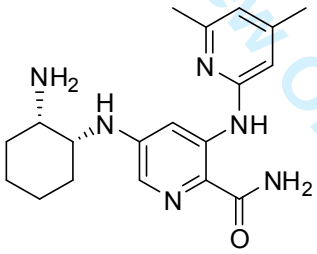
Experimental Section

Chemistry. Commercial reagents were obtained from reputable suppliers and used as received. All solvents were purchased in septum-sealed bottles stored under an inert atmosphere. All reactions were sealed with septa through which an argon atmosphere was introduced unless otherwise noted. Liquid reagents and solvents were transferred under a positive pressure of nitrogen via syringe. Reactions were conducted in microwave vials or round bottomed flasks containing Teflon-coated magnetic stir bars. Microwave reactions were performed with a Biotage Initiator Series Microwave (fixed hold time setting; reaction temperatures monitored by the internal infrared sensor).

Reactions were monitored by thin layer chromatography (TLC) on pre-coated TLC glass plates (silica gel 60 F254, 250 µm thickness) or by LC-MS (30 mm x 2 mm 2 micron column + guard; 2 mL injection; 3% to 98% MeCN/water + 0.05% TFA gradient over 2.3 minutes; 0.9 mL/min flow; APCI; positive ion mode; UV detection at 254 nM). Visualization of the developed TLC chromatogram was performed by fluorescence quenching. Silica gel column chromatography was performed on an automated purification system using pre-packed silica gel columns. ^{1}H and ^{13}C NMR were recorded on a 300, 400, 500 or 600 MHz Varian spectrometer; chemical shifts (δ) are reported relative to residual protio solvent signals. Data for NMR spectra are reported as follows: chemical shift (δ ppm), multiplicity (s = singlet, br s = broad singlet, d = doublet, t = triplet, q = quartet, dd = doublet of doublets, m = multiplet), coupling constant (Hz), integration.

All compounds reported are of at least 95% purity, as judged by LCAP (150 mm X 4.6 mm ID, 5 µm column; 5 µL injection; 10-100% MeCN/H2O + 0.05% TFA gradient over 6.75 min; 1 mL/min flow; ESI; positive ion mode; UV detection at 254 nM).



5-{[(1*R*,2*S*)-2-aminocyclohexyl]amino}-3-[(4,6-dimethylpyridin-2-yl)amino]pyridine-2-carboxamide (5)

Step 1: To a flask were added 3-bromo-5-fluoropyridine-2-carbonitrile (14.8 g, 73.5 mmol), *tert*-butyl [(1*S*,2*R*)-2-aminocyclohexyl]carbamate (15 g, 70 mmol), and diisopropylethylamine (49 mL, 280 mmol). The resulting mixture was heated at 110 °C. After 14 hours, the reaction mixture was allowed to cool to room temperature, diluted with dichloromethane, adsorbed on silica gel, and purified via silica gel column chromatography (0 to 100% EtOAc:Hexanes) to afford *tert*-butyl {(1*S*,2*R*)-2-[(5-bromo-6-cyanopyridin-3-yl)amino]cyclohexyl}carbamate (22.8 g, 82%) as a white solid. ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.06 (s, 1H), 7.22 (s, 1H), 6.86 (d, *J* = 7.8 Hz, 1H), 6.68 (d, *J* = 7.8 Hz, 1H), 3.78 (s, 1H), 3.62 (s, 1H), 1.64 – 1.44 (m, 6H), 1.30 (s, 9H), 1.14 – 1.08 (m, 2H).

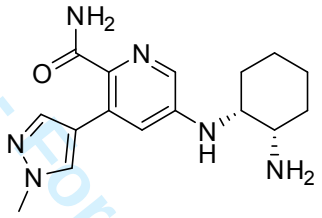
Step 2: To a flask were added *tert*-butyl {(1*S*,2*R*)-2-[(5-bromo-6-cyanopyridin-3-yl)amino]cyclohexyl}carbamate (21.5 g, 54.4 mmol), 2-amino-4,6-dimethylpyridine (9.97 g, 81.7 mmol), Tris(dibenzylideneacetone)dipalladium (0) (2.49 g, 2.72 mmol), Xantphos (3.15 g, 5.44 mmol), cesium carbonate (35.4 g, 109 mmol), and dioxane (215 mL). The mixture was sparged with nitrogen. After 15 minutes, the nitrogen purge was ceased and the reaction mixture was heated to 80 °C. After 5 hours, the reaction mixture was allowed to cool to ambient temperature, and filtered through CELITE (washing with ethyl acetate). To the filtrate was added silica gel, and the solvent was removed under reduced pressure. The residue was purified via silica gel column chromatography (0 to 100% EtOAc:Hexanes) to afford *tert*-butyl [(1*S*,2*R*)-2-({6-cyano-5-[(4,6-dimethylpyridin-2-yl)amino]pyridin-3-yl}amino)cyclohexyl]carbamate (22.0 g, 93%) as a pale brown solid. MS ESI calc'd. for $C_{24}H_{33}N_6O_2$ $[M + H]^+$ 437, found 437. ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.61 (s, 1H), 7.72 (d, *J* = 2.4 Hz, 1H), 7.52 (s, 1H), 6.60 (s, 1H), 6.54 (d, *J* = 8.4 Hz, 1H), 6.53 (s, 1H), 6.33 (d, *J* = 7.2 Hz, 1H), 3.71 (s, 1H), 3.55 (s, 1H), 2.56 (s, 3H), 2.16 (s, 3H), 1.74 – 1.66 (m, 1H), 1.64 – 1.54 (m, 2H), 1.51 – 1.42 (m, 3H), 1.32 – 1.23 (m, 10H), 1.12 – 1.08 (m, 1H).

Step 3: To a flask were added *tert*-butyl [(1*S*,2*R*)-2-({6-cyano-5-[(4,6-dimethylpyridin-2-yl)amino]pyridin-3-yl}amino)cyclohexyl]carbamate (18.9 g, 43.3 mmol) and DMSO (380 mL). The resulting mixture was cooled in an ice water bath. To the reaction mixture was added sodium hydroxide (6.0 M in water, 21.7 mL, 130 mmol) followed by the slow addition of hydrogen peroxide (35% solution in water, 7.96 mL, 91 mmol). The addition was performed at such a rate to maintain the internal temperature of the mixture between 25 °C and 30 °C. After 20 minutes, the mixture was slowly diluted with water (500 mL). After 30 minutes, the mixture was filtered, and the collected solids were washed with water and then dried for 14 hours under a nitrogen bag. The collected solids were suspended in ethyl acetate (500 mL) and the resulting slurry was seeded with a previously processed batch of *tert*-butyl [(1*S*,2*R*)-2-({6-carbamoyl-5-[(4,6-dimethylpyridin-2-yl)amino]pyridin-3-yl}amino)cyclohexyl]carbamate. After 1 hour, the mixture was diluted with hexanes (150 mL), filtered, washed with hexanes, and dried in a nitrogen bag to afford *tert*-butyl [(1*S*,2*R*)-2-({6-carbamoyl-5-[(4,6-dimethylpyridin-2-yl)amino]pyridin-3-yl}amino)cyclohexyl]carbamate (19.8 g, quant.) as a white solid. MS ESI calc'd. for $C_{24}H_{35}N_6O_3$ $[M + H]^+$ 455, found 455. ^{1}H NMR (600 MHz, DMSO-d_6) δ 11.68 (s, 1H), 8.51 (d, *J* = 1.8 Hz, 1H), 7.81 (d, *J* = 3.0 Hz, 1H), 7.55 (d, *J* = 1.8 Hz, 1H), 7.21 (s, 1H), 6.54–6.50 (m, 2H), 6.40 (s, 1H), 6.05 (d, *J* = 6.0 Hz, 1H), 3.81 (s, 1H), 3.54 (s, 1H), 2.34 (s, 3H), 2.17 (s, 3H), 1.82–1.76 (m, 1H), 1.72–1.64 (m, 1H), 1.60–1.42 (m, 4H), 1.36–1.28 (m, 1H), 1.27 (s, 9H), 1.10–1.06 (m, 1H).

