-7-yl)-5-chloropicolinamide **(21)**
A flask was charged with (S)-N-(7-amino-3-chloro-5'H-spiro[chromeno[2,3-c]pyridine-5,4'-oxazol]-2'-yl)benzamide **(20)** (0.0296 g, 0.073 mmol), 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (0.042 g, 0.218 mmol), 5-chloropyridine-2-carboxylic acid

(0.034 g, 0.218 mmol), DCM (1 mL) and DMF (0.100 mL). The reaction was stirred for 5 minutes then 1-hydroxy-1H-benzotriazole (0.029 g, 0.218 mmol) was added in one portion and the reaction was stirred at room temperature for 16 hours. The reaction was diluted with water and DCM. The organic phase was separated and concentrated under reduced pressure. The crude residue was taken up in MeOH (0.61 mL) and THF (0.12 mL). Sodium hydroxide (2 M in water, 0.11 mL, 0.22 mmol) was added. The reaction was heated to 70 °C for 3 hours, then an additional portion of sodium hydroxide (2 M in water) (0.11 mL, 0.22 mmol) was added and the reaction was continued for an additional 30 minutes. The reaction was diluted with water and EtOAc. The aqueous layer was extracted with additional EtOAc and the combined organic layers were brined, dried over magnesium sulfate and concentrated under reduced pressure. Purification via reverse phase HPLC using a gradient of 10-100% ACN : water + 0.1% TFA afforded, after neutralization, (S)-N-(2'-amino-3-chloro-5'H-spiro[chromeno[2,3-c]pyridine-5,4'-oxazol]-7-yl)-5-chloropicolinamide (11 mg, 0.026 mmol, 36 % yield) as a light yellow solid. *m/z* calculated for $C_{20}H_{13}Cl2N_5O_3$: 441.04, found 442.0 (M^+); δH NMR (300 MHz; CD_3OD; Me_4Si) 4.43 (2 H, s) 7.25 (1 H, d, J=8.92 Hz) 7.44 (1 H, s) 7.84 (1 H, dd, J=8.92, 2.63 Hz) 7.98 (1 H, d, J=2.48 Hz) 8.06 - 8.11 (1 H, m) 8.18 - 8.23 (1H, m) 8.30 (1 H, s) 8.67 - 8.77 (1H, m)

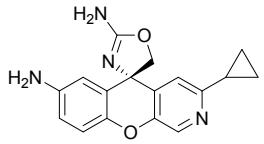




(S)-3-methyl-5'H-spiro[chromeno[2,3-c]pyridine-5,4'-oxazole]-2',7-diamine **(23)**

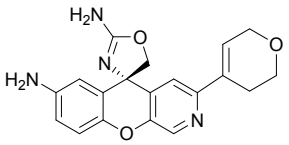
A resealable vial was charged with (S)-3-chloro-5'H-spiro[chromeno[2,3-c]pyridine-5,4'-oxazole]-2',7-diamine **(22)** (144 mg, 0.476 mmol), 1,1-bis[(di-t-butyl-p-methylaminophenyl]palladium(II) chloride (34 mg, 0.048 mmol) and potassium phosphate (303 mg, 1.43 mmol). The vial was evacuated / backfilled with nitrogen 3x. Dioxane (3 mL) was added followed by water (0.3 mL) and trimethylboroxine (0.26 mL, 1.9 mmol). The reaction was stirred in a pre-heated 110 °C oil bath for 2 hours. The reaction was cooled to room temperature before diluting with water and EtOAc. The aqueous layer was washed with additional EtOAc and the combined organic phases were washed with brine, dried over magnesium sulfate, and concentrated under reduced pressure. The crude residue was taken up in DCM (5 mL) and TFA (0.2 mL) was added. The solution was loaded onto a 25 g cation exchange column. The column was flushed with MeOH before eluting the product with 2M ammonia in MeOH. The eluate was concentrated under reduced pressure to afford (S)-3-methyl-5'H-spiro[chromeno[2,3-c]pyridine-

5,4'-oxazole]-2',7-diamine (111 mg, 0.393 mmol, 83 % yield). *m/z* calculated for $C_{15}H_{14}N_4O_2$: 282.11, found 283.1 (M^+)



