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Article

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Discovery of Tetrahydroisoquinoline-Containing CXCR4 Antagonists with Improved *in vitro* ADMET Properties

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KEYWORDS

CXCR4; CXCL12; Chemokine; Hematopoietic stem cells; Cancer; Immunomodulation;

ABSTRACT

CXCR4 is a 7-transmembrane receptor expressed by hematopoietic stem cells and progeny, as well as by ≥ 48 different cancers types. CXCL12, the only chemokine ligand of CXCR4, is secreted within the tumor microenvironment, providing sanctuary for $CXCR4^+$ tumor cells from immune surveillance and chemotherapeutic elimination by 1) stimulating pro-survival signaling and 2) recruiting $CXCR4^+$ immunosuppressive leukocytes. Additionally, distant CXCL12-rich niches attract and support $CXCR4^+$ metastatic growths. Accordingly, CXCR4 antagonists can potentially obstruct CXCR4-mediated pro-survival signaling, recondition the $CXCR4^+$ leukocyte infiltrate from immunosuppressive to immunoreactive, and inhibit $CXCR4^+$ cancer cell metastasis. Current small molecule CXCR4 antagonists suffer from poor oral bioavailability and off-target liabilities. Herein, we report a series of novel tetrahydroisoquinoline-containing CXCR4 antagonists designed to improve intestinal absorption and off-target profiles. Structure activity relationships regarding CXCR4 potency, intestinal permeability, metabolic stability, and cytochrome P450 inhibition are presented.

INTRODUCTION

Chemokines are small, soluble cytokines that bind to and signal through seven-transmembrane chemokine receptors.[1] These interactions facilitate the directional migration of chemokine receptor-expressing leukocytes along concentration gradients of chemokine ligands, and they also promote the migratory aptitude and proliferation of these cells via pro-chemotactic and pro-survival gene transcription. Although most chemokine receptors interact with numerous chemokine ligands, several others bind only to one, such as the CXC type 4 receptor (CXCR4), which interacts solely with the CXC type 12 ligand (CXCL12, also known as stromal cell-derived factor 1, or SDF-1).[2] Extracellular CXCL12 binding to CXCR4 causes a conformational change that translates to the intracellular portions of the receptor, which associate with heterotrimeric G proteins.[3] This causes dissociation of the Gα subunit from the Gβγ heterodimer. CXCR4 predominantly signals through G proteins that contain $G\alpha_{i/o}$ subunits, which are ubiquitously expressed, or $G\alpha_{15}$ subunits,[4] which are primarily expressed by cells of hematopoietic origin. Once released from Gβγ, $G\alpha_{i/o}$ proteins inhibit adenylyl cyclase (AC) and stimulate phosphoinositide 3-kinase (PI3K). Alternatively, $G\alpha_{15}$ proteins activate phospholipase C (PLC), leading to the release of intracellular Ca^{+2} ions, which is commonly measured to quantify CXCR4 activity *in vitro*. Through these signaling cascades, binding of CXCL12 to CXCR4 facilitates many cellular activities comprising chemotaxis, hematopoiesis, transcription, survival, and proliferation.[5]

CXCR4 is the only functional chemokine receptor expressed on hematopoietic stem cells[6] (HSCs). As the CXCR4/CXCL12 axis is essential for HSC colonization of fetal bone marrow during development,[7] CXCL12 or CXCR4 knockout mice are embryonic lethal, exhibiting defects in hematopoiesis, vascularization, and vital organ development.[8] In adults, high levels of

CXCL12 are secreted by stromal cells residing in the bone marrow, liver, lungs, and lymph nodes, as well as by pathogenic, damaged, and hypoxic tissue. In contrast, CXCR4 is predominantly expressed by HSCs and their lineages, including T and B lymphocytes, natural killer cells, macrophages, and neutrophils, as well as by injured or hypoxic tissue.[9] These expression profiles result in chemoattraction of $CXCR4^+$ leukocytes and progenitors to CXCL12-rich niches where they become sequestered as a result of integrin-mediated adhesion to stromal cell layers. CXCL12 also encourages $CXCR4^+$ leukocyte motility by stimulating cytoskeletal rearrangements, F-actin bundle organization, and leading edge formation CXCL12 further activates integrins and upregulates matrix metalloproteinases (MMPs), thereby facilitating attachment to and penetration through proximal circulatory vessel endothelia.

Although CXCR4 has received much attention from the drug discovery and development communities as the co-receptor for T-tropic HIV entry,[10] the CXCR4/CXCL12 axis is also integrally involved in the progression of many types of cancer.[5] CXCR4 overexpression relative to normal tissue has been detected in $\geq$ 48 different types of solid and hematological cancers.[11] Although oncogenic mutations provide the host immune system with various tumor-specific antigens, intratumoral CXCL12 instigates $CXCR4^+$ cancer cell adhesion to and migration beneath tumor-associated stroma in an integrin-dependent manner. CXCL12 also induces CXCR4-mediated transcription of growth factors, angiogenic factors, and immunosuppressive cytokines. These events promote tumor progression, evasion of immune surveillance, and resistance to chemotherapy.[9] Privileged CXCL12-rich stromal niches within the tumor microenvironment additionally provide a pro-survival sanctuary for quiescent $CXCR4^+$ cancer cell subpopulations that persist after radiation therapy and/or chemotherapy, increasing the likelihood of relapse.[12] Accordingly, high intratumoral levels of CXCR4[13] and CXCL12[14] are

independent indicators of poor clinical prognosis for patients suffering from various types of cancer.

Tumor cells additionally evade immune surveillance and chemotherapeutic elimination by hijacking CXCR4/CXCL12-mediated leukocyte trafficking to chemotactically recruit $CXCR4^+$ leukocytes and progenitors, many of which are immunosuppressive rather than immunoreactive.[15]. Although various leukocyte subsets gain access to the tumor microenvironment, many of these cells are immunosuppressive rather than immunoreactive. For example, intratumoral CXCL12 both repels cluster of differentiation 8 (CD8)-expressing effector T lymphocytes and recruits immunosuppressive forkhead box P3 (FoxP3)-expressing regulatory T lymphocytes, which facilitate both immune evasion and chemotherapeutic resistance.[16] Accordingly, low ratios of $CD8^+$ cytotoxic T lymphocytes to $FoxP3^+$ regulatory T lymphocytes correlate with poor clinical prognosis, while high ratios are associated with increased survival. CXCL12 also recruits $CXCR4^+$ pro-angiogenic cells, which support tumor vascularization, growth, and invasion.[17]

$CXCR4^+$ cancer cells further hijack the normal physiological functions of the CXCR4/CXCL12 axis to gain entry to distant CXCL12-rich tissues, where they find environments that cultivate the formation of new metastases.[18] Stromal cell secretion of CXCL12 recruits $CXCR4^+$ tumor cells to, for example, the bone marrow microenvironment, which provides a pro-survival sanctuary for these cancer cells to evade immune recognition and chemotherapeutic elimination. This migration of $CXCR4^+$ cancer cells from the primary tumor to CXCL12-rich metastatic sites occurs via a mechanism equal but opposite to normal $CXCR4^+$ leukocyte trafficking.[19] Once the primary tumor is sufficiently vascularized, $CXCR4^+$ cancer cells can enter circulation, chemotactically migrate towards distant stromal origins of CXCL12

secretion (typically the bone marrow, liver, lungs, and lymph nodes), and penetrate deep into the target organ where they engraft into pro-survival stromal compartments. Ultimately, stromal cell secretion of CXCL12 is essential for 1) pro-survival signaling within the primary $CXCR4^+$ tumor microenvironment, 2) recruitment of $CXCR4^+$ immunosuppressive leukocytes and pro-angiogenic cells to intratumoral stromal niches, and 3) $CXCR4^+$ cancer cell metastasis to distant CXCL12-rich tissues.