Step 4: *tert*-Butyl [(1*S*,2*R*)-2-({6-carbamoyl-5-[(4,6-dimethylpyridin-2-yl)amino]pyridin-3-yl}amino)cyclohexyl]carbamate (22.5 g, 49.5 mmol) was diluted with dioxane (140 mL). To this solution was added hydrochloric acid (4.0 M in dioxane, 62 mL, 250 mmol). After 30 minutes, the reaction mixture was diluted with hexanes (400 mL) and filtered. The collected solids were dried in a nitrogen bag. The solids were diluted with dioxane (140 mL) followed by hydrochloric acid (4.0 M in dioxane, 62 mL, 250 mmol). After 1 hour, the reaction mixture was diluted with hexanes (400 mL), filtered, and washed with hexanes (2 x 150 mL). The isolated solids were dried under reduced pressure, and then suspended in dichloromethane (300 mL). After stirring for 3 hours, the mixture was filtered and dried in a nitrogen bag to afford 5-

{[(1*R*,2*S*)-2-aminocyclohexyl]amino}-3-[(4,6-dimethylpyridin-2-yl)amino]pyridine-2-carboxamide hydrochloric acid salt (21.5 g, quant.) as a white solid.

Step 5: The 5-{[(1*R*,2*S*)-2-aminocyclohexyl]amino}-3-[(4,6-dimethylpyridin-2-yl)amino]pyridine-2-carboxamide hydrochloric acid salt (21.5 g, 50.3 mmol) was diluted with methanol (200 mL) and ammonia (7.0 M solution in methanol, 21.6 mL, 151 mmol). After 30 minutes, the reaction mixture was diluted with water (190 mL) and stirred. After 30 minutes, the reaction mixture was filtered, and the solids were washed with water (2 x 75 mL) and dried to afford 5-{[(1*R*,2*S*)-2-aminocyclohexyl]amino}-3-[(4,6-dimethylpyridin-2-yl)amino]pyridine-2-carboxamide (**5**, 15.0 g, 84%) as a white solid. MS ESI calc'd. for $C_{19}H_{27}N_6O$ $[M + H]^+$ 355, found 355. ^{1}H NMR (600 MHz, CD_3OD) δ 8.47 (d, J = 2.4 Hz, 1H), 7.58 (d, J = 2.4 Hz, 1H), 6.58 (s, 1H), 6.47 (s, 1H), 3.61–3.49 (m, 1H), 3.26–3.22 (m, 1H), 2.39 (s, 3H), 2.23 (s, 3H), 1.87–1.55 (m, 6H), 1.49 – 1.39 (m, 2H).



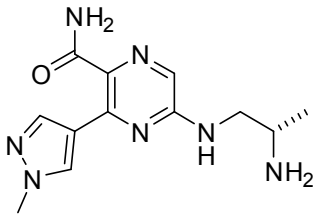
5-(((1*R*,2S)-2-aminocyclohexyl)amino)-3-(1-methyl-1*H*-pyrazol-4-yl)picolinamide (6)

Step 1: 1-Methyl-1*H*-pyrazole-4-boronic acid, pinacol ester (126 mg, 0.607 mmol), *tert*-butyl ((1*S*,2*R*)-2-((5-bromo-6-cyanopyridin-3-yl)amino)cyclohexyl)carbamate (80 mg, 0.20 mmol), palladium acetate (4.5 mg, 0.020 mmol), tricyclohexylphosphine (11.4 mg, 0.040 mmol), and potassium carbonate (56 mg, 0.405 mmol) were combined. Tetrahydrofuran (3 mL) and water (7.3 µL, 0.41 mmol) were added, and the mixture was heated to 65 °C for 6 h. The mixture was allowed to cool to rt, filtered, and concentrated in vacuo to afford 80 mg of a residue containing *tert*-Butyl ((1*S*,2*R*)-2-((6-cyano-5-(1-methyl-1*H*-pyrazol-4-yl)pyridin-3-yl)amino)cyclohexyl)carbamate that was used without further purification in the subsequent reaction.

Step 2: *tert*-Butyl ((1*S*,2*R*)-2-((6-cyano-5-(1-methyl-1*H*-pyrazol-4-yl)pyridin-3-yl)amino)cyclohexyl)carbamate (80 mg, 0.20 mmol) and trifluoroacetic acid (155 µL, 2.02 mmol) were combined in dichloromethane (1 mL) and maintained 3 h at rt. The solution was concentrated in vacuo to afford 60 mg of a residue containing 5-(((1*R*,2*S*)-2-aminocyclohexyl)amino)-3-(1-methyl-1*H*-pyrazol-4-yl)picolinonitrile that was used without further purification.

Step 3: 5-(((1*R*,2*S*)-2-aminocyclohexyl)amino)-3-(1-methyl-1*H*-pyrazol-4-yl)picolinonitrile (60 mg, 0.20 mmol) was dissolved in dioxane (3 mL). Potassium trimethylsilanolate (519 mg, 4.05 mmol) was added, and the suspension was heated to 100 °C for 3 h. The mixture was allowed to cool to rt and concentrated in vacuo. The residues, dissolved in DMSO, were filtered and submitted to purification via reverse phase HPLC (CH_3CN:H_2O with 0.1% acid modifier (TFA)). Obtained 5-(((1*R*,2S)-2-aminocyclohexyl)amino)-3-(1-methyl-1*H*-pyrazol-4-yl)picolinamide (**6**,

10 mg, 11% over 3 steps) as an off white solid. MS ESI calc'd. for $C_{16}H_{23}N_6O$ $[M + H]^+$ 315, found 315. ^1H NMR (600 MHz, DMSO-d_6) as a mixture of rotamers δ 7.98 (d, J = 2.5 Hz, 1H), 7.90 (s, 1H), 7.77 (s, 3H), 7.61 (s, 1H), 7.14 (d, J = 2.5 Hz, 1H), 3.91 (s, 1H), 3.84 (s, 3H), 3.37 (s, 1H), 1.87 – 1.48 (m, 5H), 1.39 (d, J = 8.5 Hz, 3H).



(*S*)-5-((2-aminopropyl)amino)-3-(1-methyl-1*H*-pyrazol-4-yl)pyrazine-2-carboxamide (7)

Step 1: *tert*-Butyl (*S*)-(1-aminopropan-2-yl)carbamate hydrochloride (9.8 g, 46.5 mmol) and 2,6-dichloropyrazine (7.62 g, 51.2 mmol) were combined, diluted with diisopropylethylamine (24.4 mL, 140 mmol) and heated to 115 °C. After 8 hours, the mixture was allowed to cool to room temperature, diluted with dichloromethane, adsorbed on silica and purified by silica gel column chromatography (0 to 100% EtOAc:Hexanes) to afford *tert*-butyl (*S*)-(1-((6-chloropyrazin-2-yl)amino)propan-2-yl)carbamate (13.3 g, 46.4 mmol, 99%) as a yellow solid. MS ESI calc'd. for $C_{12}H_{20}ClN_4O_2$ $[M + H]^+$ 287, found 287. ^1H NMR (600 MHz, $CDCl_3$) δ 7.72 (s, 1H), 7.69 (s, 1H), 5.79 (s, 1H), 4.72 (s, 1H), 3.88 (s, 1H), 3.45 – 3.16 (m, 2H), 1.36 (s, 9H), 1.17 (d, J = 6.8 Hz, 3H).