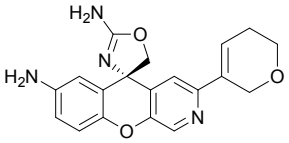
(S)-3-cyclopropyl-5'H-spiro[chromeno[2,3-c]pyridine-5,4'-oxazole]-2',7-diamine **(24)**

The title compound was prepared as described for compound **(23)** but using cyclopropylboronic acid (69% yield). *m/z* calculated for $C_{17}H_{16}N_4O_2$: 308.13, found 309.0 (M^+)



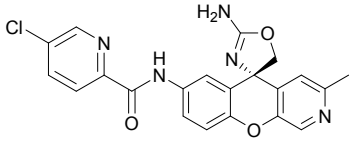
(S)-3-(3,6-dihydro-2H-pyran-4-yl)-5'H-spiro[chromeno[2,3-c]pyridine-5,4'-oxazole]-2',7-diamine **(25)**

The title compound was prepared as described for compound **(23)** but using 2-(3,6-dihydro-2H-pyran-4-yl)-4,4,5,5-tetramethyl-1,3,2-dioxaborolane (100% yield). *m/z* calculated for $C_{19}H_{18}N_4O_3$: 350.14, found 351.2 (M^+)



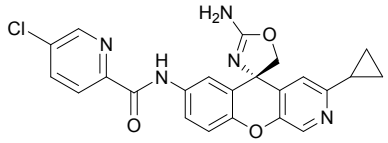
(S)-3-(5,6-dihydro-2H-pyran-3-yl)-5'H-spiro[chromeno[2,3-c]pyridine-5,4'-oxazole]-2',7-diamine **(26)**

The title compound was prepared as described for compound **(23)** but using 2-(5,6-dihydro-2H-pyran-3-yl)-4,4,5,5-tetramethyl-1,3,2-dioxaborolane (100% yield). m/z calculated for $C_{19}H_{18}N_4O_3$: 350.14, found 351.2 (M^+)



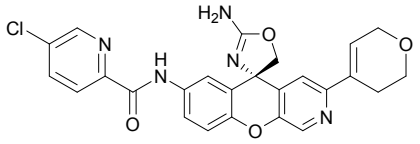
(S)-N-(2'-amino-3-methyl-5'H-spiro[chromeno[2,3-c]pyridine-5,4'-oxazol]-7-yl)-5-chloropicolinamide **(27)**

To a solution of (S)-3-methyl-5'H-spiro[chromeno[2,3-c]pyridine-5,4'-oxazole]-2',7-diamine **(23)** (0.055 g, 0.195 mmol) in THF (1 mL) and MeOH (0.5 mL) was added 5-chloropicolinic acid (0.061 g, 0.39 mmol) followed by 4-(4,6-dimethoxy-1,3,5-triazin-2-yl)-4-methylmorpholinium chloride (0.115 g, 0.390 mmol). The reaction was stirred at room temperature for 2 hours. The reaction was quenched with saturated sodium bicarbonate and diluted with water and EtOAc. The organic layer was washed with brine, dried over magnesium sulfate, and concentrated under reduced pressure. Purification via silica gel chromatography using a gradient of 30-100% EtOAc in hexanes afforded (S)-N-(2'-amino-3-methyl-5'H-spiro[chromeno[2,3-c]pyridine-5,4'-oxazol]-7-yl)-5-chloropicolinamide (0.029 g, 0.068 mmol, 35 % yield). *m/z* calculated for $C_{21}H_{16}ClN_5O_3$: 421.09, found 422.0 (M^+); δH NMR (300 MHz; $CDCl_3$; Me_4Si) (3 H, s) 4.32 - 4.42 (2 H, m) 7.15 - 7.20 (2 H, m) 7.74 - 7.79 (1H, m) 7.81 (1 H, d, J=2.48 Hz) 7.88 (1 H, dd, J=8.33, 2.34 Hz) 8.24 (1 H, d, J=8.33 Hz) 8.39 (1 H, s) 8.55 (1 H, d, J=1.90 Hz) 9.85 (1 H s)



(S)-N-(2'-amino-3-cyclopropyl-5'H-spiro[chromeno[2,3-c]pyridine-5,4'-oxazol]-7-yl)-5-chloropicolinamide **(28)**