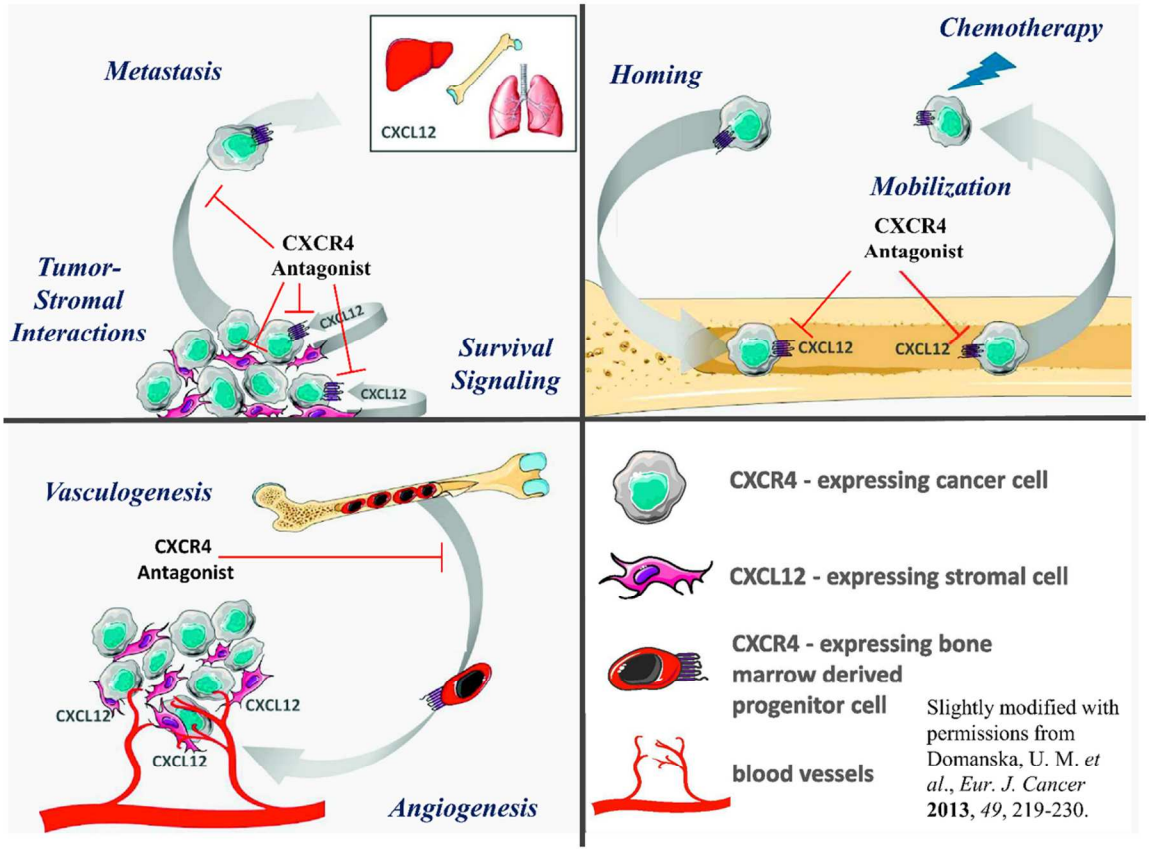


Figure 1. Potential CXCR4 mechanisms of action against cancer progression.

In principle therefore, small molecule CXCR4 antagonists can inhibit cancer progression via several mechanisms of action (**Figure 1**).[12] CXCR4 blockade can disrupt tumor-stromal interactions that confer resistance to the host immune system and to anti-cancer agents by obstructing autocrine and paracrine survival signaling mechanisms.[20] CXCR4 antagonists can

also combat cancer progression through inhibition of $CXCR4^+$ immunosuppressive leukocyte migration,[16] thereby reconditioning the tumor microenvironment in an immunomodulatory manner that induces a higher susceptibility to immune elimination. Furthermore, inhibition of $CXCR4^+$ pro-vasculogenic and pro-angiogenic progenitor trafficking from bone marrow stromal niches to the tumor microenvironment can prevent tumor cell access to systemic and lymphatic vasculature.[21] Moreover, CXCR4 antagonists have the potential to inhibit cancer metastasis to distant CXCL12-rich tissues, blocking one of the predominant factors that contributes to poor patient prognosis across many cancer types.[22] Finally, since metastatic $CXCR4^+$ tumor cells commonly find sanctuary in bone marrow compartments where they evade chemotherapeutic elimination, CXCR4 antagonists can disrupt local tumor-stromal interactions, mobilizing tumor cells into circulation where they are accessible to cytotoxic drugs.[23] Each of these mechanisms of action has been highlighted in various preclinical models of cancer by AMD3100[24] (Mozobil, Plerixafor, **Figure 2**), a potent[25] and selective[26] CXCR4 antagonist that is FDA-approved for autologous HSC mobilization and transplantation for non-Hodgkin's lymphoma and multiple myeloma. Some particularly notable preclinical anti-tumor effects of AMD3100 include delayed development of malignant ascites and an increased overall survival window as a single agent in immunocompetent murine models of ovarian cancer.[16] These results were attributed to reduced vessel density, increased apoptosis and necrosis, and 6-fold higher $CD8^+$:$FoxP3^+$ T lymphocyte ratios in the tumor microenvironment, enhancing susceptibility to the host immune system. Another example involved an immunocompetent model of hepatocellular carcinoma (HCC) and combination therapy with an antibody against immune checkpoint programmed cell death protein 1 (PD-1).[27] HCC standard of care sorafenib causes rapid clinical resistance, which begins with the induction of hypoxia. Hypoxia, in turn, upregulates CXCR4, CXCL12, and immune

checkpoint programmed death ligand 1 (PD-L1), all of which promote immunosuppression and chemotherapeutic evasion. AMD3100 in combination with sorafenib or with sorafenib and anti-PD-1 decreased both tumor volume and the number of metastatic growths relative to sorafenib alone or sorafenib and anti-PD-1 combination. In particular, the triple combination paradigm substantially improved both the $CD8^+$ cytotoxic T lymphocyte infiltrate and intratumoral apoptotic activity, emphasizing the clinical potential of combination with immune checkpoint inhibitors.

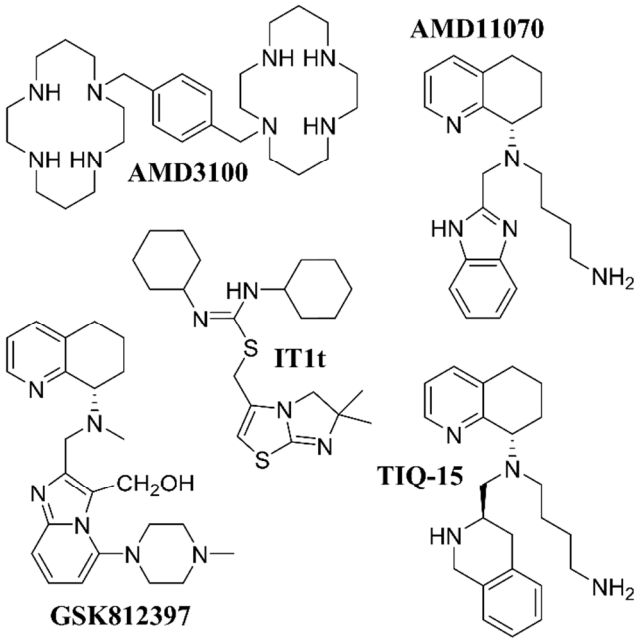


Figure 2. Non-peptide small molecule CXCR4 antagonists.

Despite these preclinical triumphs, the utility of AMD3100 in clinical oncology as an anti-tumor agent is somewhat limited due to dose-limiting cardiotoxicity and lack of oral bioavailability.[24] A second generation CXCR4 antagonist AMD11070 (**Figure 2**) with no cyclams was developed as an orally bioavailable anti-HIV treatment,[28] but potent inhibition of cytochrome P450 (CYP450) isoforms 2D6 and 3A4[29] and signs of hepatotoxicity in long-term animal studies[30] ultimately prevented clinical continuation. Various AMD11070 analogs have

since been described with particular focus on tetrahydroquinoline modifications,[31] side chain alterations,[32] and benzimidazole substitutions,[33] culminating in the discovery of advanced leads such as GSK812397.[34] Alternative scaffolds of small molecule CXCR4 antagonists include isothioureas,[35] the most characteristic of which is IT1t, cyclic peptides,[36] and other chemotypes.[37] In contrast, our research group focused on modifying the benzimidazole motif of AMD11070, replacing it with *N*-substituted piperazines[38] or with tetrahydroisoquinolines.[39] These efforts led to the discovery of TIQ-15, a highly potent (IC_{50} = 6.25 nM) and selective (< 10% inhibition of all other chemokine receptors at 10 μM) small molecule CXCR4 antagonist that exhibits robust metabolic stability in human liver microsomes ($t_{1/2}$ = 1.0 h), strong plasma stability in rats ($t_{1/2}$ = 6.0 h), and rapid mobilization of leukocytes that correlated with plasma drug concentrations in mice. Despite this impressive activity profile, TIQ-15 potently inhibits CYP450 subtype 2D6 (IC_{50} = 0.320 nM), limiting its utility in combination treatment paradigms, which are commonly employed in clinical oncology. TIQ-15 is also rapidly metabolized in mouse liver microsomes (only 17% remaining after 10 min), hindering the evaluation of anti-tumor efficacy after oral dosing regimens in preclinical mouse models of cancer. Furthermore, despite moderate oral bioavailability in rats, systemic exposure of TIQ-15 after oral administration undoubtedly remains limited by the presence of multiple basic amines. Herein, we describe the design, synthesis, and pharmacological evaluation of TIQ-15 analogs with enhanced CYP450 2D6:CXCR4 IC_{50} ratios, improved passive membrane permeability, and elevated mouse liver microsomal stability.

