Discovery of a Potent and Selective CCR4 Antagonist that Inhibits T_{reg} Trafficking into the Tumor Microenvironment

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ABSTRACT

Recruitment of suppressive $CD4^+$ $FOXP3^+$ regulatory T cells (T_{reg}) to the tumor microenvironment (TME) has the potential to weaken the anti-tumor response in patients receiving treatment with immuno-oncology (IO) agents. Human T_{reg} express CCR4 and can be recruited to the TME through the CC chemokine ligands CCL17 and CCL22. In some cancers, T_{reg} accumulation correlates with poor patient prognosis. Preclinical data suggests that preventing the recruitment of T_{reg} and increasing the population of activated effector T cells (T_{eff}) in the TME can potentiate anti-tumor immune responses. We have developed a novel series of potent, orally bioavailable small molecule antagonists of CCR4. From this series, several compounds exhibited high potency in distinct functional assays in addition to good in vitro and in vivo ADME properties. The design, synthesis, and SAR of this series and confirmation of its in vivo activity is reported.

INTRODUCTION

CC chemokine receptor 4 (CCR4) is a seven-transmembrane G protein-coupled receptor (GPCR) that is known to play a dominant role in the recruitment of highly immunosuppressive $CD4^+$, $CD25^+$, and $FOXP3^+$ regulatory T cells (T_{reg}) into the tumor microenvironment (TME).[1-2] Within the TME, dendritic cells and macrophages produce the cognate ligands for CCR4, CCL17 and CCL22.[3-4] This chemokine gradient attracts T_{reg} into the TME,[5] where they accumulate and suppress the function of $CD8^+$ effector T cells (T_{eff}), which would otherwise act to fight the tumor.[6] Several studies have shown that accumulation of T_{reg} in the TME and increased levels of CCL22 can lead to poor patient prognosis.[7-11] Thus, the antagonism of CCR4 leading to the inhibition of this T_{reg} trafficking pathway makes CCR4 an attractive target for the development of an immuno-oncology (IO) therapy.[12]

The CCR4 receptor also plays a role in the recruitment of T helper type 2 cell (Th2) subsets for autoimmune disorders such as asthma, allergic rhinitis, and atopic dermatitis.[13,14] To probe the role of CCR4 receptor antagonists in inflammatory disorders, several attempts have been made to design potent small molecule antagonists of CCR4,[15,16] leading to two series differing in their proposed binding mode.[17-20] Class I antagonists have been shown to bind to an extracellular portion of the GPCR (**1**-**3**,[21-24] Figure 1), while Class II antagonists (**4**-**6**,[25-27] Figure 1) bind intracellularly.[19] In general, Class I antagonists contain a heteroaromatic ring with a lipophilic arene, and a side chain containing a basic amine. Class II antagonists tend to be sulfonamides that are flanked by a heteroaromatic ring and a more lipophilic arene.

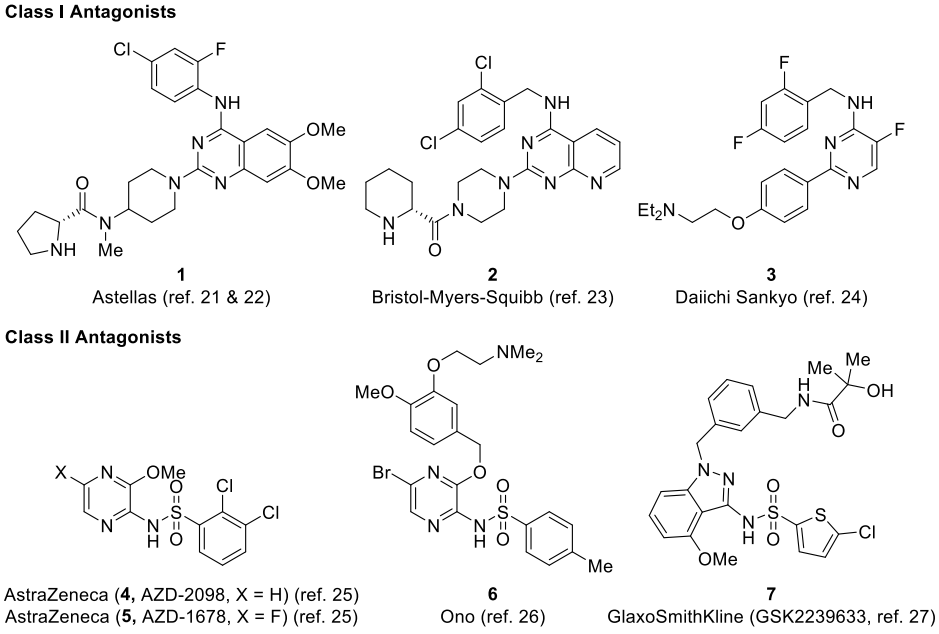


Figure 1. Representative CCR4 Antagonists

The most prominent of these antagonists is a Class II antagonist GSK2239633 (**6**), developed by GlaxoSmithKline.[27] Mentioning asthma as a possible therapeutic indication, GSK2239633 was evaluated in a Phase I clinical trial in healthy volunteers that ended in 2011.[28] In this study, the compound suffered low target engagement and low exposure in the blood

which prevented further development. AstraZeneca has also reported two Class II antagonists from their CCR4 program, identified as preclinical candidates AZD-2098 and AZD-1678 (**4** and **5**).[25] While AZD-2098 was licensed to Cancer Research UK as a potential therapy for kidney cancer in 2013,[29] no further development of this compound has been disclosed.

More recently, a CCR4-targeted cell-depleting monoclonal antibody, mogamulizumab (KY-0761, Kyowa Hakko Kirin Co., Ltd.) has received approval from the FDA to treat patients with two subtypes of relapsed Cutaneous T-Cell Lymphoma (CTCL).[30] However, treatment has shown a risk of serious side effects such as dermatologic toxicity, autoimmune issues, and complications from stem cell treatment prior to mogamulizumab administration.[31]

While targeting CCR4 via an antibody-dependent cell mediated cytotoxicity (ADCC) mechanism has shown clinical efficacy, the mechanism of action is much different compared to targeting CCR4 with a small molecule antagonist. Depletion of T_{reg} that serve other essential functions could cause a severe autoimmune reaction leading to further complications.[32-33] However, simply inhibiting T_{reg} trafficking into the TME via a small molecule antagonist may lead to tumor killing without affecting other vital immune cell functions.

To this end, we have designed novel CCR4 antagonists and evaluated their ability to inhibit T_{reg} migration into the tumor microenvironment. Herein, we highlight the SAR of a series of novel CCR4 antagonists that demonstrate best-in-class potency and pharmacokinetic properties.

RESULTS AND DISCUSSION

Structure-Activity Relationships. Upon reviewing the literature of known Class I antagonists, a pharmacophore map was built to aide in the design of a new class of CCR4 antagonists (Figure 2).[17] In general, these antagonists are comprised of a heteroarene core

structure with a side chain containing a basic nitrogen on the left-hand portion and a lipophilic arene on the northern end. The lipophilic arene and basic amine side chain are attached to the core via a 1,3-relationship.

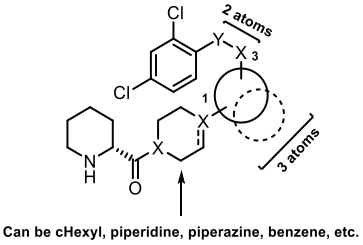


Figure 2. Pharmacophore Map of Class I CCR4 Antagonists

To assess potency of the compounds, they were first put into a calcium flux assay. Calcium flux is a robust, high-throughput functional assay that we utilized to rapidly triage compounds for in vitro cellular migration mediated by chemokine receptors.[34,35] Based on this pharmacophore model, a series of amides closely related to **8** were made which were found to inhibit calcium flux in the double-digit nM range, however these compounds suffered from low bioavailability in rats (Figure 3).[36] Upon surveying several different cores, a novel pyrazolopyrazine, compound **9**, was discovered that was within 5-fold potency of the parent compound **8**, as a racemic mixture. Additionally, this core increased the bioavailability in rats by more than 3-fold. To broaden the SAR of these antagonists, various piperidinyl-containing side chains were appended onto the left-hand side of the molecule. Replacing the amide side chain with a piperidinyl-piperidine gave **10**, which resulted in a 10-fold shift in potency. To create more conformational restriction about the 4-piperidinyl-piperidine side chain, a methyl group was installed in the 3-position of the piperidine to afford **11**. Fortunately, incorporation of the 3'-methyl-1,4'-bipiperidine was equipotent to the amide linkage found in compound to **9**. From this result, we began focusing our early stage efforts on evaluating the SAR of these substituted piperidinyl-piperidine side chains.

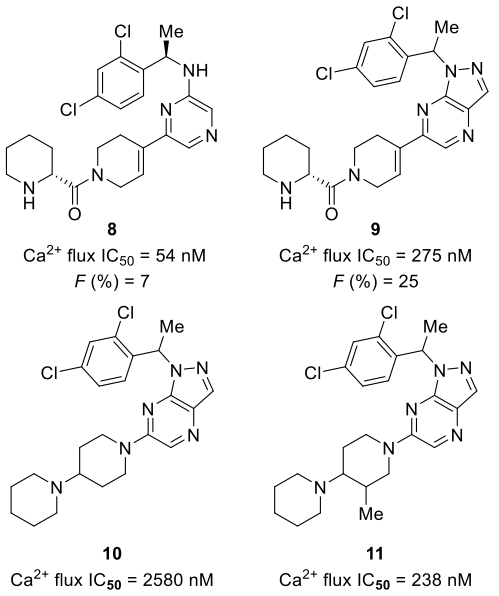
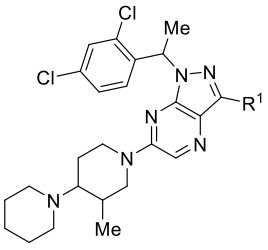


Figure 3. Discovery of a Pyrazolopyrazine Core and 3'-Methyl-1,4'-Bipiperidine Side Chain

Substitution on the C3 position of the pyrazolopyrazine core was carefully examined while leaving the left hand 3'-methyl-1,4'-bipiperidine intact. Replacing the C3-H substituent in **11** with more polar functional groups to give amide **12** and nitrile **13** resulted in compounds that were equipotent, but also suffered from extremely high hepatic clearance and low AUC_{∞} when dosed IV in rats. Installation of the more lipophilic Me (**14**) and CF_3 (**15**) groups dramatically improved the IV clearance in rats, however the C3-CF_3 was 8-fold less potent. Consequently, our SAR focused on developing new side chains to append onto the C3-methyl pyrazolopyrazine core.

Table 1. Evaluation of the C3 Position of the Pyrazolopyrazine Core



Compound	R^1	Ca^{2+} flux IC_{50} (nM)[a]	rat IV CL (mL/min/kg)[b]	rat IV AUC_∞ (hr*ng/mL)
11	H	238	121.7	68
12	$CONH_2$	228	101.7	82
13	CN	251	101.7	82
14	Me	126	38.3	217
15	CF_3	837	56.7	148

[a]Assay run in the absence of serum. [b]Dose of 0.5 mg/kg IV

Rapid evaluation of the C3-position helped to focus our efforts on confirmation of the stereochemistry required for potent CCR4 antagonism in this series. From previous literature and our extensive in-house SAR, we knew the benzylic methyl position on the northern fragment required the *R*-stereochemistry. With an enantioselective route to the C3-methyl core in hand (see experimental section for details), determination of the stereochemistry about the piperidinyl-piperidine side chain was undertaken. Chiral resolution of *trans*-3-methylpiperidin-4-yl pivalate enabled a stereoselective route for the *cis*-enantiomers of 3'-methyl-1,4'-bipiperidine (see experimental section for details). Appending these side chains onto the C3-Me-core revealed that the (*R*, *S*)-diastereomer **16** was 19-fold more potent than the (*S*, *R*)-diastereomer **17** toward inhibiting calcium flux (Figure 4).

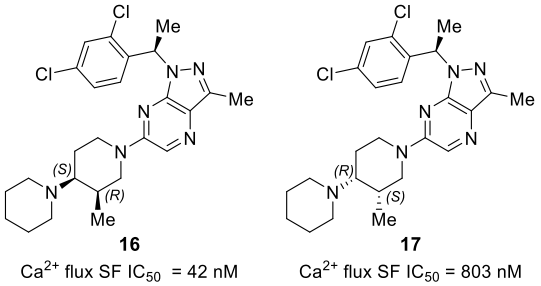


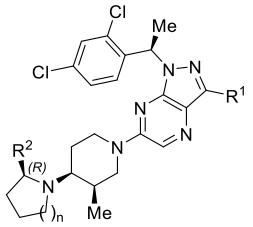
Figure 4. Stereochemical Analysis

After completing the stereochemical analysis of this series, the path was cleared to evaluate the SAR of the basic amine containing side chain. Antagonists that inhibited calcium flux in the double-digit nanomolar range were also run in a physiologically more relevant chemotaxis (CTX) assay to evaluate their ability to inhibit CCL22-mediated migration of CCR4 expressing CEM cells. This 96-well migration platform is considered the gold standard assay for interrogating in vitro cellular migration.[35] Importantly, this medium-throughput assay can be adapted for use with relevant primary immune cells (i.e. T_{reg}). Since the assay is run in 100% human serum (HS), the reported potencies reflect the protein binding of the compounds. Although calcium flux allowed us to triage compounds based on their CCR4 affinity, a significant shift in CTX potency was often observed, likely due to plasma protein binding.

Expansion or contraction of the distal piperidine ring to afford the 4-,5-,6-, and 7-membered rings (**16**, **18**-**20**) resulted in antagonists that inhibit calcium flux with similar potencies (Table 2). Unfortunately, these compounds suffered a severe shift in potency when evaluated in the CTX assay. While the size of the outermost ring had little effect on the potency of these compounds, focusing on the 5-membered pyrrolidines allowed for the installation of a variety of commercially available, enantiopure substituted heterocycles. To this end, both enantiomers of 2-methylpyrrolidine were explored to replace the simple pyrrolidine ring (**18**). While installation of the 2-methyl substituent did not suppress the shift in potency between calcium flux and CTX, the (*R*)-2-methyl-pyrrolidine used in **21** provided a 4-fold more potent compound than the (*S*)-enantiomer **22**. To probe what effect the cLogP would have on the CTX shift, several polar functionalities were evaluated including amides (**23**, **24**), sulfone (**25**), sulfonamide (**26**), nitrile (**27**), and carboxylic acid (**28**). Unfortunately, lowering the cLogP by introduction of these polar groups had little influence on the CTX shift. Surprisingly, attenuating

the oxidation state of the carboxylic acid **28** to the prolinol derived **29** produced a compound with a CTX IC_{50} = 151 nM. Further, replacement of the C3-Me core in **29** with a C3-CN core to afford **30** resulted in one of the first compounds to exhibit an extremely low CTX shift with a CTX IC_{50} = 58 nM.

Table 2. SAR of the 3,4-Substituted Piperidinyl Side Chain

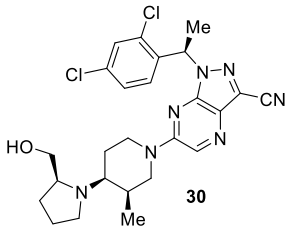


Compound	R^1	R^2	n	Ca^{2+} flux IC_{50} (nM)[a]	CTX IC_{50} (nM)[b]
16	Me	H	2	42	361
18	Me	H	1	69	N.D.
19	Me	H	0	58	218
20	Me	H	3	23	195
21	Me	Me	1	47	381
22	Me	Me (*S*)[c]	1	172	N.D.
23	Me	$C(O)NH_2$	1	170	N.D.
24	Me	$CH_2CH_2C(O)NH_2$	1	37	355
25	Me	$CH_2CH_2SO_2Me$	1	104	N.D.
26	Me	$CH_2CH_2SO_2NH_2$	1	32	259
27	Me	CH_2CH_2CN	1	41	865
28	Me	COOH	1	670	N.D.
29	Me	CH_2OH	1	32	151
30	CN	CH_2OH	1	27	58

[a]Assay run in absence of serum. [b]Assay run in 100% human serum (HS). [c]Opposite enantiomer used.

Encouraged by these results, we evaluated **30** in several in vitro and in vivo assays (Table 3). While it did not display any inhibition of CYP enzymes, when exposing compound **30** to human and rat hepatocytes, a substantial amount remained in human (74%) while rat showed a significant decrease in recovery (19%). The low in vitro metabolic stability in rat hepatocytes correlated well with the superhepatic clearance that was observed in vivo. In addition to extremely high clearance (114.6 mL/min/kg), **30** suffered from low bioavailability (%F = 7) and a high volume of distribution (13.4 L/kg).

Table 3. Profile of Piperidine **30**



30

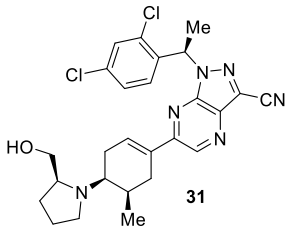
Assays	**Compound 30**
Ca^{2+} flux IC_{50} (nM)	27
CTX IC_{50} (nM)[a]	58
CYP Inhibition IC_{50} (μM)[b]	>10
% remaining hHep/rHep	74/19
rat in vivo PK[c]	
Cl (mL/min/kg)	114.6
Vss (L/kg)	13.4
$T_{1/2}$ (h)	2.1
PO $AUC_{0-\infty}$ (hr*ng/mL)	23.2
F (%)	7

[a]Assay run in 100% human serum (HS). [b]Enzymes tested: 1A2, 2C9, 2C19, 2D6, 3A4. [c]Dose of 0.5 mg/kg IV or 2 mg/kg PO

To address these issues, we hypothesized that by replacing the piperidine nitrogen attached to the core in compound **30** with a carbon, the shift in cLogP may bring about more favorable pharmacokinetics for these antagonists. Up to this point, none of the nitrogen-linked antagonists displayed acceptable pharmacokinetic properties, therefore, we integrated the carbon-carbon link similar to our earlier antagonists **8** and **9**. The requisite carbon-carbon bond to achieve this goal brought about a significant synthetic challenge, and thus we envisioned first replacing the 3-Me-piperidine ring with a 3-Me-cyclohexene ring (*vide infra*).

Given the properties of prolinol-piperidine **30**, the cyclohexenyl compound **31** was also evaluated in several in vitro and in vivo studies (Table 4). Replacement of the anilinic nitrogen connection to the core with a styrenyl bond gave a compound that was equipotent in the CTX assay, no inhibition of CYP enzymes, and similar in vitro hepatic stability in human and rat hepatocytes, 81% and 32% remaining, respectively. Interestingly, the in vivo pharmacokinetic properties of **31** were far superior to that of compound **30**, displaying a longer terminal half-life ($T_{1/2}$) and a 4-fold higher bioavailability (%F = 28).

Table 4. Profile of Cyclohexene **31**



31

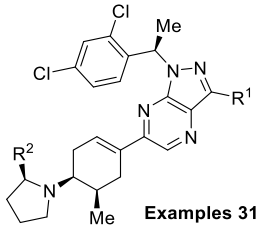
Assays	**Compound 31**
Ca^{2+} flux IC_{50} (nM)	40
CTX IC_{50} (nM)[a]	70
CYP Inhibition IC_{50} (μM)[b]	>10

% remaining hHep/rHep	81/32
rat in vivo PK[c]	
Cl (mL/min/kg)	74.7
Vss (L/kg)	9.8
$T_{1/2}$ (h)	4.1
PO $AUC_{0-\infty}$ (hr*ng/mL)	125
F (%)	28

[a]Assay run in 100% human serum (HS). [b]Enzymes tested: 1A2, 2C9, 2C19, 2D6, 3A4. [c]Dose of 0.5 mg/kg IV or 2 mg/kg PO

Encouraged by the in vivo pharmacokinetics of compound **31**, the SAR of these cyclohexenyl antagonists was further assessed (Table 5). To begin, the C3-position of the core (R^1) was again interrogated. Replacing the C3-nitrile in **31** with a C3-methyl substituent (**32**) resulted in an almost 3-fold drop in potency within the CTX assay and little change in the in vitro hepatic stability. Given these data, the C3-nitrile was conserved throughout to focus our efforts on the R^2 substituents on the pyrrolidine ring. Building steric hindrance around the primary alcohol in **31** to give the tertiary alcohol in **33** resulted in a significant loss of potency in the CTX assay. Replacement of prolinol with prolinamine (**34**) or prolinamide (**35**) increased the stability of these compounds toward human and rat hepatocytes. However, the increase in stability was accompanied by a significant decrease in potency in the CTX assay. Interestingly, installation of a 2-(*R*)-Me-pyrrolidine (**36**) resulted in a compound that was equipotent to **31** and displayed higher in vitro hepatic stability in rats. Replacement of the R^2 methyl with a more lipophilic trifluoromethyl substituent (**37**), rendered the compound completely inactive.

Table 5. Primary SAR of Cyclohexenyl Antagonists



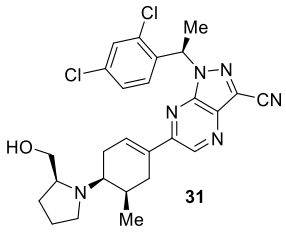
Examples 31-37

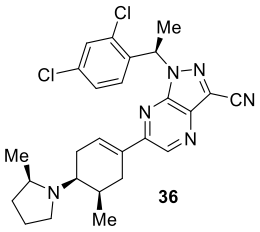
Example	R^1	R^2	Ca^{2+} flux IC_{50} (nM)[a]	CTX IC_{50} (nM)[b]	% remaining hHep/rHep
31	CN	CH_2OH	40	70	81/32
32	Me	CH_2OH	36	179	99/35
33	CN	$C(Me)_2OH$	384	1200	N.D.
34	CN	CH_2NH_2	229	638	100/100
35	CN	$C(O)NH_2$	54	1380	97/71
36	CN	Me	34	44	86/72
37	CN	CF_3	>5000	N.D.	N.D.

[a]Assay run in absence of serum. [b]Assay run in 100% human serum (HS).

Compounds **31** and **36** were the most potent analogs in their ability to inhibit CCL22-mediated CTX of CCR4 expressing CEM cells in 100% human serum. These compounds were further assessed for their ability to inhibit CTX in mouse induced T_{reg} (miT_{reg}) and for their PK characteristics in mice. Although the in vivo clearance in mice was lower for **36** (1.17 vs 12.0 mL/min/kg), leading to a substantially longer $T_{1/2}$ (30 hours vs 7.6, Table 6), the superior potency, synthetic availability, and lower lipophilicity of compound **31** ultimately led us to advance this compound into an in vivo mouse model to determine its ability to suppress T_{reg} trafficking into the tumor microenvironment.

Table 6. Comparison of Compounds **31** and **36** in iT_{reg} CTX and Mouse In Vivo PK





Example	cLogP/tPSA	miT_{reg} CTX IC_{50} (nM)	mouse in vivo PK: Cl / Vss / $T_{1/2}$ / %F
31	4.5/87.6	165	12.0 / 5.7 / 7.6 / 26
36	5.6/67.4	325	1.17 / 4.9 / 30.3 / 27

In Vivo Pharmacology in Mouse. Various murine tumor models were examined to assess their levels of CCR4 ligand (CCL17 and CCL22) expression, and it was found that the pancreatic ductal adenocarcinoma (Pan02) model displayed a high expression of these ligands.[37] Compound **31** was dosed QD for 6 days at 10 and 30 mg/kg in mice bearing Pan02 tumors (Figure 5A). Mouse T_{reg} were generated in vitro from naive $CD4^+$ T cells isolated from $Foxp3^+GFP^+$ transgenic mice (GFP^+ miT_{reg})[38,39] as described in the supporting information. These GFP^+ miT_{reg} were injected into the animals 24 h after the first dose. Inhibition of the migration of GFP^+ miT_{reg} into tumor and other tissues was compared to vehicle treated animals (Figure 5B). A significant dose-dependent decrease in the number of GFP^+ miT_{reg} was found in the tumor tissue when compared to the vehicle group. The 30 mg/kg dose showed nearly complete suppression of T_{reg} migration into the tumor. Plasma levels at 24-hr post dose for **31** showed that 30 mg/kg QD covered the approximate IC_{90} of the miT_{reg} CTX. Importantly, compound **31** was able to selectively inhibit T_{reg} trafficking into the tumor without affecting the

migration of these cells into the spleen (Figure 5), further demonstrating that these antagonists may not disrupt the functions of T_{reg} in other healthy tissues.[40]

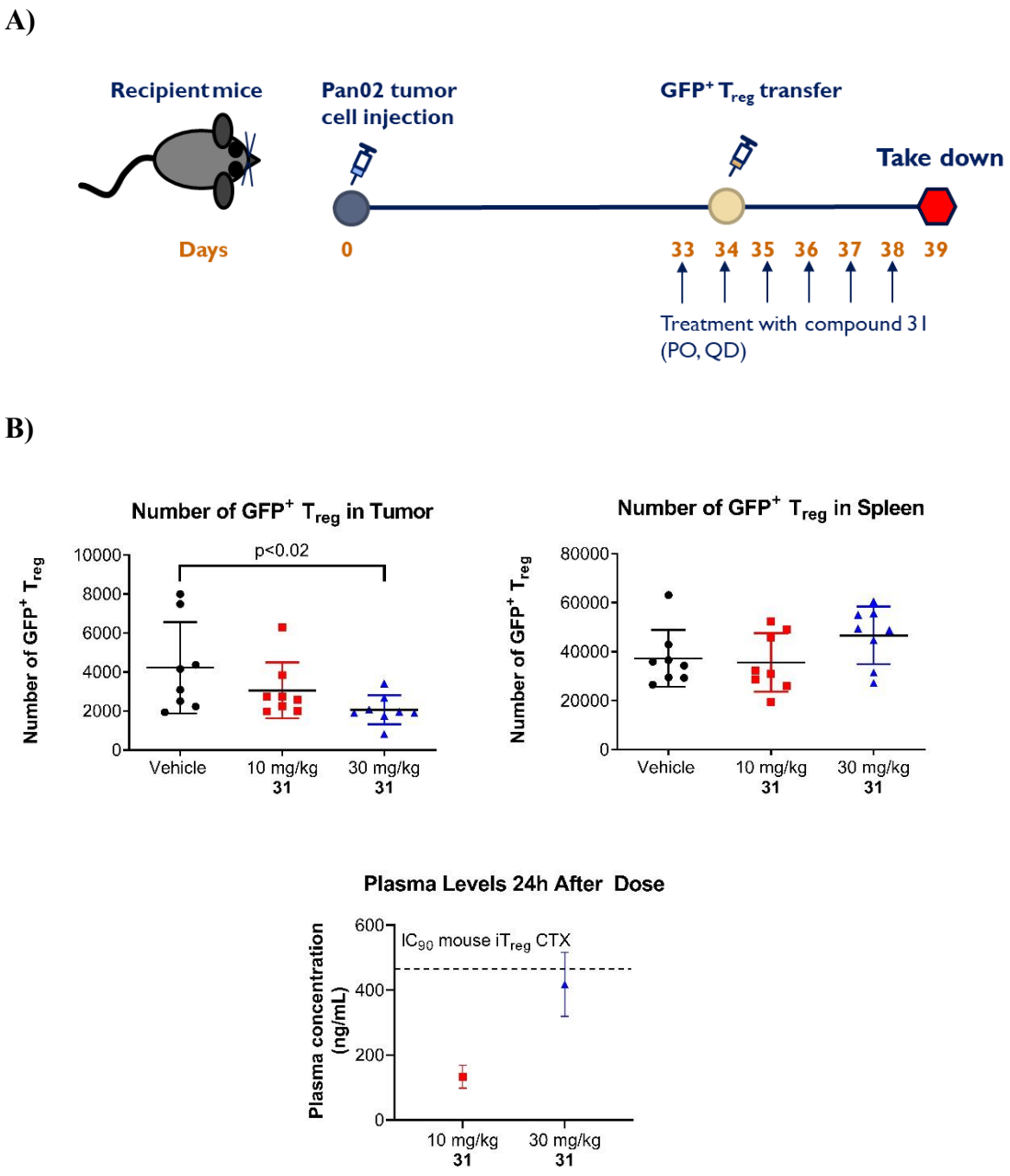


Figure 5. Treatment with Compound **31** selectively reduces T_{reg} trafficking into the tumor. **A)** Schematic outline of treatment and tumor model that is used in this study. **B)** Number of GFP^+

T_{reg} in tumor (left) and spleen (right). Plasma concentration of compound **31** is measured 24 hours after last treatment (bottom). For statistical analysis, the one-way ANOVA was used.