Step 2: *tert*-Butyl (S)-(1-((6-chloropyrazin-2-yl)amino)propan-2-yl)carbamate (13.3 g, 46.4 mmol) was diluted with DMF (100 mL), and *N*-iodosuccinimide (13.6 g, 60.5 mmol) was added. The mixture was heated to 75 °C for 8 h, then allowed to cool to room temperature. Water (100 mL) containing 2 g of disolved sodium sulfite was added. The mixture was diluted with additional water and EtOAc. The layers were separated and the organic layer was washed a second time with water. The combined aqueous layers were extracted with a second portion of EtOAc. The combined organic portions were dried with magnesium sulfate, filtered, adsorbed on silica gel and purified by silica gel column chromatography (0 to 100% EtOAc:Hexanes) to afford *tert*-butyl (*S*)-(1-((6-chloro-5-iodopyrazin-2-yl)amino)propan-2-yl)carbamate (15 g, 36.4 mmol, 78%) as a light yellow oil which solidified with standing. MS ESI calc'd. for $C_{12}H_{19}ClIN_4O_2$ $[M + H]^+$ 413, found 413. ^1H NMR (600 MHz, $CDCl_3$) δ 7.60 (s, 1H), 5.85 (s, 1H), 4.58 (s, 1H), 3.88 (s, 1H), 3.47 – 3.18 (m, 2H), 1.38 (s, 9H), 1.18 (d, J = 6.8 Hz, 3H).

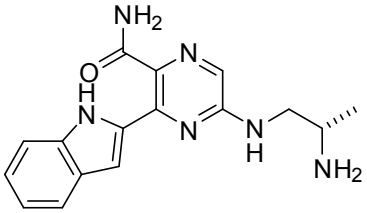
Step 3: *tert*-Butyl (*S*)-(1-((6-chloro-5-iodopyrazin-2-yl)amino)propan-2-yl)carbamate (15 g, 36.4 mmol), zinc cyanide (4.48 g, 38.2 mmol) and tetrakis(triphenylphosphine)palladium(0) (2.1 g, 1.82 mmol) were diluted under nitrogen with DMF (150 mL). The mixture was immeresed in a pre-heated oil bath at 120 °C and reaction progress followed carefully by LCMS. After 30 min, the reaction mixture was removed from the hot oil bath and immersed in a room temperature water bath. Water (300 mL) was added, resulting in a heterogenous mixture, which was filtered. The collected solid was washed with water, dried under a nitrogen bag for 2 h, then taken up in CH_2Cl_2, adsorbed on silica gel and purified by silica gel column chromatography (0 to 100% EtOAc:Hexanes) to afford *tert*-butyl (*S*)-(1-((6-chloro-5-cyanopyrazin-2-yl)amino)propan-2-

yl)carbamate (6.6 g, 21.1 mmol, 58%) as a yellow solid. MS ESI calc'd. for $C_{13}H_{19}ClN_5O_2$ $[M + H]^+$ 312, found 312. ^1H NMR (600 MHz, DMSO-d_6) δ 8.68 (s, 1H), 7.89 (s, 1H), 6.74 (d, J = 8.5 Hz, 1H), 3.68 (p, J = 7.0 Hz, 1H), 3.36 – 3.29 (m, 1H), 3.26 – 3.15 (m, 1H), 1.30 (s, 9H), 1.01 (d, J = 6.7 Hz, 3H).

Step 4: 1-Methyl-1*H*-pyrazole-4-boronic acid, pinacol ester (164 mg, 0.789 mmol), *tert*-butyl (*S*)-(1-((6-chloro-5-cyanopyrazin-2-yl)amino)propan-2-yl)carbamate (82 mg, 0.26 mmol), palladium acetate (5.9 mg, 0.026 mmol), tricyclohexylphosphine (15 mg, 0.053 mmol), and potassium carbonate (73 mg, 0.53 mmol) were combined. Tetrahydrofuran (3 mL) and water (9.5 µL, 0.53 mmol) were added, and the mixture was heated to 65 °C for 6 h. The mixture was allowed to cool to rt, filtered, and concentrated in vacuo. Purification via silica gel column chromatography (10-60% EtOAc:Hexanes) gave *tert*-butyl (*S*)-(1-((5-cyano-6-(1-methyl-1*H*-pyrazol-4-yl)pyrazin-2-yl)amino)propan-2-yl)carbamate (**7**, 78 mg, 83%) as a yellow solid. MS ESI calc'd. for $C_{17}H_{24}N_7O_2$ $[M + H]^+$ 358, found 358.

Step 5: *tert*-Butyl (*S*)-(1-((5-cyano-6-(1-methyl-1*H*-pyrazol-4-yl)pyrazin-2-yl)amino)propan-2-yl)carbamate (78 mg, 0.218 mmol) was dissolved in dichloromethane (1 mL). Trifluoroacetic acid (0.168 ml, 2.182 mmol) was added, and the resultant solution was heated to 40 °C for 1 h. The solution was allowed to cool to rt, concentrated in vacuo, and the resultant residue containing (*S*)-5-((2-aminopropyl)amino)-3-(1-methyl-1*H*-pyrazol-4-yl)pyrazine-2-carbonitrile was used without further purification in the subsequent transformation.

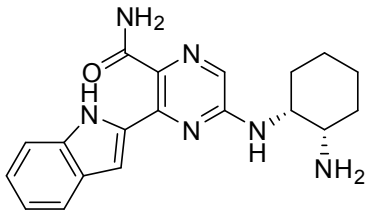
Step 6: (*S*)-5-((2-aminopropyl)amino)-3-(1-methyl-1*H*-pyrazol-4-yl)pyrazine-2-carbonitrile (56 mg, 0.218 mmol) was dissolved in DMSO (2 mL). Potassium hydroxide (61.1 mg, 1.088 mmol) and hydrogen peroxide (30% solution in water , 0.222 mL, 2.176 mmol) were added, and the suspension was stirred for 1 h at rt. The mixture was purified by preparative HPLC Reverse phase (C-18), eluting with acetonitrile/water + 0.1% trifluoroacetic acid, to give (*S*)-5-((2-aminopropyl)amino)-3-(1-methyl-1*H*-pyrazol-4-yl)pyrazine-2-carboxamide (**7**, 41 mg, 48% over 2 steps) as a yellow oil. MS ESI calc'd. for $C_{12}H_{18}N_7O$ $[M + H]^+$ 276, found 276. ^1H NMR (500 MHz, CD_3OD) δ8.21 (s, 1H), 8.03 (d, J = 0.7 Hz, 1H), 7.78 (s, 1H), 3.92 (s, 3H), 3.74 – 3.55 (m, 3H), 1.43 – 1.30 (d, J = 6.3 Hz, 3H).



(S)-5-((2-aminopropyl)amino)-3-(1H-indol-2-yl)pyrazine-2-carboxamide (8)

Using a sequence analogous to **7** afforded (*S*)-5-((2-aminopropyl)amino)-3-(1*H*-indol-2-yl)pyrazine-2-carboxamide (**8**) as a white solid. MS ESI calc'd. for $C_{16}H_{16}N_6O$ $[M + H]^+$ 311, found 311. ^1H NMR (600 MHz, CD_3OD) δ 7.84 (s, 1H), 7.58 (dt, J = 8., 1.0 Hz, 1H), 7.44 (dd, J

= 8.3, 1.0 Hz, 1H), 7.35 (d, *J* = 1.0 Hz, 1H), 7.17 (ddd, *J* = 8.1, 6.9, 1.1 Hz, 1H), 7.03 (ddd, *J* = 8.0, 7.0, 1.0 Hz, 1H), 3.83 (q, *J* = 7.8 Hz, 1H), 3.75–3.66 (m 2H), 1.41 (d, *J* = 6.2 Hz, 3H).



5-(((1*R*,2*S*)-2-aminocyclohexyl)amino)-3-(1*H*-indol-2-yl)pyrazine-2-carboxamide (11)

Step 1: To a mixture of 2,6-dichloropyrazine (1.68 g, 11.3 mmol) and *tert*-butyl ((1*S*,2*R*)-2-aminocyclohexyl)carbamate (2.2 g, 10.3 mmol) was added diisopropylethylamine (2.69 mL, 15.4 mmol), and the resulting slurry was heated to 115 °C and left stirring for 18 h. The reaction mixture was allowed to cool to room temperature, diluted with dichloromethane and adsorbed on silica gel then purified by silica gel column chromatography (0 to 100% EtOAc/Hex) to afford *tert*-butyl ((1*S*,2*R*)-2-((6-chloropyrazin-2-yl)amino)cyclohexyl)carbamate (2.94 g, 9.0 mmol, 88%) as a yellow solid. MS ESI calc'd. for $C_{15}H_{25}ClN_4O_2$ $[M + H]^+$ 327, found 327. ^1H NMR (600 MHz, DMSO-d_6) δ 7.89 (s, 1H), 7.59 (s, 1H), 7.10 (d, *J* = 7.8 Hz, 1H), 6.55 (d, *J* = 8.3 Hz, 1H), 4.00 (s, 1H), 3.68 (s, 1H), 1.74–1.02 (m, 8H), 1.29 (s, 9H).