RESULTS

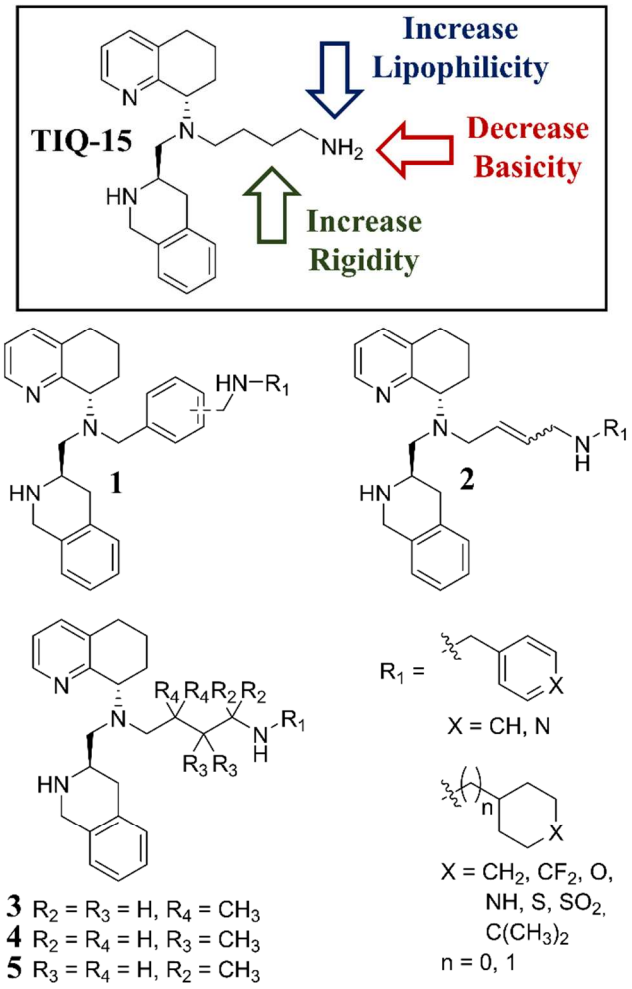
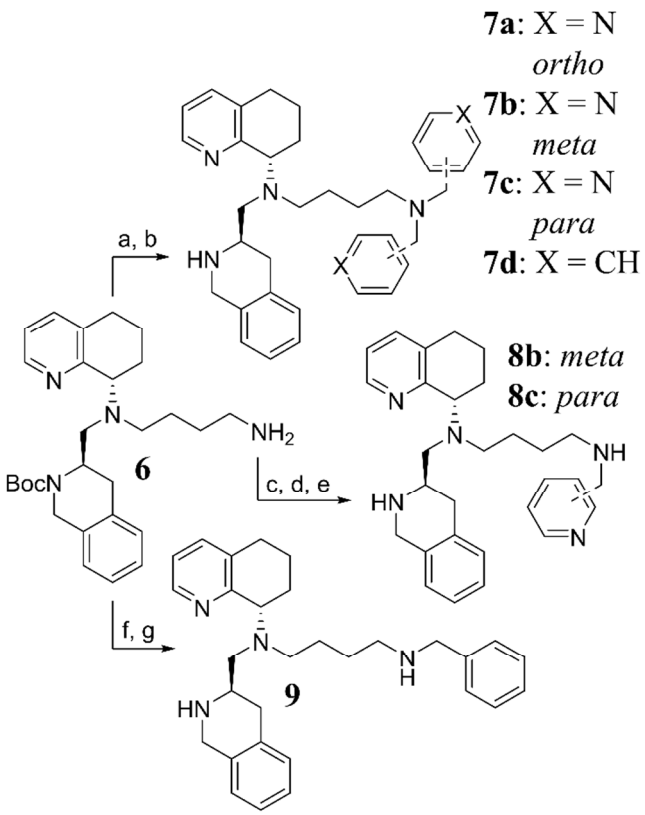


Figure 3. Design principles of TIQ-15 analogs.

To surmount the indicated shortcomings of TIQ-15, a series of conformationally restricted TIQ-15 derivatives with decreased basicity and/or increased lipophilicity were designed (**Figure 3**). While improving basicity and lipophilicity parameters was expected to increase intestinal permeability, rigidifying the butylamine side chain was anticipated to maximize CXCR4 specificity and to limit CYP450 2D6 inhibition and mouse metabolism. These design principles led to the conception of TIQ-15 analogs bearing benzylic amine (**1**), allylic amine (**2**), or *gem*-dimethylbutylamine (**3-5**) side chains, as well as carbocyclic or heterocyclic amine functionalization. Synthesis of these compounds was envisioned to be conducted in a modular fashion, quite similar to the previously described preparation of TIQ-15 analogs,[39] which utilized butylamine **6** as a common synthetic intermediate (**Scheme 1**).

Scheme 1. Arylmethyl functionalization of the butylamine side chain[a]

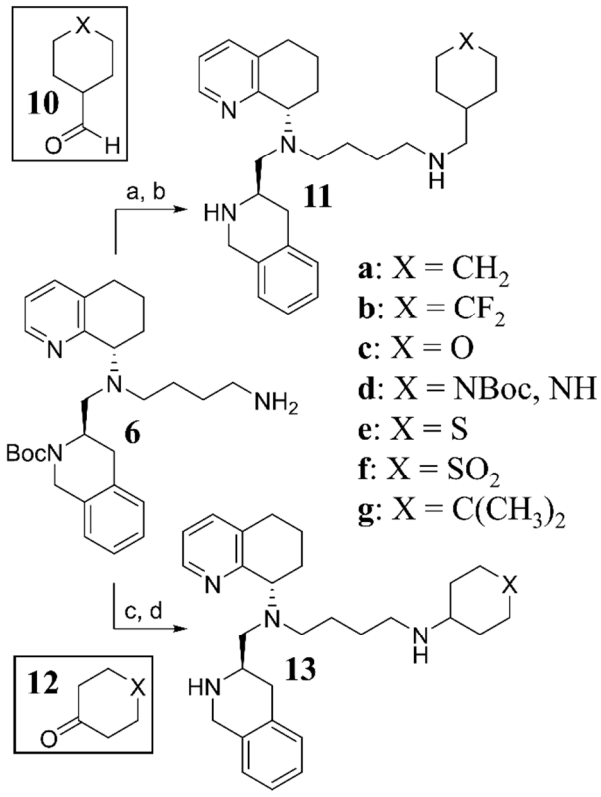


[a]Reagents and Conditions: a) C_5H_5NCHO or PhCHO, $Na(OAc)_3BH$, DCM, rt, 24 h, 26-68% yield; b) TFA, DCM, rt, 24 h, 67-88% yield; c) C_5H_5NCHO, $Na(OAc)_3BH$, DCM, rt, 5 h; d) BOC_2O, DMAP, DIPEA, DCM, rt, 6 h, 78-79% yield over 2 steps; e) TFA, DCM, rt, 24 h, 14-24% yield; f) PhCHO, $Ti(O\textit{i}Pr)_4$, $NaBH_4$, DCM, MeOH, rt, 4 h, 44% yield; g) TFA, DCM, rt, 24 h, 20% yield.