Pharmacokinetic Profiling of 31. Results from the T_{reg} migration study were encouraging and led to further profiling the in vivo pharmacokinetics of compound **31** in rat, dog, and cyno (Table 7). Additionally, the predicted hepatic clearance of **31** was found across species by monitoring the in vitro metabolic stability of **31** in a time-course assay with hepatocytes. The in vivo CL of all species were within 2-fold of their predicted hepatic CL, demonstrating good in vivo/in vitro correlation. When comparing the in vivo hepatic clearance across species, **31** suffered from very high clearance in rats while displaying lower clearance in dog and cynomolgus monkeys.

Table 7. PK Profile of **31** Across Species[a]

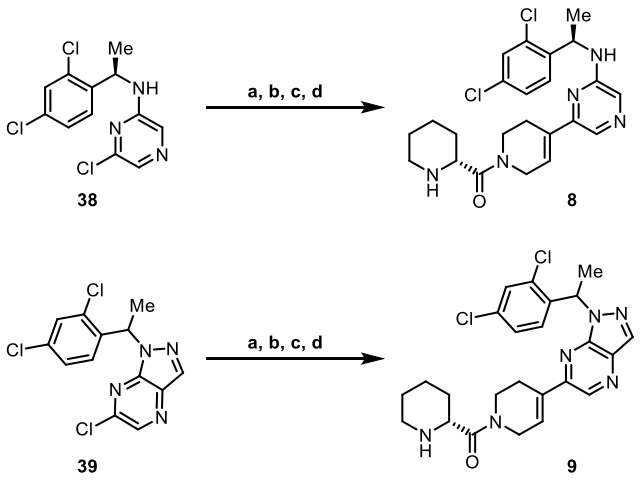
	rat[b]	dog[b]	cyno[b]	human
Pred. Hepatic CL (mL/min/kg)	45.0	18.3	12.5	7.2
in vivo CL (mL/min/kg)	74.7	10.7	11.3	---
IV Terminal Half Life (hr)	2.2	16.4	9.9	---
F (%)	28	75	30	---

[a]Three animals per group were used in each study. [b] Dose of 0.5 mg/kg IV or 2 mg/kg PO

Synthetic Chemistry. Synthetic efforts began by devising similar routes for piperidinyl amides **8** and **9**. Coupling of pyrazinyl chlorides **38** and **39** to *tert*-butyl 4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-3,6-dihydropyridine-1(2*H*)-carboxylate via a Pd-catalyzed Suzuki coupling in the presence of aqueous base yielded the desired C-C bond formation (Scheme 1). Removal of the Boc-protecting group followed by a HATU coupling resulted in the installation

of the basic piperidyl left hand piece. Finally, treatment with 4N HCl in dioxane revealed the basic amine to furnish compounds **8** and **9**.

Scheme 1. Synthesis of Compounds **8** and **9**[a]

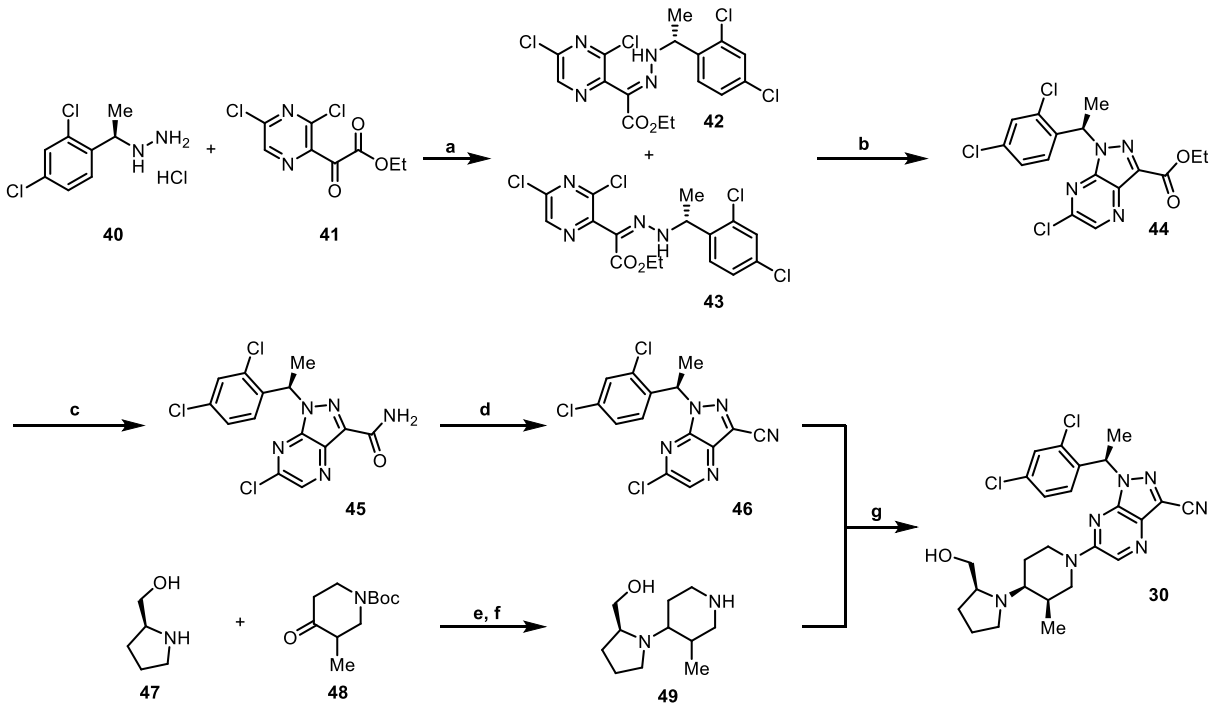


[a]Reagents and conditions: (a) *tert*-butyl 4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-3,6-dihydropyridine-1(2*H*)-carboxylate, 1M Na_2CO_3 (aq.), $Pd(dppf)Cl_2$, THF, 80-100 °C; (b) 4N HCl (in dioxane), MeOH, rt; (c) HATU, DIPEA, DMF, rt; (d) 4N HCl (in dioxane), MeOH, rt.

As a representative example of the piperidyl-pyrrolidine series, the synthesis of compound **30** is shown (Scheme 2). The core structure was prepared by first heating hydrazine **40** (see experimental section for synthesis) with glyoxylate **41** in THF to form a mixture of hydrazones **42** and **43**. Treating these hydrazones with NaH in THF initiated an intramolecular S_NAr to form the pyrazolo portion of the core structure in **44**. Conversion of ester **44** to the final nitrile core **46** proceeded cleanly upon stirring with NH_4OH to form **45**, followed by dehydration with Burgess reagent. Reductive amination of prolinol **47** with ketone **48** followed by treatment with TFA to remove the Boc-protecting group provided the requisite amino side chain **49** as a mixture of stereoisomers. Completion of compound **30** was achieved via simple S_NAr of **49** with the chloropyrazine core **46** under thermal conditions to afford the desired antagonist **30** after

chiral separation. Stereochemical confirmation for these antagonists was confirmed via an enantioselective synthesis of a similar analogue (see experimental section for details).

Scheme 2. Synthesis of Compound **30**[a]

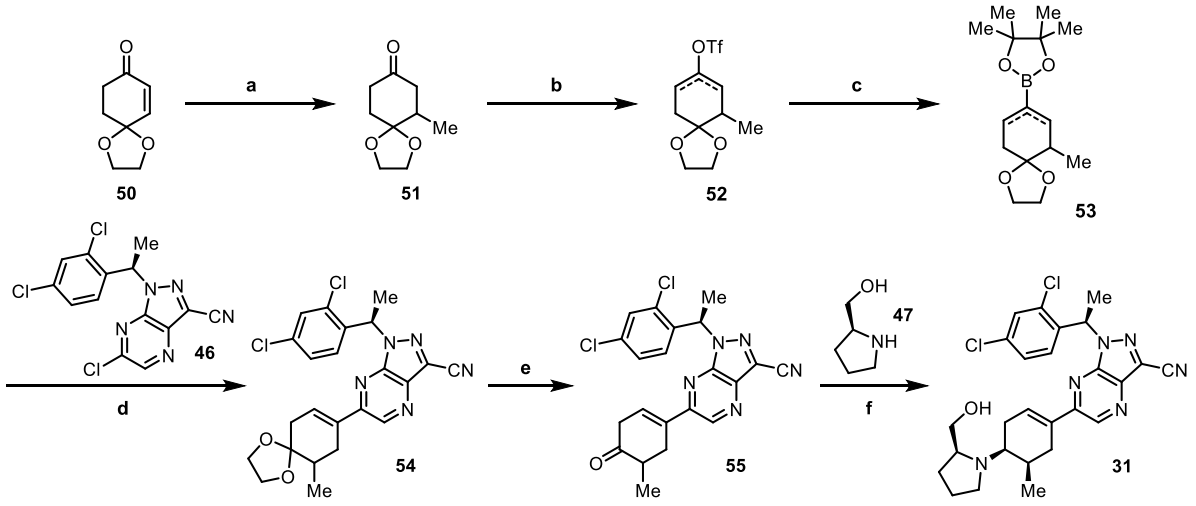


[a]Reagents and conditions: (a) THF, 80 °C; (b) NaH, THF, 0 °C to rt, 50% yield over two steps; (c) NH_4OH (29% in water), dioxane, rt, 95% yield; (d) Burgess Reagent, CH_2Cl_2, rt, 88%; (e) $Na(OAc)_3BH$, AcOH, 1,2-DCE, rt; (f) TFA, CH_2Cl_2, 40% over two steps (g) DIPEA, DMF, 100 °C, 10%.

Synthesis of the cyclohexenyl CCR4 antagonists created a significant challenge and is represented in the synthesis of compound **31** (Scheme 3). Gilman addition of methyl cuprate to the α, β-unsaturated ketone **50** was accomplished to form **51**. Deprotonation of **51** with LDA and treatment with phenyl triflimide gave triflate **52** as a mixture of olefin regio-isomers, which underwent a palladium catalyzed formation of the Bpin-**53**. Separation of the desired olefin isomer followed by Suzuki-Miyaura coupling with chloride **46** gave ketal **54**, followed by

treatment with TFA to unmask ketone **55**. Formation of the final analogue was completed via a simple reductive amination with (*R*)-prolinol (**47**) and ketone **55**, followed by chiral separation to afford compound **31**.

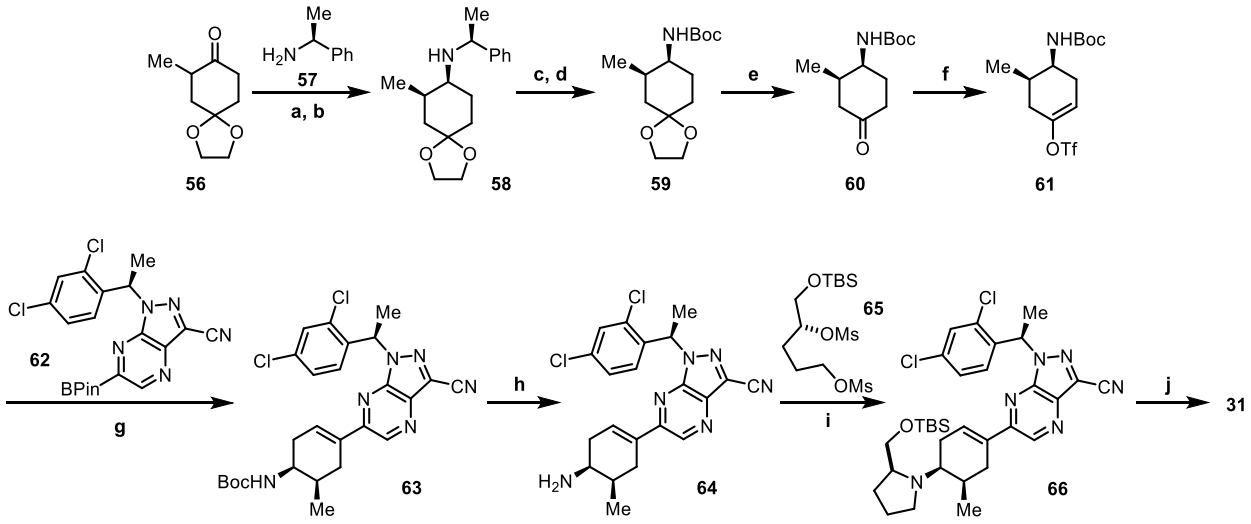
Scheme 3. Synthesis of Cyclohexenyl CCR4 Antagonist **31**[a]



[a]Reagents and conditions: (a) MeLi, CuCN, TMSCl, Et_2O:THF, -40 °C; (b) LDA, then $PhNTf_2$, THF, -78 °C, 53% over two steps; (c) $Bpin_2$, $Pd(dppf)Cl_2$, KOAc, KBr, dioxane, 95 °C; (d) $Pd(dppf)Cl_2$, Na_2CO_3, THF:H_2O, 90 °C; (e) TFA, CH_2Cl_2, rt; (f) $NaBH_3CN$, AcOH, 1,2-DCE, rt.

For larger scale synthesis of **31**, a more selective route was needed that avoided chiral separations in the final step (Scheme 4). Reductive amination between ketone **56** and (*R*)-a-methylbenzylamine (**57**) set the stereocenters on the cyclohexane ring in >10:1 selectivity, with the desired isomer being easily isolated by silica gel chromatography. Subsequent protecting group manipulation and formation of the vinyl triflate proceeded in >30:1 regioselectivity of olefin **61**. Suzuki coupling with Bpin-**62** (obtained from **46**) provided compound **63** in good yield. Removal of the Boc group and bisalkylation with **65** afforded the desired protected prolinol **66**, which after a final TBS-deprotection yielded compound **31** as a single isomer.

Scheme 4. 2nd Generation Synthesis of Cyclohexenyl CCR4 Antagonist **31**[a]



[a]Reagents and conditions: (a) benzene, 95 °C, quant. yield; (b) $NaBH(OAc)_3$, CH_2Cl_2:AcOH (10:1), rt, 65%; (c) $Pd(OH)_2$, H_2, MeOH, rt, 97%; (d) Boc_2O, Et_3N, CH_2Cl_2, rt, 98%; (e) TsOH, THF:H_2O, rt, 70%; (f) LiHMDS, $PhNTf_2$, THF, -78 °C, 97%; (g) $Pd(PPh_3)_4$, Na_2CO_3, toluene:EtOH:H_2O, 90 °C, 76%; (h) 4 N HCl in dioxane, CH_2Cl_2, rt, quant. yield; (i) DIPEA, MeCN, rt, 75%; (j) 4 N HCl in dioxane, CH_2Cl_2, rt, 82%.

CONCLUSIONS

A novel, orally bioavailable cyclohexenyl series of CCR4 antagonists has been discovered that is potent and selective (see Supporting Information for data) against CCR4. Compound **31** exhibits good in vitro and in vivo ADME properties, but importantly, it inhibits T_{reg} trafficking into the tumor microenvironment without suppressing the number of T_{reg} in healthy tissues. In order to achieve near complete blockade of this migration, we targeted trough concentrations that approximated the IC_{90} in a mouse chemotaxis assay. This is consistent with other PK/PD relationships observed for other chemokine receptor antagonists.[41] These studies helped lead to the design and discovery of FLX475, which is currently in human clinical trials.

EXPERIMENTAL METHODS

General Methods for Chemistry. All commercial reagents and solvents were used as received unless otherwise noted. An inert atmosphere of nitrogen was used for reactions involving air or moisture sensitive reagents. Analytical thin layer chromatography (TLC) was performed using 2.5 x 7.5 cm Merck silica gel 60 F_{254} thin layer plates (EMD Millipore 1.15341.0001) visualized using combinations of UV visualization, p-anisaldehyde, potassium permanganate, and iodine staining. Silica gel column chromatography was performed using Teledyne ISCO RediSep Rf normal phase (35−70 μm) silica gel columns on a Teledyne ISCO CombiFlash Rf or CombiFlash Rf+ purification system (detection at 254 nm). Reverse phase preparative HPLC was carried out using a Gemini-NX-C18 column (10 μm, 250 x 30 mm, Phenomenex, Torrance, CA) eluting with a linear gradient from 5 to 100% acetonitrile in water containing 0.1% trifluoroacetic acid over 30 minutes on a Teledyne ISCO EZ Prep, Teledyne ISCO ACCQPrep HP125, or Agilent 1200 Series purification system. Analytical reverse phase HPLC was performed using a Gemini-NX-C18 column (5 μm, 250 x 4.6 mm, Phenomenex, Torrance, CA) eluting with MeCN in water with 0.1% TFA on an Agilent 1200 Series purification system (detection at 254 nm). Proton NMR spectra were recorded on a Varian Oxford 400 MHz spectrometer and carbon NMR spectra were recorded at 101 MHz. Chemical shifts are expressed in δ ppm referenced to tetramethylsilane (δ = 0 ppm). Abbreviations used in describing peak signal multiplicity are as follows: s = singlet, d = doublet, dd = double doublets, t = triplet, q = quartet, m = multiplet, br = broad peak. Analytical LC-MS was performed using a ZORBAX SB-C18 column (1.8 μm, 2.1 x 50 mm, 600 bar, Agilent, Santa Clara, CA) eluting with a linear gradient from 0% to 100% B over 2 min and then 100% B for 3 min (A = 5% MeCN in H2O with 0.1% formic acid, B = MeCN + 0.1% formic acid, flow rate 0.4 mL/min) using an Agilent 1260 Infinity II LC System (detection at 254 nm) equipped with an Agilent

6120 Quadrupole LC/MS in electrospray ionization mode (ESI+). The purity of all compounds used in bioassays was determined by this method to be >95% pure.

General Procedure A for S_nAr. The appropriate heteroaryl chloride and amine (1.0 to 2.0 equiv) were dissolved in DMF, DMSO, or CH_2Cl_2 and *N*,*N*-diisopropylethylamine (2.0 to 3.0 equiv) was added. The reaction was heated to 40 – 100 °C until the complete consumption of starting material was observed by either LC-MS or TLC. After cooling, the mixture was diluted with water and ethyl acetate and the layers were separated. The organic layer was washed with brine solution, dried over sodium sulfate, concentrated via rotary evaporation, and the residue was purified by silica gel chromatography or reverse phase preparative HPLC.

General Procedure B for Suzuki Coupling. The appropriate aryl halide and aryl or alkenyl pinacol boronate (1.0 to 1.5 equiv) were dissolved in THF (0.2 M) and 1 M aqueous Na_2CO_3 (2.0 to 5.0 equiv) was added. [1,1'-bis(diphenylphosphino)ferrocene]dichloropalladium (II) (15 mol%) was added and the reaction was heated to 80 – 100 °C until the complete consumption of starting material was observed by LC-MS or TLC. After cooling, the mixture was diluted with water and ethyl acetate and the layers were separated. The organic layer was washed with brine solution, dried over sodium sulfate, concentrated via rotary evaporation, and the residue was purified by silica gel chromatography unless otherwise noted.

General Procedure C for HATU Coupling. The appropriate carboxylic acid and amine (1.0 to 2.0 equiv) were dissolved in DMF (0.2 M) and *N*,*N*-diisopropylethylamine (3.0 to 5.0 equiv) was added. HATU (1.1 equiv) was added and the reaction was stirred at room temperature until complete consumption of the starting material was observed by LC-MS or TLC. The mixture was diluted with ethyl acetate, washed with water, washed with brine solution, dried

over sodium sulfate, and concentrated by rotary evaporation. The residue was purified by silica gel chromatography or reverse phase preparative HPLC.

General Procedure D for reductive amination. The appropriate ketone and amine (1.2 equiv) were dissolved in 1,2-dichloroethane (0.3 M) then acetic acid (1.5 equiv) and sodium triacetoxyborohydride or sodium cyanoborohydride (1.5 eq) were added. The mixture was stirred at room temperature until the desired product was formed, monitoring by LC-MS. The reaction was quenched with saturated aqueous sodium bicarbonate solution and extracted with dichloromethane. The combined organic layers were dried over sodium sulfate and concentrated via rotary evaporation to afford the desired product, which was purified by silica gel chromatography, preparative reverse phase HPLC, or used without further purification.

(4-(6-(((*R*)-1-(2,4-dichlorophenyl)ethyl)amino)pyrazin-2-yl)-3,6-dihydropyridin-1(2*H*)-yl)((*R*)-piperidin-2-yl)methanone hydrochloride (8). Step 1. The Suzuki coupling was performed according to general procedure B using (*R*)-6-chloro-*N*-(1-(2,4-dichlorophenyl)ethyl)pyrazin-2-amine (1.0 g, 3.30 mmol), and *tert*-butyl 4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-3,6-dihydropyridine-1(2*H*)-carboxylate (1.0 equiv) at 100 °C for 18 h. The residue was purified by silica gel chromatography (50% ethyl acetate in hexanes) to afford *tert*-butyl (*R*)-4-(6-((1-(2,4-dichlorophenyl)ethyl)amino)pyrazin-2-yl)-3,6-dihydropyridine-1(2*H*)-carboxylate (67% yield). LRMS-ESI$^+$: *m/z* calcd for $C_{22}H_{26}Cl_2N_4O_2$ $[M+H]^+$ = 449.2; found, 449.

Step 2. T*ert*-butyl (*R*)-4-(6-((1-(2,4-dichlorophenyl)ethyl)amino)pyrazin-2-yl)-3,6-dihydropyridine-1(2*H*)-carboxylate (414 mg, 0.92 mmol) was dissolved in MeOH (15 mL) and HCl (4N in 1,4-dioxane, 5 mL) was added. The reaction was stirred at 23 °C for 2 h. The mixture was basified with 1 N NaOH, diluted with CH_2Cl_2, and the layers were separated. The organic

layer was washed with brine solution, dried over sodium sulfate, concentrated via rotary evaporation, and used in the next step without purification. The HATU coupling was performed according to general procedure C using the deprotected amine (275 mg, 0.79 mmol), (*R*)-1-(*tert*-butoxycarbonyl)piperidine-2-carboxylic acid (1.0 equiv), *N,N*-diisopropylethylamine (2.0 equiv), and HATU (1.1 equiv) in DMF (2.5 mL) at 23 °C for 14 h. The residue was purified by silica gel chromatography (0 to 100% ethyl acetate in hexanes) to afford *tert*-butyl (*R*)-2-(4-(6-(((*R*)-1-(2,4-dichlorophenyl)ethyl)amino)pyrazin-2-yl)-1,2,3,6-tetrahydropyridine-1-carbonyl)piperidine-1-carboxylate (69% yield). LRMS-ESI$^+$: *m/z* calcd for $C_{28}H_{35}Cl_2N_5O_3$ $[M+H]^+$ = 560.2; found, 560.

Step 3. *Tert*-butyl (*R*)-2-(4-(6-(((*R*)-1-(2,4-dichlorophenyl)ethyl)amino)pyrazin-2-yl)-1,2,3,6-tetrahydropyridine-1-carbonyl)piperidine-1-carboxylate (305 mg, 0.54 mmol) was dissolved in MeOH (10 mL) and HCl (4N in 1,4-dioxane, 5 mL) was added. The mixture was stirred at 23 °C for 5 h. The solution was basified with 1 N NaOH and extracted with CH_2Cl_2. The organic layer was washed with brine solution, dried over sodium sulfate, and concentrated via rotary evaporation and used in the next step without further purification. The deprotected amine (250 mg, 0.543 mmol) was dissolved in CH_2Cl_2 (10 mL) and HCl (4N in 1,4-dioxane, 0.136 mL, 0.543 mmol) was added dropwise. The reaction was stirred for 10 minutes and concentrated via rotary evaporation to afford (4-(6-(((*R*)-1-(2,4-dichlorophenyl)ethyl)amino)pyrazin-2-yl)-3,6-dihydropyridin-1(2*H*)-yl)((*R*)-piperidin-2-yl)methanone hydrochloride (**8**, 100% yield). ^{1}H NMR (400 MHz, $CDCl_3$; HCl Salt) δ 7.89 (d, J = 3.2 Hz, 1H), 7.66 (d, J = 3.3 Hz, 1H), 7.38 (d, J = 2.1 Hz, 1H), 7.31 (d, J = 8.4 Hz, 1H), 6.69 – 6.38 (m, 1H), 5.37 – 5.17 (m, 1H), 5.08 (d, J = 5.6 Hz, 1H), 3.87 – 3.54 (m, 2H), 3.28 – 2.96 (m, 3H), 2.70 (q, J = 11.6, 11.1 Hz, 1H), 2.53 (s, 2H), 1.92 (d, J = 9.8 Hz, 1H), 1.76 (d, J = 12.6 Hz,

1H), 1.60 (t, J = 13.7 Hz, 1H), 1.53 (d, J = 6.8 Hz, 3H), 1.41 (dd, J = 26.8, 12.7 Hz, 1H), 1.31 – 1.19 (m, 3H). LRMS-ESI$^+$: m/z calcd for $C_{23}H_{27}Cl_2N_5O$ $[M+H]^+$ = 460.2; found, 460.

(4-(1-(1-(2,4-dichlorophenyl)ethyl)-1*H*-pyrazolo[3,4-*b*]pyrazin-6-yl)-3,6-dihydropyridin-1(2*H*)-yl)((*R*)-piperidin-2-yl)methanone 2,2,2-trifluoroacetate (9). Step 1. The Suzuki coupling was performed according to general procedure B using 6-chloro-1-(1-(2,4-dichlorophenyl)ethyl)-1*H*-pyrazolo[3,4-*b*]pyrazine (0.275 mmol), and *tert*-butyl 4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-3,6-dihydropyridine-1(2*H*)-carboxylate (1.0 equiv) at 100 °C for 18 h. The residue was purified by silica gel chromatography (0 to 100% ethyl acetate in hexanes) to afford *tert*-butyl 4-(1-(1-(2,4-dichlorophenyl)ethyl)-1*H*-pyrazolo[3,4-*b*]pyrazin-6-yl)-3,6-dihydropyridine-1(2*H*)-carboxylate (60% yield). LRMS-ESI$^+$: m/z calcd for $C_{23}H_{25}Cl_2N_5O_2$ $[M+H]^+$ = 474.2; found, 474.

Step 2. *Tert*-butyl 4-(1-(1-(2,4-dichlorophenyl)ethyl)-1*H*-pyrazolo[3,4-*b*]pyrazin-6-yl)-3,6-dihydropyridine-1(2*H*)-carboxylate (0.120 mmol) was dissolved in CH_2Cl_2 (2 mL) and TFA (0.5 mL) was added. The reaction was stirred at 23 °C for 2 h. The mixture was basified with 1 N NaOH, diluted with CH_2Cl_2, and the layers were separated. The organic layer was washed with brine solution, dried over sodium sulfate, concentrated via rotary evaporation, and used in the next step without purification. The HATU coupling was performed according to general procedure C using the deprotected amine (275 mg, 0.79 mmol), (*R*)-1-(*tert*-butoxycarbonyl)piperidine-2-carboxylic acid (180 mg, 0.79 mmol), *N*,*N*-diisopropylethylamine (0.14 mL, 1.58 mmol), and HATU (1.1 equiv) in DMF (2.5 mL). The residue was purified by silica gel chromatography (0 to 100% ethyl acetate in hexanes) to afford *tert*-butyl (*R*)-2-(4-(6-(((*R*)-1-(2,4-dichlorophenyl)ethyl)amino)pyrazin-2-yl)-1,2,3,6-tetrahydropyridine-1-carbonyl)piperidine-1-carboxylate. The residue was dissolved in CH_2Cl_2 (2 mL) and TFA (0.5

mL) was added. The reaction was stirred 1 h at room temperature and concentrated to afford (4-(1-(1-(2,4-dichlorophenyl)ethyl)-1*H*-pyrazolo[3,4-*b*]pyrazin-6-yl)-3,6-dihydropyridin-1(2*H*)-yl)((*R*)-piperidin-2-yl)methanone 2,2,2-trifluoroacetate (**9**, 69% yield). ^{1}H-NMR (400 MHz; $CDCl_3$; TFA Salt) δ 8.78 (br s, 2H), 8.30 (s, 1H), 7.43 – 7.41 (m, 2H), 7.20 – 7.17 (m, 1H), 6.76 – 6.63 (m, 2H), 5.49 (br s, 1H), 3.80 – 3.72 (m, 4H), 3.14 – 2.79 (m, 2H), 2.05 – 1.97 (m, 11 H), 1.26 – 1.19 (m, 3 H). LRMS-ESI$^+$: m/z calcd for $C_{24}H_{27}Cl_2N_6O$ $[M+H]^+$ = 485.2; found, 485.