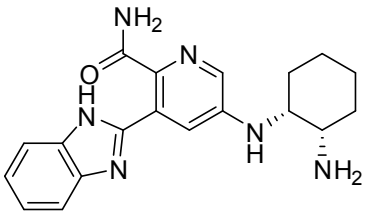
Step 2: To a solution of *tert*-butyl ((1*S*,2*R*)-2-((6-chloropyrazin-2-yl)amino)cyclohexyl)carbamate (2.85 g, 8.72 mmol) in DMF (28.5 mL) was added *N*-iodosuccinimide (2.16 g, 9.59 mmol), and the resulting mixture was heated at 65 °C. After 3 h, additional *N*-iodosuccinimide (0.39 g, 1.74 mmol) was added in 30 min intervals four times to achieve full conversion. The reaction mixture was allowed to cool to room temperature, then water containing sodium sulfite was added followed by EtOAc. The layers were separated, the organic washed a second time with water then dried with $MgSO_4$, filtered and adsorbed on silica gel then purified by silica gel column chromatography (0 to 100% EtOAc/Hex) to afford *tert*-butyl ((1*S*,2*R*)-2-((6-chloro-5-iodopyrazin-2-yl)amino)cyclohexyl)carbamate (3.58 g, 8.7 mmol, 91%) as a yellow solid. MS ESI calc'd. for $C_{15}H_{24}ClIN_4O_2$ $[M + H]^+$ 453, found 453. ^1H NMR (600 MHz, DMSO-d_6) δ 7.75 (s, 1H), 7.24 (d, *J* = 7.6 Hz, 1H), 6.58 (d, *J* = 8.3 Hz, 1H), 3.94 (s, 1H), 3.67 (s, 1H), 1.71–1.00 (m, 8H), 1.29 (s, 9H).

Step 3: A suspension of *tert*-butyl ((1*S*,2*R*)-2-((6-chloro-5-iodopyrazin-2-yl)amino)cyclohexyl)carbamate (3.50 g, 7.7 mmol), zinc cyanide (1.04 g, 8.89 mmol) and tetrakis(triphenylphosphine)palladium(0) (0.45 g, 0.39 mmol) in DMF (35 mL) was immersed in a pre-heated oil bath at 120 °C and left stirring for 30 min. It was then cooled quickly by immersion in a room temperature water bath; water (70 mL) was added dropwise to the reaction mixture, and the resulting slurry was filtered and washed with water. The collected solid was dissolved with dichloromethane, adsorbed on silica gel and purified by silica gel column chromatography to afford *tert*-butyl ((1*S*,2*R*)-2-((6-chloro-5-cyanopyrazin-2-yl)amino)cyclohexyl)carbamate (2.25 g, 6.40 mmol, 83%) as a light yellow solid. MS ESI calc'd. for $C_{16}H_{24}ClN_5O_2$ $[M + H]^+$ 352, found 352. ^1H NMR (600 MHz, $CDCl_3$) δ 7.72 (s, 1H), 7.02 (s, 1H), 4.82 (s, 1H), 3.96 (s, 2H), 2.09–1.12 (m, 8H), 1.41 (s, 9H).

Step 4: To a flask containing *tert*-butyl ((1*S*,2*R*)-2-((6-chloro-5-cyanopyrazin-2-yl)amino)cyclohexyl)carbamate (100 mg, 0.28 mmol), 1*H*-indole-2-boronic acid pinacol ester (138 mg, 0.568 mmol), palladium (II) acetate (6.4 mg, 0.028 mmol), tricyclohexylphosphine (16 mg, 0.057 mmol), and potassium carbonate (79 mg, 0.57 mmol) was added tetrahydrofuran (2.5 mL) and water (10 µL, 0.57 mmol). The mixture was degassed by sparging with argon (5 min) and heated to 65 °C for 6 h. The mixture was allowed to cool to rt, filtered, and concentrated in vacuo to afford *tert*-butyl ((1*S*,2*R*)-2-((5-cyano-6-(1*H*-indol-2-yl)pyrazin-2-yl)amino)cyclohexyl)carbamate. The residue was used without further purification in the subsequent transformation. MS ESI calc'd. for $C_{24}H_{29}N_6O_2$ $[M + H]^+$ 433, found 433.

Step 5: *tert*-Butyl ((1*S*,2*R*)-2-((5-cyano-6-(1*H*-indol-2-yl)pyrazin-2-yl)amino)cyclohexyl)carbamate (123 mg, 0.284 mmol) was dissolved in dichloromethane (2 mL). Trifluoroacetic acid (0.219 mL, 2.84 mmol) was added, and the solution was maintained 1 h at rt. The mixture was concentrated in vacuo, and the resultant residue was dissolved in MeOH, filtered, and submitted to purification via reverse phase HPLC (CH_3CN:H_2O with 0.1% acid modifier (TFA)) to afford 5-(((1*R*,2*S*)-2-aminocyclohexyl)amino)-3-(1*H*-indol-2-yl)pyrazine-2-carbonitrile as the corresponding trifluoroacetic acid salt, which was used directly in the subsequent transformation. MS ESI calc'd. for $C_{19}H_{21}N_6$ $[M + H]^+$ 333, found 333.

Step 6: 5-(((1*R*,2*S*)-2-Aminocyclohexyl)amino)-3-(1*H*-indol-2-yl)pyrazine-2-carbonitrile (241 mg, 0.725 mmol) was dissolved in DMSO (3.5 mL). Potassium hydroxide (203 mg, 3.63 mmol) and hydrogen peroxide (30% in water, 0.741 mL, 7.25 mmol) were added sequentially, and the resultant suspension was stirred vigorously for 1 h at rt. The mixture was filtered, and the resultant solution was submitted to purification via reverse phase HPLC (CH_3CN:H_2O with 0.1% acid modifier (TFA)) to afford a solid. The solid was suspended in 10% IPA:$CHCl_3$ (10 mL). Saturated aqueous sodium bicarbonate (10 mL) was added, and the layers were separated. The aqueous portion was then extracted with 10% IPA:$CHCl_3$ (2 x 10 mL). The combined organic extracts were dried over sodium sulfate, filtered, and concentrated in vacuo. The resultant solid was washed with acetonitrile and water (30 mL) and dried to afford 5-(((1*R*,2*S*)-2-aminocyclohexyl)amino)-3-(1*H*-indol-2-yl)pyrazine-2-carboxamide (**11**, 160 mg, 48%). MS ESI calc'd. for $C_{19}H_{23}N_6O$ $[M + H]^+$ 351, found 351. ^{1}H NMR (500 MHz, DMSO-d_6) δ 12.51 (s, 1H), 8.05 (s, 1H), 7.92 (s, 1H), 7.60 (s, 1H), 7.57 (d, J = 8.0 Hz, 1H), 7.48 (d, J = 8.2 Hz, 1H), 7.26 (s, 1H), 7.20 (s, 1H), 7.13 (t, J = 7.6 Hz, 1H), 6.99 (t, J = 7.3 Hz, 1H), 4.21 (s, 1H), 3.06 (s, 1H), 1.73 (d, J = 8.7 Hz, 1H), 1.66–1.45 (m, 5H), 1.38–1.34 (m, 2H).