Sodium triacetoxyborohydride (STAB)-mediated reductive aminations involving amine **6** and benzaldehyde or 2-, 3-, or 4-pyridinecarboxaldehydes were anticipated to deliver the mono-substituted analogs after BOC group removal. In contrast, the corresponding bis(pyridylmethyl) (**7a-c**) and dibenzyl (**7d**) analogs predominated over the desired mono-substituted derivatives, even when only 0.95 eq of aldehyde were added, indicating that the mono-substituted products retained comparable reactivity to the unsubstituted starting material. Although the disubstituted compounds were undesired byproducts, they were still considered useful probes to investigate the CXCR4 binding pocket that interacts with the butylamine side chain of TIQ-15. While STAB-mediated reductive amination of butylamine **6** and pyridinecarboxaldehydes favored disubstituted over mono-substituted products, column chromatography provided ample quantities of pure disubstituted products for biological evaluation, as well as smaller quantities of difficult to separate mixtures (~2:1) of mono- to bis(pyridylmethyl) congeners. This permitted selective masking of the only remaining nucleophilic amines via DMAP-catalyzed BOC protection, which enabled chromatographic purification of the desired mono-pyridylmethyl BOC-protected amines. BOC protecting groups were subsequently removed using TFA, which cleanly provided the desired mono-pyridylmethyl TIQ-15 analogs **8a-c**[40]. While this BOC protection strategy delivered the desired compounds in the face of unexpected overreactivity during STAB-mediated reductive amination, this was clearly a problem that needed to be addressed for future analogs. A particularly attractive option was to first preform the imine intermediate and subsequently add a reducing agent.[41] Accordingly, butylamine **6** was stirred with benzaldehyde and titanium isopropoxide to ensure complete imine formation, followed by addition of sodium borohydride

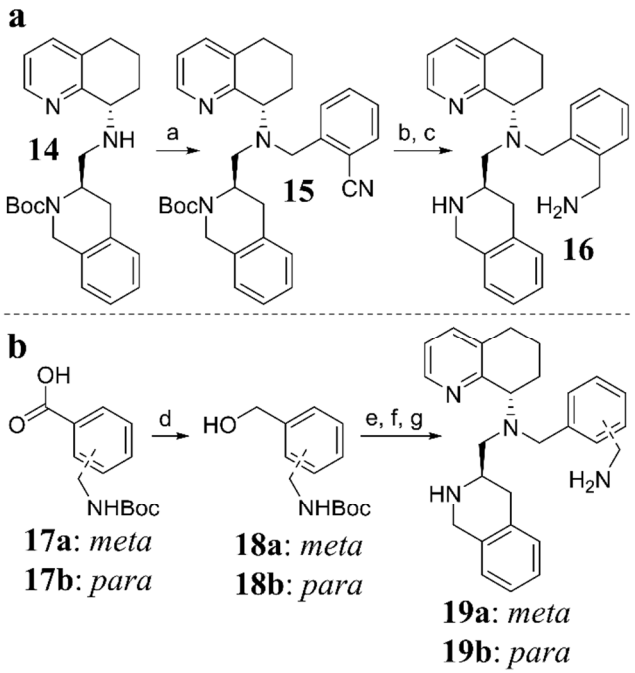
to rapidly reduce both the imine and any remaining benzaldehyde to prevent overreactivity. This protocol facilitated isolation of the desired mono-benzyl product, which was then subjected to BOC deprotection conditions, generating the desired mono-benzyl TIQ-15 analog **9**. This titanium isopropoxide/sodium borohydride procedure was additionally applied toward the syntheses of carbocyclic and heterocyclic TIQ-15 derivatives (**Scheme 2**). Butylamine **6** underwent titanium isopropoxide/sodium borohydride-mediated reductive amination with aldehydes **10a-f**, producing the corresponding mono-substituted products without detection of any undesired disubstituted byproducts. These intermediates were then treated with TFA to remove the BOC protecting groups, delivering the desired mono-substituted TIQ-15 analogs **11a-f**. Finally, reductive amination between butylamine **6** and ketones **12a-g** did not require the titanium isopropoxide/sodium borohydride protocol, but was rather afforded using STAB with acetic acid as an additive, generating the intermediate mono-substituted products. Typical BOC deprotection was carried out to yield the resulting mono-substituted TIQ-15 analogs **13a-g**, thereby furnishing this series of substituted-butylamine derivatives of TIQ-15.

Scheme 2. Saturated cyclic functionalization of the butylamine side chain[a]



[a]Reagents and Conditions: a) **10**, $Ti(OiPr)_4$, $NaBH_4$, DCM, MeOH, rt, 4 h, 27-62% yield; b) TFA, DCM, rt, 24 h, 42-98% yield; c) **12**, $Na(OAc)_3BH$, AcOH, DCM, rt, 24 h, 40-55% yield; d) TFA, DCM, rt, 24 h, 19-98% yield.

Scheme 3. Synthesis of benzylic amine side chain analogs[a]

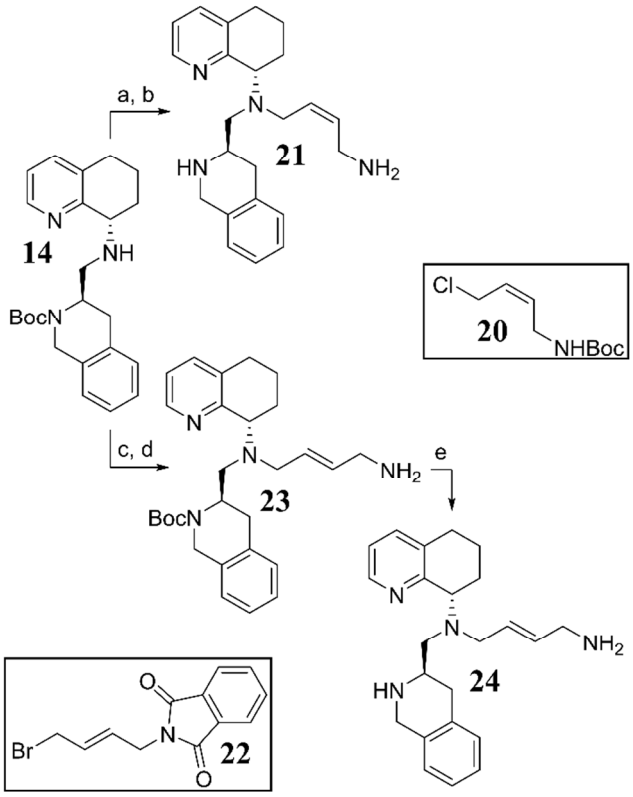


[a]Reagents and Conditions: a) 2-Cyanobenzaldehyde, $Na(OAc)_3BH$, DCM, rt, 4 h, 64% yield; b) $NiCl_2$-$(H_2O)_6$, $NaBH_4$, BOC_2O, $NH(CH_2CH_2NH_2)_2$, MeOH, 0˚C to rt, 96 h, 26% yield; c) TFA, DCM, rt, 24 h, 62% yield; d) BH_3-Me_2S, DCM, rt, 24 h, 49-74% yield; e) Dess-Martin periodinane, DCM, rt, 2.5 h; f) **14**, $Na(OAc)_3BH$, AcOH, DCM, rt, 24 h, 51-55% yield over 2 steps; g) TFA, DCM, rt, 24 h, 49-65% yield.

Secondary amine **14**[39] was used as the common starting material for the syntheses of benzylic and allylic amine side chain analogs. First, secondary amine **14** underwent STAB-mediated reductive amination with 2-cyanobenzaldehyde to produce the corresponding nitrile **15** in 64% yield (**Scheme 3a**). The nitrile was subsequently reduced using nickel(II) chloride hexahydrate and sodium borohydride, and the forming amine was trapped *in situ* with BOC_2O.[42] The resulting intermediate was deprotected using TFA to deliver the desired *ortho*-benzylic amine side chain analog **16** in 62% yield. Alternatively, the *meta*- and *para*-benzylic amine side chain derivatives **19a** and **19b** were prepared using a different approach (**Scheme 3b**). Commercially available carboxylic acids **17a** and **17b** were reduced to the corresponding alcohols **18a** and **18b**[43] using borane-dimethylsulfide complex, followed by Dess-Martin oxidation[44] of the resulting alcohols to the analogous aldehydes. With the *meta*- and *para*-substituted benzaldehyde derivatives in

hand, each of them was subjected to STAB-mediated reductive amination with secondary amine **14** using an acetic acid additive to yield the desired bis-BOC-protected intermediates. BOC group removal using TFA generated *meta*- and *para*-benzylic amine congeners **19a** and **19b**, respectively. To prepare the *cis*-allylic and *trans*-allylic amine side chain analogs of TIQ-15, secondary amine **14** was reacted with *cis*-allylic chloride **20**[32] or *trans*-allylic bromide **22**[45] in S_N2 fashion under Finkelstein-like conditions[32] to generate the corresponding tertiary amines (**Scheme 4**). The BOC protecting groups of the resulting *cis*-allylic amine were removed using TFA to deliver derivative **21** in 59% yield. Alternatively, the amines of the *trans*-allylic intermediate were differentially protected as phthalimide and BOC. Accordingly, the phthalimide group was removed using aqueous hydrazine, generating allylic amine **23**, followed by BOC group deprotection using TFA to afford *trans*-allylic amine analog **24**.