6-([1,4'-bipiperidin]-1'-yl)-1-(1-(2,4-dichlorophenyl)ethyl)-1*H*-pyrazolo[3,4-*b*]pyrazine 2,2,2-trifluoroacetate (10). The SnAr was performed according to general procedure A using 1,4'-bipiperidine (2.0 equiv), 6-chloro-1-(1-(2,4-dichlorophenyl)ethyl)-1*H*-pyrazolo[3,4-*b*]pyrazine (**39**, 90 mg, 0.275 mmol), and *N*,*N*-diisopropylethylamine (2.0 equiv) in DMF (5 mL) at 100 °C for 2 h. Purified by reverse phase preparative HPLC (Gemini-NX, 10 μm, 250 x 30 mm, C18 column, Phenomenex, Torrance, CA), eluent: 0 to 100% acetonitrile in water, both eluents containing 0.1% TFA, gradient elution over 30 minutes to afford 6-([1,4'-bipiperidin]-1'-yl)-1-(1-(2,4-dichlorophenyl)ethyl)-1*H*-pyrazolo[3,4-*b*]pyrazine 2,2,2-trifluoroacetate (**10**) as a mixture of diastereomers (7% yield). ^{1}H NMR (400 MHz, $CDCl_3$; TFA Salt) δ 11.21 (bs, 1H), 8.21 (s, 1H), 8.10 (s, 1H), 7.38 (d, J = 2.2 Hz, 1H), 7.37 (d, J = 4.1 Hz, 1H), 7.17 (dd, J = 8.5, 2.1 Hz, 1H), 6.41 (q, J = 7.1 Hz, 1H), 4.63 (t, J = 12.3 Hz, 2H), 3.62 – 3.42 (m, 3H), 3.09 – 2.98 (m, 2H), 2.82 – 2.65 (m, 2H), 2.20 (t, J = 12.4 Hz, 2H), 2.12 – 1.98 (m, 2H), 1.94 (d, J = 7.1 Hz, 3H), 1.92 – 1.87 (m, 2H), 1.83 – 1.66 (m, 2H), 1.48 – 1.22 (m, 2H). LRMS-ESI$^+$: *m/z* calcd for $C_{23}H_{28}Cl_2N_6$ $[M+H]^+$ = 459.2; found, 459.

1-(1-(2,4-dichlorophenyl)ethyl)-6-(3'-methyl-[1,4'-bipiperidin]-1'-yl)-1*H*-pyrazolo[3,4-*b*]pyrazine hydrochloride (11). The SnAr was performed according to general procedure A using 3'-methyl-1,4'-bipiperidine (2.0 equiv), 6-chloro-1-(1-(2,4-

dichlorophenyl)ethyl)-1*H*-pyrazolo[3,4-*b*]pyrazine (**39**, 90 mg, 0.275 mmol), and *N*,*N*-diisopropylethylamine (2.0 equiv) in DMF (5 mL) at 100 °C for 2 h. Purified by reverse phase preparative HPLC (Gemini-NX, 10 µm, 250 x 30 mm, C18 column, Phenomenex, Torrance, CA), eluent: 0 to 100% acetonitrile in water, both eluents containing 0.1% TFA, gradient elution over 30 minutes. The TFA salt was neutralized by passing it through a basification column (PL-HCO3 MP SPE, 500 mg per 5 mL tube, Agilent) using dichloromethane and methanol (4:1) as the eluent and concentrated under reduced pressure. HCl (1 N in diethyl ether, 1 mL) was added and stirred 10 minutes. The mixture was concentrated under reduced pressure to afford 1-(1-(2,4-dichlorophenyl)ethyl)-6-(3'-methyl-[1,4'-bipiperidin]-1'-yl)-1*H*-pyrazolo[3,4-*b*]pyrazine hydrochloride (**11**) as a mixture of diastereomers (6% yield). ^{1}H NMR (400 MHz, $CDCl_3$; HCl Salt) δ 11.22 (bs, 1H), 8.21 (s, 0.66H), 8.20 (s, 0.34H), 8.09 (s, 0.66H), 8.09 (s, 0.34H), 7.40 – 7.35 (m, 2H), 7.17 (dd, *J* = 8.5, 2.0 Hz, 1H), 6.39 (q, *J* = 7.2 Hz, 1H), 4.86 – 4.63 (m, 3H), 4.57 – 4.47 (m, 1H), 3.88 – 3.78 (m, 1H), 3.73 – 3.64 (m, 1H), 3.34 – 3.23 (m, 1H), 3.13 – 3.04 (m, 1H), 2.98 – 2.88 (m, 1H), 2.83 – 2.69 (m, 2H), 2.64 – 2.54 (m, 1H), 2.26 – 2.00 (m, 3H), 1.93 (d, *J* = 7.0 Hz, 3H), 1.91 – 1.88 (m, 2H), 1.52 – 1.40 (m, 1H), 1.21 (d, *J* = 6.6 Hz, 2H), 1.13 (d, *J* = 6.6 Hz, 1H). LRMS-ESI$^+$: *m/z* calcd for $C_{24}H_{30}Cl_2N_6$ $[M+H]^+$ = 473.2; found, 473.

1-(1-(2,4-dichlorophenyl)ethyl)-6-((3'*R*,4'*S*)-3'-methyl-[1,4'-bipiperidin]-1'-yl)-1*H*-pyrazolo[3,4-*b*]pyrazine-3-carbonitrile hydrochloride (12). Step 1. To a solution of *n*-BuLi (2.5 M in hexanes, 132 mL, 0.329 mol) was added 2,2,6,6-tetramethylpiperidine (56 mL, 0.329 mol) in THF (200 mL) at -78 °C over 15 min. The yellow slurry was stirred at -78 °C for 15 min. In a separate flask, diethyl oxalate (41 mL, 0.302 mol) and 2,6-dichloropyrazine (40 g, 0.274 mol) were dissolved in THF (685 mL) and cooled to -78 °C. The lithium 2,2,6,6-tetramethylpiperidine solution was added to the 2,6-dichloropyrazine solution via cannula over

15 min at -78 °C. The reaction was stirred at -78 °C for 30 min before the addition of AcOH (20 mL). The mixture was warmed to room temperature and quenched with saturated aqueous NH_4Cl. The mixture was extracted with EtOAc and combined organic layers were washed with saturated aqueous NH_4Cl, dried over $MgSO_4$ and concentrated under reduced pressure. The residue was dissolved in EtOH (50 mL) and (1-(2,4-dichlorophenyl)ethyl)hydrazine hydrochloride (9.83 g, 40.7 mmol) was added. The reaction was stirred at room temperature for 16 h and saturated aqueous $NaHCO_3$ was added. The mixture was concentrated under reduced pressure to remove the EtOH and the aqueous layer was extracted with dichloromethane. The combined organic layers were dried over Na_2SO_4 and concentrated under reduced pressure. The residue was purified by silica gel chromatography (0 to 20% ethyl acetate in hexanes) to afford ethyl (*E*)-2-(2-(1-(2,4-dichlorophenyl)ethyl)hydrazineylidene)-2-(3,5-dichloropyrazin-2-yl)acetate and ethyl (*Z*)-2-(2-(1-(2,4-dichlorophenyl)ethyl)hydrazineylidene)-2-(3,5-dichloropyrazin-2-yl)acetate as a mixture of isomers (57% yield). LRMS-ESI$^+$: *m/z* calcd for $C_{16}H_{14}Cl_4N_4O_2$ $[M+H]^+$ = 435.0; found, 435.

Step 2. A mixture of ethyl (*E*)-2-(2-(1-(2,4-dichlorophenyl)ethyl)hydrazono)-2-(3,5-dichloropyrazin-2-yl)acetate and ethyl (*Z*)-2-(2-(1-(2,4-dichlorophenyl)ethyl)hydrazono)-2-(3,5-dichloropyrazin-2-yl)acetate (54 g, 126 mmol) was dissolved in THF (500 mL) and cooled to 5 °C. NaH (60% dispersion in oil, 5.56 g, 139 mmol) was added portion wise and the mixture was warmed to room temperature then stirred for 1 h. An additional portion of NaH (60% dispersion in oil, 5.0 g, 126 mmol) was added portion-wise and the mixture was stirred at room temperature for 2 h. *Tert*-BuOH (5 drops) was added and the mixture was stirred at room temperature for 10 d. The mixture was diluted with saturated aqueous NH_4Cl and extracted with ethyl acetate. The organic layer was dried over $MgSO_4$ and concentrated under reduced pressure. The residue was

recrystallized from dichloromethane and hexanes to afford ethyl 6-chloro-1-(1-(2,4-dichlorophenyl)ethyl)-1*H*-pyrazolo[3,4-*b*]pyrazine-3-carboxylate (60% yield). LRMS-ESI$^+$: *m/z* calcd for $C_{16}H_{13}Cl_3N_4O_2$ $[M+H]^+$ = 399.0; found, 399.

Step 3. The SnAr was performed following general procedure A using ethyl 6-chloro-1-(1-(2,4-dichlorophenyl)ethyl)-1*H*-pyrazolo[3,4-*b*]pyrazine-3-carboxylate (0.388 mmol), 3'-methyl-1,4'-bipiperidine 2,2,2-trifluoroacetate (0.388 mmol), *N*,*N*-diisopropylethylamine (0.776 mmol) in DMSO (1.0 mL) at room temperature for 16 h. The residue was purified by silica gel chromatography (10% MeOH in CH_2Cl_2) to afford ethyl 1-(1-(2,4-dichlorophenyl)ethyl)-6-(3'-methyl-[1,4'-bipiperidin]-1'-yl)-1*H*-pyrazolo[3,4-*b*]pyrazine-3-carboxylate (50% yield). LRMS-ESI$^+$: *m/z* calcd for $C_{27}H_{34}Cl_2N_6O_2$ $[M+H]^+$ = 545.2; found, 545.

Step 4. Ethyl 1-(1-(2,4-dichlorophenyl)ethyl)-6-(3'-methyl-[1,4'-bipiperidin]-1'-yl)-1*H*-pyrazolo[3,4-*b*]pyrazine-3-carboxylate (35 mg, 0.064 mmol) was suspended in a mixture of EtOH (0.26 mL), THF (0.26 mL), and water (0.13 mL). Lithium hydroxide (12 mg, 0.52 mmol) was added and the mixture was heated to 60 °C for 16 h. The reaction was cooled to room temperature and the solvent was removed under reduced pressure to afford 1-(1-(2,4-dichlorophenyl)ethyl)-6-(3'-methyl-[1,4'-bipiperidin]-1'-yl)-1*H*-pyrazolo[3,4-*b*]pyrazine-3-carboxylic acid which was used without further purification. To a solution of the carboxylic acid (13 mg, 0.025 mmol) in DMF (0.15 mL) was added HATU (12 mg, 0.030 mmol) and *N,N*-diisopropylethylamine (9 μL, 0.050 mmol). Ammonia gas was bubbled through the solution for 2 min, then the solution was stirred under ammonia atmosphere for 10 min. 0.5 M aqueous HCl solution (10 mL) was added, the solid was collected by filtration and washed with additional 0.5 M aqueous HCl solution. The solid was dried under reduced pressure to afford 1-(1-(2,4-dichlorophenyl)ethyl)-6-(3'-methyl-[1,4'-bipiperidin]-1'-yl)-1*H*-pyrazolo[3,4-*b*]pyrazine-3-

carboxamide hydrochloride (**12**) as a mixture of diastereomers (98% yield). ^{1}H NMR (400 MHz, CD_3OD, HCl salt; 1:1 mix of isomers) δ 8.47 (s, 0.5H), 8.46 (s, 0.5H), 7.50 – 7.38 (m, 2H), 7.30 – 7.24 (m, 1H), 6.51 – 6.44 (m, 1H), 3.79 – 3.61 (m, 2H), 3.54 – 3.46 (m, 1H), 3.20 – 3.12 (m, 1H), 3.11 – 2.88 (m, 4H), 2.68 – 2.58 (m, 1H), 2.24 (d, J = 12.6 Hz, 1H), 2.01 (s, 2H), 1.95 (dd, J = 7.0, 1.1 Hz, 3H), 1.91 – 1.65 (m, 4H), 1.58 (s, 1H), 1.28 (s, 1H), 1.03 (d, J = 6.9 Hz, 1.5H), 0.94 (d, J = 7.0 Hz, 1.5H). LRMS-ESI$^+$: *m/z* calcd for $C_{25}H_{32}Cl_2N_7O$ $[M+H]^+$ = 516.2; found, 516.

1-(1-(2,4-dichlorophenyl)ethyl)-6-(3'-methyl-[1,4'-bipiperidin]-1'-yl)-1*H*-pyrazolo[3,4-*b*]pyrazine-3-carbonitrile 2,2,2-trifluoroacetate (13). Step 1. To a solution of 1-(1-(2,4-dichlorophenyl)ethyl)-6-(3'-methyl-[1,4'-bipiperidin]-1'-yl)-1*H*-pyrazolo[3,4-*b*]pyrazine-3-carboxamide hydrochloride (**12**, 12 mg, 0.023 mmol) was added *N,N*-diisopropylethylamine (6 μL, 0.035 mmol) and methyl *N*-(triethylammoniosulfonyl)carbamate (36 mg, 0.15 mmol). The reaction mixture was stirred at room temperature for 16 h before and concentrated under reduced pressure. The residue was purified by reverse phase preparative HPLC (Gemini-NX, 10 μm, 250 x 30 mm, C18 column, Phenomenex, Torrance, CA), eluent: 0 to 100% acetonitrile in water, both eluents containing 0.1% TFA, gradient elution over 30 minutes to afford 1-(1-(2,4-dichlorophenyl)ethyl)-6-(3'-methyl-[1,4'-bipiperidin]-1'-yl)-1*H*-pyrazolo[3,4-*b*]pyrazine-3-carbonitrile 2,2,2-trifluoroacetate (**13**) as a mixture of diastereomers (30% yield). ^{1}H NMR (400 MHz, CD_3OD, TFA salt; 1:1 mix of isomers) δ 8.52 (d, J = 5.3 Hz, 1H), 7.49 (td, J = 1.4, 0.7 Hz, 1H), 7.42 – 7.31 (m, 2H), 6.48 (q, J = 7.1 Hz, 1H), 3.79 – 3.62 (m, 2H), 3.55 – 3.47 (m, 1H), 3.24 – 2.89 (m, 4H), 2.68 – 2.58 (m, 1H), 2.25 (d, J = 12.8 Hz, 1H), 2.07 – 1.96 (m, 2H), 1.93 (dd, J = 7.1, 0.8 Hz, 3H), 1.90 – 1.51 (m, 4H), 1.31 – 1.26 (m, 1H), 1.02 (d, J = 6.9 Hz, 1.5H), 0.92 (d, J = 6.9 Hz, 1.5H). LRMS-ESI$^+$: *m/z* calcd for $C_{25}H_{29}Cl_2N_7$ $[M+H]^+$ = 498.2; found, 498.

1-(1-(2,4-dichlorophenyl)ethyl)-3-methyl-6-(3'-methyl-[1,4'-bipiperidin]-1'-yl)-1*H*-pyrazolo[3,4-*b*]pyrazine 2,2,2-trifluoroacetate (14). Step 1. (1-(2,4-dichlorophenyl)ethyl)hydrazine hydrochloride (5.0 g, 20.7 mmol) was dissolved in ethanol (35 mL) at room temperature and 1-(3,5-dichloropyrazin-2-yl)ethan-1-one (3.6 g, 18.8 mmol) was added. The mixture was stirred at room temperature for 8 h and then concentrated under reduced pressure. The residue was suspended in 20% ethyl acetate in hexanes (20 mL) and filtered through a silica gel plug, eluting with 20% ethyl acetate in hexanes. The filtrate was concentrated under reduced pressure to give (*Z*)-3,5-Dichloro-2-(1-(2-(1-(2,4-dichlorophenyl)ethyl)hydrazono)ethyl)pyrazine and (*E*)-3,5-dichloro-2-(1-(2-(1-(2,4-dichlorophenyl)ethyl)hydrazono)ethyl)pyrazine (9:1) as a viscous orange oil which were used in the next step without further purification. LRMS-ESI$^+$: *m/z* calcd for $C_{14}H_{12}Cl_4N_4$ $[M+H]^+$ = 377.0; found, 377.

Step 2. A mixture of (*Z*)-3,5-dichloro-2-(1-(2-(1-(2,4-dichlorophenyl)ethyl)hydrazono)ethyl)pyrazine and (*E*)-3,5-dichloro-2-(1-(2-(1-(2,4-dichlorophenyl)ethyl)hydrazono)ethyl)pyrazine (9:1) (3.4 g, 9.2 mmol) was dissolved in *N*-methyl-2-pyrrolidone (20 mL) at room temperature and 2,6-lutidine (3.2 mL, 27.6 mmol) was added. The mixture was degassed with nitrogen and then heated to 100 °C under nitrogen for 8 h. The reaction mixture was cooled to room temperature and poured into a separatory funnel containing 50 mL of 1 M HCl in water and 50 mL of ethyl acetate. The layers were separated, and the organic layer was washed with 50 mL of 1M HCl in water, dried over sodium sulfate, and concentrated under reduced pressure. The residue was purified by silica gel chromatography (0 to 20% (1:1 MTBE:CH_2Cl_2) in hexanes) to provide 6-chloro-1-(1-(2,4-dichlorophenyl)ethyl)-

3-methyl-1*H*-pyrazolo[3,4-*b*]pyrazine as off-white solid (65% yield). LRMS-ESI$^+$: *m/z* calcd for $C_{14}H_{11}Cl_3N_4$ $[M+H]^+$ = 341.0; found, 341.

Step 3. The SnAr was performed following general procedure A using 6-chloro-1-(1-(2,4-dichlorophenyl)ethyl)-3-methyl-1*H*-pyrazolo[3,4-*b*]pyrazine (181 mg, 0.53 mmol), 3'-methyl-1,4'-bipiperidine (146 mg, 0.80 mmol), and *N*,*N*-diisopropylethylamine (0.50 mL, 3.18 mmol) in DMSO (2.0 mL) at 100 °C for 2 h. The residue was purified by reverse phase preparative HPLC (Gemini-NX, 10 μm, 250 x 30 mm, C18 column, Phenomenex, Torrance, CA), eluent: 0 to 100% acetonitrile in water, both eluents containing 0.1% TFA, gradient elution over 30 minutes, to afford 1-(1-(2,4-dichlorophenyl)ethyl)-3-methyl-6-(3'-methyl-[1,4'-bipiperidin]-1'-yl)-1*H*-pyrazolo[3,4-*b*]pyrazine 2,2,2-trifluoroacetate (**14**) as a 1.6:1 mixture of diastereomers (10% yield). ^{1}H NMR (400 MHz, CD_3OD; TFA Salt) δ 8.27 (s, 0.6H), 8.25 (s, 0.4H), 7.45 – 7.43 (m, 1H), 7.40 (d, *J* = 8.5 Hz, 0.6H), 7.34 (d, *J* = 8.5 Hz, 0.4H), 7.28 – 7.23 (m, 1H), 6.30 (q, *J* = 7.1 Hz, 1H), 4.82 – 4.70 (m, 1H), 4.70 – 4.55 (m, 1H), 3.75 (d, *J* = 12.4 Hz, 1H), 3.65 (m, *J* = 12.6 Hz, 1H), 3.49 (dt, *J* = 12.4, 4.0 Hz, 1H), 3.17 – 3.08 (m, 1H), 3.05 – 2.88 (m, 3H), 2.64 – 2.55 (m, 1H), 2.50 (s, 3H), 2.22 (d, *J* = 12.4 Hz, 1H), 2.06 – 1.94 (m, 2H), 1.90 – 1.68 (m, 7H), 1.62 – 1.48 (m, 1H), 1.02 (d, *J* = 6.8 Hz, 1.8H), 0.94 (d, *J* = 6.9 Hz, 1.2H). LRMS-ESI$^+$: *m/z* calcd for $C_{25}H_{32}Cl_2N_6$ $[M+H]^+$ = 487.2; found, 487.

1-(1-(2,4-dichlorophenyl)ethyl)-3-methyl-6-(3'-methyl-[1,4'-bipiperidin]-1'-yl)-1*H*-pyrazolo[3,4-*b*]pyrazine hydrochloride (15). Step 1. To a solution of 2,2,6,6-tetramethylpiperidine (13.7 mL, 80.6 mmol) in THF (200 mL) at -40 °C was added *n*-BuLi (2.5 M in hexanes, 34.9 mL, 87.3 mmol). The mixture was stirred at -40 °C for 30 min. In a separate flask, ethyl 2,2,2-trifluoroacetate (10.4 mL, 87.3 mmol) and 2,6-dichloropyrazine (10.0 g, 67.1 mmol) were dissolved in THF (200 mL) and cooled to -90 °C. The lithium 2,2,6,6-

tetramethylpiperidine solution was added to the 2,6-dichloropyrazine solution via cannula over 30 min at -90 °C. The mixture was stirred at -90 °C for 30 min and then (1-(2,4-dichlorophenyl)ethyl)hydrazine hydrochloride (9.73 g, 40.3 mmol) was added, and the mixture was warmed to room temperature. The mixture was concentrated under reduced pressure, then ethanol (200 mL) was added and the mixture was stirred at room temperature for 24 h. The mixture was concentrated under reduced pressure and the residue was purified by silica gel chromatography (0 to 100% ethyl acetate in hexanes) to provide (*E*)-3,5-Dichloro-2-(1-(2-(1-(2,4-dichlorophenyl)ethyl)hydrazono)-2,2,2-trifluoroethyl)pyrazine and (*Z*)-3,5-dichloro-2-(1-(2-(1-(2,4-dichlorophenyl)ethyl) hydrazono)-2,2,2-trifluoroethyl)pyrazine (21% yield) as a viscous orange oil. LRMS-ESI$^+$: *m/z* calcd for $C_{14}H_9Cl_4F_3N_4$ $[M+H]^+$ = 431.0; found, 431.

Step 2. (*E*)-3,5-dichloro-2-(1-(2-(1-(2,4-dichlorophenyl)ethyl)hydrazono)-2,2,2-trifluoroethyl)pyrazine and (*Z*)-3,5-dichloro-2-(1-(2-(1-(2,4-dichlorophenyl)ethyl) hydrazono)-2,2,2-trifluoroethyl)pyrazine (2.5 g, 5.79 mmol) were dissolved in THF (58 mL) and the solution was cooled to 0 °C. 1,8-Diazabicyclo[5.4.0]undec-7-ene (1.73 mL, 11.6 mmol) was added dropwise. After the addition was complete, the mixture warmed to room temperature and stirred for 10 h. The mixture was concentrated under reduced pressure and the residue was purified by silica gel chromatography (0 to 20% ethyl acetate in hexanes) to provide 6-chloro-1-(1-(2,4-dichlorophenyl)ethyl)-3-(trifluoromethyl)-1*H*-pyrazolo[3,4-*b*]pyrazine (79% yield) as a light orange oil. LRMS-ESI$^+$: *m/z* calcd for $C_{14}H_8Cl_2F_3N_4$ $[M+H]^+$ = 395.0; found, 395.

Step 3. The SnAr was performed following general procedure A using 6-chloro-1-(1-(2,4-dichlorophenyl)ethyl)-3-(trifluoromethyl)-1*H*-pyrazolo[3,4-*b*]pyrazine (0.253 mmol), 3'-methyl-1,4'-bipiperidine (51 mg, 0.278 mmol), and *N*,*N*-diisopropylethylamine (88 μL, 0.506 mmol) in dichloromethane (5 mL) at room temperature for 8 h. The residue was purified by

silica gel chromatography (0 to 20% methanol in dichloromethane). After concentrating the fractions containing the desired product, HCl (1 N in diethyl ether, 1 mL) was added and stirred 1 minute. The mixture was concentrated under reduced pressure to provide 1-(1-(2,4-dichlorophenyl)ethyl)-3-methyl-6-(3'-methyl-[1,4'-bipiperidin]-1'-yl)-1*H*-pyrazolo[3,4-*b*]pyrazine hydrochloride (**15**) as a mixture of diastereomers (78% yield) as a yellow solid. ^{1}H NMR (400 MHz, $CDCl_3$; HCl Salt) δ 8.28 (s, 0.6H), 8.28 (s, 0.4H), 7.41 – 7.33 (m, 2H), 7.22 – 7.15 (m, 1H), 6.49 – 6.40 (m, 1H), 4.59 (d, *J* = 13.2 Hz, 1H), 4.40 (t, *J* = 13.1 Hz, 1H), 3.06 (d, *J* = 12.9 Hz, 1H), 2.88 (t, *J* = 13.1 Hz, 1H), 2.55 – 2.38 (m, 4H), 2.36 – 2.19 (m, 2H), 1.93 (d, *J* = 7.1 Hz, 3H), 1.60 (m, 6H), 1.50 – 1.41 (m, 2H), 0.91 (d, *J* = 6.9 Hz, 1.8H), 0.84 (d, *J* = 6.8 Hz, 1.2H). LRMS-ESI$^+$: *m/z* calcd for $C_{25}H_{29}Cl_2F_3N_6$ $[M+H]^+$ = 541.2; found, 541.

1-((*R*)-1-(2,4-dichlorophenyl)ethyl)-3-methyl-6-((3'*R*,4'*S*)-3'-methyl-[1,4'-bipiperidin]-1'-yl)-1*H*-pyrazolo[3,4-*b*]pyrazine hydrochloride (16). Step 1. To a solution of *trans*-methylpiperidin-4-yl pivalate (1.93 g, 9.65 mmol) in EtOAc (8 mL) at room temperature was added a solution of (+)-*O,O'*-dibenzoyl-*D*-tartaric acid (1.73 g, 4.8 mmol) in EtOAc (13 mL). A precipitate formed immediately and the solution was then cooled to 0 °C then filtered through a Buchner funnel. The crystals were washed with cold EtOAc and dried in vacuo. The crystals were recrystallized in refluxing MeOH (14 mL), slowly cooled to room temperature, and filtered with a Buchner funnel to afford a white solid which was washed with minimal cold MeOH. The product was dried in vacuo, taken up in diethyl ether, and washed with 1N NaOH three times. The organic layer was dried over sodium sulfate, filtered, and concentrated in vacuo to afford (3*R*,4*R*)-3-methylpiperidin-4-yl pivalate (38% yield). ^{1}HNMR and LCMS matched literature values previously reported.[42] Stereochemistry was confirmed by obtained a single

crystal structure of the tartrate salt (Cambridge Crystallographic Data Centre Deposition Number 1915201, authors will release the atomic coordinates upon article publication).

Step 2. To a solution of (3*R*,4*R*)-3-methylpiperidin-4-yl pivalate (0.73 g, 3.65 mmol) and benzaldehyde (0.46 g, 4.4 mmol) in dichloromethane (10 mL) at room temperature was added sodium triacetoxyborohydride. The solution was stirred overnight. The reaction was quenched with sat. aq. $NaHCO_3$, extracted with EtOAc, dried over sodium sulfate, filtered, and concentrated via rotary evaporation to afford the desired product which was used without further purification. This crude oil was then taken up in MeOH (20 mL) and treated with NaOMe (25% in MeOH, 4 mL). The solution was refluxed overnight, cooled to room temperature, and concentrated. The crude material was diluted with diethyl ether, washed with 1N NaOH (x2), dried over sodium sulfate, filtered, and concentrated in vacuo. The residue was purified via silica gel chromatography (0 to 20% MeOH in dichloromethane) to afford (3*R*,4*R*)-1-benzyl-3-methylpiperidin-4-ol (33% yield). Ee: 83% Chiralpak® IF-3; 250mmx4.6mm, 10% EtOH in heptanes (with 0.1% Et_2NH); flow rate 1 mL/min; detection at 254 nm; t_1 = 4.3 min (minor) t_2 = 6.2 min (major). LRMS-ESI$^+$: *m/z* calcd for $C_{13}H_{19}NO$ $[M+H]^+$ = 206.2; found, 206.