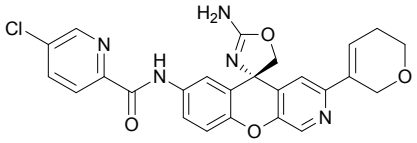
The title compound was prepared as described for compound **(27)** but using (S)-3-cyclopropyl-5'H-spiro[chromeno[2,3-c]pyridine-5,4'-oxazole]-2',7-diamine **(24)** (45 % yield)**.** *m/z* calculated for $C_{23}H_{18}ClN_5O_3$: 447.11, found 448.1 (M^+); δH NMR (300 MHz; $CDCl_3$; Me_4Si) 0.92 - 1.04 (4 H, m) 2.01 - 2.11 (1 H, m) 4.33 - 4.42 (2H, m) 7.15 - 7.20 (2 H, m) 7.74 - 7.83 (2 H, m) 7.89 (1 H, dd, J=8.33, 2.34 Hz) 8.25 (1 H, d, J=7.89 Hz,) 8.34 (1H, s,) 8.56 (1 H, d, J=1.90 Hz) 9.86 (1 H, s)



(S)-N-(2'-amino-3-(3,6-dihydro-2H-pyran-4-yl)-5'H-spiro[chromeno[2,3-c]pyridine-5,4'-oxazol]-7-yl)-5-chloropicolinamide **(29)**

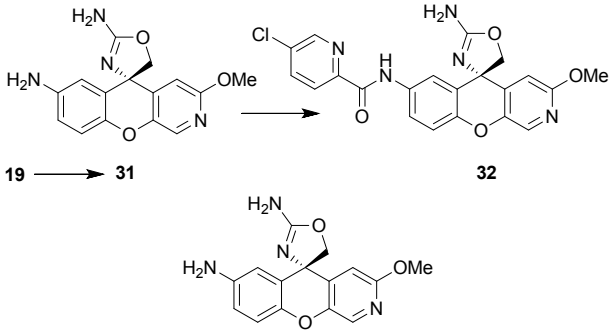
The title compound was prepared as described for compound **(27)** but using (S)-3-(3,6-dihydro-2H-pyran-4-yl)-5'H-spiro[chromeno[2,3-c]pyridine-5,4'-oxazole]-2',7-diamine **(25)** (52 % yield)**.** *m/z* calculated for $C_{25}H_{20}ClN_5O_4$: 489.12, found 490.1 (M^+); δH NMR (300 MHz; $CDCl_3$; Me_4Si) 2.62 - 2.69 (2 H, m) 3.96 (2 H, t, J=5.48 Hz) 4.36 - 4.42 (3 H, m) 4.44 - 4.48 (1 H, m) 6.61 - 6.66 (1 H, m) 7.20 (1 H, d, J=8.77 Hz) 7.38 (1 H, s) 7.79 (1 H, dd, J=8.77, 2.63 Hz) 7.85 (1 H, d,

J=2.34 Hz) 7.89 (1 H, dd, J=8.33, 2.34 Hz) 8.25 (1 H, d, J=8.62 Hz) 8.46 (1 H, s) 8.57 (1H, d, J=2.34 Hz) 9.88 (1 H, s)



(S)-N-(2'-amino-3-(5,6-dihydro-2H-pyran-3-yl)-5'H-spiro[chromeno[2,3-c]pyridine-5,4'-oxazol]-7-yl)-5-chloropicolinamide **(30)**

The title compound was prepared as described for compound **(27)** but using (S)-3-(5,6-dihydro-2H-pyran-3-yl)-5'H-spiro[chromeno[2,3-c]pyridine-5,4'-oxazole]-2',7-diamine **(26)** (26% yield)**.** *m/z* calculated for $C_{25}H_{20}ClN_5O_4$: 489.12, found 490.1 (M^+); δH NMR (300 MHz; DMSO-d6; Me_4Si) 2.23 - 2.36 (2 H, m) 3.75 (2 H, t, J=5.4 Hz) 4.16 - 4.22 (1 H, m) 4.26 - 4.33 (1 H, m) 4.44 - 4.61 (2 H, m) 6.55 (2 H, br, s) 6.59 - 6.66 (1 H, m) 7.20 (1 H, d, J=8.92 Hz) 7.40 (1 H, s) 7.85 (1 H, dd, J=8.92, 2.48 Hz) 8.07 (1 H, d, J=2.48 Hz) 8.12 - 8.25 (2 H, m) 8.42 (1 H, s) 8.78 (1 H, d, J=1.61 Hz) 10.78 (1 H, s)