Scheme 4. Synthesis of allylic amine side chain analogs[a]

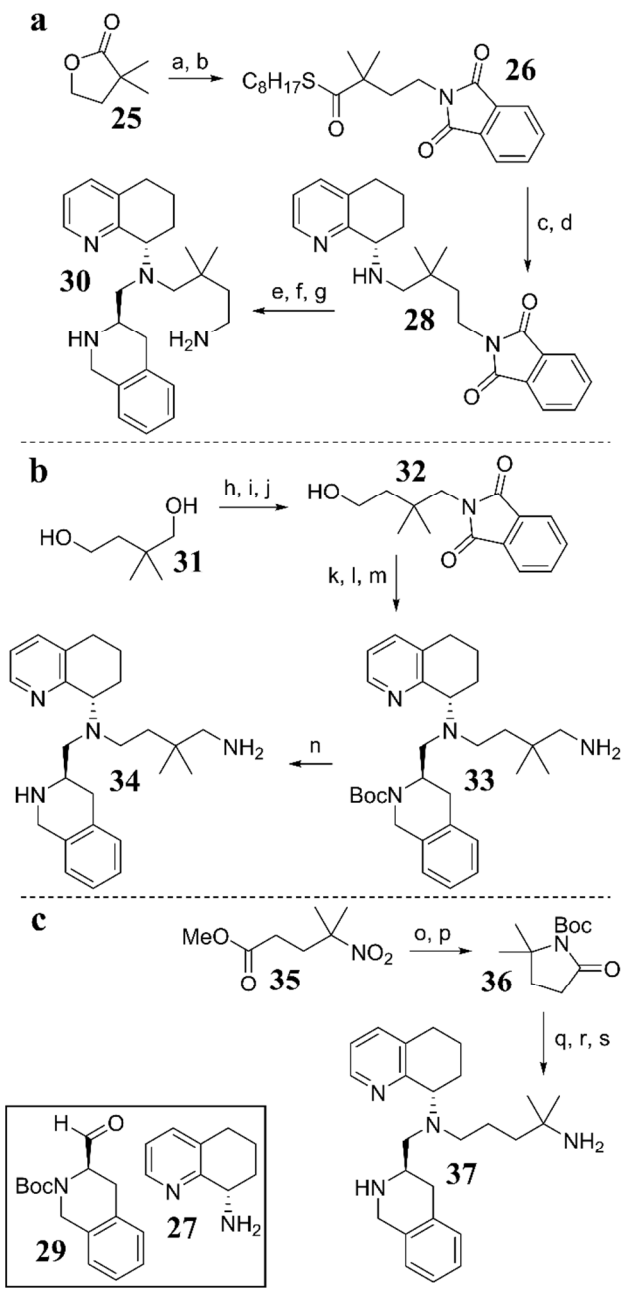


[a]Reagents and Conditions: a) **20**, KI, DIPEA, MeCN, 50˚C, 72 h, 69% yield; b) TFA, DCM, rt, 24 h, 59% yield; c) **22**, KI, DIPEA, 50˚C, 24 h, 51% yield; d) NH_2-NH_2 (aq), MeOH, rt, 24 h, 76% yield; e) TFA, DCM, rt, 24 h, 25% yield.

To prepare the 2,2-dimethylbutyl side chain analog, lactone **25** preliminarily underwent trimethylaluminum-mediated ring opening with 1-octanethiol[46] (**Scheme 5a**), followed by Mitsunobu reaction[47] of the resulting alcohol with nucleophilic phthalimide to yield desired intermediate **26**. Fukuyama reduction[48] of the thioester to the corresponding aldehyde proceeded cleanly, and subsequent STAB-mediated reductive amination with amine **27** generated anticipated secondary amine **28** in high yield. Ensuing reductive amination with aldehyde **29** using titanium isopropoxide and STAB furnished the differentially protected scaffold of the 2,2-dimethylbutyl side chain analog. Phthalimide deprotection with aqueous hydrazine, followed by TFA-mediated BOC group removal, yielded derivative **30**. To prepare the 3,3-dimethylbutyl side chain analog, the least sterically-encumbered alcohol of 2,2-dimethylbutane-1,4-diol was

selectively silyl protected in 67% isolated yield[49] (**Scheme 5b**). The remaining alcohol was subjected to Mitsunobu reaction[47] using phthalimide as a nucleophile, which was followed by silyl group removal using aqueous hydrochloric acid to generate desired intermediate **32**. Sulfur trioxide-pyridine complex was subsequently employed in a Parikh-Doering oxidation[50] to deliver the corresponding aldehyde, which was then subjected to reductive amination involving secondary amine **14** and STAB, followed by aqueous hydrazine-mediated phthalimide deprotection to yield the desired primary amine **33**. Finally, analog **34** was afforded via removal of the BOC protecting group with TFA. Reduction of nitropentanoate **35** via Raney Nickel-catalyzed hydrogenolysis generated the corresponding amine *in situ*, which spontaneously cyclized onto the ester to deliver the related pyrrolidinone[51] (**Scheme 5c**). BOC protection of this γ-lactam furnished desired intermediate **36**. DIBALH-mediated amide reduction to corresponding hemiaminal proceeded cleanly in 71% isolated yield. Subsequent STAB-mediated reductive amination with secondary amine **14** delivered the resulting bis-BOC protected scaffold, which was finally deprotected with TFA to yield the desired 4,4-dimethylbutylamine **37**.

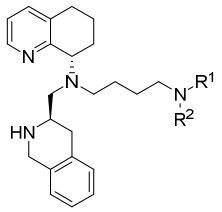
Scheme 5. Synthesis of *gem*-dimethylbutylamine side chain analogs[a]



[a]Reagents and Conditions: a) HSC_8H_{17}, Me_3Al, DCM, 0˚C to rt, 12 h, 66% yield; b) PPh_3, DIAD, phthalimide, THF, rt, 2.5 h, 93% yield; c) $PdCl_2$, Et_3SiH, Et_3N, DCM, rt, 20 min, 86% yield; d) **27**, $Na(OAc)_3BH$, DCE, rt, 1 h, 99% yield; e) **29**, $Ti(OiPr)_4$, $Na(OAc)_3BH$, DCM, rt, 12 h, 56% yield; f) NH_2-NH_2 (aq), MeOH, rt, 12 h; g) TFA, DCM, rt, 24 h, 85% over 2 steps; h) TBSCl, imidazole, DMF, 0˚C to rt, 1 h, 67% yield; i) PPh_3, DIAD, phthalimide, THF, rt, 36 h; j) HCl (aq), THF, rt, 2 h, 84% yield over 2 steps; k) SO_3-C_5H_5N, Et_3N, DCM, DMSO, 0˚C, 3 h, 77% yield; l) **14**, $Na(OAc)_3BH$, AcOH, DCE, rt, 12 h, 81% yield; m) NH_2-NH_2 (aq), MeOH, rt, 24 h; n) TFA, DCM, rt, 24 h, 91% yield over 2 steps; o) Raney Ni, H_2, EtOH, rt, 18 h, 88% yield; p) BOC_2O, DMAP, Et_3N, DCM, 18 h, 40˚C, 82% yield; q) DIBALH, Et_2O, -78˚C to rt, 6

h, 71% yield; r) **14**, $Na(OAc)_3BH$, AcOH, DCM, rt, 60 h, 30% yield; s) TFA, DCM, rt, 24 h, 36% yield.