Step 3. To a solution of (3*R*,4*R*)-1-benzyl-3-methylpiperidin-4-ol (690 mg, 3.2 mmol) in dichloromethane (10 mL) at 0 °C was added triethylamine (0.49 mL, 3.5 mmol) and methanesulfonyl chloride (0.25 mL, 3.2 mmol) in that order. After 1 hr the solution was treated with sat. aq. $NaHCO_3$, extracted with dichloromethane, dried over sodium sulfate, filtered, and concentrated in vacuo. The crude product was purified via silica gel chromatography (0 to 100% EtOAc in hexanes) to afford (3R,4R)-1-benzyl-3-methylpiperidin-4-yl methanesulfonate (70% yield). To a solution of (3*R*,4*R*)-1-benzyl-3-methylpiperidin-4-yl methanesulfonate (636 mg, 2.24 mmol) in DMF (10 mL) was added sodium azide (291 mg, 4.48 mmol) at room temperature

and the reaction was heated to 60 °C for 18 h. The solution was cooled to room temperature, diluted with water, and extracted with diethyl ether (x3). The combined organic phases were dried over sodium sulfate, filtered, and concentrated in vacuo. The residue was purified via silica gel chromatography (0 to 100% EtOAc in hexanes) to afford (3*R*,4*S*)-4-azido-1-benzyl-3-methylpiperidine (39% yield). LRMS-ESI$^+$: *m/z* calcd for $C_{13}H_{18}N_4$ $[M+H]^+$ = 231.2; found, 231.

Step 4. To a solution of (3*R*,4*S*)-4-azido-1-benzyl-3-methylpiperidine (200 mg, 0.87 mmol) in diethyl ether (16 mL) at 0 °C was added lithium aluminum hydride (1.0 mL, 4M in diethyl ether) and the solution was left to slowly warm to room temperature overnight. The reaction mixture was quenched using the standard Fieser workup, filtered through a plug of celite with EtOAc and concentrated in vacuo to afford (3*R*,4*S*)-1-benzyl-3-methylpiperidin-4-amine which was used without further purification. To a solution of the crude amine (30 mg, 0.15 mmol) in acetonitrile (2 mL) was added 1,5-dibromopentane (18 µL, 0.14 mmol) and potassium carbonate (37 mg, 0.27 mmol) at room temperature. The solution was heated to 60 °C for 18 h, cooled to room temperature, and filtered through a pad of celite with MeOH. The mixture was concentrated in vacuo and purified via reversed phase preparative HPLC (Gemini-NX, 10 µm, 250 x 30 mm, C18 column, Phenomenex, Torrance, CA), eluent: 0 to 100% acetonitrile in water, both eluents containing 0.1% TFA, gradient elution over 30 minutes, to afford (3'*R*,4'*S*)-1'-benzyl-3'-methyl-1,4'-bipiperidine (22% yield). LRMS-ESI$^+$: *m/z* calcd for $C_{18}H_{28}N_2$ $[M+H]^+$ = 273.2; found, 273.

Step 5. A solution of (3'*R*,4'*S*)-1'-benzyl-3'-methyl-1,4'-bipiperidine (50 mg, 0.18 mmol) in MeOH (0.8 mL), AcOH (0.2 mL), and EtOAc (1.0 mL) was sparged with argon gas for 10 minutes. To this solution was added palladium hydroxide (3 mg, 10 mol%) and the solution was sparged with hydrogen gas for 10 minutes. The reaction as stirred under a hydrogen atmosphere

overnight at room temperature. The suspension was sparged with argon gas for 10 minutes then filtered through a pad of celite with MeOH to afford crude (3'*R*,4'*S*)-3'-methyl-1,4'-bipiperidine which was used in the next step without further purification. LRMS-ESI$^+$: *m/z* calcd for $C_{11}H_{22}N_2$ $[M+H]^+$ = 183.2; found, 183.

Step 6. The SnAr was performed following general procedure A using (*R*)-6-chloro-1-(1-(2,4-dichlorophenyl)ethyl)-3-methyl-1*H*-pyrazolo[3,4-*b*]pyrazine (**67**, 180 mg, 0.51 mmol), (3'*R*,4'*S*)-3'-methyl-1,4'-bipiperidine (95 mg, 0.51 mmol), and *N*,*N*-diisopropylethylamine (0.17 mL, 1.0 mmol) in DMF (2 mL) at 80 °C for 16 h. The residue was purified by reverse phase preparative HPLC (Gemini-NX, 10 µm, 250 x 30 mm, C18 column, Phenomenex, Torrance, CA), eluent: 0 to 100% acetonitrile in water, both eluents containing 0.1% TFA, gradient elution over 30 minutes to give a mixture of stereoisomers. The mixture of stereoisomers was basified using a basification column (PL-HCO3 MP SPE, 500 mg per 5 mL tube, Agilent) using dichloromethane and methanol (4:1) as the eluent and concentrated under reduced pressure. The residue was dissolved in ethanol, treated with 2 N HCl in Et_2O (0.2 mL), and concentrated under reduced pressure to afford 1-((*R*)-1-(2,4-dichlorophenyl)ethyl)-3-methyl-6-((3'*R*,4'*S*)-3'-methyl-[1,4'-bipiperidin]-1'-yl)-1*H*-pyrazolo[3,4-*b*]pyrazine (**16**, 46% yield). ^{1}H NMR (400 MHz, CD_3CN; HCl Salt) δ 8.17 (s, 1H), 7.47 (d, *J* = 2.2 Hz, 1H), 7.41 (d, *J* = 8.6 Hz, 1H), 7.26 (dd, *J* = 8.5, 2.2 Hz, 1H), 6.25 (q, *J* = 7.1 Hz, 1H), 4.53 (ddt, *J* = 13.3, 4.8, 2.5 Hz, 1H), 4.40 (dt, *J* = 13.5, 2.7 Hz, 1H), 3.00 (dd, *J* = 13.5, 2.9 Hz, 1H), 2.82 (td, *J* = 13.2, 3.1 Hz, 1H), 2.43 (s, 3H), 2.42 – 2.35 (m, 4H), 2.31 – 2.21 (m, 1H), 2.20 – 2.15 (m, 1H), 1.88 – 1.82 (m, 1H), 1.84 (d, *J* = 7.1 Hz, 3H), 1.58 – 1.47 (m, 4H), 1.47 – 1.35 (m, 3H), 0.81 (d, *J* = 7.0 Hz, 3H). LRMS-ESI$^+$: *m/z* calcd for $C_{25}H_{32}Cl_2N_6$ $[M+H]^+$ = 487.2; found, 487.

1-((*R*)-1-(2,4-dichlorophenyl)ethyl)-3-methyl-6-((3'*S*,4'*R*)-3'-methyl-[1,4'-bipiperidin]-1'-yl)-1*H*-pyrazolo[3,4-*b*]pyrazine hydrochloride (17). The inactive isomer was prepared via the same route, however during the chiral resolution (-)-*O,O'*-dibenzoyl-*L*-tartaric acid was used in place of (+)-*O,O'*-dibenzoyl-*D*-tartaric acid to afford (3*S*,4*S*)-3-methylpiperidin-4-yl pivalate (a single crystal x-ray of this tartrate was also obtained, Cambridge Crystallographic Data Centre Deposition Number 1915202, authors will release the atomic coordinates upon article publication). This side chain was found to yield the less active isomer **17**, and thus the stereochemistry of this series was based on these results. ^{1}H NMR (400 MHz, CD_3CN; HCl Salt) δ 8.16 (s, 1H), 7.46 (d, *J* = 2.2 Hz, 1H), 7.39 (dd, *J* = 8.5, 2.2 Hz, 1H), 7.25 (dd, *J* = 8.5, 2.2 Hz, 1H), 6.25 (q, *J* = 7.1 Hz, 1H), 4.53 (ddt, *J* = 13.1, 4.5, 2.4 Hz, 1H), 4.45 (dt, *J* = 13.5, 2.7 Hz, 1H), 3.00 (dd, *J* = 13.4, 2.8 Hz, 1H), 2.82 (td, *J* = 13.2, 3.1 Hz, 1H), 2.43 (s, 3H), 2.46 – 2.37 (m, 4H), 2.29 – 2.22 (m, 1H), 2.21 – 2.15 (m, 1H), 1.90 – 1.84 (m, 1H), 1.83 (d, *J* = 7.1 Hz, 3H), 1.57 – 1.47 (m, 4H), 1.47 – 1.36 (m, 3H), 0.75 (d, *J* = 6.9 Hz, 3H). LRMS-ESI^{+}: *m/z* calcd for $C_{25}H_{32}Cl_2N_6$ $[M+H]^+$ = 487.2; found, 487.

1-((*R*)-1-(2,4-dichlorophenyl)ethyl)-3-methyl-6-((3*R*,4*S*)-3-methyl-4-(pyrrolidin-1-yl)piperidin-1-yl)-1*H*-pyrazolo[3,4-*b*]pyrazine hydrochloride (18). Step 1. The reductive amination was performed following general procedure D using 1-*tert*-butoxycarbonyl-3-methyl-4-piperidone (213 mg, 1.0 mmol), pyrrolidine (0.12 mL, 1.5 mmol), sodium triacetoxyborohydride (316 mg, 1.5 mmol) in 1,2-dichloroethane (2.5 mL) at room temperature for 16 h. The crude residue was dissolved in methanol (0.5 mL) and HCl (4 N in 1,4-dioxane, 2 mL, 8 mmol) was added. The mixture was stirred at room temperature for 1 h and then concentrated under reduced pressure to afford 3-methyl-4-(pyrrolidin-1-yl)piperidine

hydrochloride which was used without further purification. LRMS-ESI$^+$: *m/z* calcd for $C_{10}H_{20}N_2$ $[M+H]^+$ = 168.2; found, 168.

Step 2. The SnAr was performed following general procedure A using (*R*)-6-chloro-1-(1-(2,4-dichlorophenyl)ethyl)-3-methyl-1*H*-pyrazolo[3,4-*b*]pyrazine (**67**, 75 mg, 0.22 mmol), 3-methyl-4-(pyrrolidin-1-yl)piperidine hydrochloride (41 mg, 0.2 mmol), and *N*,*N*-diisopropylethylamine (0.15 mL, 0.86 mmol) in DMF (1.0 mL) at 80 °C for 1 h. The residue was purified by reverse phase preparative HPLC (Gemini-NX, 10 μm, 250 x 30 mm, C18 column, Phenomenex, Torrance, CA), eluent: 0 to 100% acetonitrile in water, both eluents containing 0.1% TFA, gradient elution over 30 minutes to give a mixture of stereoisomers. The stereoisomers were basified using a basification column (PL-HCO3 MP SPE, 500 mg per 5 mL tube, Agilent) using dichloromethane and methanol (4:1) as the eluent and concentrated under reduced pressure. The residue was azeotroped with ethanol and the residue was further purified using chiral HPLC (OZ-H, Chiralpak®, Daicel Corporation, West Chester, PA), eluent: 20% ethanol in heptanes, with heptanes containing 0.1% diethylamine, 30 min. The first eluting isomer was isolated and the free base was dissolved in ethanol, treated with 2 N HCl in Et_2O (0.2 mL), and concentrated under reduced pressure to afford 1-((*R*)-1-(2,4-dichlorophenyl)ethyl)-3-methyl-6-((3*R*,4*S*)-3-methyl-4-(pyrrolidin-1-yl)piperidin-1-yl)-1*H*-pyrazolo[3,4-*b*]pyrazine hydrochloride (**18**, 7% yield). ^{1}H NMR (400 MHz, CD_3OD; HCl salt) δ 8.31 (s, 1H), 7.46 (d, *J* = 2.1 Hz, 1H), 7.43 (d, *J* = 8.5 Hz, 1H), 7.28 (dd, *J* = 8.5, 2.1 Hz, 1H), 6.32 (q, *J* = 6.9 Hz, 1H), 4.75 (dm, *J* = 14.1 Hz, 1H), 4.60 (dm, *J* = 13.8 Hz, 1H), 3.76 – 3.67 (m, 2H), 3.52 – 3.45 (m, 1H), 3.26 – 3.11 (m, 3H), 3.01 (td, *J* = 13.6, 3.0 Hz, 1H), 2.57 – 2.50 (m, 1H), 2.52 (s, 3H), 2.24 – 2.16 (m, 2H), 2.14 – 1.98 (m, 3H), 1.96 – 1.83 (m, 1H), 1.89 (d, *J* = 7.1 Hz, 3H), 1.05 (d, *J* = 6.9 Hz, 3H). LRMS-ESI$^+$: *m/z* calcd for $C_{24}H_{30}Cl_2N_6$ $[M+H]^+$ = 473.2; found, 473.

6-((3*R*,4*S*)-4-(azetidin-1-yl)-3-methylpiperidin-1-yl)-1-((*R*)-1-(2,4-dichlorophenyl)ethyl)-3-methyl-1*H*-pyrazolo[3,4-*b*]pyrazine hydrochloride (19). Step 1. The reductive amination was performed following general procedure D using 1-*tert*-butoxycarbonyl-3-methyl-4-piperidone (213 mg, 1.0 mmol), azetidine (0.10 mL, 1.5 mmol), and sodium triacetoxyborohydride (316 mg, 1.5 mmol) in 1,2-dichloroethane (2.5 mL) at room temperature for 16 h. The crude residue was dissolved in methanol (0.5 mL) and HCl (4 N in 1,4-dioxane, 2 mL, 8 mmol) was added. The mixture was stirred at room temperature for 1 h and then concentrated under reduced pressure to afford 4-(azetidin-1-yl)-3-methylpiperidine hydrochloride which was used without further purification. LRMS-ESI$^+$: *m/z* calcd for $C_9H_{18}N_2$ $[M+H]^+$ = 155.2; found, 155.

Step 2. The SnAr was performed following general procedure A using (*R*)-6-chloro-1-(1-(2,4-dichlorophenyl)ethyl)-3-methyl-1*H*-pyrazolo[3,4-*b*]pyrazine (**67**, 75 mg, 0.22 mmol), 4-(azetidin-1-yl)-3-methylpiperidine hydrochloride (38 mg, 0.2 mmol), and *N,N*-diisopropylethylamine (0.15 mL, 0.86 mmol) in DMF (1.0 mL) at 80 °C for 1 h. The residue was purified by reverse phase preparative HPLC (Gemini-NX, 10 µm, 250 x 30 mm, C18 column, Phenomenex, Torrance, CA), eluent: 0 to 100% acetonitrile in water, both eluents containing 0.1% TFA, gradient elution over 30 minutes to afford a mixture of stereoisomers. The stereoisomers were basified using a basification column (PL-HCO3 MP SPE, 500 mg per 5 mL tube, Agilent) using dichloromethane and methanol (4:1) as the eluent and concentrated under reduced pressure. The residue was azeotroped with ethanol and the residue was further purified using chiral HPLC (OZ-H, Chiralpak®, Daicel Corporation, West Chester, PA), eluent: 20% ethanol in heptanes, with heptanes containing 0.1% diethylamine, 30 min. The first eluting isomer was isolated and the free base was dissolved in ethanol, treated with 2 N HCl in Et_2O (0.2

mL), and concentrated under reduced pressure to afford 6-((3*R*,4*S*)-4-(azetidin-1-yl)-3-methylpiperidin-1-yl)-1-((*R*)-1-(2,4-dichlorophenyl)ethyl)-3-methyl-1*H*-pyrazolo[3,4-*b*]pyrazine hydrochloride (**19**, 5% yield). ^{1}H NMR (400 MHz, CD_3OD; HCl salt) δ 8.32 (s, 1H), 7.46 (s, *J* = 2.1 Hz, 1H), 7.44 (d, *J* = 8.5 Hz, 1H), 7.29 (dd, *J* = 8.5, 2.1 Hz, 1H), 6.31 (q, *J* = 7.1 Hz, 1H), 4.70 (dm, *J* = 13.5 Hz, 1H), 4.57 (dt, *J* = 13.8, 2.4 Hz, 1H), 4.37 – 4.09 (m, 4H), 3.64 (dt, *J* = 12.2, 4.3 Hz, 1H), 3.18 (dd, *J* = 14.0, 2.6 Hz, 1H), 3.06 – 2.97 (m, 1H), 2.74 – 2.58 (m, 1H), 2.52 (s, 3H), 2.47 – 2.32 (m, 2H), 1.96 – 1.88 (m, 1H),. 1.89 (d, *J* = 7.1 Hz, 3H), 1.71 – 1.56 (m, 1H), 0.92 (d, *J* = 7.0 Hz, 3H). LRMS-ESI$^+$: *m/z* calcd for $C_{23}H_{28}Cl_2N_6$ $[M+H]^+$ = 459.2; found, 459.

6-((3*R*,4*S*)-4-(azepan-1-yl)-3-methylpiperidin-1-yl)-1-((*R*)-1-(2,4-dichlorophenyl)ethyl)-3-methyl-1*H*-pyrazolo[3,4-*b*]pyrazine hydrochloride (20). Step 1. The reductive amination was performed following general procedure D using 1-*tert*-butoxycarbonyl-3-methyl-4-piperidone (213 mg, 1.0 mmol), azepane (0.17 mL, 1.5 mmol), and sodium triacetoxyborohydride (316 mg, 1.5 mmol) in 1,2-dichloroethane (2.5 mL) at room temperature for 16 h. The crude residue was dissolved in methanol (0.5 mL) and HCl (4 N in 1,4-dioxane, 2 mL, 8 mmol) was added. The mixture was stirred at room temperature for 1 h and then concentrated under reduced pressure to afford 1-(3-methylpiperidin-4-yl)azepane hydrochloride which was used without further purification. LRMS-ESI$^+$: *m/z* calcd for $C_{12}H_{24}N_2$ $[M+H]^+$ = 197.2; found, 197.

Step 2. The SnAr was performed following general procedure A (*R*)-6-chloro-1-(1-(2,4-dichlorophenyl)ethyl)-3-methyl-1*H*-pyrazolo[3,4-*b*]pyrazine (**67**, 75 mg, 0.22 mmol), 1-(3-methylpiperidin-4-yl)azepane hydrochloride (47 mg, 0.2 mmol), and *N,N*-diisopropylethylamine (0.15 mL, 0.86 mmol) in DMF (1.0 mL) at 80 °C for 1 h. The residue was purified by reverse phase preparative HPLC (Gemini-NX, 10 µm, 250 x 30 mm, C18 column, Phenomenex,

Torrance, CA), eluent: 0 to 100% acetonitrile in water, both eluents containing 0.1% TFA, gradient elution over 30 minutes to give a mixture of stereoisomers. The stereoisomers were basified using a basification column (PL-HCO3 MP SPE, 500 mg per 5 mL tube, Agilent) using dichloromethane and methanol (4:1) as the eluent and concentrated under reduced pressure. The residue was azeotroped with ethanol and the residue was further purified using chiral HPLC (OZ-H, Chiralpak®, Daicel Corporation, West Chester, PA), eluent: 20% ethanol in heptanes, with heptanes containing 0.1% diethylamine, 30 min. The first eluting isomer was isolated and the free base was dissolved in ethanol, treated with 2 N HCl in Et_2O (0.2 mL), and concentrated under reduced pressure to afford 6-((3*R*,4*S*)-4-(azepan-1-yl)-3-methylpiperidin-1-yl)-1-((*R*)-1-(2,4-dichlorophenyl)ethyl)-3-methyl-1*H*-pyrazolo[3,4-*b*]pyrazine hydrochloride (**20**, 10% yield). ^{1}H NMR (400 MHz, CD_3OD; HCl salt) δ 8.11 (s, 1H), 7.21 (d, *J* = 2.1 Hz, 1H), 7.14 (d, *J* = 8.5 Hz, 1H), 7.04 (dd, *J* = 8.5, 2.2 Hz, 1H), 6.09 (q, *J* = 7.2 Hz, 1H), 4.56 – 4.38 (m, 2H), 3.42 (dt, *J* = 7.0, 3.7 Hz, 1H), 3.35 – 3.07 (m, 4H), 2.95 (dd, *J* = 14.0, 2.2 Hz, 1H), 2.87 – 2.78 (m, 1H), 2.37 (s, 1H), 2.33 – 2.27 (m, 1H), 2.29 (s, 3H), 1.94 (br d, *J* = 11.0 Hz, 1H), 1.74 (d, *J* = 25.2 Hz, 4H), 1.65 (d, *J* = 7.1 Hz, 3H), 1.61 – 1.45 (m, 4H), 0.73 (d, *J* = 6.9 Hz, 3H). LRMS-ESI$^+$: *m/z* calcd for $C_{26}H_{34}Cl_2N_6$ $[M+H]^+$ = 501.2; found, 501.

1-((*R*)-1-(2,4-dichlorophenyl)ethyl)-3-methyl-6-((3*R*,4*S*)-3-methyl-4-((*R*)-2-methylpyrrolidin-1-yl)piperidin-1-yl)-1*H*-pyrazolo[3,4-*b*]pyrazine hydrochloride (21). Step 1. The reductive amination was performed following general procedure D using 1-*tert*-butoxycarbonyl-3-methyl-4-piperidone (213 mg, 1.0 mmol), (*R*)-2-methylpyrrolidine (128 mg, 1.5 mmol), and sodium triacetoxyborohydride (316 mg, 1.5 mmol) in 1,2-dichloroethane (2.5 mL) at room temperature for 16 h. The crude residue was dissolved in methanol (0.5 mL) and HCl (4 N in 1,4-dioxane, 2 mL, 8 mmol) was added. The mixture was stirred at room

temperature for 1 h and then concentrated under reduced pressure to give 3-methyl-4-((*R*)-2-methylpyrrolidin-1-yl)piperidine hydrochloride which was used without further purification. LRMS-ESI$^+$: *m/z* calcd for $C_{11}H_{22}N_2$ $[M+H]^+$ = 183.2; found, 183.

Step 2. The SnAr was performed following general procedure A using (*R*)-6-chloro-1-(1-(2,4-dichlorophenyl)ethyl)-3-methyl-1*H*-pyrazolo[3,4-*b*]pyrazine (**67**, 75 mg, 0.22 mmol), 3-methyl-4-((*R*)-2-methylpyrrolidin-1-yl)piperidine hydrochloride (44 mg, 0.2 mmol), and *N,N*-diisopropylethylamine (0.15 mL, 0.86 mmol) in DMF (1.0 mL) at 80 °C for 1 h. The residue was purified by reverse phase preparative HPLC (Gemini-NX, 10 µm, 250 x 30 mm, C18 column, Phenomenex, Torrance, CA), eluent: 0 to 100% acetonitrile in water, both eluents containing 0.1% TFA, gradient elution over 30 minutes to give a mixture of stereoisomers. The stereoisomers were basified using a basification column (PL-HCO3 MP SPE, 500 mg per 5 mL tube, Agilent) using dichloromethane and methanol (4:1) as the eluent and concentrated under reduced pressure. The residue was azeotroped with ethanol and the residue was further purified using chiral HPLC (ID-3, Chiralpak®, Daicel Corporation, West Chester, PA), eluent: 20% ethanol in heptanes, with heptanes containing 0.1% diethylamine, 30 min. The first eluting isomer was isolated and the free base was dissolved in ethanol, treated with 2 N HCl in Et_2O (0.2 mL), and concentrated under reduced pressure to afford 1-((*R*)-1-(2,4-dichlorophenyl)ethyl)-3-methyl-6-((3*R*,4*S*)-3-methyl-4-((*R*)-2-methylpyrrolidin-1-yl)piperidin-1-yl)-1*H*-pyrazolo[3,4-*b*]pyrazine hydrochloride (**21**, 5% yield). ^{1}H NMR (400 MHz, $CDCl_3$; HCl salt): δ 8.09 (s, 1H), 7.40 (d, *J* = 8.5 Hz, 1H), 7.35 (d, *J* = 2.1 Hz, 1H), 7.14 (dd, *J* = 8.5, 2.2 Hz, 1H), 6.31 (q, *J* = 7.1 Hz, 1H), 4.55 – 4.47 (m, 1H), 4.37 – 4.31 (m, 1H), 3.15 – 3.07 (m, 1H), 3.05 – 2.98 (m, 1H), 2.86 (td, *J* = 12.8, 3.6 Hz, 1H), 2.79 – 2.72 (m, 1H), 2.68 – 2.60 (m, 1H), 2.58 – 2.53 (m, 4H),

2.18 – 2.11 (m, 1H), 1.90 (d, *J* = 7.1 Hz, 3H), 1.83 – 1.63 (m, 5H), 1.50 – 1.42 (m, 1H), 0.99 – 0.93 (m, 6H). LRMS-ESI$^+$: *m/z* calcd for $C_{25}H_{32}Cl_2N_6$ $[M+H]^+$ = 487.2; found, 487.

1-((*R*)-1-(2,4-dichlorophenyl)ethyl)-3-methyl-6-((3*R*,4*S*)-3-methyl-4-((*S*)-2-methylpyrrolidin-1-yl)piperidin-1-yl)-1*H*-pyrazolo[3,4-*b*]pyrazine hydrochloride (22). Step 1. The reductive amination was performed following general procedure D using 1-*tert*-butoxycarbonyl-3-methyl-4-piperidone (213 mg, 1.0 mmol), (*S*)-2-methylpyrrolidine (128 mg, 1.5 mmol), and sodium triacetoxyborohydride (316 mg, 1.5 mmol) in 1,2-dichloroethane (2.5 mL) at room temperature for 16 h. The crude residue was dissolved in methanol (0.5 mL) and HCl (4 N in 1,4-dioxane, 2 mL, 8 mmol) was added. The mixture was stirred at room temperature for 1 h and then concentrated under reduced pressure to give 3-methyl-4-((*S*)-2-methylpyrrolidin-1-yl)piperidine hydrochloride which was used without further purification. LRMS-ESI$^+$: *m/z* calcd for $C_{11}H_{22}N_2$ $[M+H]^+$ = 183.2; found, 183.

Step 2. The SnAr was performed following general procedure A using (*R*)-6-chloro-1-(1-(2,4-dichlorophenyl)ethyl)-3-methyl-1*H*-pyrazolo[3,4-*b*]pyrazine (**67**, 75 mg, 0.22 mmol), 3-methyl-4-((*S*)-2-methylpyrrolidin-1-yl)piperidine hydrochloride (44 mg, 0.2 mmol), and *N,N*-diisopropylethylamine (0.15 mL, 0.86 mmol) in DMF (1.0 mL) at 80 °C for 1 h. The residue was purified by reverse phase preparative HPLC (Gemini-NX, 10 µm, 250 x 30 mm, C18 column, Phenomenex, Torrance, CA), eluent: 0 to 100% acetonitrile in water, both eluents containing 0.1% TFA, gradient elution over 30 minutes to give a mixture of stereoisomers. The stereoisomers were basified using a basification column (PL-HCO3 MP SPE, 500 mg per 5 mL tube, Agilent) using dichloromethane and methanol (4:1) as the eluent and concentrated under reduced pressure. The residue was azeotroped with ethanol and the residue was further purified using chiral HPLC (ID-3, Chiralpak®, Daicel Corporation, West Chester, PA), eluent: 20%

ethanol in heptanes, with heptanes containing 0.1% diethylamine, 30 min. The first eluting isomer was isolated and the free base was dissolved in ethanol, treated with 2 N HCl in Et_2O (0.2 mL), and concentrated under reduced pressure to afford 1-((*R*)-1-(2,4-dichlorophenyl)ethyl)-3-methyl-6-((3*R*,4*S*)-3-methyl-4-((*S*)-2-methylpyrrolidin-1-yl)piperidin-1-yl)-1*H*-pyrazolo[3,4-*b*]pyrazine hydrochloride (**22**, 5% yield). ^{1}H NMR (400 MHz, $CDCl_3$; HCl salt): δ 8.08 (s, 1H), 7.37 (d, *J* = 8.5 Hz, 1H), 7.34 (d, *J* = 2.1 Hz, 1H), 7.15 – 7.11 (m, 1H), 6.35 – 6.29 (m, 1H), 4.58 – 4.51 (m, 1H), 4.38 – 4.33 (m, 1H), 3.18 – 2.98 (m, 2H), 2.92 – 2.61 (m, 3H), 2.56 (s, 3H), 2.19 – 2.10 (m, 1H), 1.90 (d, *J* = 7.1 Hz, 4H), 1.83 – 1.60 (m, 2H), 1.49 – 1.42 (m, 1H), 1.32 – 1.23 (m, 3H), 0.95 (d, *J* = 6.3 Hz, 3H), 0.87 (d, *J* = 6.7 Hz, 3H). LRMS-ESI$^+$: *m/z* calcd for $C_{25}H_{32}Cl_2N_6$ $[M+H]^+$ = 487.2; found, 487.