5-(((1*R*,2*S*)-2-aminocyclohexyl)amino)-3-(1*H*-benzo[*d*]imidazol-2-yl)picolinamide (12)

Step 1: 2-Chloro-5-fluoronicotinic acid (1.0 g, 5.7 mmol) was suspended in dichloromethane (6 mL). DMF (50 µL, 0.65 mmol) and oxalyl chloride (550 µL, 6.3 mmol) were added sequentially with stirring. The mixture was heated to 35 °C for 1 hour until homogeneous. The solution was allowed to cool to rt and concentrated in vacuo. Toluene (10 mL) was added followed by benzene-1,2-diamine (924 mg, 8.5 mmol), and the mixture was heated to 110 °C for 6 hours. The mixture was allowed to cool to room temperature and filtered. The mother liquor was concentrated in vacuo and purified by silica gel column chromatography (0 to 100% EtOAc/Hex) to afford 2-(2-chloro-5-fluoropyridin-3-yl)-1*H*-benzo[*d*]imidazole (350 mg, 25%) as a white solid. MS ESI calc'd. for $C_{12}H_7ClFN_3O$ $[M + H]^+$ 248, found 248. ^1H NMR (600 MHz, DMSO-d_6) δ 12.90 (s, 1H), 8.61 (d, *J* = 3.0, 1H), 8.33 (dd, *J* = 3.0, 8.6 Hz, 1H), 7.65 (br s, 2H), 7.25 (s, 2H).

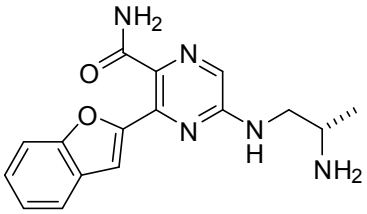
Step 2: 2-(2-Chloro-5-fluoropyridin-3-yl)-1*H*-benzo[*d*]imidazole (100 mg, 0.40 mmol), zinc cyanide (95 mg, 0.81 mmol), and tetrakis(triphenylphosphine)palladium(0) (93 mg, 0.081 mmol) were combined in *N*-methyl-2-pyrrolidone (5 mL). The mixture was heated to 130 °C for 30 minutes. Water was added to the reaction mixture, and the resulting slurry was filtered and washed with water. The collected solid was dissolved with dichloromethane, adsorbed on silica gel and purified by silica gel column chromatography (0 to 100% EtOAc:Hexanes) to afford 3-(1*H*-benzo[*d*]imidazol-2-yl)-5-fluoropicolinonitrile (67 mg, 70%) as a white solid. MS ESI calc'd. for $C_{13}H_7FN_4$ $[M + H]^+$ 239, found 239. ^1H NMR (600 MHz, DMSO-d_6) δ 8.88 (d, *J* = 2.2 Hz, 1H), 8.47 (dd, *J* = 2.1, 9.1 Hz, 1H), 7.69 (s, 2H), 7.29 (dd, *J* = 2.8, 5.5 Hz, 2H).

Step 3: 3-(1*H*-benzo[*d*]imidazol-2-yl)-5-fluoropicolinonitrile (80 mg, 0.34 mmol) was dissolved in DMSO (1 mL). Sodium hydroxide (168 µL, 1.0 mmol, 6 M in water) and hydrogen peroxide (35% in water, 176 µL, 2.0 mmol) were added sequentially, and the resultant suspension was stirred vigorously for 1 h at rt. The mixture was diluted with EtOAc and water. The layers were separated, and the aqueous portion was extracted with EtOAc (2X). The combined organic extracts were dried over magnesium sulfate, filtered, and concentrated in vacuo. Purification via silica gel column chromatography (0–10% MeOH:dichloromethane) gave 3-(1*H*-benzo[*d*]imidazol-2-yl)-5-fluoropicolinamide (60 mg, 70%) as a white solid. MS ESI calc'd. for $C_{13}H_9FN_4O$ $[M + H]^+$ 257, found 257. ^1H NMR (600 MHz, DMSO-d_6) δ 12.82 (s, 1H), 8.71 (d, *J* = 2.7 Hz, 1H), 8.22 (dd, *J* = 2.7, 9.5 Hz, 1H), 8.14 (s, 1H), 7.68 (s, 1H), 7.65 (s, 2H), 7.20 (s, 2H).

Step 4: To a mixture of 3-(1*H*-benzo[*d*]imidazol-2-yl)-5-fluoropicolinamide (34 mg, 0.13 mmol) and *tert*-butyl ((1*S*,2*R*)-2-aminocyclohexyl)carbamate (43 mg, 0.20 mmol) in *N*-methyl-2-pyrrolidone (500 µL) was added diisopropylethylamine (70 µL, 0.40 mmol), and the resulting slurry was heated to 150 °C and left stirring for 8 h. The reaction mixture was allowed to cool to room temperature and concentrated in vacuo to afford *tert*-butyl ((1*S*,2*R*)-2-((5-(1*H*-benzo[*d*]imidazol-2-yl)-6-carbamoylpyridin-3-yl)amino)cyclohexyl)carbamate, which was used without further purification in the subsequent transformation. MS ESI calc'd. for $C_{24}H_{30}N_6O_3$ $[M + H]^+$ 451, found 451.

Step 5: *tert*-Butyl ((1*S*,2*R*)-2-((5-(1*H*-benzo[*d*]imidazol-2-yl)-6-carbamoylpyridin-3-yl)amino)cyclohexyl)carbamate (60 mg, 0.13 mmol) was dissolved in dichloromethane (2 mL). Trifluoroacetic acid (0.10 mL, 1.3 mmol) was added, and the solution was maintained 1 h at rt.

The mixture was concentrated in vacuo, and the resultant residue was dissolved in MeOH, filtered, and submitted to purification via reverse phase HPLC (CH_3CN:H_2O with 0.1% acid modifier (TFA)) to afford **5**-(((1*R*,2*S*)-2-aminocyclohexyl)amino)-3-(1*H*-benzo[*d*]imidazol-2-yl)picolinamide (**54**, 9 mg, 18%) as an off-white solid. MS ESI calc'd. for $C_{19}H_{22}N_6O$ $[M + H]^+$ 351, found 351. ^1H NMR (500 MHz, DMSO-d_6) δ 8.17 (d, *J* = 2.0 Hz, 1H), 7.62–7.58 (m, 2H), 7.48 (d, *J* = 2.4 Hz, 1H), 7.26 (dd, *J* = 3.1, 6.0 Hz, 2H), 3.80–3.76 (m, 1H), 3.21–3.15 (m, 1H), 1.84–1.61 (m, 6H), 1.53–1.40 (m, 2H).



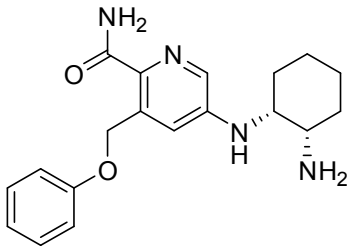
(*S*)-5-((2-aminopropyl)amino)-3-(benzofuran-2-yl)pyrazine-2-carboxamide (13)

tert-Butyl (*S*)-(1-((5-chloro-6-cyanopyridin-3-yl)amino)propan-2-yl)carbamate (62 mg, 0.2 mmol), benzofuran-2-ylboronic acid (65 mg, 0.400 mmol), SiliaCat® DPP-Pd (79 mg, 0.030 mmol. 0.38mmol/g), sodium carbonate (85 mg, 0.800 mmol), water (400 µL), and dioxane (1 mL) were combined. The mixture was heated at 150 °C for 5 min in the microwave. Supplemental benzofuran-2-ylboronic acid (32 mg, 0.200 mmol) was added, and the mixture was again heated for 5 min in the microwave at 150 °C. The mixture was diluted with THF, filtered, and concentrated in vacuo. Trifluoroacetic acid (3 mL) was then added to the residue and stirred until the residue was dissolved. The mixture was concentrated in vacuo. The residue obtained was then dissolved in DMSO (1 mL) and sodium hydroxide (0.200 ml, 2.00 mmol, 10 M in water) was added. Hydrogen peroxide (30% solution in water, 0.175 ml, 2.000 mmol) was added dropwise and the slurry was diluted with methanol (1 mL) and filtered. Concentrated in vacuo and the compound was submitted to mass directed reverse phase HPLC purification (CH_3CN:H_2O 0.1% TFA) to afford (*S*)-5-((2-aminopropyl)amino)-3-(benzofuran-2-yl)pyrazine-2-carboxamide (22 mg, 26%) as a light colored solid. MS ESI calc'd. for $C_{16}H_{18}N_5O_2$ $[M + H]^+$ 312, found 312 ^1H NMR (500 MHz, DMSO-d_6): δ 7.93 (s, 1H), 7.87 (s, 2H), 7.81 (d, *J* = 7.6 Hz, 2H), 7.71 (d, *J* = 7.7 Hz, 1H), 7.56–7.54 (m, 1H), 7.44–7.43 (m, 2H), 7.37–7.34 (m, 1H), 7.29–7.26 (m, 1H), 3.52 (m, 3H), 1.23 (d, *J* = 6.1 Hz, 3H).