Table 1. Activity profiles of mono- and bis(arylmethyl)-substituted butylamines



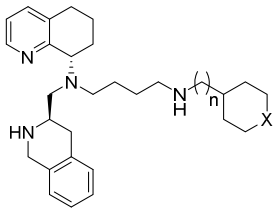
ID	R^1	R^2	CXCR4 Ca^{+2} Flux	mAChR Ca^{+2} Flux	PAMPA (nm/s)[a]		CYP450 IC_{50} (μM)[b]		Metabolic Stability (% Remaining)[b]	
			IC_{50} (nM)[a]	*IC_{50} (μM)[b]*	*pH = 7.4*	*pH = 5.5*	*2D6*	*3A4*	*Human*	*Mouse*
TIQ-15	H	H	6.25 ± 2.05	> 30.0	0[b]	6.00[b]	0.320	> 20.0	77.0	17.0
7a	2-Pyridylmethyl	2-Pyridylmethyl	664 ± 123	12.9	294 ± 128	48.0 ± 7.00	1.16	6.01	0.548	0.374
7b	3-Pyridylmethyl	3-Pyridylmethyl	471 ± 190	~11.5	435 ± 14.0	91.0 ± 23.0	0.348	1.29	0.432	1.40
7c	4-Pyridylmethyl	4-Pyridylmethyl	832 ± 522	11.4	774 ± 303	180 ± 7.00	0.120	0.622	0.521	1.55
7d	Benzyl	Benzyl	> 16,700[b]	> 33.3	ND	39.0 ± 51.0	0.494	2.24	4.84	0.298
8a	H	2-Pyridylmethyl	89.5 ± 24.8	> 30.0	109[b]	58.0[b]	0.350	4.50	26.0	8.60
8b	H	3-Pyridylmethyl	15.3 ± 5.00	~19.9	0	0	0.217	8.45	21.4	10.2
8c	H	4-Pyridylmethyl	22.7[b]	> 33.3	0	6.50 ± 5.00	0.145	4.50	1.92	6.44
9	H	Benzyl	20.1 ± 10.5	~3.43	ND	0	0.141	6.15	71.7	31.2

[a]n=2; [b]n=1; ND = not determined; Reported error represents standard deviation.

Each of these final compounds was evaluated *in vitro* for CXCR4 inhibition using a fluorometric Ca^{+2} flux assay,[52] for intestinal permeability via parallel artificial membrane permeability assay[53] (PAMPA), for CYP450 isoform inhibition using a fluorescence-based assay,[54] and for metabolic stability via human and mouse liver microsome assays using liquid chromatography tandem-mass spectrometry (LC-MS/MS) quantification.[55] Muscarinic acetylcholine receptors (mAChR) were considered surrogates for off-target activity due to previously observed CXCR4 antagonist promiscuity at these receptors (unpublished data), and inhibition was measured using fluorometric Ca^{+2} flux assays. **Table 1** includes the results obtained for the mono- and bis(arylmethyl) substituted analogs. Amongst this initial series of TIQ-15 derivatives, mono-3-pyridylmethyl (**8b**, IC_{50} = 15.3 ± 5.00 nM), mono-4-pyridylmethyl

(**8c**, IC_{50} = 22.7 nM), and mono-benzyl (**9**, IC_{50} = 20.1 ± 10.5 nM) analogs demonstrated the most potent inhibition of CXCR4, while bis(pyridylmethyl) analogs were much less potent (471 ± 190 nM ≤ IC_{50} ≤ 832 ± 522 nM), and dibenzyl congener **7d** was completely inactive. None of these compounds strongly inhibited mAChR activity (IC_{50} > 10 μM) except for the mono-benzyl analog, which still proved relatively inactive (**9**, IC_{50} ≈ 3.43 μM). Although the mono-substituted derivatives poorly permeated artificial intestinal membranes, disubstituted amines were substantially more permeable, likely due to increased lipophilicity and decreased amine basicity. None of the mono-arylmethyl derivatizations of the TIQ-15 butylamine improved the CYP450 profile, each potently inhibiting the 2D6 isoform with IC_{50} values in the nanomolar range and picking up micromolar activity against the 3A4 isoform. Perhaps not surprisingly, the disubstituted analogs not only retained potent 2D6 and modest 3A4 inhibition, but also introduced antagonist activity at several other CYP450 isoforms (unpublished data) and suffered from extremely rapid metabolism by both human and mouse liver microsomes. Although mono-benzyl congener **9** demonstrated reasonable metabolic stability for both species relative to TIQ-15, the other mono-substituted analogs were metabolized much more rapidly. Despite suboptimal PAMPA, CYP450, and metabolic properties, mono-pyridylmethyl functionalization produced the most interesting and attractive activity profiles relative to other compounds within this series, marking them for further investigation with alternative side chains.

Table 2. Activity profiles of saturated carbocyclic and heterocyclic substituted butylamines



ID	X	n	CXCR4 Ca^{+2} Flux	mAChR Ca^{+2} Flux	PAMPA (nm/s)[a]		CYP450 IC_{50} (μM)[b]		Metabolic Stability (% Remaining)[b]	
			IC_{50} (nM)[a]	*IC_{50} (μM)[b]*	*pH = 7.4*	*pH = 5.5*	*2D6*	*3A4*	*Human*	*Mouse*
TIQ-15	NA	NA	6.25 ± 2.05	> 30.0	0[b]	6.00[b]	0.320	> 20.0	77.0	17.0
11a	CH_2	1	158 ± 22.8	> 16.7	9.00 ± 13.0	21.0 ± 7.00	0.354	2.78	50.0	41.8
11b	CF_2	1	168 ± 83.1	> 16.7	ND	ND	0.538	19.2	49.2	37.7
11c	O	1	52.9 ± 45.0	> 33.3	0	6.00 ± 8.00	2.72	> 20.0	57.6	48.5
11d	NH	1	36.5[b]	> 16.7	ND	0	2.53	> 20.0	84.6	37.9
11e	S	1	69.6 ± 2.78	> 16.7	ND	ND	0.629	> 20.0	49.4	2.82
11f	SO_2	1	17.0 ± 13.4	> 16.7	ND	0	1.17	> 20.0	74.0	39.3
13a	CH_2	0	75.2 ± 53.9	> 33.3	ND	14.5 ± 11.0	0.524	11.7	46.1	43.8
13b	CF_2	0	3.71 ± 0.620	~28.2	32.0 ± 9.00	17.0 ± 9.00	0.189	11.3	57.2	34.8
13c	O	0	29.4 ± 16.5	> 33.3	0	0	0.657	> 20.0	53.0	55.8
13d	NH	0	63.4 ± 48.7	> 33.3	ND	19.0 ± 26.0	2.61	> 20.0	100	38.0
13e	S	0	54.7 ± 1.93	> 33.3	2.00 ± 3.00	46.0 ± 17.0	0.434	> 20.0	39.9	23.2
13f	SO_2	0	45.5 ± 28.8	> 33.3	0	0	0.853	> 20.0	11.7	17.6
13g	$C(CH_3)_2$	0	77.6[b]	~19.9	3.00 ± 4.00	0	0.090	12.9	30.1	50.0

[a]n=2; [b]n=1; ND = not determined; Reported error represents standard deviation.

Activity profiles of final compounds containing saturated carbocyclic and heterocyclic substituted butylamines are outlined in **Table 2**. All TIQ-15 analogs in this second series demonstrated poor passive permeability, as well as virtual inactivity at mAChR. In contrast, each of these congeners potently inhibited CXCR4 activity (IC_{50} < 100 nM), except for cyclohexylmethyl (**11a**, IC_{50} = 158 ± 22.8 nM) and difluorocyclohexylmethyl (**11b**, IC_{50} = 168 ± 83.1 nM) derivatives. Notably, difluorocyclohexyl analog **13b** and sulfonyl analog **11f** were the only compounds with comparable CXCR4 potency (IC_{50} = 3.71 ± 0.620 nM and 17.0 ± 13.4 nM, respectively) to TIQ-15 (IC_{50} = 6.25 ± 2.05 nM). All other derivatives amongst this series exhibited relatively similar CXCR4 antagonist activity (29.4 ± 16.5 nM ≤ IC_{50} ≤ 77.6 nM).