(*S*)-1-((3*R*,4*S*)-1-(1-((*R*)-1-(2,4-dichlorophenyl)ethyl)-3-methyl-1*H*-pyrazolo[3,4-*b*]pyrazin-6-yl)-3-methylpiperidin-4-yl)pyrrolidine-2-carboxamide hydrochloride (23). Step 1. The reductive amination was performed following general procedure D using 1-*tert*-butoxycarbonyl-3-methyl-4-piperidone (1.5 g, 7.03 mmol), *L*-prolinamide (1.2 g, 10.5 mmol), AcOH (0.6 mL, 10.5 mmol), and sodium triacetoxyborohydride (2.22 g, 10.7 mmol) in 1,2-dichloroethane (20 mL) at room temperature 16 h. The residue was purified by silica gel chromatography (0 to 5% methanol in dichloromethane) to afford a mixture of diastereomers. The residue was dissolved in dichloromethane (20 mL) and HCl (4 N in 1,4-dioxane, 5 mL) was added. The mixture was stirred at room temperature for 1 h and then concentrated under reduced pressure to afford (2*S*)-1-(3-methylpiperidin-4-yl)pyrrolidine-2-carboxamide hydrochloride which was used in the next step without further purification. LRMS-ESI$^+$: *m/z* calcd for $C_{11}H_{20}N_2O_2$ $[M+H]^+$ = 212.2; found, 212.

Step 2. The SnAr was performed following general procedure A using (*R*)-6-chloro-1-(1-(2,4-dichlorophenyl)ethyl)-3-methyl-1*H*-pyrazolo[3,4-*b*]pyrazine (**67**, 204 mg, 0.60 mmol), (2*S*)-1-(3-methylpiperidin-4-yl)pyrrolidine-2-carboxamide hydrochloride (150 mg, 0.61 mmol), and *N,N*-diisopropylethylamine (0.31 mL, 1.8 mmol) in DMSO (1.2 mL) at 80 °C for 16 h. The residue was purified by silica gel chromatography (0% to 10% methanol in dichloromethane) to afford a mixture of diastereomers. The mixture was further purified by preparative SFC (AD-H (2 x 25 cm), 35% isopropanol with 0.1% DEA and CO_2 at 100 bar, 55 mL/min). The first eluting isomer was isolated from this purification and converted to the HCl salt by diluting in dichloromethane (2 mL), adding HCl (2 N in diethyl ether, 0.5 mL), and concentrating to afford 1-((*R*)-1-(2,4-dichlorophenyl)ethyl)-6-((4*S*,5*R*)-4-((*S*)-2-(hydroxymethyl)pyrrolidin-1-yl)-5-methylcyclohex-1-en-1-yl)-1*H*-pyrazolo[3,4-*b*]pyrazine-3-carbonitrile hydrochloride (**23**, 15% yield). ^{1}H NMR (400 MHz, $CDCl_3$; TFA Salt) δ 8.07 (s, 1H), 7.39 (d, *J* = 8.3 Hz, 1H), 7.36 (d, *J* = 2.1 Hz, 1H), 7.13 (dd, *J* = 8.5, 2.1 Hz, 1H), 7.08 (d, *J* = 5.8 Hz, 1H), 6.30 (q, *J* = 7.1 Hz, 1H), 5.37 (d, *J* = 5.7 Hz, 1H), 4.49 (d, *J* = 13.4 Hz, 1H), 4.37 (d, *J* = 13.6 Hz, 1H), 4.07 – 3.95 (m, 1H), 3.48 (s, 1H), 3.32 – 3.15 (m, 2H), 3.04 (dd, *J* = 13.5, 2.9 Hz, 1H), 2.82 (td, *J* = 12.9, 3.2 Hz, 1H), 2.45 – 2.30 (m, 1H), 2.28 – 2.11 (m, 2H), 2.02 – 1.94 (m, 1H), 1.89 (d, *J* = 7.2 Hz, 3H), 1.88 – 1.56 (m, 4H), 1.35 (d, *J* = 4.4 Hz, 1H), 1.23 -1.16 (m, 1H), 0.99 (d, *J* = 6.9 Hz, 3H). LRMS-ESI$^+$: *m/z* calcd for $C_{25}H_{31}Cl_2N_7O_2$ $[M+H]^+$ = 516.2; found, 516.

3-((*S*)-1-((3*R*,4*S*)-1-(1-((*R*)-1-(2,4-dichlorophenyl)ethyl)-3-methyl-1*H*-pyrazolo[3,4-*b*]pyrazin-6-yl)-3-methylpiperidin-4-yl)pyrrolidin-2-yl)propenamide hydrochloride (24). Step 1. To a cooled suspension of sodium *tert*-butoxide (2.41 g, 25.1 mmol) in THF (100 mL) at 0 °C was added trimethylphosphonoacetate (4.5 mL, 27.6 mmol). After 1 h, the solution was transferred via cannula into a solution of *N*-Boc-*L*-prolinal (5.0 g, 25.1 mmol) in THF (50 mL).

After 30 min, the mixture was poured into saturated aqueous sodium bicarbonate solution and the aqueous layer was extracted with ethyl acetate. The combined organic layers were dried over sodium sulfate and concentrated under reduced pressure to afford the intermediate tert-butyl (*S*,*E*)-2-(3-methoxy-3-oxoprop-1-en-1-yl)pyrrolidine-1-carboxylate which was used without further purification (99% yield). LRMS-ESI$^+$: *m/z* calcd for $C_{13}H_{21}NO_4$ $[M+H]^+$ = 256.2; found, 256.

Step 2. A solution of *tert*-butyl (*S*,*E*)-2-(3-methoxy-3-oxoprop-1-en-1-yl)pyrrolidine-1-carboxylate (6.38 g, 25.0 mmol) and 10% palladium on carbon (1.3 g, 1.25 mmol) in methanol (100 mL) was stirred under a hydrogen atmosphere for 16 h. Nitrogen gas was bubbled through the solution for 15 minutes before it was carefully filtered through Celite using MeOH and concentrated under reduced pressure to afford *tert*-butyl (*S*)-2-(3-methoxy-3-oxopropyl)pyrrolidine-1-carboxylate which was used without further purification. To a cooled solution of *tert*-butyl (*S*)-2-(3-methoxy-3-oxopropyl)pyrrolidine-1-carboxylate (25.0 mmol) in dichloromethane (100 mL) at 0 °C was added trifluoroacetic acid (19 mL, 250 mmol). The mixture was stirred at room temperature for 16 h and concentrated via rotary evaporation. The residue was re-dissolved in dichloromethane, and potassium carbonate (17.3 g, 125 mmol) was added. After 30 min of stirring, the solution was filtered and the solvent was removed under reduced pressure to afford methyl (*S*)-3-(pyrrolidin-2-yl)propanoate which was used without further purification (99% yield). LRMS-ESI$^+$: *m/z* calcd for $C_8H_{15}NO_2$ $[M+H]^+$ = 158.1; found, 158.

Step 3. The reductive amination was performed following general procedure D using *tert*-butyl 3-methyl-4-oxopiperidine-1-carboxylate (2.24 g, 10.6 mmol), methyl (*S*)-3-(pyrrolidin-2-yl)propanoate (2 g, 12.73 mmol), sodium triacetoxyborohydride (3.4 g, 15.8 mmol), and acetic

acid (0.9 mL, 15.8 mmol) in dichloromethane (25 mL) at room temperature for 16 h. The residue was loaded onto silica gel and purified by normal-phase column chromatography (0 to 50% ethyl acetate in hexanes) to afford *tert*-butyl 4-((*S*)-2-(3-methoxy-3-oxopropyl)pyrrolidin-1-yl)-3-methylpiperidine-1-carboxylate (75% yield). LRMS-ESI$^+$: *m/z* calcd for $C_{19}H_{34}N_2O_4$ $[M+H]^+$ = 355.3; found, 355.

Step 4. To a solution of *tert*-butyl 4-((*S*)-2-(3-methoxy-3-oxopropyl)pyrrolidin-1-yl)-3-methylpiperidine-1-carboxylate (2.03 g, 5.97 mmol) in THF (20 mL), and water (10 mL) was added lithium hydroxide (215 mg, 8.96 mmol). The reaction was heated to 50 ºC for 16 h, cooled to room temperature and the solvent was removed under reduced pressure to afford 3-((2*S*)-1-(1-(*tert*-butoxycarbonyl)-3-methylpiperidin-4-yl)pyrrolidin-2-yl)propanoic acid which was used without further purification. The residue was dissolved in dichloromethane (30 mL) and trifluoroacetic acid (4.6 mL, 59.7 mmol) was added. The reaction was stirred at room temperature for 16 h and concentrated via rotary evaporation to afford 3-((2*S*)-1-(3-methylpiperidin-4-yl)pyrrolidin-2-yl)propanoic acid 2,2,2-trifluoroacetate which was used without further purification (99% yield). LRMS-ESI$^+$: *m/z* calcd for $C_{19}H_{34}N_2O_4$ $[M+H]^+$ = 355.3; found, 355.

Step 5. The SnAr was performed following general procedure A using (*R*)-6-chloro-1-(1-(2,4-dichlorophenyl)ethyl)-3-methyl-1*H*-pyrazolo[3,4-*b*]pyrazine (**67**, 512 mg, 1.50 mmol), 3-((2*S*)-1-(3-methylpiperidin-4-yl)pyrrolidin-2-yl)propanoic acid 2,2,2-trifluoroacetate (721 mg, 3.00 mmol), and *N*,*N*-diisopropylethylamine (0.78 mL, 4.50 mmol) in DMSO (3.0 mL) at 80 ºC for 16 h. The residue was purified by reverse phase preparative HPLC (Gemini-NX, 10 μm, 250 x 30 mm, C18 column, Phenomenex, Torrance, CA), eluent: 35 to 40 % acetonitrile in water, both eluents containing 0.1% ammonium formate, gradient over 20 minutes, to afford the

formate salt of the desired product as the second eluting isomer. The formate salt was suspended in saturated aqueous sodium bicarbonate solution and extracted three times with dichloromethane. The combined organic layers were dried over sodium sulfate and concentrated under reduced pressure. The residue was re-dissolved in dichloromethane (3.0 mL), treated with 2M HCl in Et_2O (0.3 mL), and concentrated under reduced pressure to afford 3-((*S*)-1-((3*R*,4*S*)-1-(1-((*R*)-1-(2,4-dichlorophenyl)ethyl)-3-methyl-1*H*-pyrazolo[3,4-*b*]pyrazin-6-yl)-3-methylpiperidin-4-yl)pyrrolidin-2-yl)propanoic acid hydrochloride (17% yield). ^{1}H NMR (400 MHz, CD_3CN, HCl salt) δ 8.31 (s, 1H), 8.17 (s, 1H), 7.46 (d, *J* = 2.2 Hz, 1H), 7.43 (d, *J* = 8.5 Hz, 1H), 7.27 – 7.22 (m, 1H), 6.26 (q, *J* = 7.1 Hz, 1H), 4.63 – 4.53 (m, 1H), 4.45 (dt, *J* = 13.6, 2.6 Hz, 1H), 3.66 – 3.57 (m, *J* = 5.9, 3.2 Hz, 1H), 3.31 – 3.20 (m, 1H), 3.06 – 2.80 (m, 4H), 2.49 (ddd, *J* = 17.6, 9.9, 3.0 Hz, 1H), 2.43 (s, *J* = 1.4 Hz, 3H), 2.41 – 2.33 (m, 2H), 2.15 – 1.96 (m, 3H), 1.93 – 1.85 (m, 2H), 1.83 (d, *J* = 7.1 Hz, 3H), 1.81 – 1.68 (m, 3H), 0.98 (d, *J* = 6.9 Hz, 3H). LRMS-ESI$^+$: *m/z* calcd for $C_{27}H_{35}Cl_2N_6O_2$ $[M+H]^+$ = 545.2; found, 545.

Step 6. The HATU coupling was performed following general procedure C using 3-((*S*)-1-((3*R*,4*S*)-1-(1-((*R*)-1-(2,4-dichlorophenyl)ethyl)-3-methyl-1*H*-pyrazolo[3,4-*b*]pyrazin-6-yl)-3-methylpiperidin-4-yl)pyrrolidin-2-yl)propanoic acid hydrochloride (30 mg, 0.055 mmol), ammonia gas (excess), *N,N*-diisopropylethylamine (19 μL, 0.110 mmol), and HATU (23 mg, 0.061 mmol) in DMF (1.0 mL) at room temperature for 10 min. The residue was purified by reverse phase preparative HPLC (Gemini-NX, 10 μm, 250 x 30 mm, C18 column, Phenomenex, Torrance, CA), eluent: 0 to 100% acetonitrile in water, both eluents containing 0.1% TFA, gradient elution over 30 minutes to afford 3-((*S*)-1-((3*R*,4*S*)-1-(1-((*R*)-1-(2,4-dichlorophenyl)ethyl)-3-methyl-1*H*-pyrazolo[3,4-*b*]pyrazin-6-yl)-3-methylpiperidin-4-

yl)pyrrolidin-2-yl)propenamide (**24**, 99% yield).). LRMS-ESI$^+$: *m/z* calcd for $C_{27}H_{35}Cl_2N_7O$ $[M+H]^+$ = 544.2; found, 544.

1-((*R*)-1-(2,4-dichlorophenyl)ethyl)-3-methyl-6-((3*R*,4*S*)-3-methyl-4-((*S*)-2-(2-(methylsulfonyl)ethyl)pyrrolidin-1-yl)piperidin-1-yl)-1*H*-pyrazolo[3,4-*b*]pyrazine hydrochloride (25). Step 1. Dimethyl ((methylsulfonyl)methyl)phosphonate (1.22 g, 6.02 mmol) was dissolved in THF (20 mL) and the solution was cooled to 0 °C. Sodium *tert*-butoxide (0.579 g, 6.02 mmol) was added and then the mixture was stirred for 30 min at 0 °C. *Tert*-Butyl (*S*)-2-formylpyrrolidine-1-carboxylate was then added (dissolved in 5 mL of THF). The reaction was stirred vigorously for 2 h at room temperature and then quenched with saturated aqueous sodium bicarbonate. The mixture was extracted with CH_2Cl_2 and the combined organic fractions were dried over Na_2SO_4, filtered and concentrated under reduced pressure to provide *tert*-butyl (*S*,*E*)-2-(2-(methylsulfonyl)vinyl)pyrrolidine-1-carboxylate (98% yield) as a colorless oil which was used without further purification. LRMS-ESI$^+$: *m/z* calcd for $C_{12}H_{21}NO_4S$ $[M+H]^+$ = 276.1; found, 276.

Step 2. *Tert*-Butyl (*S*,*E*)-2-(2-(methylsulfonyl)vinyl)pyrrolidine-1-carboxylate (1.2 g, 4.36 mmol) was dissolved in MeOH (20 mL) and then PtO_2 (9.9 mg, 0.044 mmol) was added. The mixture was purged twice with nitrogen and then twice with hydrogen. The reaction was then placed under an atmosphere of hydrogen and stirred for 1 h at room temperature. The mixture was flushed with nitrogen and then filtered carefully through a silica gel plug and concentrated under reduced pressure to provide *tert*-butyl (*S*)-2-(2-(methylsulfonyl)ethyl)pyrrolidine-1-carboxylate (99% yield) as a colorless oil which was used in the next step without further purification. LRMS-ESI$^+$: *m/z* calcd for $C_{12}H_{23}NO_4S$ $[M+H]^+$ = 278.1; found, 278.

Step 3. *Tert*-Butyl (*S*)-2-(2-(methylsulfonyl)ethyl)pyrrolidine-1-carboxylate (1.3 g, 4.69 mmol) was dissolved in CH_2C1_2 (10 mL) and HCl (4 N in 1,4-dioxane, 3.5 mL, 14.1 mmol) was added. The reaction was stirred for 4 h at room temperature. The mixture was diluted with dichloromethane and washed with saturated aqueous sodium carbonate. The organic fraction was dried over Na_2SO_4, filtered and concentrated under reduced pressure to provide (*S*)-2-(2-(methylsulfonyl)ethyl)pyrrolidine hydrochloride (99% yield) which was used without further purifiaction. LRMS-ESI$^+$: *m/z* calcd for $C_7H_{15}NO_2S$ $[M+H]^+$ = 178.1; found, 178.

Step 4. The reductive amination was performed following general procedure D using 1-*tert*-butoxycarbonyl-3-methyl-4-piperidone (1.0 g, 4.69 mmol), *(S)*-2-(2-(methylsulfonyl)ethyl)pyrrolidine hydrochloride (1.0 g, 4.69 mmol), and sodium triacetoxyborohydride (2.98 g, 14.07 mmol) in 1,2-dichloroethane (10 mL) at room temperature for 6 h. The crude residue was used without further purification. The residue was dissolved in dichloromethane (20 mL) and HCl (4 N in 1,4-dioxane, 3.5 mL) was added. The mixture was stirred at room temperature for 4 h and then concentrated under reduced pressure to afford (2*S*)-1-(3-methylpiperidin-4-yl)pyrrolidine-2-carboxamide hydrochloride (96% yield) which was used in the next step without further purification. LRMS-ESI$^+$: *m/z* calcd for $C_{13}H_{26}N_2O_2S$ $[M+H]^+$ = 275.2; found, 275.

Step 5. The SnAr was performed following general procedure A using (*R*)-6-chloro-1-(1-(2,4-dichlorophenyl)ethyl)-3-methyl-1*H*-pyrazolo[3,4-*b*]pyrazine (**67**, 500 mg, 1.46 mmol), (2*S*)-1-(3-methylpiperidin-4-yl)pyrrolidine-2-carboxamide hydrochloride (500 mg, 1.61 mmol), and *N*,*N*-diisopropylethylamine (0.76 mL, 4.39 mmol) in DMF (15 mL) at 80 °C for 8 h. The residue was purified by reverse phase preparative HPLC (Gemini-NX, 10 µm, 250 x 30 mm, C18 column, Phenomenex, Torrance, CA), eluent: 0 to 100% acetonitrile in water, both eluents

containing 0.1% TFA, gradient elution over 30 minutes to give a mixture of stereoisomers. The stereoisomers were basified using a basification column (PL-HCO3 MP SPE, 500 mg per 5 mL tube, Agilent) using dichloromethane and methanol (4:1) as the eluent and concentrated under reduced pressure. The residue was azeotroped with ethanol and the residue was further purified using chiral HPLC (IF-3, Chiralpak®, Daicel Corporation, West Chester, PA), eluent: 45% ethanol in heptanes, with heptanes containing 0.1% diethylamine, 30 min. The first eluting isomer was isolated and the free base was dissolved in ethanol, treated with 2 N HCl in Et_2O (0.2 mL), and concentrated under reduced pressure to afford 1-((*R*)-1-(2,4-dichlorophenyl)ethyl)-3-methyl-6-((3*R*,4*S*)-3-methyl-4-((*R*)-2-methylpyrrolidin-1-yl)piperidin-1-yl)-1*H*-pyrazolo[3,4-*b*]pyrazine hydrochloride (**25**, 6% yield). ^{1}H NMR (400 MHz, $CDCl_3$; HCl Salt) δ 8.09 (s, 1H), 7.41 (d, J = 8.5 Hz, 1H), 7.37 (d, J = 2.0 Hz, 1H), 7.15 (dd, J = 8.5, 2.0 Hz, 1H), 6.31 (q, J = 7.1 Hz, 1H), 4.52 (d, J = 13.3 Hz, 1H), 4.35 (d, J = 13.3 Hz, 1H), 3.22 – 3.07 (m, 2H), 3.06 – 2.92 (m, 3H), 2.90 (s, 3H), 2.89 – 2.80 (m, 1H), 2.57 (s, 3H), 2.49 – 2.43 (m, 1H), 2.21 – 2.09 (m, 1H), 2.00 – 1.92 (m, 3H), 1.91 (d, J = 7.1 Hz, 3H), 1.87 – 1.62 (m, 5H), 1.59 – 1.50 (m, 1H), 0.95 (d, J = 6.8 Hz, 3H). LRMS-ESI$^+$: *m/z* calcd for $C_{27}H_{36}Cl_2N_6O_2S$ $[M+H]^+$ = 579.2; found, 579.

2-((*S*)-1-((3*R*,4*S*)-1-(1-((*R*)-1-(2,4-dichlorophenyl)ethyl)-3-methyl-1*H*-pyrazolo[3,4-*b*]pyrazin-6-yl)-3-methylpiperidin-4-yl)pyrrolidin-2-yl)ethane-1-sulfonamide (26). Step 1. Triethylamine (1.0 mL, 7.4 mmol) was added to *tert*-butyl (*S*)-2-(2-hydroxyethyl)pyrrolidine-1-carboxylate (800 mg, 3.7 mmol) in dichloromethane (10 mL). The mixture was cooled in to 0 °C and methanesulfonyl chloride (0.35 mL, 4.44 mmol) was added. After 1.5 h, the reaction mixture was quenched with water (10 mL) and the layers were separated. The organic layer was dried over sodium sulfate, filtered, and concentrated via rotary evaporation to afford an oil which was

used directly in the following step without further purification. The intermediate mesylate (~3.7 mmol) was dissolved in DMF (10 mL) and potassium thioacetate (845 mg, 7.4 mmol) was added. The mixture was heated to 50 °C, upon which copious precipitate appeared. An additional 10 mL of DMF was added to the mixture and the reaction was stirred for an additional 1 h. The reaction mixture was then partitioned between 50% ethyl acetate in hexanes (100 mL) and water (100 mL). The organic layer was washed with water (2 x 50 mL), dried over sodium sulfate, filtered, and concentrate via rotary evaporation. The residue was purified by silica gel chromatography (0 to 50% ethyl acetate in hexanes) to afford *tert*-butyl (*S*)-2-(2-(acetylthio)ethyl)pyrrolidine-1-carboxylate as a white solid (68% over 2 steps). LRMS-ESI$^+$: *m/z* calcd for $C_{13}H_{23}NO_3S$ $[M+H]^+$ = 274.2; found, 274.

Step 2. *N*-chlorosuccinimide (1.33 g, 10 mmol) was added to a mixture of 2 N aqueous HCl (1.24 mL, 2.5 mmol) in acetonitrile (16.5 mL) at room temperature. The mixture was stirred for 10 min before being cooled to 0 °C. T*ert*-butyl (*S*)-2-(2-(acetylthio)ethyl)pyrrolidine-1-carboxylate (678 mg, 2.5 mmol) was then added to the reaction mixture dropwise as a solution in acetonitrile (4 mL). The reaction was warmed to room temperature and stirred for 10 min before diluting with ethyl acetate (100 mL) and water (100 mL). The organic layer was washed with brine (50 mL), dried over sodium sulfate, filtered, and concentrated via rotary evaporation to afford the sulfonyl chloride intermediate as a white solid which was used in the next step without further purification. The sulfonyl chloride intermediate (~2.5 mmol) was dissolved in acetonitrile (16.5 mL) and concentrated aqueous ammonia (33 %, 2.48 mL) was added. The mixture was stirred for 15 min and then diluted with ethyl acetate (50 mL) and water (50 mL). The organic layer was dried over sodium sulfate, filtered, and concentrated via rotary evaporation. The residue was treated with HCl (4 N in 1,4-dioxane, 10 mL) and stirred overnight. After

concentrating, the solid was taken up in methanol (10 mL) and filtered through a basification column (PL-HCO3 MP SPE, Agilent) to afford (*S*)-2-(pyrrolidin-2-yl)ethane-1-sulfonamide which was used directly in the next step without further purification (93% yield). LRMS-ESI$^+$: *m/z* calcd for $C_6H_{14}N_2O_2S$ $[M+H]^+$ = 179.1; found, 179.

Step 3. The reductive amination was performed following general procedure D using 1-*tert*-butoxycarbonyl-3-methyl-4-piperidone (213 mg, 1.0 mmol), (*S*)-2-(pyrrolidin-2-yl)ethane-1-sulfonamide (267 mg, 1.5 mmol), and sodium triacetoxyborohydride (316 mg, 1.5 mmol) in 1,2 dichloroethane at room temperature for 16 h. The crude residue was dissolved in dichloromethane (6 mL) and trifluoroacetic acid (2 mL) was added. The reaction was stirred at room temperature for 1 h and concentrated by rotary evaporation. The residue was purified by silica gel chromatography (0 to 30% methanol in dichloromethane) to afford 2-((*S*)-1-((3*R*,4*S*)-3-methylpiperidin-4-yl)pyrrolidin-2-yl)ethane-1-sulfonamide as the first eluting diastereomer (22% yield). LRMS-ESI$^+$: *m/z* calcd for $C_6H_{14}N_2O_2S$ $[M+H]^+$ = 275.2; found, 275.

Step 4. The SnAr was performed following general procedure A using (*R*)-6-chloro-1-(1-(2,4-dichlorophenyl)ethyl)-3-methyl-1*H*-pyrazolo[3,4-*b*]pyrazine (**67**, 75 mg, 0.22 mmol), 2-((*S*)-1-((3*R*,4*S*)-3-methylpiperidin-4-yl)pyrrolidin-2-yl)ethane-1-sulfonamide (55 mg, 0.2 mmol), and *N*,*N*-diisopropylethylamine (0.15 mL, 0.861 mmol) in DMF (0.5 mL) at 80 °C for 1 h. The residue was purified by reverse phase preparative HPLC (Gemini-NX, 10 µm, 250 x 30 mm, C18 column, Phenomenex, Torrance, CA), eluent: 0 to 100% acetonitrile in water, both eluents containing 0.1% TFA, gradient elution over 30 minutes to afford the trifluoroacetate salt. The salt was dissolved in methanol, filtered through a basification column (PL-HCO3 MP SPE, Agilent), the filtrate was treated with HCl (2 N in diethyl ether, 0.5 mL), and concentrated under reduced pressure to afford 2-((*S*)-1-((3*R*,4*S*)-1-(1-((*R*)-1-(2,4-dichlorophenyl)ethyl)-3-methyl-

1*H*-pyrazolo[3,4-*b*]pyrazin-6-yl)-3-methylpiperidin-4-yl)pyrrolidin-2-yl)ethane-1-sulfonamide (**26**, 15% yield). ^{1}H NMR (400 MHz, CD_3OD, HCl salt) δ 8.25 (s, 1H), 7.40 (d, *J* = 2.1 Hz, 1H), 7.37 (d, *J* = 8.5 Hz, 1H), 7.22 (dd, *J* = 8.5, 2.1 Hz, 1H), 6.28 (q, *J* = 7.1 Hz, 1H), 4.75 (dm, *J* = 13.7 Hz, 1H), 4.59 (dm, *J* = 13.8 Hz, 1H), 4.19 – 4.04 (m, 1H), 3.78 – 3.57 (m, 2H), 3.43 – 3.32 (m, 1H), 3.29 – 3.25 (m, 2H), 3.15 (br d, *J* = 13.6 Hz, 1H), 3.02 (br t, *J* = 11.9 Hz, 1H), 2.55 (br s, 1H), 2.49 (s, 3H), 2.39 – 1.91 (m, 10H), 1.85 (d, *J* = 7.1 Hz, 3H), 1.07 (d, *J* = 6.9 Hz, 3H). LRMS-ESI$^+$: *m/z* calcd for $C_{26}H_{35}Cl_2N_7O_2S$ $[M+H]^+$ = 580.2; found, 580.

3-((*S*)-1-((3*R*,4*S*)-1-(1-((*R*)-1-(2,4-dichlorophenyl)ethyl)-3-methyl-1*H*-pyrazolo[3,4-*b*]pyrazin-6-yl)-3-methylpiperidin-4-yl)pyrrolidin-2-yl)propanenitrile 2,2,2-trifluoroacetate (27). To a solution of 3-((*S*)-1-((3*R*,4*S*)-1-(1-((*R*)-1-(2,4-dichlorophenyl)ethyl)-3-methyl-1*H*-pyrazolo[3,4-*b*]pyrazin-6-yl)-3-methylpiperidin-4-yl)pyrrolidin-2-yl)propanamide (**24**, 30 mg, 0.055 mmol) was added Burgess reagent (26 mg, 0.110 mmol). The reaction was stirred at room temperature for 16 h before being concentrated under reduced pressure. The residue was purified by reverse phase preparative HPLC (Gemini-NX, 10 µm, 250 x 30 mm, C18 column, Phenomenex, Torrance, CA), eluent: 0 to 100% acetonitrile in water, both eluents containing 0.1% TFA, gradient elution over 30 minutes to afford 3-((*S*)-1-((3*R*,4*S*)-1-(1-((*R*)-1-(2,4-dichlorophenyl)ethyl)-3-methyl-1*H*-pyrazolo[3,4-*b*]pyrazin-6-yl)-3-methylpiperidin-4-yl)pyrrolidin-2-yl)propanenitrile 2,2,2-trifluoroacetate (**27**, 30% yield). ^{1}H NMR (400 MHz, $CDCl_3$, TFA salt) δ 8.11 (s, 1H), 7.39 – 7.34 (m, 2H), 7.15 (dd, J = 8.5, 2.1 Hz, 1H), 6.31 (q, J = 7.1 Hz, 1H), 4.65 (d, J = 13.7 Hz, 1H), 4.52 (d, J = 13.8 Hz, 1H), 3.89 (s, 1H), 3.66 (s, 1H), 3.47 (d, J = 11.9 Hz, 1H), 3.15 (s, 1H), 3.06 (d, J = 13.6 Hz, 1H), 2.97 (t, J = 12.9 Hz, 1H), 2.79 – 2.66 (m, 1H), 2.58 (s, 3H), 2.50 – 1.92 (m, 10H), 1.90 (d, J = 7.1 Hz, 3H), 1.20 (d, J = 6.7 Hz, 3H). LRMS-ESI$^+$: *m/z* calcd for $C_{27}H_{34}Cl_2N_7$ $[M+H]^+$ = 526.2; found, 526.