5-(((1*R*,2*S*)-2-aminocyclohexyl)amino)-3-((*E*)-styryl)picolinamide (14)

Using a sequence analogous to **6** afforded 5-(((1*R*,2*S*)-2-aminocyclohexyl)amino)-3-((*E*)-styryl)picolinamide (**14**) as a white solid. MS ESI calc'd. for $C_{20}H_{25}N_4O$ $[M + H]^+$ 337, found

337. ^{1}H NMR (500 MHz, CD_3OD) δ 8.18 (d, J = 16.4 Hz, 1H), 8.08 (d, J = 2.7 Hz, 1H), 7.60 – 7.54 (m, 2H), 7.46 (d, J = 2.7 Hz, 1H), 7.36 (dd, J = 8.3, 7.0 Hz, 2H), 7.31 – 7.25 (m, 1H), 7.12 (d, J = 16.3 Hz, 1H), 4.11 (s, 1H), 3.59 – 3.51 (m, 1H), 1.97 – 1.64 (m, 6H), 1.64 – 1.47 (m, 2H).



5-(((1*R*,2*S*)-2-aminocyclohexyl)amino)-3-(phenoxymethyl)picolinamide (15)

Step 1: To a solution of 5-bromo-3-methylpicolinonitrile (2.08 g, 10 mmol), *N*-bromosuccinimide (2.06 g, 11 mmol) in carbon tetrachloride (20 mL) was added azobisisobutyronitrile (173 mg, 1.0 mmol) and the mixture was stirred overnight at rt. Then the mixture was filtered and concentrated in vacuo to give 5-bromo-3-(bromomethyl)picolinonitrile (1.4 g, 48%). MS ESI calc'd. for $C_7H_5Br_2N_2$ $[M + H]^+$ 275, found 275, 277, 279. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.74 (d, J = 8.0 Hz, 1H), 8.14 (d, J = 8.0 Hz, 1H), 4.65 (s, 2H).

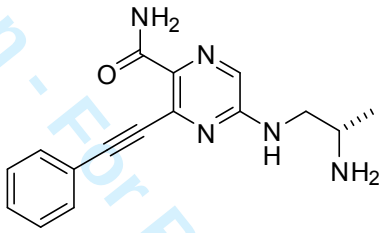
Step 2: To a solution of 5-bromo-3-(bromomethyl)picolinonitrile (1.41 g, 5.1 mmol) in acetonitrile (15 mL) were added potassium carbonate (2.13 g, 15 mmol) and phenol (0.53 g, 5.7 mmol) and the mixture was stirred at rt for 45 min. Then water and ethyl acetate were added and the layers were separated. The organic layer was concentrated in vacuo to give 5-bromo-3-(phenoxymethyl)picolinonitrile (1.35 g, 91%). MS ESI calc'd. for $C_{13}H_{10}BrN_2O$ $[M + H]^+$ 289, found 289, 291. ^{1}H NMR: (300MHz, $CDCl_3$) δ 8.75 (d, J = 8.0 Hz, 1H), 8.25 (d, J = 8.0 Hz, 1H), 7.44–7.28 (m, 2H), 7.14–6.95 (m, 3H), 5.25 (s, 2H).

Step 3: A mixture of 5-bromo-3-(phenoxymethyl)picolinonitrile (250 mg, 0.87 mmol), *N*-Boc-1*S*,2*R*-diaminocyclohexane (185 mg, 0.87 mmol), Tris(dibenzylideneacetone)dipalladium (0) (100 mg, 0.17 mmol), Xantphos (201 mg, 0.35 mmol), and cesium carbonate (850 mg, 2.6 mmol) was stirred in a dry flask and sparged with nitrogen for 5 min. To this mixture was added dioxane (2 mL) followed by an addtional 5 min of nitrogen sparging. The reaction mixture was then stirred at 95 °C for 24 h under a nitrogen atmosphere before being diluted with water and extracted with ethyl acetate (3X). The combined organic layers were dried over sodium sulfate, filtered, and concentrated in vacuo. The resultant residue was purified by *prep*-TLC to give *tert*-butyl ((1*S*,2*R*)-2-((6-cyano-5-(phenoxymethyl)pyridin-3-yl)amino)cyclohexyl)carbamate (27 mg, 8%). MS ESI calc'd. for $C_{24}H_{31}N_4O_3$ $[M + H]^+$ 423, found 423. ^{1}H NMR: (400 MHz, $CDCl_3$) δ 8.01 (s, 1H), 7.42–7.22 (m, 3H), 7.09–7.01 (m, 3H), 5.22 (s, 2H), 4.72 (s, 1H), 4.04 (s, 1H), 3.82 (s, 1H), 3.64 (s, 1H), 1.82–1.24 (m, 17H).

Step 4: A mixture of *tert*-butyl ((1*S*,2*R*)-2-((6-cyano-5-(phenoxymethyl)pyridin-3-yl)amino)cyclohexyl)carbamate (27 mg, 0.06 mmol), potassium carbonate (36 mg, 0.26 mmol) and hydrogen peroxide (30% solution in water , 55 µL, 0.52 mmol) in DMSO (10 mL) was stirred at rt for 1 h. The mixture was then diluted with water and extracted with ethyl acetate (3X). The combined organic layers were dried over sodium sulfate, filtered, and concentrated in

vacuo. The residue was purified by *prep*-TLC to give *tert*-butyl ((1*S*,2*R*)-2-((6-carbamoyl-5-(phenoxymethyl)pyridin-3-yl)amino)cyclohexyl)carbamate (27 mg, 96%). MS ESI calc'd. for $C_{24}H_{33}N_4O_4$ $[M + H]^+$ 441, found 441. ^{1}H NMR (400MHz, $CDCl_3$): δ 7.84 (s, 1H), 7.73 (s, 1H), 7.34 (s, 2H), 7.03 (s, 2H), 6.94 (s, 1H), 5.65 (s, 2H), 4.78 (s, 1H), 3.93 (s, 1H), 3.75 (s, 1H), 1.54–1.42 (m, 8H), 1.42–1.34 (m, 9H).

Step 5: To a mixture of *tert-butyl* ((1*S*,2*R*)-2-((6-carbamoyl-5-(phenoxymethyl)pyridin-3-yl)amino)cyclohexyl)carbamate (27 mg, 0.06 mmol) in dichloromethane (3 mL) was added trifluoroacetic acid (1 mL, 13.1 mmol), and the mixture was stirred at rt for 2 h under a nitrogen atmosphere. The reaction mixture was then adjusted to pH 7 and purified by *prep*-HPLC to give 5-(((1*R*,2*S*)-2-aminocyclohexyl)amino)-3-(phenoxymethyl)picolinamide (9 mg, 77%) as a white solid. MS ESI calc'd. for $C_{19}H_{25}N_4O_2$ $[M + H]^+$ 341, found 341. ^{1}H NMR: (300 MHz, $CDCl_3$) δ 8.11 (s, 1H), 7.52 (s, 1H), 7.38–7.32 (m, 2H), 7.10–7.05 (m, 3H), 5.67 (s, 2H), 4.08 (s, 1H), 3.55 (s, 1H), 1.93–1.58 (m, 8H).