Attractively, none of these congeners inhibited CYP450 1A4, 2C19, 2C8, or 2C9 isoforms (unpublished data), nor did any of them substantially inhibit the 3A4 isoform (IC_{50} values > 10 μM), except for cyclohexylmethyl analog **11a** (IC_{50} = 2.78 μM). Although each of the compounds retained antagonist activity against the 2D6 isoform, piperidinyl (**13d**), piperidinylmethyl (**11d**), and tetrahydropyranylmethyl (**11c**) modifications in particular limited this activity (2.53 μM ≤ IC_{50} ≤ 2.72 μM). Furthermore, none of these compounds demonstrated metabolic liabilities on the order of the arylmethyl analogs. Rather, metabolic stability was considerably improved across the board, perhaps with the exception of sulfonyl analog **13f**, which was metabolized relatively extensively. While piperidinyl and piperidinylmethyl derivatives **13d** and **11d** were particularly resistant to human microsomal metabolism, tetrahydropyranyl (**13c**), tetrahydropyranylmethyl (**11c**), and dimethylcyclohexyl (**13g**) congeners were the least susceptible to mouse microsomal metabolism relative to the rest of the compounds in this series. Although piperidinyl and piperidinylmethyl functionalization of the butylamine resulted in reasonably attractive activity profiles, each of these modifications introduces an additional basic amine, which would likely make robust oral bioavailability extremely difficult to achieve. Accordingly, these compounds represent important data points, but were not marked for further investigation. In contrast, difluorocyclohexyl (**13b**, most potent against CXCR4), dimethylcyclohexyl (**13g**, good mouse metabolic stability), tetrahydropyranyl (**13c**, good mouse metabolic stability), and tetrahydropyranylmethyl (**11c**, good mouse metabolic stability and most optimal 2D6 activity) modifications warranted further exploration with alternative side chains due to relatively attractive full activity profiles with particular emphasis on the indicated properties.

Table 3. Activity profiles of TIQ-15 amine side chain analogs

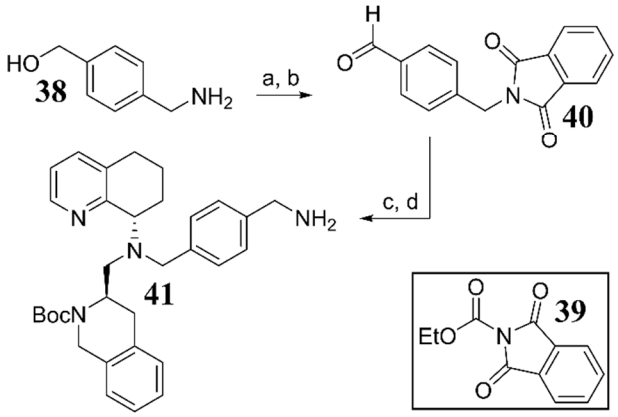
ID	Amine Side Chain	CXCR4 Ca^{+2} Flux	mAChR Ca^{+2} Flux	PAMPA (nm/s)[a]		CYP450 IC_{50} (μM)[b]		Metabolic Stability (% Remaining)[b]	
		IC_{50} (nM)[a]	*IC_{50} (μM)*[b]	*pH = 7.4*	*pH = 5.5*	*2D6*	*3A4*	*Human*	*Mouse*
TIQ-15	*Butyl*	6.25 ± 2.05	> 30.0	0[b]	6.00[b]	0.320	> 20.0	77.0	17.0
16	*ortho*-Benzylic	50.8 ± 37.1	1.20	214 ± 1.00	184 ± 41.5	0.318	15.2	50.5	1.29
19a	*meta*-Benzylic	292 ± 82.9	~25.7	ND	ND	0.032	4.63	97.3	89.2
19b	*para*-Benzylic	115 ± 85.9	> 33.3	301 ± 22.5	337 ± 61.0	0.048	7.44	100	94.2
21	*cis*-Allylic	9.26[b]	0.605 ± 0.287[a]	569 ± 129	39.0 ± 5.00	0.333	12.7	100	4.36
24	*trans*-Allylic	129 ± 109	> 16.7	829 ± 100	264 ± 27.0	0.510	9.29	100	10.7
30	2,2-Dimethylbutyl	> 33,300	> 33.3	255 ± 106	268 ± 95.0	0.197	10.8	76.1	5.22
34	3,3-Dimethylbutyl	69.0 ± 54.4	> 16.7	405 ± 144	0	0.498	> 20.0	88.7	51.2
37	4,4-Dimethylbutyl	24.6 ± 8.46	> 16.7	0	20.0 ± 28.0	0.345	> 20.0	73.5	3.32

[a]n=2; [b]n=1; ND = not determined; Reported error represents standard deviation.

Activity profiles of the TIQ-15 side chain analogs are presented in **Table 3**. This series of compounds retained moderately potent CXCR4 antagonist activity (50.8 ± 37.1 nM ≤ IC_{50} ≤ 129 ± 109 nM), with the exceptions of 2,2-dimethylbutyl analog **30** (IC_{50} > 33.3 μM) and the *meta*-benzylic amine **19a** (IC_{50} = 292 ± 82.9 nM), which were particularly weak inhibitors, as well as *cis*-allylic amine **21** and 4,4-dimethylbutyl derivative **37**, which were particularly potent (IC_{50} = 9.26 nM and 24.6 ± 8.46 nM, respectively). All of these congeners, except for *cis*-allylic amine **21** (IC_{50} = 0.605 ± 0.287 nM) and *ortho*-benzylic amine **16** (IC_{50} = 1.20 μM), were virtually inactive at mAChR (IC_{50} ≥ 16.7 μM). Notably, intestinal permeability was dramatically improved across the board, with the interesting exception of 4,4-dimethylbutyl congener **37**, which marked only a marginal improvement over TIQ-15 passive membrane diffusion. Although potent inhibition of the CYP450 2D6 isoform was demonstrated by all of the compounds within this series, only analogs containing benzylic amine side chains inhibited other CYP450 isoforms (unpublished data). Inhibition of the 3A4 isoform was variable across the series, but only *meta*-benzylic amine **19a** (IC_{50} = 4.63 μM) exhibited < 5 μM antagonist activity. Furthermore, each of

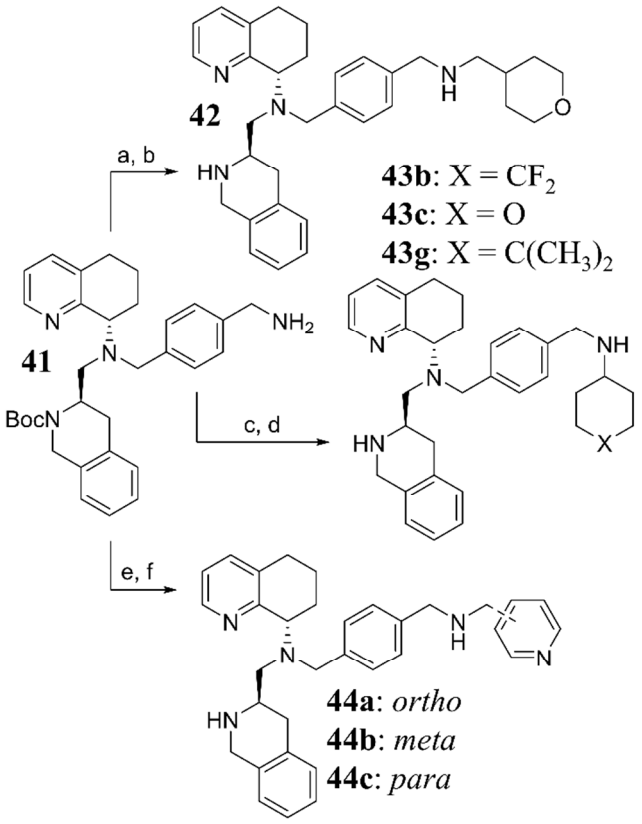
the compounds amongst this series showed robust human metabolic stability, the most susceptible of which was *ortho*-benzylic amine **16** (50.5% remaining after 10 min). In stark contrast, mouse microsomal stability was particularly poor (< 10% remaining after 10 min) for the *cis*-allylic amine (**21**), *ortho*-benzylic amine (**16**), 4,4-dimethylbutyl (**37**), and 2,2-dimethylbutyl (**30**) side chain derivatives. Although *trans*-allylic amine **24** was only modestly more stable in mouse liver microsomes than these compounds, 3,3-dimethylbutyl congener **34**, as well as the *meta*- and *para*-benzylic amines **19a** and **19b** were quite stable in comparison. Ultimately, the *para*-benzylic amine (**19b**), *trans*-allylic amine (**24**), and 3,3-dimethylbutylamine (**34**) side chains elicited more attractive full activity profiles than *cis*-allylic amine **21**, as well as the other *gem*-dimethylbutyl and benzylic amine side chain analogs, marking these three particular side chains to be incorporated in subsequent series of TIQ-15 analogs.