((3*R*,4*S*)-1-(1-((*R*)-1-(2,4-dichlorophenyl)ethyl)-3-methyl-1*H*-pyrazolo[3,4-*b*]pyrazin-6-yl)-3-methylpiperidin-4-yl)-*L*-proline 2,2,2-trifluoroacetate (28). Step 1. The reductive amination was performed following general procedure D using 1-*tert*-butoxycarbonyl-3-methyl-4-piperidone (2.83 g, 13.3 mmol), *L*-proline (1.84 g, 16 mmol), AcOH (1.14 mL, 20 mmol), and sodium triacetoxyborohydride (4.24 g, 20 mmol) in 1,2-dichloroethane (30 mL) at room temperature for 16 h. The residue was purified by silica gel chromatography (0 to 50% ethyl acetate in hexanes) to afford a mixture of diastereomers. The residue was dissolved in dichloromethane (50 mL) and trifluoroacetic acid (16.5 mL) was added. The mixture was stirred at room temperature for 16 h and then concentrated under reduced pressure to afford (3-methylpiperidin-4-yl)-*L*-proline trifluoroacetate which was used in the next step without further purification. LRMS-ESI$^+$: *m/z* calcd for $C_{11}H_{20}CN_2O_2$ $[M+H]^+$ = 213.2; found, 213.

Step 2. The SnAr was performed following general procedure A using (*R*)-6-chloro-1-(1-(2,4-dichlorophenyl)ethyl)-3-methyl-1*H*-pyrazolo[3,4-*b*]pyrazine (**67**, 75 mg, 0.22 mmol), (3-methylpiperidin-4-yl)-*L*-proline trifluoroacetate (65 mg, 0.2 mmol), and *N,N*-diisopropylethylamine (0.15 mL, 0.86 mmol) in DMSO (1.0 mL) at 80 °C for 1 h. The residue was purified by reverse phase preparative HPLC (Gemini-NX, 10 μm, 250 x 30 mm, C18 column, Phenomenex, Torrance, CA), eluent: 0 to 100% acetonitrile in water, both eluents containing 0.1% TFA, gradient elution over 30 minutes to afford ((3*R*,4*S*)-1-(1-((*R*)-1-(2,4-dichlorophenyl)ethyl)-3-methyl-1*H*-pyrazolo[3,4-*b*]pyrazin-6-yl)-3-methylpiperidin-4-yl)-*L*-proline trifluoroacetate as the second eluting isomer (**28**, 7% yield). ^{1}H NMR (400 MHz, CD_3CN; TFA salt): δ 8.20 (s, 1H), 7.48 – 7.43 (m, 2H), 7.27 (dd, *J* = 8.5, 2.1 Hz, 1H), 6.29 (q, 1H), 4.66 – 4.51 (m, 2H), 4.47 (dd, *J* = 10.2, 4.0 Hz, 1H), 3.90 – 3.80 (m, 1H), 3.63 – 3.50 (m, 1H), 3.32 – 3.17 (m, 1H), 3.07 (d, *J* = 13.7 Hz, 1H), 2.97 – 2.85 (m, 1H), 2.56 – 2.46 (m, 2H),

2.46 (s, 3H), 2.35 – 2.24 (m, 1H), 2.20 – 2.09 (m, 1H), 2.06 – 1.95 (m, 1H), 1.93 – 1.87 (m, 2H), 1.85 (d, *J* = 7.1 Hz, 3H), 1.09 (d, *J* = 6.9 Hz, 3H). LRMS-ESI$^+$: *m/z* calcd for $C_{25}H_{30}Cl_2N_6O_2$ $[M+H]^+$ = 517.2; found, 517.

((S)-1-((3R,4S)-1-(1-((R)-1-(2,4-dichlorophenyl)ethyl)-3-methyl-1H-pyrazolo[3,4-b]pyrazin-6-yl)-3-methylpiperidin-4-yl)pyrrolidin-2-yl)methanol (29). Step 1. The reductive amination was performed following general procedure D using 1-*tert*-butoxycarbonyl-3-methyl-4-piperidone (213 mg, 1.0 mmol), (*S*)-pyrrolidin-2-ylmethanol (152 mg, 1.5 mmol), and sodium triacetoxyborohydride (316 mg, 1.5 mmol) in 1,2-dichloroethane (2.5 mL) at room temperature for 16 h. The residue was purified by silica gel chromatography (0 to 20% methanol in dichloromethane) to afford the *cis*-diastereomer of *tert*-butyl 4-((*S*)-2-(hydroxymethyl)pyrrolidin-1-yl)-3-methylpiperidine-1-carboxylate (40% yield). The residue was dissolved in dichloromethane (2.0 mL) and trifluoroacetic acid (0.5 mL) was added. The mixture was stirred at room temperature for 1 h and then concentrated under reduced pressure to afford the *cis*-diastereomer of ((2*S*)-1-(3-methylpiperidin-4-yl)pyrrolidin-2-yl)methanol 2,2,2-trifluoroacetate which was used without further purification. LRMS-ESI$^+$: *m/z* calcd for $C_{11}H_{22}N_2O$ $[M+H]^+$ = 199.2; found, 199.

Step 2. The SnAr was performed following general procedure A using (*R*)-6-chloro-1-(1-(2,4-dichlorophenyl)ethyl)-3-methyl-1*H*-pyrazolo[3,4-*b*]pyrazine (**67**, 75 mg, 0.22 mmol), the *cis*-diastereomer of ((2*S*)-1-(3-methylpiperidin-4-yl)pyrrolidin-2-yl)methanol 2,2,2-trifluoroacetate (62 mg, 0.2 mmol), and *N*,*N*-diisopropylethylamine (0.15 mL, 0.86 mmol) in DMF (1.0 mL) at 80 °C for 1 h. The residue was purified by reverse phase preparative HPLC (Gemini-NX, 10 µm, 250 x 30 mm, C18 column, Phenomenex, Torrance, CA), eluent: 0 to 100% acetonitrile in water, both eluents containing 0.1% TFA, gradient elution over 30 minutes

to afford the 2,2,2-trifluoroacetate salt of the title compound. The residue was dissolved in dichloromethane, washed with saturated aqueous sodium bicarbonate, and concentrated under reduced pressure. The organic layer was treated with HCl (2 N in diethyl ether, 1.0 mL) and the volatiles were removed to afford ((*S*)-1-((3*R*,4*S*)-1-(1-((*R*)-1-(2,4-dichlorophenyl)ethyl)-3-methyl-1*H*-pyrazolo[3,4-*b*]pyrazin-6-yl)-3-methylpiperidin-4-yl)pyrrolidin-2-yl)methanol (**29**, 15% yield). ^{1}H NMR (400 MHz, CD_3CN; HCl salt): δ 8.20 (s, 1H), 7.47 – 7.42 (m, 2H), 7.26 (dd, *J* = 8.5, 2.2 Hz, 1H), 6.27 (q, *J* = 7.1 Hz, 1H), 4.66 – 4.57 (m, 1H), 4.52 (dt, *J* = 13.8, 2.5, 1H), 4.02 – 3.92 (m, 1H), 3.71 (dd, *J* = 12.3, 4.2 Hz, 1H), 3.66 – 3.52 (m, 2H), 3.46 – 3.35 (m, 1H), 3.34 – 3.22 (m, 1H), 3.04 (dd, *J* = 13.7, 2.4 Hz, 1H), 2.53 – 2.46 (m, 1H), 2.44 (s, 3H), 2.19 – 2.08 (m, 2H), 2.06 – 1.85 (m, 4H), 1.84 (d, *J* = 7.1 Hz, 3H), 1.85 – 1.77 (m, 1H), 1.01 (d, *J* = 6.9 Hz, 3H). LRMS-ESI$^+$: *m/z* calcd for $C_{25}H_{32}Cl_2N_6O$ $[M+H]^+$ = 503.2; found, 503.

1-((*R*)-1-(2,4-dichlorophenyl)ethyl)-6-((3*R*,4*S*)-4-((*S*)-2-(hydroxymethyl)pyrrolidin-1-yl)-3-methylpiperidin-1-yl)-1*H*-pyrazolo[3,4-*b*]pyrazine-3-carbonitrile (30). The S_nAr was performed following general procedure A using (*R*)-6-chloro-1-(1-(2,4-dichlorophenyl)ethyl)-1*H*-pyrazolo[3,4-*b*]pyrazine-3-carbonitrile (**46**, 75 mg, 0.22 mmol), the cis-diastereomer of ((2*S*)-1-(3-methylpiperidin-4-yl)pyrrolidin-2-yl)methanol 2,2,2-trifluoroacetate (42, 62 mg, 0.2 mmol), and *N*,*N*-diisopropylethylamine (0.15 mL, 0.86 mmol) in DMF (1.0 mL). The mixture was heated at 80 °C for 1 h and concentrated. The residue was purified by reverse phase preparative HPLC (Gemini-NX, 10 μm, 250 x 30 mm, C18 column, Phenomenex, Torrance, CA), eluent: 0 to 100% acetonitrile in water, both eluents containing 0.1% TFA, gradient elution over 30 minutes. The residue was dissolved in dichloromethane, washed with saturated aqueous sodium bicarbonate, and concentrated under reduced pressure. The organic layer was treated with HCl (2 N in diethyl ether, 1.0 mL) and the volatiles were removed to afford 1-((*R*)-1-(2,4-

dichlorophenyl)ethyl)-6-((3*R*,4*S*)-4-((*S*)-2-(hydroxymethyl)pyrrolidin-1-yl)-3-methylpiperidin-1-yl)-1*H*-pyrazolo[3,4-*b*]pyrazine-3-carbonitrile hydrochloride (**30**, 10% yield). ^{1}H NMR (400 MHz, CD_3CN; HCl salt): δ 8.43 (s, 1H), 7.52 (d, J = 2.2 Hz, 1H), 7.46 (d, J = 8.5 Hz, 1H), 7.33 (dd, J = 8.5, 2.2 Hz, 1H), 6.45 (q, J = 7.0 Hz, 1H), 4.72 – 4.54 (m, 2H), 4.02 – 3.90 (m, 1H), 3.74 – 3.68 (m, 2H), 3.65 – 3.55 (m, 1H), 3.46 – 3.36 (m, 1H), 3.35 – 3.25 (m, 1H), 3.09 (dd, J = 13.7, 1.9 Hz, 1H), 2.99 (dt, J = 13.4, 3.0 Hz, 1H), 2.59 – 2.50 (m, 1H), 2.28 – 2.20 (m, 1H), 2.19 – 1.93 (m, 4H), 1.91 (d, J = 7.1 Hz, 3H), 1.88 – 1.78 (m, 1H), 1.06 (d, J = 6.9 Hz, 3H). LRMS-ESI$^+$: m/z calcd for $C_{25}H_{29}Cl_2N_7O$ $[M+H]^+$ = 514.2; found, 514.

1-((*R*)-1-(2,4-dichlorophenyl)ethyl)-6-((4*S*,5*R*)-4-((*S*)-2-(hydroxymethyl)pyrrolidin-1-yl)-5-methylcyclohex-1-en-1-yl)-1*H*-pyrazolo[3,4-*b*]pyrazine-3-carbonitrile hydrochloride (31). 1-((*R*)-1-(2,4-dichlorophenyl)ethyl)-6-(5-methyl-4-oxocyclohex-1-en-1-yl)-1*H*-pyrazolo[3,4-*b*]pyrazine-3-carbonitrile (**55**, 350 mg, 0.82 mmol), L-prolinol (**47**, 330 mg, 3.2 mmol), $NaBH_3CN$ (130 mg, 1.98 mmol) and AcOH (0.146 mL) were dissolved in 1,2-dichloroethane (3 mL) and stirred at room temperature for 16 h. After concentrating, the residue was purified by reverse phase preparative HPLC (Gemini-NX, 10 µm, 250 x 30 mm, C18 column, Phenomenex, Torrance, CA), eluent: 0 to 100% acetonitrile in water, both eluents containing 0.1% TFA, gradient elution over 30 minutes to afford a 4:1:1 mixture of diastereomers. The mixture was further purified by preparative SFC (AD-H (2 x 25 cm), 40% isopropanol with 0.1% DEA and CO_2 at 100 bar, 50 mL/min). The third eluting isomer was isolated from this purification and converted to the HCl salt by diluting in dichloromethane (2 mL), adding HCl (2 N in diethyl ether, 0.5 mL), and concentrating to afford 1-((*R*)-1-(2,4-dichlorophenyl)ethyl)-6-((4*S*,5*R*)-4-((*S*)-2-(hydroxymethyl)pyrrolidin-1-yl)-5-methylcyclohex-1-en-1-yl)-1*H*-pyrazolo[3,4-*b*]pyrazine-3-carbonitrile hydrochloride (**31**, 10% yield). ^{1}H NMR

(400 MHz, CD_3OD; HCl salt) δ 9.08 (s, 1H), 7.46 (dd, J = 6.7, 2.1 Hz, 1H), 7.43 (d, J = 8.5 Hz, 1H), 7.31 (dd, J = 8.5, 2.1 Hz, 1H), 7.02 – 6.92 (m, 1H), 6.72 (q, J = 7.0 Hz, 1H), 4.01 (tt, J = 8.7, 4.2 Hz, 1H), 3.81 (dd, J = 12.0, 4.8 Hz, 1H), 3.71 (dd, J = 12.0, 7.8 Hz, 2H), 3.65 – 3.57 (m, 1H), 3.44 (td, J = 10.6, 6.4 Hz, 1H), 3.02 (dt, J = 10.7, 3.8 Hz, 1H), 2.97 – 2.48 (m, 4H), 2.31 – 2.02 (m, 2H), 1.99 (d, J = 7.0 Hz, 3H), 1.97 – 1.87 (m, 2H), 1.14 (d, J = 6.7 Hz, 3H); m/z 511.1 ($M+H^+$). ^{13}C NMR (101 MHz; $CDCl_3$; HCl salt) δ 152.2, 142.0, 141.5, 136.2, 135.0, 133.4, 133.0, 132.5, 129.6, 129.1, 128.8, 128.0, 118.6, 111.9, 72.1, 66.7, 62.0, 53.6, 52.9, 33.4, 28.0, 26.8, 26.6, 24.7, 20.3, 13.6. LRMS-ESI^+: m/z calcd for $C_{26}H_{28}Cl_2N_6O$ $[M+H]^+$ = 511.2; found, 511.

2nd Generation Synthesis of 1-((*R*)-1-(2,4-dichlorophenyl)ethyl)-6-((4*S*,5*R*)-4-((*S*)-2-(hydroxymethyl)pyrrolidin-1-yl)-5-methylcyclohex-1-en-1-yl)-1*H*-pyrazolo[3,4-*b*]pyrazine-3-carbonitrile hydrochloride (31). To a solution of 6-((4*S*,5*R*)-4-((*S*)-2-(((tert-butyldimethylsilyl)oxy)methyl)pyrrolidin-1-yl)-5-methylcyclohex-1-en-1-yl)-1-((*R*)-1-(2,4-dichlorophenyl)ethyl)-1*H*-pyrazolo[3,4-*b*]pyrazine-3-carbonitrile (**66**, 432 mg, 0.69 mmol) in dichloromethane (6.9 mL) was added 4 N HCl in 1,4-dioxane (0.69 mL, 2.76 mmol). After 4 h at room temperature, the solution was concentrated under reduced pressure, and the residue was purified by silica gel chromatography (0 to 20% methanol in dichloromethane) to afford 1-((*R*)-1-(2,4-dichlorophenyl)ethyl)-6-((4*S*,5*R*)-4-((*S*)-2-(hydroxymethyl)pyrrolidin-1-yl)-5-methylcyclohex-1-en-1-yl)-1*H*-pyrazolo[3,4-*b*]pyrazine-3-carbonitrile (**31***,* 82% yield). *^1H NMR and LCMS data matched previous prepared lots.*

((2*S*)-1-(4-(1-((*R*)-1-(2,4-dichlorophenyl)ethyl)-3-methyl-1*H*-pyrazolo[3,4-*b*]pyrazin-6-yl)-6-methylcyclohex-3-en-1-yl)pyrrolidin-2-yl)methanol 2,2,2-trifluoroacetate (32). Step 1. A 300 mL sealed tube was charged with a mixture of 4,4,5,5-tetramethyl-2-(10-methyl-1,4-

dioxaspiro[4.5]dec-7-en-8-yl)-1,3,2-dioxaborolane and 4,4,5,5-tetramethyl-2-(6-methyl-1,4-dioxaspiro[4.5]dec-7-en-8-yl)-1,3,2-dioxaborolane (**53**, 1.40 g, 5.00 mmol), (*R*)-6-chloro-1-(1-(2,4-dichlorophenyl)ethyl)-3-methyl-1*H*-pyrazolo[3,4-*b*]pyrazine (**67**, 1.14 g, 3.33 mmol), $Pd(dppf)Cl_2$ (183 mg, 0.250 mmol), 4:1 THF:H_2O (11.2 mL), and Na_2CO_3 (882 mg, 8.33 mmol). Nitrogen gas was bubbled through the reaction mixture for 35 min before placing the reaction vessel in a pre-heated oil bath at 90 °C for 4 h. The reaction mixture was quenched with saturated aqueous $NaHCO_3$ and extracted with EtOAc (2 x). The combined organic layers were washed with brine, dried over sodium sulfate, concentrated, and purified by silica gel chromatography (10% to 25% EtOAc in hexanes) to afford 1-((*R*)-1-(2,4-dichlorophenyl)ethyl)-3-methyl-6-(10-methyl-1,4-dioxaspiro[4.5]dec-7-en-8-yl)-1*H*-pyrazolo[3,4-*b*]pyrazine as the second eluting peak (21% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.69 (d, J = 1.1 Hz, 1H), 7.41 (dd, J = 8.4, 5.9 Hz, 1H), 7.35 (d, J = 2.1 Hz, 1H), 7.16 – 7.12 (m, 1H), 6.71 – 6.67 (m, 1H), 6.54 (q, J = 7.1 Hz, 1H), 4.06 – 3.96 (m, 4H), 3.03 – 2.87 (m, 1H), 2.65 (s, 2H), 2.63 – 2.42 (m, 3H), 2.23 – 2.12 (m, 1H), 1.94 (d, J = 7.1 Hz, 3H), 1.06 (d, J = 6.8, 3.4, 3H). LRMS-ESI$^+$: *m/z* calcd for $C_{23}H_{24}Cl_2N_4O_2$ $[M+H]^+$ = 459.1; found, 459.

Step 2. 1-((*R*)-1-(2,4-dichlorophenyl)ethyl)-3-methyl-6-(10-methyl-1,4-dioxaspiro[4.5]dec-7-en-8-yl)-1*H*-pyrazolo[3,4-*b*]pyrazine (320 mg, 0.670 mmol) was diluted with dichloromethane (6 mL) and TFA (1.5 mL). The resulting clear, red solution was stirred at room temperature for 20 h. The reaction was then carefully quenched with saturated aqueous $NaHCO_3$ and extracted with dichloromethane (3 x). The combined organic layers were dried over Na_2SO_4 and concentrated to afford 4-(1-((*R*)-1-(2,4-dichlorophenyl)ethyl)-3-methyl-1*H*-pyrazolo[3,4-*b*]pyrazin-6-yl)-6-methylcyclohex-3-en-1-one (92% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.74 (d, J = 1.4 Hz, 1H), 7.45 (dd, J = 8.5, 1.4 Hz, 1H), 7.37 (dd, J = 2.9, 2.1 Hz, 1H),

7.21 – 7.14 (m, 1H), 6.79 (ddt, J = 5.9, 4.0, 2.0 Hz, 1H), 6.57 (qd, J = 7.0, 1.2 Hz, 1H), 3.45 – 3.32 (m, 1H), 3.31 – 3.07 (m, 2H), 2.80 (dq, J = 12.3, 6.3 Hz, 1H), 2.68 (d, J = 0.5 Hz, 3H), 2.65 – 2.53 (m, 1H), 1.96 (d, J = 7.1 Hz, 3H), 1.24 (d, J = 6.6 Hz, 3H). LRMS-ESI$^+$: *m/z* calcd for $C_{21}H_{20}Cl_2N_4O_1$ $[M+H]^+$ = 415.1; found, 415.

Step 3. The reductive amination was performed following general procedure D using 4-(1-((*R*)-1-(2,4-dichlorophenyl)ethyl)-3-methyl-1*H*-pyrazolo[3,4-*b*]pyrazin-6-yl)-6-methylcyclohex-3-en-1-one (287 mg, 0.690 mmol), *L*-prolinol (349 mg, 3.46 mmol), $NaBH_3CN$ (87 mg, 1.4 mmol), and AcOH (0.19 mL) in 1,2-dichloroethane (2 mL) at room temperature overnight. The residue was purified by reverse phase preparative HPLC (Gemini-NX, 10 µm, 250 x 30 mm, C18 column, Phenomenex, Torrance, CA), eluent: 45% to 75% acetonitrile in water, both eluents containing 0.1% TFA, gradient elution over 30 min to afford ((2*S*)-1-(4-(1-((*R*)-1-(2,4-dichlorophenyl)ethyl)-3-methyl-1*H*-pyrazolo[3,4-*b*]pyrazin-6-yl)-6-methylcyclohex-3-en-1-yl)pyrrolidin-2-yl)methanol 2,2,2-trifluoroacetate (**32**, 5% yield). ^{1}H NMR (400 MHz, CD_3OD; TFA salt) δ 8.81 (s, 0.5H), 8.80 (s, 0.5H), 7.54 – 7.40 (m, 2H), 7.27 (ddd, *J* = 8.5, 3.7, 2.0 Hz, 1H), 6.88 – 6.75 (m, 1H), 6.54 (qd, *J* = 7.1, 2.5 Hz, 1H), 4.15 – 3.35 (m, 6H), 3.13 – 2.73 (m, 3.5H), 2.73 – 2.50 (m, 4H), 2.38 – 1.99 (m, 3.5H), 1.99 – 1.84 (m, 4H), 1.22 – 1.01 (m, 3H). LRMS-ESI$^+$: *m/z* calcd for $C_{26}H_{31}Cl_2N_5O$ $[M+H]^+$ = 500.2; found, 500.

1-((*R*)-1-(2,4-dichlorophenyl)ethyl)-6-(4-((*S*)-2-(2-hydroxypropan-2-yl)pyrrolidin-1-yl)-5-methylcyclohex-1-en-1-yl)-1*H*-pyrazolo[3,4-*b*]pyrazine-3-carbonitrile 2,2,2-trifluoroacetate (33). The reductive amination was performed following general procedure D using 1-((*R*)-1-(2,4-dichlorophenyl)ethyl)-6-(5-methyl-4-oxocyclohex-1-en-1-yl)-1*H*-pyrazolo[3,4-*b*]pyrazine-3-carbonitrile (**55**, 256 mg, 0.60 mmol), (*S*)-2-(pyrrolidin-2-yl)propan-2-ol (78 mg, 0.60 mmol), and sodium triacetoxyborohydride (381 mg, 1.8 mmol) in 1,2-

dichloroethane (2.0 mL) at room temperature for 16 h. The residue was purified by reverse phase preparative HPLC (Gemini-NX, 10 µm, 250 x 30 mm, C18 column, Phenomenex, Torrance, CA), eluent: 0 to 100% acetonitrile in water, both eluents containing 0.1% TFA, gradient elution over 30 minutes to afford 1-((*R*)-1-(2,4-dichlorophenyl)ethyl)-6-(4-((*S*)-2-(2-hydroxypropan-2-yl)pyrrolidin-1-yl)-5-methylcyclohex-1-en-1-yl)-1*H*-pyrazolo[3,4-*b*]pyrazine-3-carbonitrile trifluoroacetate as a mixture of diastereomers (**33**, 3% yield). ^{1}H NMR (400 MHz, $CDCl_3$; TFA Salt) δ 9.10 (s, 1H), 7.52 – 7.49 (m, 1H), 7.47 – 7.41 (m, 1H), 7.36 – 7.31 (m, 1H), 7.04 – 6.95 (m, 1H), 6.74 (q, *J* = 7.0 Hz, 1H), 4.04 – 3.88 (m, 1H), 3.72 – 3.60 (m, 2H), 3.56 – 3.40 (m, 1H), 3.23 – 2.92 (m, 2H), 2.91 – 2.62 (m, 2H), 2.33 – 2.20 (m, 2H), 2.19 – 2.03 (m, 4H), 2.00 (d, *J* = 7.2 Hz, 3H), 1.41 – 1.27 (m, 9H). LRMS-ESI$^+$: *m/z* calcd for $C_{28}H_{32}Cl_2F_3N_6O$ $[M+H]^+$ = 538.2; found, 538.

6-((4*S*,5*R*)-4-((*S*)-2-(aminomethyl)pyrrolidin-1-yl)-5-methylcyclohex-1-en-1-yl)-1-((*R*)-1-(2,4-dichlorophenyl)ethyl)-1*H*-pyrazolo[3,4-*b*]pyrazine-3-carbonitrile hydrochloride (34). The reductive amination was performed following general procedure D using 1-((*R*)-1-(2,4-dichlorophenyl)ethyl)-6-(5-methyl-4-oxocyclohex-1-en-1-yl)-1*H*-pyrazolo[3,4-*b*]pyrazine-3-carbonitrile (**55**, 120 mg, 0.28 mmol), *tert*-butyl (*S*)-(pyrrolidin-2-ylmethyl)carbamate (62 mg, 0.31 mmol), and sodium triacetoxyborohydride (120 mg, 0.56 mmol) in 1,2-dichloroethane (1.1 mL) at room temperature for 16 h. The residue was purified by reverse phase preparative HPLC (Gemini-NX, 10 µm, 250 x 30 mm, C18 column, Phenomenex, Torrance, CA), eluent: 0 to 100% acetonitrile in water, both eluents containing 0.1% TFA, gradient elution over 30 minutes to afford the desired isomer as the second eluting peak. The residue was diluted with dichloromethane, washed with saturated aqueous sodium bicarbonate, dried over sodium sulfate, and concentrated by rotary evaporation. The free base was treated with HCl (4 N in 1,4-dioxane,

1 mL), stirred for 1 h, and concentrated under reduced pressure to afford 6-((4*S*,5*R*)-4-((*S*)-2-(aminomethyl)pyrrolidin-1-yl)-5-methylcyclohex-1-en-1-yl)-1-((*R*)-1-(2,4-dichlorophenyl)ethyl)-1*H*-pyrazolo[3,4-*b*]pyrazine-3-carbonitrile hydrochloride (**34**, 5% yield). ^{1}H NMR (400 MHz; CD_3OD; HCl salt) δ 9.10 (s, 1H), 7.50 (d, *J* = 2.1 Hz, 1H), 7.45 (d, *J* = 8.5 Hz, 1H), 7.34 (dd, *J* = 8.5, 2.1 Hz, 1H), 6.99 – 6.93 (m, 1H), 6.74 (q, *J* = 7.0 Hz, 1H), 4.27 – 4.18 (m, 1H), 3.86 – 3.77 (m, 2H), 3.77 – 3.70 (m, 2H), 3.69 – 3.63 (m, 4H), 3.60 – 3.54 (m, 2H), 3.49 – 3.38 (m, 2H), 2.96 – 2.82 (m, 2H), 2.74 – 2.62 (m, 1H), 2.44 – 2.30 (m, 1H), 2.28 – 2.08 (m, 2H), 2.01 (d, *J* = 7.1 Hz, 3H), 1.18 (d, *J* = 6.0 Hz, 3H). LRMS-ESI$^+$: *m/z* calcd for $C_{26}H_{29}Cl_2N_7$ $[M+H]^+$ = 510.2; found, 510.