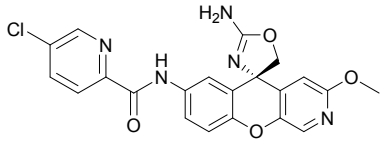
(S)-7-bromo-3-methoxy-5'H-spiro[chromeno[2,3-c]pyridine-5,4'-oxazol]-2'-amine **(31)**

Step 1: To a microwave vial was added (S)-N-(7-bromo-3-chloro-5'H-spiro[chromeno[2,3-c]pyridine-5,4'-oxazol]-2'-yl)benzamide **(19)** (0.35 g, 0.73 mmol) and sodium methoxide (0.4 mL, 7 mmol). The vial was sealed before adding DMSO (5 mL). The reaction was heated to 130 °C for 45 minutes in a Biotage microwave reactor. The reaction was diluted with EtOAc and water. The organic layer was separated, washed with brine, dried over magnesium sulfate, and concentrated under reduced pressure. Purification via silica gel chromatography using a gradient of 20-70% EtOAc in hexanes afforded (S)-N-(7-bromo-3-methoxy-5'H-spiro[chromeno[2,3-c]pyridine-5,4'-oxazol]-2'-yl)benzamide (0.19 g, 0.41 mmol, 55 % yield).

Step 2: A resealable vial was charged with (S)-N-(7-bromo-3-methoxy-5'H-spiro[chromeno[2,3-c]pyridine-5,4'-oxazol]-2'-yl)benzamide (0.171 g, 0.367 mmol), tris(dibenzylideneacetone)dipalladium (0) (0.012 g, 0.013 mmol), and (2-biphenyl)dicyclohexylphosphine (9.0 mg, 0.026 mmol). The vial was sealed and evacuated / backfilled with nitrogen 3x. Lithium bis(trimethylsilyl)amide (1 M in THF, 1.0 mL, 1.0 mmol) was added. The reaction was stirred in a pre-heated 65 °C oil bath for 16 hours. The reaction

was cooled to room temperature then quenched with saturated ammonium chloride and diluted with water and EtOAc. The organic phase was separated, washed with brine, dried over magnesium sulfate and concentrated under reduced pressure. Purification via silica gel chromatography using a gradient of 20-100% EtOAc in hexanes afforded (S)-N-(7-amino-3-methoxy-5'H-spiro[chromeno[2,3-c]pyridine-5,4'-oxazol]-2'-yl)benzamide (0.10 g, 0.26 mmol, 71 % yield).