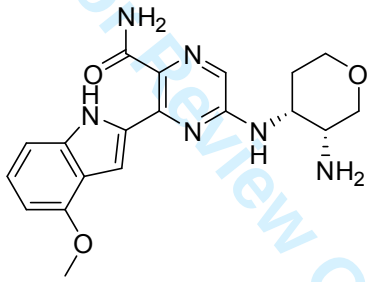
(*S*)-5-((2-aminopropyl)amino)-3-(phenylethynyl)pyrazine-2-carboxamide (16)

Step 1: To a mixture of *tert*-butyl (*S*)-(1-((6-chloro-5-cyanopyrazin-2-yl)amino)propan-2-yl)carbamate (4.0 g, 12.8 mmol), copper iodide (0.24 g, 1.28 mmol), tetrakis(triphenylphosphine)palladium(0) (1.0 g, 0.90 mmol) under nitrogen was added acetonitrile (30 mL), triethylamine (2.68 mL, 19.3 mmol) and phenylacetylene (1.69 mL, 15.4 mmol). The mixture was heated to 60 °C for 3 h, then allowed to cool to room temperature, adsorbed on silica gel, and purified by silica gel column chromatography (0 to 100% EtOAc:Hexanes). The isolate was disssolved in EtOAc (5 mL), and hexanes (35 mL) was introduced dropwise to the resulting stirred solution. A heterogenous mixture was formed which was filtered, and the collected solid was dried under a nitrogen bag to afford *tert*-butyl (*S*)-(1-((5-cyano-6-(phenylethynyl)pyrazin-2-yl)amino)propan-2-yl)carbamate (4.2 g, 11.1 mmol, 87%) as a light yellow solid. MS ESI calc'd. for $C_{21}H_{24}N_5O_2$ $[M + H]^+$ 378, found 378. ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.33 (s, 1H), 7.93 (s, 1H), 7.60 (d, J = 7.4 Hz, 2H), 7.55 – 7.41 (m, 3H), 6.74 (d, J = 8.4 Hz, 1H), 3.75 – 3.59 (m, 1H), 3.35 (q, J = 6.4, 5.3 Hz, 1H), 3.26 – 3.15 (m, 1H), 1.31 (s, 9H), 1.03 (d, J = 6.7 Hz, 3H).

Step 2: To a solution of *tert*-butyl (*S*)-(1-((5-cyano-6-(phenylethynyl)pyrazin-2-yl)amino)propan-2-yl)carbamate (300 mg, 0.80 mmol) in DMSO (1 mL) immersed in a room temperature water bath was added 6N NaOH (0.40 mL, 2.38 mmol) followed by the dropwise addition of 30% aqueous H_2O_2 (0.42 mL, 4.88 mmol). After 20 minutes, water (3 mL) was added slowly resulting in a heterogenous mixture, which was filtered. The collected solid was washed with water, then dried under a nitrogen bag to afford *tert*-butyl (*S*)-(1-((5-carbamoyl-6-

(phenylethynyl)pyrazin-2-yl)amino)propan-2-yl)carbamate (245 mg, 0.62 mmol, 78%) as a yellow solid. MS ESI calc'd. for $C_{21}H_{26}N_5O_3$ $[M + H]^+$ 396, found 396. ^{1}H NMR (600 MHz, DMSO-d_6) δ 7.87 (s, 1H), 7.69 (s, 1H), 7.62 (s, 1H), 7.57 – 7.47 (m, 2H), 7.45 – 7.35 (m, 3H), 7.18 (s, 1H), 6.75 (d, J = 8.4 Hz, 1H), 3.72 – 3.62 (m, 1H), 3.28 – 3.21 (m, 2H), 1.32 (s, 9H), 1.03 (d, J = 6.6 Hz, 3H).

Step 3: *tert*-Butyl (*S*)-(1-((5-carbamoyl-6-(phenylethynyl)pyrazin-2-yl)amino)propan-2-yl)carbamate (200 mg, 0.51 mmol) was diluted with dioxane (1 mL). 4N HCl in dioxane (0.44 mL, 1.77 mmol) was added. The reaction mixture was warmed to 40 °C and maintained for 90 min resulting in a slurry. The mixture was allowed to cool to room temperature. Hexanes (3 mL) was added, and the mixture was filtered. The collected solid was washed with hexanes and dried under a nitrogen bag. The crude solid was submitted to purification via reverse phase HPLC (CH_3CN:H_2O with 0.1% acid modifier (TFA)) to afford (*S*)-5-((2-aminopropyl)amino)-3-(phenylethynyl)pyrazine-2-carboxamide 2,2,2-trifluoroacetate (**16**, 66 mg, 0.16 mmol, 32%) as a yellow solid. MS ESI calc'd. for $C_{16}H_{18}N_5O$ $[M + H]^+$ 296, found 296. ^{1}H NMR (600 MHz, CD_3OD) δ 7.91 (s, 1H), 7.64 – 7.58 (m, 2H), 7.42 – 7.35 (m, 3H), 3.64 (dd, J = 5.8, 3.4 Hz, 2H), 3.58 (q, J = 6.3 Hz, 1H), 1.34 (d, J = 6.6 Hz, 3H).



5-(((3*R*,4*R*)-3-aminotetrahydro-2H-pyran-4-yl)amino)-3-(4-methoxy-1H-indol-2-yl)pyrazine-2-carboxamide (17)

5-(((3*R*,4*R*)-3-aminotetrahydro-2H-pyran-4-yl)amino)-3-(4-methoxy-1H-indol-2-yl)pyrazine-2-carboxamide (**17**) as a white soild was prepared in analogous fashion to **11**. MS ESI calc'd. for $C_{19}H_{22}N_6O_3$ $[M + H]^+$ 383, found 383. ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.97 (d, J = 1.9 Hz, 1H), 7.98 (d, J = 14.8 Hz, 1H), 7.96 (s, 3H), 7.84 (s, 1H), 7.65 (s, 1H), 7.57 (d, J = 5.6 Hz, 1H), 7.20 (d, J = 2.0 Hz, 1H), 7.10–7.03 (m, 2H), 6.53–6.45 (m, 1H), 4.49 (dd, J = 4.4, 11.7 Hz, 1H), 3.99 (d, J = 11.7 Hz, 1H), 3.93 (d, J = 10.7 Hz, 1H), 3.86 (s, 3H), 3.85–3.76 (m, 2H), 3.69–3.56 (m, 2H), 2.08–1.93 (m, 1H), 1.78 (d, J = 8.9 Hz, 1H).

In vitro kinase assay

The *in vitro* enzymatic potency of compounds described herein was determined using a time-resolved fluorescence resonance energy transfer (TR-FRET) assay kit (QSS Assist SYK_TRFRET Kit, Carna Biosciences). Compound was solvated in DMSO and serially diluted in DMSO from 10,000 nM to 0.508 nM or 100 nM to 0.0508 nM. An acoustic dispenser (Labcyte) was utilized to transfer 10 nL of compound or DMSO into each well of an empty 384

well white Proxiplate (Perkin Elmer). Syk enzyme was diluted in assay buffer (15 mM Tris-HCl, pH 7.5, 0.01% Tween 20, 2 mM DTT, 5 mM magnesium chloride) to 50 pM. The ATP/substrate solution provided with the assay kit was diluted to 3.3x concentration in the above buffer. To each well of the assay plate already containing DMSO and compounds, 7 µL of diluted full length GST-tagged human Syk enzyme were added. Compound / DMSO and Syk were allowed to pre-incubate for 10 minutes at room temperature then 3 µL of diluted ATP/substrate was added to initiate the reaction (giving a final concentration of 250 nM of proprietary peptide substrate, 25 µM ATP, and 35 pM Syk in the final reaction).