(*S*)-1-((1*S*,6*R*)-4-(3-cyano-1-((*R*)-1-(2,4-dichlorophenyl)ethyl)-1*H*-pyrazolo[3,4-*b*]pyrazin-6-yl)-6-methylcyclohex-3-en-1-yl)pyrrolidine-2-carboxamide hydrochloride (35). The reductive amination was performed following general procedure D using 1-((*R*)-1-(2,4-dichlorophenyl)ethyl)-6-(5-methyl-4-oxocyclohex-1-en-1-yl)-1*H*-pyrazolo[3,4-*b*]pyrazine-3-carbonitrile (**55**, 120 mg, 0.28 mmol), *L*-prolinamide (46 mg, 0.40 mmol), and sodium triacetoxyborohydride (119 mg, 0.56 mmol) in 1,2-dichloroethane (1.1 mL) at room temperature for 16 h. The residue was purified by reverse phase preparative HPLC (Gemini-NX, 10 µm, 250 x 30 mm, C18 column, Phenomenex, Torrance, CA), eluent: 0 to 100% acetonitrile in water, both eluents containing 0.1% TFA, gradient elution over 30 minutes to afford the desired isomer. The residue was diluted with dichloromethane, washed with saturated aqueous sodium bicarbonate, dried over sodium sulfate, and concentrated by rotary evaporation. The free base was treated with HCl (4 N in 1,4-dioxane, 1 mL), stirred for 1 h, and concentrated under reduced pressure to afford (*S*)-1-((1*S*,6*R*)-4-(3-cyano-1-((*R*)-1-(2,4-dichlorophenyl)ethyl)-1*H*-pyrazolo[3,4-*b*]pyrazin-6-yl)-6-methylcyclohex-3-en-1-yl)pyrrolidine-2-carboxamide

hydrochloride (**35**, 8% yield). ^{1}H NMR (400 MHz, CD_3CN; HCl salt) δ 9.02 (s, 1H), 7.55 – 7.48 (m, 2H), 7.36 (dd, *J* = 8.5, 2.2 Hz, 1H), 7.08 (s, 1H), 6.92 – 6.78 (m, 1H), 6.77 – 6.63 (m, 2H), 4.43 (dd, *J* = 10.4, 3.6 Hz, 1H), 4.02 – 3.89 (m, 1H), 3.69 – 3.61 (m, 1H), 2.89 – 2.35 (m, 8H), 2.25 – 2.13 (m, 2H), 1.99 (d, *J* = 7.0 Hz, 3H), 1.14 (d, *J* = 6.9 Hz, 3H). LRMS-ESI$^+$: *m/z* calcd for $C_{26}H_{27}Cl_2N_7O$ $[M+H]^+$ = 524.2; found, 524.

1-((*R*)-1-(2,4-dichlorophenyl)ethyl)-6-((4*S*,5*R*)-5-methyl-4-((*R*)-2-methylpyrrolidin-1-yl)cyclohex-1-en-1-yl)-1*H*-pyrazolo[3,4-*b*]pyrazine-3-carbonitrile hydrochloride (36). The reductive amination was performed following general procedure D using 1-((*R*)-1-(2,4-dichlorophenyl)ethyl)-6-(5-methyl-4-oxocyclohex-1-en-1-yl)-1*H*-pyrazolo[3,4-*b*]pyrazine-3-carbonitrile (**55**, 120 mg, 0.28 mmol), (*R*)-2-methylpyrrolidine (34 mg, 0.4 mmol), and sodium triacetoxyborohydride (119 mg, 0.56 mmol) in 1,2-dichloroethane (1.1 mL) at room temperature for 16 h. The residue was purified by reverse phase preparative HPLC (Gemini-NX, 10 µm, 250 x 30 mm, C18 column, Phenomenex, Torrance, CA), eluent: 0 to 100% acetonitrile in water, both eluents containing 0.1% TFA, gradient elution over 30 minutes to afford a mixture of diastereomers. The mixture was further purified using chiral preparative SFC (OD-H (2 x 25 cm), 30% methanol with 0.1% diethyl amine and CO_2 at 100 bar, 60 mL/min). The third eluting isomer was isolated from this purification and converted to the HCl salt by diluting in dichloromethane (2 mL), adding HCl (2 N in diethyl ether, 0.5 mL), and concentrating to afford 1-((*R*)-1-(2,4-dichlorophenyl)ethyl)-6-((4*S*,5*R*)-5-methyl-4-((*R*)-2-methylpyrrolidin-1-yl)cyclohex-1-en-1-yl)-1*H*-pyrazolo[3,4-*b*]pyrazine-3-carbonitrile hydrochloride (**36**, 7% yield). ^{1}H NMR (400 MHz, $CDCl_3$,HCl salt) δ 8.94 (s, 1H), 7.43 (d, *J* = 8.5 Hz, 1H), 7.39 (d, *J* = 2.2 Hz, 1H), 7.22 (dd, *J* = 8.5, 2.1 Hz, 1H), 6.90 – 6.84 (m, 1H), 6.74 (q, *J* = 7.1 Hz, 1H), 3.34 – 3.07 (m, 1H), 2.86 – 2.24 (m, 8H), 1.99 (d, *J* = 7.1 Hz, 4H), 1.90 – 1.71 (m, 2H), 1.55 – 1.42 (m,

1H), 0.99 (t, *J* = 7.4 Hz, 6H). ^{13}C NMR (101 MHz; $CDCl_3$; HCl salt) δ 152.4, 142.0, 141.5, 136.1, 134.9, 133.4, 132.8, 132.3, 129.6, 129.2, 129.1, 128.0, 118.4, 111.9, 67.1, 60.1, 57.6, 53.6, 49.6, 32.6, 30.1, 28.1, 25.7, 20.3, 19.9, 13.7, 13.5. LRMS-ESI$^+$: *m/z* calcd for $C_{26}H_{28}Cl_2N_6$ $[M+H]^+$ = 495.2; found, 495.

1-((*R*)-1-(2,4-dichlorophenyl)ethyl)-6-(5-methyl-4-((*S*)-2-(trifluoromethyl)pyrrolidin-1-yl)cyclohex-1-en-1-yl)-1*H*-pyrazolo[3,4-*b*]pyrazine-3-carbonitrile hydrochloride (37). The reductive amination was performed following general procedure D using 1-((*R*)-1-(2,4-dichlorophenyl)ethyl)-6-(5-methyl-4-oxocyclohex-1-en-1-yl)-1*H*-pyrazolo[3,4-*b*]pyrazine-3-carbonitrile (**55**, 120 mg, 0.28 mmol), (*S*)-2-(trifluoromethyl)pyrrolidine (43 mg, 0.31 mmol), and sodium triacetoxyborohydride (120 mg, 0.56 mmol) in 1,2-dichloroethane (1.1 mL) at room temperature for 16 h. The residue was purified by reverse phase preparative HPLC (Gemini-NX, 10 µm, 250 x 30 mm, C18 column, Phenomenex, Torrance, CA), eluent: 0 to 100% acetonitrile in water, both eluents containing 0.1% TFA, gradient elution over 30 minutes to afford the desired product as a mixture of isomers. The residue was diluted with dichloromethane, washed with saturated aqueous sodium bicarbonate, dried over sodium sulfate, and concentrated by rotary evaporation. The free base was treated with HCl (4 N in 1,4-dioxane, 1 mL), stirred for 1 h, and concentrated under reduced pressure to afford 1-((*R*)-1-(2,4-dichlorophenyl)ethyl)-6-(5-methyl-4-((*S*)-2-(trifluoromethyl)pyrrolidin-1-yl)cyclohex-1-en-1-yl)-1*H*-pyrazolo[3,4-*b*]pyrazine-3-carbonitrile hydrochloride (**37**, 6% yield). ^{1}H NMR (400 MHz, CD_3OD; HCl Salt) δ 9.04 (s, 1H), 7.47 (d, *J* = 2.1 Hz, 1H), 7.45 (d, *J* = 8.5 Hz, 1H), 7.31 (dd, *J* = 8.5, 2.1 Hz, 1H), 7.01 (dd, *J* = 5.9, 2.1 Hz, 1H), 6.72 (q, *J* = 7.0 Hz, 1H), 4.39 – 4.23 (m, 1H), 3.54 – 3.43 (m, 1H), 3.28 – 3.21 (m, 1H), 3.20 – 3.09 (m, 1H), 2.98 – 2.88 (m, 2H), 2.70 – 2.56 (m, 1H), 2.30 – 2.11

(m, 3H), 2.10 – 2.00 (m, 2H), 1.99 (d, *J* = 7.1 Hz, 3H), 1.99 – 1.85 (m, 1H). LRMS-ESI$^+$: *m/z* calcd for $C_{26}H_{25}Cl_2F_3N_6$ $[M+H]^+$ = 549.2; found, 549.

(*R*)-6-chloro-*N*-(1-(2,4-dichlorophenyl)ethyl)pyrazin-2-amine (38). The S_NAr was performed according to general procedure A using 2,6-dichloropyrazine (3.0 g, 20.1 mmol) and (*R*)-1-(2,4-dichlorophenyl)ethan-1-amine (1.0 equiv) in DMSO (40 mL) along with the addition of CsF (3.0 equiv) at 75 °C for 45 minutes. The residue was purified by silica gel chromatography (50% ethyl acetate in hexanes) to afford (*R*)-6-chloro-*N*-(1-(2,4-dichlorophenyl)ethyl)pyrazin-2-amine (**38**, 79% yield). LRMS-ESI$^+$: m/z calcd for $C_{12}H_{10}Cl_3N_3$ $[M+H]^+$ = 302.0; found, 302.

6-chloro-1-(1-(2,4-dichlorophenyl)ethyl)-1*H*-pyrazolo[3,4-*b*]pyrazine (39). Step 1. To a solution 1-(2,4-dichlorophenyl)ethan-1-one (10.3 g, 54.4 mmol) in EtOH (50 mL) at room temperature was added tert-butyl carbazate (21.6 g, 163.5 mmol). The reaction was stirred at room temperature for 2 d, then at 50 °C for 1 h. The mixture was cooled to 0 °C and filtered. The filtrate was concentrated, and the residue was recrystallized from EtOH. The solids were combined and purified using silica gel chromatography (0 to 25% ethyl acetate in hexanes). The residue was dissolved in EtOH (125 mL) and one crystal of bromocresol green was added. $NaCNBH_3$ (11.8 g, 188 mmol) was added and AcOH was added dropwise to maintain a yellow color. The reaction was stirred at 60 °C for 2 d and AcOH was added dropwise to maintain a yellow color. The mixture was cooled to room temperature, concentrated, and purified by silica gel chromatography (10 to 20% MTBE in hexanes). The residue was dissolved in dioxane (0.17 M) and HCl (4 N in 1,4-dioxane, 10 equiv) was added. The mixture was stirred at 50 °C for 16 h, and a white precipitate formed. The mixture was cooled to room temperature and concentrated

under reduced pressure to afford (1-(2,4-dichlorophenyl)ethyl)hydrazine hydrochloride (75% yield). LRMS-ESI$^+$: m/z calcd for $C_8H_{10}Cl_2N_2$ $[M+H]^+$ = 205.0; found, 205.

Step 2. To a solution of 2,2,6,6-tetramethylpiperidine (22.9 mL, 134.3 mmol) in THF (200 mL) at -40 °C was added n-BuLi (2.5 M in hexanes, 56.4 mL, 141.0 mmol). The mixture was stirred at -40 °C for 30 min. In a separate flask, ethyl formate (10.9 mL, 134.3 mmol) and 2,6-dichloropyrazine (10.0 g, 67.1 mmol) were dissolved in THF (200 mL) and cooled to -90 °C. The lithium 2,2,6,6-tetramethylpiperidine solution was added to the 2,6-dichloropyrazine solution via cannula over 30 min at -90 °C. The mixture was stirred at -90 °C for 1 h and then acetic acid (7.68 mL, 134.3 mmol) was added, followed by (1-(2,4-dichlorophenyl)ethyl)hydrazine hydrochloride (8.11 g, 33.56 mmol). The reaction was warmed to room temperature and stirred at room temperature for 6 h. The mixture was filtered through a silica gel-celite plug, concentrated under reduced pressure and then the residue was purified by normal-phase column chromatography on silica gel (20 to 100% ethyl acetate in hexanes) to afford a mixture of (*Z*)-3,5-dichloro-2-((2-(1-(2,4-dichlorophenyl)ethyl)hydrazono)methyl)pyrazine and (*E*)-3,5-dichloro-2-((2-(1-(2,4-dichlorophenyl)ethyl)hydrazono)methyl)pyrazine (25% yield) as a viscous orange oil. LRMS-ESI$^+$: m/z calcd for $C_{13}H_{10}Cl_4N_4$ $[M+H]^+$ = 363.0; found, 363.

Step 3. (*Z*)-3,5-dichloro-2-((2-(1-(2,4-dichlorophenyl)ethyl)hydrazono)methyl)pyrazine and (*E*)-3,5-dichloro-2-((2-(1-(2,4-dichlorophenyl)ethyl)hydrazono)methyl)pyrazine (1.0 g, 2.75 mmol) were dissolved in DMF (50 mL) and the solution was degassed. 1,8-Diazabicyclo[5.4.0]undec-7-ene (0.82 mL, 5.49 mmol) was added and the mixture was heated to 140 °C for 2 h. After cooling, the mixture was diluted with water and ethyl acetate and the layers were separated. The organic layer was washed with brine solution, dried over sodium sulfate,

concentrated via rotary evaporation, and the residue was purified by silica gel chromatography (0 to 100% ethyl acetate in hexanes) to afford 6-chloro-1-(1-(2,4-dichlorophenyl)ethyl)-1*H*-pyrazolo[3,4-*b*]pyrazine (**39**, 10% yield). LRMS-ESI$^+$: m/z calcd for $C_{13}H_9Cl_3N_4$ $[M+H]^+$ = 327.0; found, 327.

(*R*)-(1-(2,4-dichlorophenyl)ethyl)hydrazine hydrochloride (40). Step 1. To a 6 L flask equipped with overhead stirrer was added (*R*)-1-(2,4-dichlorophenyl)ethan-1-amine (211 g, 1.11 mol), water (3.4 L), and concentrated HCl (92.5 mL, 1.11 mol). To the slurry was added solid KOCN (90 g, 1.11 mol) in one portion at room temperature. All solids went into solution and a white precipitate began to form after 1 h. The white precipitate was isolated by filtration. The filtrate was left standing at room temperature and more precipitate formed. The precipitate was again isolated by filtration. This was repeated several times until no more precipitate formed in the filtrate upon standing at room temperature for 1 d. All the solids were combined and dried under high vacuum yielding (*R*)-1-(1-(2,4-dichlorophenyl)ethyl)urea (78% yield). LRMS-ESI$^+$: *m/z* calcd for $C_9H_{10}N_2O$ $[M+H]^+$ = 232.0; found, 232.

Step 2. (*R*)-1-(1-(2,4-dichlorophenyl)ethyl)urea (50 g, 214.6 mmol) was milled into a fine powder and placed into an oven dried 2 L flask. The 2 L flask was purged with nitrogen gas and a degassed mixture of 1 L of toluene and 375 mL of *t*-BuOH was added via cannula under nitrogen. Solid *t*-BuOK (240.3 g, 2146 mmol) was milled into fine powder and added to a separate 5 L, 3 neck flask. The 5 L flask was purged with nitrogen, and a degassed mixture of 1 L of toluene and 650 mL of *t*-BuOH was added via cannula under nitrogen gas. The 2 L and the 5 L mixtures were slurries and were cooled to -20 °C. The lights inside the hood were turned off before *t*BuOCl (23.18 g, 24 mL, 214.6 mmol) was added to the 2 L flask at -20 °C. The -20 °C bath was removed and the mixture was placed in a 0 °C bath. As soon as the slurry went all into

solution, the mixture was transferred to the 5 L flask via cannula under nitrogen at -20 °C. The lights in the hood were turned on, the -20 °C bath was removed, and the mixture was placed into 0 °C bath. The mixture was stirred at 0 °C for 10 min and then warmed to room temperature, at which time the mixture was poured onto ice. The mixture was extracted with EtOAc (x2) and the combined organic layers were washed with 1 L of water, 500 mL of saturated sodium thiosulfate, and 1 L of brine solution. The solvents were concentrated under reduced pressure to afford *tert*-butyl (*R*)-2-(1-(2,4-dichlorophenyl)ethyl)hydrazine-1-carboxylate (99% yield). Ee: 99% Chiralpak® IF-3; 250mmx4.6mm, 5% iPrOH in heptanes; flow rate 1 mL/min; detection at 254 nm; t_1 = 4.5 min (minor) t_2 = 5.3 min (major). LRMS-ESI$^+$: *m/z* calcd for $C_{13}H_{18}Cl_2N_2O_2$ $[M+H]^+$ = 306.2; found, 306. Note: vigorous stirring of the solution is critical for the success of this reaction.

The residue was dissolved in 250 mL of dioxane and HCl (4 N in 1,4-dioxane, 161 mL, 643.8 mmol) was added at room temperature. The mixture was stirred at room temperature overnight and then concentrated under reduced pressure. The residue was triturated from 25% ethyl acetate in hexanes (1 mL of solvent per 1 g of residue) to afford *(R)*-(1-(2,4-dichlorophenyl)ethyl)hydrazine hydrochloride (**40**, 78% yield). ^{1}H NMR (300 MHz, Methanol-*d*) δ 7.63 (d, *J* = 8.4 Hz, 1H), 7.52 (d, *J* = 2.1 Hz, 1H), 7.42 (dd, *J* = 8.4, 2.1 Hz, 1H), 4.67 (q, *J* = 6.9 Hz, 1H), 1.40 (d, *J* = 6.9 Hz, 3H). Ee: 98% Chiralpak® IF-3; 250mmx4.6mm, 20% iPrOH in heptanes; flow rate 1 mL/min; detection at 254 nm; t_1 = 4.7 min (major) t_2 = 6.9 min (minor). LRMS-ESI$^+$: *m/z* calcd for $C_8H_{10}Cl_2N2$ $[M+H]^+$ = 206.2; found, 206.

(*R*)-6-chloro-1-(1-(2,4-dichlorophenyl)ethyl)-1*H*-pyrazolo[3,4-*b*]pyrazine-3-carboxylate (44). Step 1. Ethyl 2-(3,5-dichloropyrazin-2-yl)-2-oxoacetate (**41**, 14.5 g, 58.4 mmol) and (*R*)-(1-(2,4-dichlorophenyl)ethyl)hydrazine hydrochloride (**40**, 11.7 g, 48.7 mmol)

were dissolved in THF (97 mL), heated to 80 °C for 2 h, and stirred at room temperature for 16 h. The mixture was diluted with brine, extracted with ethyl acetate, and the combined organic layers were dried over magnesium sulfate and concentrated to afford a mixture of ethyl (*R*,*E*)-2-(2-(1-(2,4-dichlorophenyl)ethyl)hydrazineylidene)-2-(3,5-dichloropyrazin-2-yl)acetate (**42**) and (*R*,*Z*)-2-(2-(1-(2,4-dichlorophenyl)ethyl)hydrazineylidene)-2-(3,5-dichloropyrazin-2-yl)acetate (**43**) as a red oil, which was used directly in the next reaction without further purification. LRMS-ESI$^+$: m/z calcd for $C_{16}H_{14}Cl_4N_4O_2$ $[M+H]^+$ = 435.0; found, 435.

Step 2. The mixture of ethyl (*R*,*E*)-2-(2-(1-(2,4-dichlorophenyl)ethyl)hydrazono)-2-(3,5-dichloropyrazin-2-yl)acetate (**42**) and (*R*,*Z*)-2-(2-(1-(2,4-dichlorophenyl)ethyl)hydrazineylidene)-2-(3,5-dichloropyrazin-2-yl)acetate (**43**) was diluted with THF (240 mL), cooled to 0 °C, and NaH (60% dispersion in mineral oil, 3.89 g, 97.4 mmol) was added carefully. The reaction was stirred overnight, monitoring by TLC and LC-MS. The reaction was slowly poured into a mixture of crushed ice (~500 mL) and saturated aqueous ammonium chloride (300 mL) under vigorous stirring. The aqueous layer was extracted with EtOAc (2 x 400mL). The combined organic layers were dried over sodium sulfate and concentrated by rotary evaporation. The residue was purified by silica gel chromatography (10% to 20% ethyl acetate in hexanes) to afford ethyl (*R*)-6-chloro-1-(1-(2,4-dichlorophenyl)ethyl)-1*H*-pyrazolo[3,4-*b*]pyrazine-3-carboxylate (**44**) as a sticky orange oil (50% yield). LRMS-ESI$^+$: m/z calcd for $C_{16}H_{13}Cl_3N_4O_2$ $[M+H]^+$ = 399.0; found, 399.

(*R*)-6-chloro-1-(1-(2,4-dichlorophenyl)ethyl)-1*H*-pyrazolo[3,4-*b*]pyrazine-3-carboxamide (45). Ethyl (*R*)-6-chloro-1-(1-(2,4-dichlorophenyl)ethyl)-1*H*-pyrazolo[3,4-*b*]pyrazine-3-carboxylate (**44**, 4.0 g, 10 mmol) was dissolved in 1,4-dioxane (40 mL) in a sealed tube. The reaction solution was diluted with 29% ammonium hydroxide in water (40 mL), and

the tube was quickly sealed. The reaction was stirred at room temperature for 3 days, monitoring by LC-MS. The reaction was driven to completion by bubbling ammonia gas gently through the solution. The crude mixture was poured into 200 mL of water and a white precipitate formed. The white precipitate was collected and rinsed with 200 mL of water and 50 mL of hexanes. The resulting solid was dried overnight on high vacuum to afford (*R*)-6-chloro-1-(1-(2,4-dichlorophenyl)ethyl)-1*H*-pyrazolo[3,4-*b*]pyrazine-3-carboxamide (**45**) as an off-white powder (95% yield). LRMS-ESI$^+$: m/z calcd for $C_{14}H_{10}Cl_3N_5O$ $[M+H]^+$ = 370.0; found, 370.

(*R*)-6-chloro-1-(1-(2,4-dichlorophenyl)ethyl)-1*H*-pyrazolo[3,4-*b*]pyrazine-3-carbonitrile (46). (*R*)-6-chloro-1-(1-(2,4-dichlorophenyl)ethyl)-1*H*-pyrazolo[3,4-*b*]pyrazine-3-carboxamide (**45**, 4.47 g, 12.1 mmol) was dissolved in dry dichloromethane (30 mL) under argon gas before adding Burgess' reagent (4.3 g, 18.1 mmol). The reaction was stirred at room temperature and monitored by TLC. Upon the disappearance of starting material, the reaction was diluted with saturated aqueous sodium bicarbonate and dichloromethane. The organic layer was separated, and the aqueous layer was extracted with dichloromethane. The combined organic layers were dried over magnesium sulfate and concentrated. The crude product was purified by silica gel chromatography (5% to 20% ethyl acetate in hexanes) to afford (*R*)-6-chloro-1-(1-(2,4-dichlorophenyl)ethyl)-1*H*-pyrazolo[3,4-*b*]pyrazine-3-carbonitrile (**46**) as a clear, colorless, sticky solid (88% yield). ^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.69 (s, 1H), 7.43 (d, *J* = 8.5 Hz, 1H), 7.41 (d, *J* = 2.2 Hz, 1H), 7.27 (dd, *J* = 8.5, 2.2 Hz, 1H), 6.68 (q, *J* = 7.0 Hz, 1H), 1.98 (d, *J* = 7.0 Hz, 3H). LRMS-ESI$^+$: m/z calcd for $C_{14}H_8Cl_3N_5$ $[M+H]^+$ = 352.0; found, 352.

((2*S*)-1-(3-methylpiperidin-4-yl)pyrrolidin-2-yl)methanol 2,2,2-trifluoroacetate (49). To a solution of L-prolinol (**47**, 152 mg, 1.5 mmol) and 1-*tert*-butoxycarbonyl-3-methyl-4-piperidone (**48**, 213 mg, 1.0 mmol) in 1,2-dichloroethane (0.3 M) was added acetic acid (1.5

equiv) and sodium triacetoxyborohydride (316 mg, 1.5 mmol). The solution was stirred at room temperature for 16 h and the residue was purified by silica gel chromatography (0 to 20% methanol in dichloromethane) to afford the *cis*-diastereomer of *tert*-butyl 4-((*S*)-2-(hydroxymethyl)pyrrolidin-1-yl)-3-methylpiperidine-1-carboxylate (40% yield). The residue was dissolved in dichloromethane (2.0 mL) and trifluoroacetic acid (0.5 mL) was added. The mixture was stirred at room temperature for 1 h and then concentrated under reduced pressure to afford the *cis*-diastereomer of ((2*S*)-1-(3-methylpiperidin-4-yl)pyrrolidin-2-yl)methanol 2,2,2-trifluoroacetate (**49**) which was used without further purification. LRMS-ESI$^+$: *m/z* calcd for $C_{11}H_{22}N_2O$ $[M+H]^+$ = 199.2; found, 199.

6-methyl-1,4-dioxaspiro[4.5]decan-8-one (51). To a 2 L flask equipped with a stir bar was added CuCN (58.1 g, 0.649 mol) and Et_2O (1.6 L). The solution was cooled to -40 °C before slowly adding MeLi (209 mL, 3.1 M in DME, 0.649 mol). As time passed the reaction produced a bright yellow precipitate. In a separate 5 L flask, 1,4-dioxaspiro[4.5]dec-6-en-8-one (**50**, 50 g, 0.324 mol) was dissolved in a mixture of Et_2O (640 mL) and THF (640 mL). The solution was cooled to -40 °C before slowly adding TMSCl (80 mL, 0.63 mol). After the cuprate solution had stirred for 30 min, it was cannulated into the enone solution at -40 °C (some yellow solid remained in cuprate flask). The reaction was stirred at -40 °C for 1 h, then quenched with 800 mL of 3:1 saturated aqueous NH_4Cl/NH_4OH solution and slowly warmed to room temperature. The organic layer was separated, and the aqueous layer was extracted with EtOAc. The combined organic layers were washed with brine, dried over Na_2SO_4, and concentrated via rotary evaporation. The residue was purified by silica gel chromatography (50% EtOAc in hexanes) to afford a mixture of desired product and the corresponding silyl enol ether. The mixture was resuspended dichloromethane and washed twice with 1 N aqueous HCl solution, dried over

Na_2SO_4, and concentrated to afford 6-methyl-1,4-dioxaspiro[4.5]decan-8-one (**51**) as an orange oil, which was carried forward without further purification (98% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.03 (s, 4H), 2.57 – 2.31 (m, 4H), 2.29 – 2.15 (m, 1H), 2.10 – 1.98 (m, 1H), 1.88 – 1.78 (m, 1H), 0.96 (d, J = 6.7 Hz, 3H). LRMS-ESI$^+$: m/z calcd for $C_9H_{15}O_3$ $[M+H]^+$ = 171.1; found, 171.

1-((*R*)-1-(2,4-dichlorophenyl)ethyl)-6-(10-methyl-1,4-dioxaspiro[4.5]dec-7-en-8-yl)-1*H*-pyrazolo[3,4-*b*]pyrazine-3-carbonitrile (54). Step 1. To a solution of *i*-Pr_2NH (53.5 mL, 0.381 mol) in THF (200 mL) cooled to -78 °C was added *n*-BuLi (152 mL, 2.5 M in hexanes, 0.380 mol). The mixture was warmed to 0 °C and stirred for 30 min. In a separate flask 6-methyl-1,4-dioxaspiro[4.5]decan-8-one (**51**, 54 g, 0.317 mol) was dissolved in THF (1.15 L). The solution was cooled to -78 °C before slowly adding the LDA solution. After stirring for 30 min, a solution of $PhNTf_2$ (136 g, 0.381 mol) in THF (400 mL) was added and the resulting mixture was warmed to room temperature and stirred overnight. The reaction was partitioned between EtOAc and saturated aqueous $NaHCO_3$. The organic layer was separated, and the aqueous layer was extracted with EtOAc. The combined organic layers were dried over Na_2SO_4 and concentrated by rotary evaporation. The residue was purified by silica gel chromatography (5 to 10% EtOAc in hexanes) to provide a mixture of desired product and PhNHTf. The mixture was resuspended in EtOAc, washed three times with 1 N aqueous NaOH, washed with brine, dried over Na_2SO_4, and concentrated to afford a 2:1 mixture of olefin isomers 10-methyl-1,4-dioxaspiro[4.5]dec-7-en-8-yl trifluoromethanesulfonate and 6-methyl-1,4-dioxaspiro[4.5]dec-7-en-8-yl trifluoromethanesulfonate (**52**) as a clear, colorless oil (36% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.61 (tt, J = 3.7, 1.4 Hz, 0.66H; diagnostic peak for major isomer), 5.56 (dt, J = 3.6, 1.4 Hz, 0.34H; diagnostic peak for minor isomer), 4.04 – 3.92 (m, 4H), 2.64 – 2.56 (m, 1H), 2.54 –

2.35 (m, 1H), 2.34 – 2.25 (m, 1H), 2.18 – 1.74 (m, 2H), 1.06 (d, J = 7.1 Hz, 1H; diagnostic peak for minor isomer), 1.00 (d, J = 6.8 Hz, 2H; diagnostic peak for major isomer). LRMS-ESI$^+$: m/z calcd for $C_{10}H_{14}F_3O_5S$ $[M+H]^+$ = 303.1; found, 303.