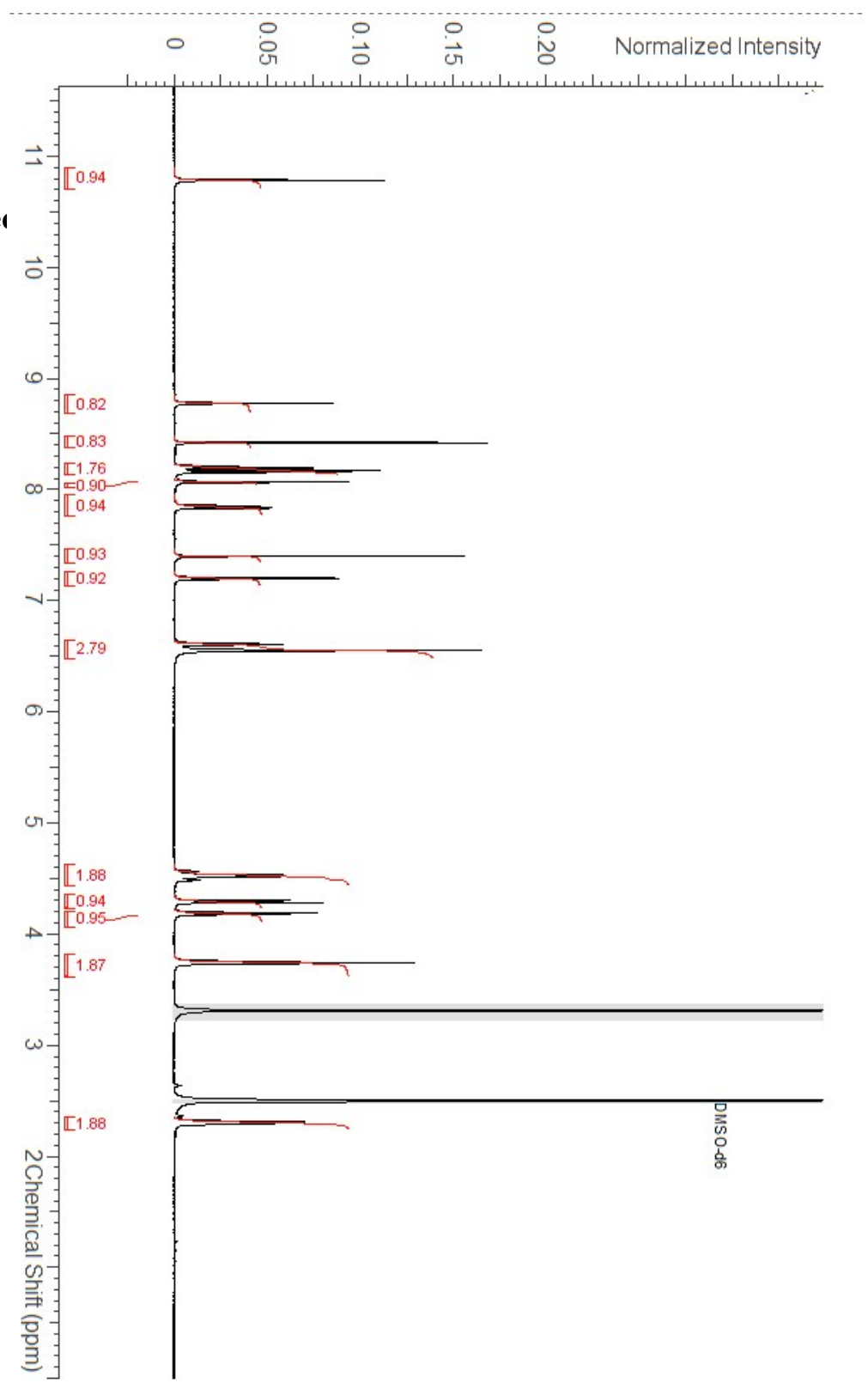
Step 3: To a solution of (S)-N-(7-amino-3-methoxy-5'H-spiro[chromeno[2,3-c]pyridine-5,4'-oxazol]-2'-yl)benzamide (0.10 g, 0.26 mmol) in MeOH (2 mL) and THF (1 mL) was added sodium hydroxide (2 M in water, 0.8 mL, 1.7 mmol). The reaction was heated to 65 °C for 16 hours. The reaction was diluted with saturated ammonium chloride and extracted with EtOAc (3x). The combined organic phases were washed with brine, dried over magnesium sulfate, and concentrated under reduced pressure. The crude material was taken up in DCM (5 mL) and TFA (0.2 mL) was added. The solution was loaded onto a cation exchange column. The column was flushed with MeOH before eluting the title compound with 2M ammonia in MeOH. The eluate was concentrated under reduced pressure to afford (S)-3-methoxy-5'H-spiro[chromeno[2,3-c]pyridine-5,4'-oxazole]-2',7-diamine (0.070 g, 0.23 mmol, 90 % yield). *m/z* calculated for $C_{15}H_{14}N_4O_3$: 298.11, found 299.1 (M^+)