The reaction plate was incubated at room temperature for 45 minutes after which 10 µL of stop/detection mix ((2 nM Eu-labeled antiphosphotyrosine antibody (Perkin Elmer) and 70 nM streptavadin labeled allophycocyanin (Perkin-Elmer) in 15 mM Tris pH 7.5, 40 mM EDTA, 0.01% Tween 20) was added and allowed to incubate for 30 minutes. The plate was read on the Envision reader (Perkin Elmer) using the following parameters: excitation wavelength of 320 nm, and dual emission monitoring at 616 and 665 nm. The delay time was 20 µs with a 200 µs window time. The time between flashes was 16600 µs and 20 flashes were used per well.

The ratio of fluorescence at 665nm:620nm was measured for each well and the reading corrected for background by subtraction of the average signal of the negative control wells. Inhibition by compound was measured as the ratio of the signal from the compound-treated wells to the positive control wells (which received enzyme and DMSO, but no compound).The IC_{50} values were determined by fitting dose response curve data to the four-parameter logistic equation (reference Prism):

$$FractionalActivity = Minimum - \frac{(Maximum - Minimum)}{1 + 10^{(Log(IC_{50}) - Log([Inhibitor]) * Slope)}}$$

Generation of activated ZAP70. ZAP70 protein was purified as a GST fusion from SF9 insect cells. The protein was phosphorylated *in vitro* using recombinant LCK. Briefly, ZAP70 was dialyzed into 50 mM HEPES, pH 7.0 50 mM NaCl, 5% glycerol, 2 mM dithiothreitol over the course of 1 hour. The protein was diluted to 0.2 mg/mL and 0.1 molar equivalents of His-tagged LCK (Life Technologies) were added along with 1mM ATP and 10 mM magnesium chloride. The protein was incubated at room temperature for 1.5 hours and phosphorylation was confirmed by LCMS. The phosphorylated protein was repurified over GST-Trap resin to remove LCK.

ZAP70 kinase assay. Potency of compounds against ZAP70 was measured in an assay similar to that used for Syk. Compounds and assay plates were prepared identically to that described above. ZAP70 enzyme was diluted in assay buffer (1x kinase buffer (Life Technologies) supplemented with 2 mM DTT) to 28.5 pM. A separate 3.33x solution of ATP (116.7 uM) and biotin-EQEDEPEGDYFEWLE-CONH2 peptide (3.33 uM) was prepared in the same buffer. To each well of the assay plate already containing DMSO and compounds, 7 µL of diluted phosphorylated ZAP70 enzyme was added. Compound / DMSO and ZAP70 were allowed to pre-incubate for 10 minutes at room temperature then 3 µL of the ATP/substrate was added to initiate the reaction (giving a final concentration of 20 pM ZAP70 1 uM peptide substrate and 35 µM ATP). The remainder of the assay was performed and analyzed as described above for SYK.

In vitro selectivity assay

Compounds were profiled for their *in vitro* activity against up to 266 protein kinases using the Invitrogen SelectScreen protein kinase profiling service (www.invitrogen.com). All assays were performed at ATP concentrations near Km(ATP). Compounds were tested at 10 μM, 1 μM, and 0.1 μM and the percent inhibition observed at the three concentrations were fitted to the four-parameter logistic equation above in which maximum percent inhibition, minimum percent inhibition, and slope were held constant
at 100, 0, and 1.0 respectively.

Assay for Inhibition of IgE-dependent basophil activation in human whole blood.[i]

Human blood was collected from healthy volunteers free of medication for 7 days into vacutainers containing Na-heparin. Basophils were primed for activation with recombinant human IL-3 (5 ng/mL) (PerroTech) for 15 minutes at 37 ^{o}C in 5% CO_2 incubator. 5 μL of 600 ng/mL anti-human IgE antibody diluted in PBS-0.1% BSA (Bethyl Laboratories) was added to 55 μL of primed blood and incubated for 20 minutes at 37 ^{o}C in 5% CO_2 incubator to activate basophils. Samples were then centrifuged at 1000 g for 5 minutes at 4 ^{o}C to separate cells from serum. Maxisorp assay plates (Nunc) were pre-coated with 100 μL of 5 μg/mL of mouse IgG (H+L)F(ab)' (KPL) overnight at room temperature and blocked with 110μl of 1 X EIA buffer (Cayman) at 4 ^{o}C for 24 hours. After aspirating blocking buffer, 25 μL each of 1:10 diluted serum (in 1X EIA buffer, Cayman), 1:1000 diluted LTC_4 alkaline phosphatase conjugate (AssayDesign) and 1:300,000 diluted anti-CysLT (AssayDesign) were added and incubated for 18 hours at room temperature on a plate shaker. Plate was then washed 5 times with 100 μL washing buffer -0.05% Tween 20 (Cayman) before addition of 60 μL of alkaline phosphatase substrate pNpp (AssayDesign). Following 2 hour incubation for color development at room temperature, 15 μL of stop solution (AssayDesign) was added and plate was read on Envision (PerkinElmer) at 405 nM absorbance.

A 1000 pg/mL to 11.56 pg/mL 2:3 fold serial diluted CysLT standard curve from 4 parameter logistic fitting was generated on each assay plate. The concentration of CysLT in serum samples was calculated from the standard curve. Maximum and minimum CysLT release in blood sample was determined either in the presence or absence of anti-IgE stimulation and was used to calculate % of inhibition by compound treatment. Inhibition of CysLT release from IgE activated basophils in human whole blood was evaluated by pre-incubation of compounds with IL-3 primed blood for 30 min at 37 ^{o}C in a 5% CO_2 incubator before stimulation. IC_{50} was determined following 10-dose titration and four parameter logistic curve fitting.

Crystallization conditions for compound 8

Crystals of Syk construct containing residues 363 to 639 were grown using the sitting drop method of vapor diffusion. Briefly, the frozen protein at 10 mg/mL in buffer containing 10mM HEPES pH 7.5, 150 mM NaCl, 10% glycerol, 5mM DTT, and 10mM methionine was thawed and mixed with **8** to a final concentration of 0.6mM **8**. The protein solution was mixed 1:1 with reservoir solution containing 16% PEG3350 and 0.1M Tris pH 7.1-7.4. Wells were sealed and incubated at 4 °C. Co-crystals were cryoprotected in buffer containing 20% PEG3350, 0.1M Tris

pH 7.2, and 15% glycerol prior to flash cooling in liquid nitrogen and data collection. X-ray data sets were collected at the IMCA-CAT Beamline 17-ID (Advanced Photon Source, Argonne National Laboratory, Argonne, IL). The complex between the human Syk kinase domain and compound **8** has been deposited in the Protein Data Bank (PDB) with accession code 5TIU.

Crystallographic data

Data collection	
Space group	$P2_12_12_1$
Cell dimensions	
a, b, c (Å)	39.8, 85.5, 89.4
α, β, γ (°)	90.0, 90.0, 90.0
Wavelength	1.00
Resolution (Å)	42.74-1.49 (1.57-1.49)[a]
R_{merge}	0.035 (0.463)
$I/\sigma(I)$	23.5 (3.3)
Completeness (%)	99.9 (100.0)
Redundancy	6.4 (6.5)
Refinement	
Resolution (Å)	21.82-1.49
No. reflections	50257
R_{work}/R_{free}	18.5/20.3
# Atoms/ *B* factors	
Protein atoms	2150/28.4
Solvent Atoms	248/37.3
Ligand Atoms	23/20.4
r.m.s. deviations	
bond lengths (Å)	0.010
bond angles (deg)	1.01

[a] number in parentheses represents highest resolution shell

[i] The potency shift from the Syk enzymatic assay to the human whole blood assay can be rationalized by considering two factors. First, we measured the K_m of Syk for ATP to be ~10 μM, which would lead to a shift versus the cellular ATP concentration (~1–5 mM) for our ATP-competitive inhibitors. Secondly, the plasma protein binding of the test compound also leads to a shift, as only unbound inhibitor is effective in the human whole blood assay. We have found that the shift from the enzymatic to the whole blood assay can be explained considering these two parameters.

Supplementary Figure S1.

Predicted ground state geometry for the core of 8. Note the pseudo 7-membered ring formed through intramolecular hydrogen bonding.

S1