Step 2. To a solution of 10-methyl-1,4-dioxaspiro[4.5]dec-7-en-8-yl trifluoromethanesulfonate (**52**, 6.5 g, 21.5 mmol) in 1,4-dioxane (108 mL) was added bis(pinacolato)diboron (13.7 g, 53.8 mmol), KOAc (6.33 g, 64.5 mmol), and KBr (2.81 g, 23.7 mmol). The mixture was degassed by bubbling nitrogen gas through the solution for 10 min, then Pd(dppf)Cl_2 (787 mg, 1.08 mmol) was added and the resulting solution was degassed for another 15 min. The reaction was heated to 95 °C for 16 h. After the mixture was cooled to room temperature, it was partitioned between EtOAc and water. The organic layer was separated, and the aqueous layer was extracted with EtOAc. The combined organic layers were washed with brine, dried over Na_2SO_4, and concentrated by rotary evaporation. The residue was purified by silica gel chromatography (0 to 10% EtOAc in dichloromethane) to afford a 2:1 mixture of olefin isomers 4,4,5,5-tetramethyl-2-(10-methyl-1,4-dioxaspiro[4.5]dec-7-en-8-yl)-1,3,2-dioxaborolane and 4,4,5,5-tetramethyl-2-(6-methyl-1,4-dioxaspiro[4.5]dec-7-en-8-yl)-1,3,2-dioxaborolane (**53**) as a yellow oil (81% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.41 (tt, J = 3.9, 1.9 Hz, 0.66H; diagnostic peak for major isomer), 6.32 (dt, J = 3.2, 2.0 Hz, 0.33H; diagnostic peak for minor isomer), 4.01 – 3.90 (m, 4H), 2.53 – 2.21 (m, 3H), 2.17 – 2.07 (m, 1H), 2.00 – 1.55 (m, 1H), 1.24 (s, 12H), 1.02 (d, J = 7.3 Hz, 1H; diagnostic peak for minor isomer), 0.92 (d, J = 6.7 Hz, 2H; diagnostic peak for major isomer). LRMS-ESI$^+$: m/z calcd for $C_{15}H_{26}BO_4$ $[M+H]^+$ = 281.2; found, 281.

Step 3. To a solution of (*R*)-6-chloro-1-(1-(2,4-dichlorophenyl)ethyl)-1*H*-pyrazolo[3,4-*b*]pyrazine-3-carbonitrile (**46**, 1.67 g, 4.74 mmol), 4,4,5,5-tetramethyl-2-(10-methyl-1,4-

dioxaspiro[4.5]dec-7-en-8-yl)-1,3,2-dioxaborolane (**53**, 1.99 g, 7.10 mmol), and 1 N aqueous Na_2CO_3 (14.2 mL, 14.2 mmol) in 1,4-dioxane (15.8 mL) was added $Pd(dppf)_2Cl_2$ (0.20 g, 0.240 mmol). The reaction mixture was degassed by bubbling nitrogen through the solution for 15 min before heating the reaction to 95 °C for 75 min. The reaction was concentrated to dryness and the residue was partitioned between EtOAc and saturated aqueous $NaHCO_3$. The organic layer was separated, and the aqueous was extracted with EtOAc. The combined organic layers were washed with brine, dried over Na_2SO_4, and concentrated by rotary evaporation. The residue was purification by silica gel chromatography (20 to 30% EtOAc in pentanes) to afford 1-((*R*)-1-(2,4-dichlorophenyl)ethyl)-6-(10-methyl-1,4-dioxaspiro[4.5]dec-7-en-8-yl)-1*H*-pyrazolo[3,4-*b*]pyrazine-3-carbonitrile (**54**) as the second eluting isomer as a pale orange foam (47% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.95 (d, J = 1.8 Hz, 1H), 7.42 (dd, J = 8.5, 4.5 Hz, 1H), 7.39 (dd, J = 2.2, 0.4 Hz, 1H), 7.23 (ddt, J = 8.5, 2.2, 0.5 Hz, 1H), 6.82 (tt, J = 4.2, 2.2 Hz, 1H), 6.73 (q, J = 7.0 Hz, 1H), 4.10 – 3.95 (m, 4H), 3.03 – 2.86 (m, 1H), 2.66 – 2.46 (m, 3H), 2.19 (h, J = 7.2 Hz, 1H), 1.99 (d, J = 7.1 Hz, 3H), 1.07 (dd, J = 6.8, 3.8 Hz, 3H). LRMS-ESI$^+$: m/z calcd for $C_{23}H_{21}Cl_2N_5O_2$ $[M+H]^+$ = 470.1; found, 470.

1-((*R*)-1-(2,4-dichlorophenyl)ethyl)-6-(5-methyl-4-oxocyclohex-1-en-1-yl)-1*H*-pyrazolo[3,4-*b*]pyrazine-3-carbonitrile (55). To a solution of 1-((*R*)-1-(2,4-dichlorophenyl)ethyl)-6-(10-methyl-1,4-dioxaspiro[4.5]dec-7-en-8-yl)-1*H*-pyrazolo[3,4-*b*]pyrazine-3-carbonitrile (**54**, 6.1 g, 13.0 mmol) in dichloromethane (173 mL) was added trifluoroacetic acid (29.6 mL, 389 mmol). The reaction was stirred 16 h at room temperature. The mixture was concentrated to ~20% of its original volume, diluted with EtOAc (200 mL), and quenched with saturated aqueous $NaHCO_3$. The organic layer was separated, and the aqueous layer was extracted with EtOAc. The combined organic layers were washed with brine, dried

over Na_2SO_4, and concentrated by rotary evaporation. The residue was purification by silica gel chromatography (25 to 30% EtOAc in hexanes) to afford 1-((*R*)-1-(2,4-dichlorophenyl)ethyl)-6-(5-methyl-4-oxocyclohex-1-en-1-yl)-1*H*-pyrazolo[3,4-*b*]pyrazine-3-carbonitrile (**55**) as an orange solid (87% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.00 (d, J = 2.7 Hz, 1H), 7.45 – 7.43 (m, 1H), 7.41 (dd, J = 3.6, 2.1 Hz, 1H), 7.26 – 7.23 (m, 1H), 6.92 (ddt, J = 6.4, 4.1, 2.1 Hz, 1H), 6.76 (q, J = 7.1 Hz, 1H), 3.45 – 3.10 (m, 3H), 2.86 – 2.75 (m, 1H), 2.69 – 2.54 (m, 1H), 2.00 (dd, J = 7.1, 0.9 Hz, 3H), 1.26 (d, J = 6.6 Hz, 3H). LRMS-ESI$^+$: m/z calcd for $C_{21}H_{18}Cl_2N_5O_2$ $[M+H]^+$ = 426.1; found, 426.

(7*R*,8*S*)-7-methyl-*N*-((*S*)-1-phenylethyl)-1,4-dioxaspiro[4.5]decan-8-amine (58). To a solution of 7-methyl-1,4-dioxaspiro[4.5]decan-8-one (**56**, 20.0 g, 118 mmol) in benzene (14.7 mL) was added (1*S*)-1-phenylethanamine (**57**, 15.2 mL, 118 mmol). The solution was heated to reflux overnight using a Dean-Stark apparatus for removal of water. After 24 h, the solution was concentrated under reduced pressure to afford (*R*,*E*)-7-methyl-*N*-((*S*)-1-phenylethyl)-1,4-dioxaspiro[4.5]decan-8-imine which was used without further purification. Sodium triacetoxyborohydride (316 mg, 1.5 mmol) was added portion-wise to a solution of (*R*,*E*)-7-methyl-*N*-((*S*)-1-phenylethyl)-1,4-dioxaspiro[4.5]decan-8-imine (31.7 g, 117 mmol) in dichloromethane (283 mL) and acetic acid (10 mL). The resulting solution was stirred for 16 h at room temperature before being diluted with dichloromethane (300 mL) and quenched with saturated aqueous $NaHCO_3$. The organic layer was washed with brine, dried over Na_2SO_4, and concentrated by rotary evaporation. The residue was purified by silica gel chromatography (20 to 30% EtOAc in hexanes) to afford (7*R*,8*S*)-7-methyl-*N*-((*S*)-1-phenylethyl)-1,4-dioxaspiro[4.5]decan-8-amine (**58**, 65% yield; 10:1 dr) as a pale yellow oil. ^{1}H NMR (400 MHz, Methanol-d4) δ 7.35 – 7.25 (m, 4H), 7.22 – 7.16 (m, 1H), 3.91 – 3.79 (m, 4H), 3.76 (q, J = 7.1

Hz, 1H), 2.57 – 2.52 (m, 1H), 1.70 – 1.49 (m, 3H), 1.47 – 1.32 (m, 3H), 1.31 (d, J = 6.6 Hz, 3H), 1.25 – 1.20 (m, 1H), 1.01 (d, J = 7.1 Hz, 3H; diagnostic peak for major isomer), 0.90 (d, *J* = 7.1 Hz, 0.3H; diagnostic peak for minor isomer). LRMS-ESI$^+$: m/z calcd for $C_{17}H_{26}NO_2$ $[M+H]^+$ = 276.2; found, 276.

Tert-butyl ((7*R*,8*S*)-7-methyl-1,4-dioxaspiro[4.5]decan-8-yl)carbamate (59). A suspension of (7*R*,8*S*)-7-methyl-*N*-((*S*)-1-phenylethyl)-1,4-dioxaspiro[4.5]decan-8-amine (**58**, 12 g, 43.6 mmol) and 20% $Pd(OH)_2$ on carbon (1.2 g, 1.71 mmol, 0.04 equiv) in methanol (80 mL) was placed under a hydrogen atmosphere using a Parr apparatus (40 psi) for 72 h. After sparging with nitrogen gas for 30 minutes, the mixture was filtered through Celite with methanol and the filtrate was concentrated under reduced pressure to afford (7*R*,8*S*)-7-methyl-1,4-dioxaspiro[4.5]decan-8-amine (99% yield) as a colorless oil. LRMS-ESI$^+$: m/z calcd for $C_9H_{18}NO_2$ $[M+H]^+$ = 172.1; found, 172. To a solution of (7*R*,8*S*)-7-methyl-1,4-dioxaspiro[4.5]decan-8-amine (7.88 g, 46.0 mmol) and triethylamine (6.4 mL, 46.0 mmol) in THF (92 mL) was added di-tert-butyl dicarbonate (10.0 g, 46.0 mmol) at room temperature. The resulting solution was stirred for 16 h at room temperature before being diluted with water (200 mL). The aqueous layer was extracted 3 times with dichloromethane, the combined organic layers were dried over Na_2SO_4 and concentrated by rotary evaporation to afford tert-butyl ((7*R*,8*S*)-7-methyl-1,4-dioxaspiro[4.5]decan-8-yl)carbamate (**59**, 98% yield) as a white solid that was used without further purification. LRMS-ESI$^+$: m/z calcd for $C_{14}H_{26}NO_4$ $[M+H]^+$ = 272.2; found, 272.

Tert-butyl *N*-[(1*S*,2*R*)-2-methyl-4-oxo-cyclohexyl]carbamate (60). To a solution of tert-butyl ((7*R*,8*S*)-7-methyl-1,4-dioxaspiro[4.5]decan-8-yl)carbamate (**59**, 6.54 g, 24.0 mmol) in THF (42 mL) and water (42 mL) was added *p*-toluenesulfonic acid monohydrate (9.67 g, 50.8

mmol) at room temperature. The resulting solution was stirred for 16 h at room temperature before being quenched with saturated aqueous $NaHCO_3$. The aqueous layer was extracted twice with ethyl acetate, then the combined organic layers were dried over Na_2SO_4 and concentrated by rotary evaporation. The residue was purified by silica gel chromatography (0 to 100% EtOAc in hexanes) to afford tert-butyl *N*-[(1*S*,2*R*)-2-methyl-4-oxo-cyclohexyl]carbamate (**60** 73% yield) as a white amorphous solid. 1H NMR (400 MHz, $CDCl_3$) δ 5.28 (br s, 1H), 3.82 – 3.76 (m, 1H), 2.25 – 1.95 (m, 5H), 1.85 – 1.74 (m, 1H), 1.73 – 1.63 (m, 1H), 1.20 (s, 9H), 0.72 (d, J = 6.5 Hz, 3H). LRMS-ESI$^+$: m/z calcd for $C_{12}H_{22}NO_3$ $[M+H]^+$ = 228.2; found, 228.

(4*S*,5*R*)-4-((tert-butoxycarbonyl)amino)-5-methylcyclohex-1-en-1-yl trifluoromethanesulfonate (61). To a cooled (-78 ºC) solution of tert-butyl *N*-[(1*S*,2*R*)-2-methyl-4-oxo-cyclohexyl]carbamate (**60**, 1.68 g, 7.39 mmol) in THF (25 mL) was added LiHMDS (1 M in THF, 18.5 mL, 18.5 mmol) dropwise. After 45 min at this temperature, a solution of $PhNTf_2$ (3.17 g, 8.87 mmol) in THF (10 mL) was added. The reaction was stirred for 15 mins at −78 ºC and it was warmed to room temperature. After stirring for 1 h at room temperature, the reaction was quenched with water. The aqueous layer was extracted twice with ethyl acetate, then the combined organic layers were washed with 1 M aq. NaOH (5 times) and brine (once), dried over Na_2SO_4 and concentrated by rotary evaporation to afford (4*S*,5*R*)-4-((tert-butoxycarbonyl)amino)-5-methylcyclohex-1-en-1-yl trifluoromethanesulfonate (**61**, 97% yield, >20:1 dr) as a tan colored solid that was used without further purification. ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.66 (br s, 1H), 4.51 (d, J = 9.2 Hz, 1H), 3.95 – 3.83 (m, 1H), 2.51 – 2.42 (m, 2H), 2.21 – 2.04 (m, 3H), 1.44 (s, 9H), 0.98 (d, J = 6.3 Hz, 3H). LRMS-ESI$^+$: m/z calcd for $C_{13}H_{21}F_3NO_5S$ $[M+H]^+$ = 360.1; found, 360.

(*R*)-1-(1-(2,4-dichlorophenyl)ethyl)-6-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1*H*-pyrazolo[3,4-*b*]pyrazine-3-carbonitrile (62). A sealed tube was charged with a solution of 6-chloro-1-[(1*R*)-1-(2,4-dichlorophenyl)ethyl]pyrazolo[3,4-*b*]pyrazine-3-carbonitrile (**46**, 17.6 g, 49.8 mmol) in 1,4-dioxane (245 mL), followed by potassium acetate (16.7 g, 170 mmol), bis(pinacolato)diboron (12.7 g, 49.8 mmol), and 1,1'-Bis(diphenylphosphino)ferrocene-palladium(II) dichloride (1.82 g, 2.49 mmol). Nitrogen gas was bubbled through the reaction solution for 15 min. The sealed tube was then placed in an oil bath and heated to 90 °C for 2 h. After cooling to room temperature, the reaction mixture was filtered through a pad of Celite and silica gel (1:1), and the pad was rinsed with ethyl acetate (1 L). The residue was purified by silica gel chromatography (0 to 100% EtOAc in hexanes) to afford (*R*)-1-(1-(2,4-dichlorophenyl)ethyl)-6-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1*H*-pyrazolo[3,4-*b*]pyrazine-3-carbonitrile (**62**, 72% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.12 (s, 1H), 7.48 (d, J = 8.5 Hz, 1H), 7.39 (d, J = 2.1 Hz, 1H), 7.26 (dd, J = 8.5, 2.1 Hz, 1H), 6.95 (q, J = 7.0 Hz, 1H), 1.97 (d, J = 7.0 Hz, 3H), 1.43 (s, 12H). LRMS-ESI$^+$: m/z calcd for $C_{20}H_{21}BCl_2N_5O_2$ $[M+H]^+$ = 444.1; found, 444.

Tert-butyl ((1*S*,6*R*)-4-(3-cyano-1-((*R*)-1-(2,4-dichlorophenyl)ethyl)-1*H*-pyrazolo[3,4-*b*]pyrazin-6-yl)-6-methylcyclohex-3-en-1-yl)carbamate (63). A sealed tube was charged with a solution of 1-[(1*R*)-1-(2,4-dichlorophenyl)ethyl]-6-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)pyrazolo[3,4-b]pyrazine-3-carbonitrile (**62**, 8.2 g, 18.5 mmol) and [(4*S*,5*R*)-4-(tert-butoxycarbonylamino)-5-methyl-cyclohexen-1-yl] trifluoromethanesulfonate (**61**, 6.64g, 18.5 mmol) in water (6 mL) and toluene (62 mL). Sodium carbonate (5.87 g, 55.4 mmol) and 1,1'-bis(diphenylphosphino)ferrocene-palladium(II) dichloride (1.35 g, 1.85 mmol) were added and nitrogen gas was bubbled through the reaction solution for 15 min. The sealed tube was then

placed in an oil bath and heated to 90 °C for 16 h. After cooling to room temperature, the reaction mixture was filtered through a pad of Celite and silica gel (1:1), and the pad was rinsed with ethyl acetate (500 mL). The residue was purified by silica gel chromatography (30 to 100% EtOAc in hexanes) to afford tert-butyl ((1*S*,6*R*)-4-(3-cyano-1-((*R*)-1-(2,4-dichlorophenyl)ethyl)-1*H*-pyrazolo[3,4-*b*]pyrazin-6-yl)-6-methylcyclohex-3-en-1-yl)carbamate (**63**, 83% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.94 (s, 1H), 7.43 (d, J = 8.5 Hz, 1H), 7.40 (d, J = 2.1 Hz, 1H), 7.23 (dd, J = 8.5, 2.1 Hz, 1H), 6.88 (s, 1H), 6.74 (q, J = 7.1 Hz, 1H), 4.58 (d, J = 9.3 Hz, 1H), 4.04 – 3.96 (m, 1H), 2.80 – 2.70 (m, 1H), 2.69 – 2.59 (m, 1H), 2.46 – 2.28 (m, 2H), 1.99 (d, J = 7.1 Hz, 3H), 1.45 (s, 9H), 1.07 (d, J = 6.8 Hz, 3H). LRMS-ESI$^+$: m/z calcd for $C_{26}H_{29}Cl_2N_6O_2$ $[M+H]^+$ = 527.2; found, 527.

6-((4*S*,5*R*)-4-amino-5-methylcyclohex-1-en-1-yl)-1-((*R*)-1-(2,4-dichlorophenyl)ethyl)-1*H*-pyrazolo[3,4-*b*]pyrazine-3-carbonitrile hydrochloride (64). To a solution of tert-butyl ((1*S*,6*R*)-4-(3-cyano-1-((*R*)-1-(2,4-dichlorophenyl)ethyl)-1*H*-pyrazolo[3,4-*b*]pyrazin-6-yl)-6-methylcyclohex-3-en-1-yl)carbamate (**63**, 0.6 g, 1.14 mmol) in dichloromethane (8 mL) was added 4 N HCl in 1,4-dioxane (1.3 mL, 5.05 mmol). After 2 h at room temperature, the solution was concentrated under reduced pressure to afford 6-((4*S*,5*R*)-4-amino-5-methylcyclohex-1-en-1-yl)-1-((*R*)-1-(2,4-dichlorophenyl)ethyl)-1*H*-pyrazolo[3,4-*b*]pyrazine-3-carbonitrile hydrochloride (**64**, 100% yield) which was used without further purification. LRMS-ESI$^+$: m/z calcd for $C_{21}H_{21}Cl_2N_6$ $[M+H]^+$ = 427.1; found, 427.

[(4*R*)-5-[tert-butyl(dimethyl)silyl]oxy-4-methylsulfonyloxy-pentyl] methanesulfonate (65). To a cooled (0 ºC) solution of (4*R*)-5-[tert-butyl(dimethyl)silyl]oxypentane-1,4-diol[43] (2.14 g, 9.13 mmol) and triethylamine (3.8 mL, 27.4 mmol) in dichloromethane (33 mL) was added methanesulfonyl chloride (1.3 mL, 16.6 mmol)

dropwise. The reaction mixture was warmed to room temperature and stirred for 16 h before quenching with saturated aqueous $NaHCO_3$. The aqueous layer was extracted with ethyl acetate, the combined organic layers were dried over Na_2SO_4, and concentrated by rotary evaporation. The residue was purified by silica gel chromatography (0 to 100% EtOAc in hexanes) to afford [(4*R*)-5-[tert-butyl(dimethyl)silyl]oxy-4-methylsulfonyloxy-pentyl] methanesulfonate (**65**, 79% yield) as a white amorphous solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.70 – 4.63 (m, 1H), 4.30 – 4.18 (m, 2H), 4.11 – 4.04 (m, 1H), 3.76 – 3.66 (m, 2H), 3.04 (s, 3H), 2.98 (s, 3H), 1.95 – 1.67 (m, 4H), 0.86 (s, 9H), 0.05 (s, 3H), 0.04 (s, 3H). LRMS-ESI$^+$: m/z calcd for $C_{13}H_{31}O_7S_2Si$ $[M+H]^+$ = 391.1; found, 391.

6-((4*S*,5*R*)-4-((*S*)-2-(((tert-butyldimethylsilyl)oxy)methyl)pyrrolidin-1-yl)-5-methylcyclohex-1-en-1-yl)-1-((*R*)-1-(2,4-dichlorophenyl)ethyl)-1*H*-pyrazolo[3,4-*b*]pyrazine-3-carbonitrile (66). To a solution of 6-[(4*S*,5*R*)-4-amino-5-methyl-cyclohexen-1-yl]-1-[(1*R*)-1-(2,4-dichlorophenyl)ethyl]pyrazolo[3,4-*b*]pyrazine-3-carbonitrile (**64**, 482 mg, 1.13 mmol) and [(4*R*)-5-[tert-butyl(dimethyl)silyl]oxy-4-methylsulfonyloxy-pentyl] methanesulfonate (**65**, 881 mg, 2.26 mmol) in acetonitrile (5 mL) was added *N*,*N*-diisopropylethylamine (1.0 mL, 5.64 mmol). The solution was heated to reflux for 24 h before cooling to room temperature and concentrating under reduced pressure. The residue was purified by silica gel chromatography (0 to 20% methanol in dichloromethane) to afford 6-((4*S*,5*R*)-4-((*S*)-2-(((tert-butyldimethylsilyl)oxy)methyl)pyrrolidin-1-yl)-5-methylcyclohex-1-en-1-yl)-1-((*R*)-1-(2,4-dichlorophenyl)ethyl)-1*H*-pyrazolo[3,4-*b*]pyrazine-3-carbonitrile (**66**, 75% yield). LRMS-ESI$^+$: m/z calcd for $C_{32}H_{43}Cl_2N_6OSi$ $[M+H]^+$ = 625.3; found, 625.

(*R*)-6-chloro-1-(1-(2,4-dichlorophenyl)ethyl)-3-methyl-1H-pyrazolo[3,4-*b*]pyrazine (67). Step 1. (*R*)-(1-(2,4-dichlorophenyl)ethyl)hydrazine hydrochloride (**40**, 47.3 g, 195.8 mmol)

was dissolved in ethanol (356 mL) at room temperature and 1-(3,5-dichloropyrazin-2-yl)ethan-1-one (34.0 g, 178.0 mmol) was added. The mixture was stirred at room temperature for 8 h and then concentrated under reduced pressure. The residue was suspended in 20% ethyl acetate in hexanes (200 mL) and filtered through a silica gel plug eluting with 20% ethyl acetate in hexanes. The filtrate was concentrated under reduced pressure to give (*R*,*Z*)-3,5-dichloro-2-(1-(2-(1-(2,4-dichlorophenyl)ethyl)hydrazono)ethyl)pyrazine and (*R*,*E*)-3,5-dichloro-2-(1-(2-(1-(2,4-dichlorophenyl)ethyl)hydrazono)ethyl)pyrazine (9:1) as a viscous orange oil. LRMS-ESI$^+$: *m/z* calcd for $C_{14}H_{12}Cl_4N_4$ $[M+H]^+$ = 377.0; found, 377.

Step 2. A mixture of (*R*,*Z*)-3,5-dichloro-2-(1-(2-(1-(2,4-dichlorophenyl)ethyl)hydrazono)ethyl)pyrazine and (*R,E*)-3,5-dichloro-2-(1-(2-(1-(2,4-dichlorophenyl)ethyl)hydrazono)ethyl)pyrazine (9:1) (33 g, 87.3 mmol) was dissolved in *N*-methyl-2-pyrrolidone (218 mL) at room temperature and 2,6-lutidine (30.3 mL, 261.9 mmol) was added. The mixture was degassed with nitrogen and heated to 100 °C under nitrogen for 8 h. The reaction mixture was cooled to room temperature and poured into a separatory funnel containing 500 mL of 1 N HCl in water and 500 mL of ethyl acetate. The layers were separated, and the organic layer was washed with 500 mL of 1 N HCl in water, dried over sodium sulfate, and concentrated under reduced pressure. The residue was purified by silica gel chromatography (0 to 20% (1:1 MTBE:dichloromethane) in hexanes) to provide (*R*)-6-chloro-1-(1-(2,4-dichlorophenyl)ethyl)-3-methyl-1H-pyrazolo[3,4-*b*]pyrazine (**67**) as off-white solid (67% yield). Ee: 98% Chiralpak® OZ-H; 250mmx4.6mm, 5% iPrOH in heptanes; flow rate 1 mL/min; detection at 254 nm; R_t = 6.9 min. ^{1}H NMR (300 MHz, Chloroform-*d*) δ 8.45 (s, 1H), 7.43 (d, *J* = 8.4 Hz, 1H), 7.38 (d, *J* = 2.1 Hz, 1H), 7.19 (dd, *J* = 8.4, 2.1 Hz, 1H), 6.48 (q, *J* = 6.9 Hz, 1H), 1.93 (d, *J* = 6.9 Hz, 3H). LRMS-ESI$^+$: *m/z* calcd for $C_{14}H_{11}Cl_3N_4$ $[M+H]^+$ = 341.0; found, 341.

ASSOCIATED CONTENT

Supporting Information. The Supporting Information is available free of charge via the Internet at the ACS Publications website.

Assay conditions; hepatocyte stability assay; in vitro safety panel; X-ray structures of synthetic intermediates of **16** and **17**; Molecular Formula Strings

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Notes

X-ray structures of intermediates for compounds **16** and **17** were deposited in the Cambridge Crystallographic Data Centre under deposition numbers 1915201 (**16**) and 1915202 (**17**). The authors will release the atomic coordinates upon article publication. RAPT Therapeutics was formerly known as FLX Bio Inc. The authors declare the following competing financial interests: All authors of this manuscript are or were employees of RAPT Therapeutics.

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ABBREVIATIONS

CCR4, CC chemokine receptor 4; CCL17, CC chemokine ligand 17; CCL22, CC chemokine ligand 22; T_{reg}, regulatory T cells; IO, immuno-oncology; T_{eff}, effector T cells; TME, tumor microenvironment; Th2, T helper type 2 cell; GPCR, G protein-coupled receptor; CTCL,

Cutaneous T-Cell Lymphoma; ADCC, antibody-dependent cell mediated cytotoxicity; CTX, chemotaxis; CD4, cluster of differentiation 4; CD8, cluster of differentiation 8; CD25, cluster of differentiation 25; FOXP3, forkhead box P3; HS, human serum; hHep, human hepatocytes; rHep, rat hepatocytes; Vss, volume of distribution; CL, clearance; miTreg, mouse induced regulatory T cells; GFP, green fluorescent protein; HATU, 1-[Bis(dimethylamino)methylene]-1H-1,2,3-triazolo[4,5-b]pyridinium 3-oxid hexafluorophosphate; HCl, hydrochloric acid; Pd, palladium; DIPEA, *N*,*N*-diisopropylethylamine; NaH, sodium hydride; NH_4OH, ammonium hydroxide; S_NAr, nucleophilic aromatic substitution.

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