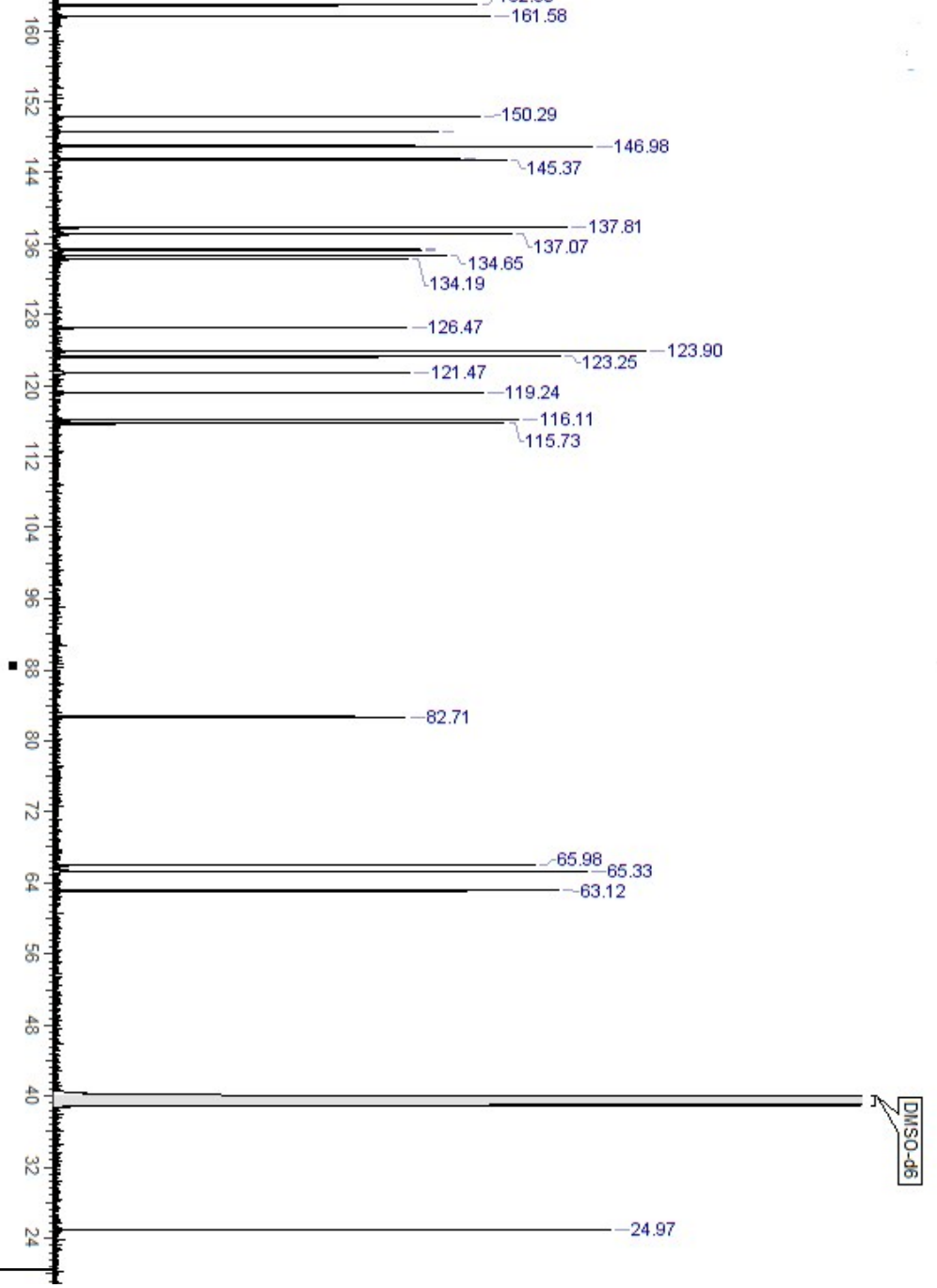


(S)-N-(2'-amino-3-methoxy-5'H-spiro[chromeno[2,3-c]pyridine-5,4'-oxazol]-7-yl)-5-chloropicolinamide **(32)**

The title compound was prepared as described for compound **(27)** but using (S)-3-methoxy-5'H-spiro[chromeno[2,3-c]pyridine-5,4'-oxazole]-2',7-diamine **(31)** (65% yield)**.** *m/z* calculated for $C_{21}H_{16}ClN_5O_4$: 437.09, found 438.0 (M^+); δH NMR (300 MHz; $CDCl_3$; Me_4Si) 3.93 (3 H, s) 4.33 (2 H, s) 6.80 (1 H, s) 7.16 (1 H, d, J=8.5 Hz) 7.76 - 7.83 (2 H, m) 7.87 (1 H, dd, J=8.3, 2.3 Hz) 8.08 (1 H, s) 8.24 (1H, d, J=8.3 Hz) 8.54 (1 H, d, J=2.3 Hz) 9.85 (1 H, s)

^{1}H and ^{13}C NMR Spec





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