Scheme 6. Preparation of the *para*-benzylic amine side chain scaffold[a]



[a]Reagents and Conditions: a) **39**, Et_3N, THF, rt, 24 h; b) Dess-Martin periodinane, DCM, rt, 3 h; c) **14**, $Na(OAc)_3BH$, DCM, rt, 24 h, 65% yield over 3 steps; d) NH_2-NH_2 (aq), MeOH, rt, 24 h, 55% yield.

Scheme 7. Functionalization of the *para*-benzylic amine side chain[a]

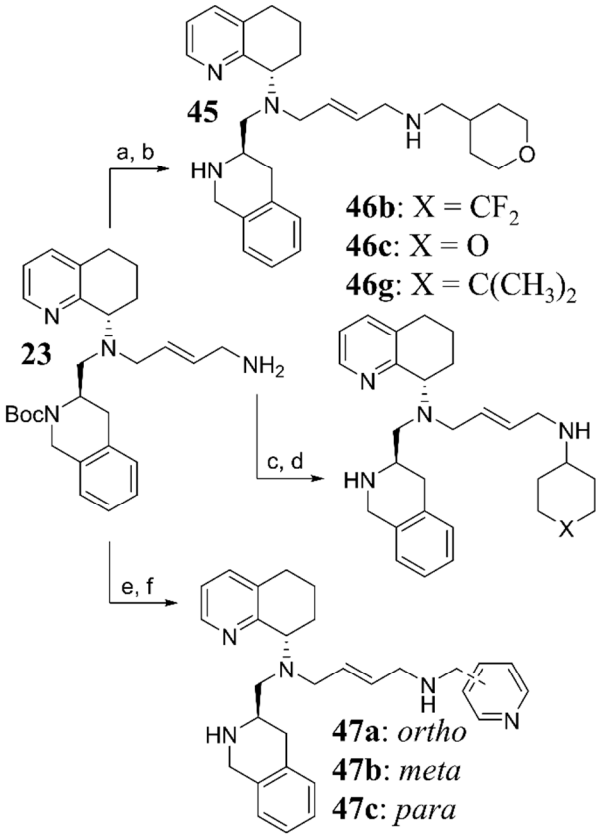


[a]Reagents and Conditions: a) **10c**, Ti(O*i*Pr)$_4$, NaBH$_4$, DCM, MeOH, rt, 4 h, 37% yield; b) TFA, DCM, rt, 24 h, 88% yield; c) **12b**, **12c**, or **12g**, Na(OAc)$_3$BH, AcOH, DCM, rt, 24 h, 59-84% yield; d) TFA, DCM, rt, 24 h, 71-87% yield; e) C_5H_5NCHO, Ti(O*i*Pr)$_4$, NaBH$_4$, DCM, MeOH, rt, 4 h, 61-65% yield; f) TFA, DCM, rt, 24 h, 70-92% yield.

At this point, three arylmethyl substitutions (2-, 3-, and 4-mono-pyridylmethyl), four saturated carbocyclic and heterocyclic modifications (difluorocyclohexyl, dimethylcyclohexyl, tetrahydropyranyl, and tetrahydropyranylmethyl), and three side chains (*para*-benzylic amine, *trans*-allylic amine, and 3,3-dimethylbutylamine) were selected as the subject of second generation investigation. Accordingly, amine **38** and phthalimide **39** underwent phthalimide transfer reaction[56] (**Scheme 6**), which was followed by Dess-Martin oxidation[44] of the remaining benzylic alcohol to corresponding aldehyde **40**. Subsequent reductive amination with secondary

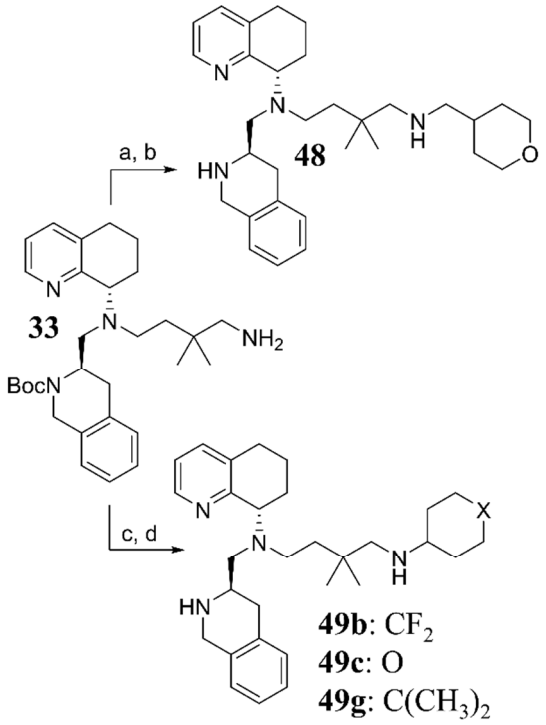
amine **14** and STAB furnished the differentially protected scaffold of the *para*-benzylic amine side chain analog of TIQ-15. Subsequent phthalimide protecting group removal using aqueous hydrazine delivered corresponding primary amine **41**, which was primed for selective functionalization of the benzylic amine. Amine **41** and aldehyde **10c** were then subjected to reductive amination using titanium isopropoxide and sodium borohydride to yield the corresponding mono-adduct, which was subsequently deprotected with TFA to deliver the desired tetrahydropyranylmethyl benzylic amine **42** (**Scheme 7**). Alternatively, amine **41** underwent STAB-mediated reductive amination with difluorocyclohexanone **12b**, tetrahydropyranone **12c**, and dimethylcyclohexanone **12g**, followed by TFA-mediated BOC deprotection to furnish the corresponding substituted benzylic amines **43b**, **43c**, and **43g**. Benzylic amine **41** and 2-, 3-, and 4-pyridinecarboxaldehydes were also subjected to titanium isopropoxide and sodium borohydride reductive amination conditions to generate solely the corresponding mono-adducts, which were then deprotected using TFA to afford the desired bis(arylmethyl) amines **44a-c**. In parallel, second generation substituted *trans*-allylic amines (**Scheme 8**) and substituted 3,3-dimethylbutylamines (**Scheme 9**) were prepared in similar fashion. All final compounds were pharmacologically evaluated according to the previously described assay paradigm, and activity profiles of these second generation TIQ-15 analogs are presented in **Table 4**.

Scheme 8. Functionalization of the *trans*-allylic amine side chain[a]



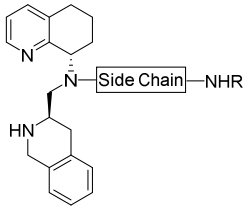
[a]Reagents and Conditions: a) **10c**, Ti(O*i*Pr)$_4$, NaBH$_4$, DCM, MeOH, rt, 4 h, 40% yield; b) TFA, DCM, rt, 24 h, 75% yield; c) **12b**, **12c**, or **12g**, Na(OAc)$_3$BH, AcOH, DCM, rt, 24 h, 51-63% yield; d) TFA, DCM, rt, 24 h, 19-98% yield; e) C_5H_5NCHO, Ti(O*i*Pr)$_4$, NaBH$_4$, DCM, MeOH, rt, 4 h, 54-63% yield; f) TFA, DCM, rt, 24 h, 70-92% yield.

Scheme 9. Functionalization of the 3,3-dimethylbutylamine side chain[a]



[a]Reagents and Conditions: a) **10c**, Ti(O*i*Pr)$_4$, NaBH$_4$, DCM, MeOH, rt, 4 h, 73% yield; b) TFA, DCM, rt, 24 h, 74% yield; c) **12b**, **12c**, or **12g**, Na(OAc)$_3$BH, AcOH, DCM, rt, 24 h, 54-60% yield; d) TFA, DCM, rt, 24 h, 96-98% yield.

Table 4. Activity profiles of second generation TIQ-15 analogs



ID	Amine Side Chain	R	CXCR4 Ca^{+2} Flux	mAChR Ca^{+2} Flux	PAMPA (nm/s)[a]		CYP450 IC_{50} (μM)[b]		Metabolic Stability (% Remaining)[b]	
			IC_{50} (nM)[a]	*IC_{50} (μM)[b]*	*pH = 7.4*	*pH = 5.5*	*2D6*	*3A4*	*Human*	*Mouse*
TIQ-15	*Butyl*	H	6.25 ± 2.05	> 30.0	0[b]	6.00[b]	0.320	> 20.0	77.0	17.0
42	*para*-Benzylic	4-Tetrahydropyranylmethyl	183 ± 156	> 16.7	ND	9.00 ± 4.00	0.403	0.994	2.71	45.0
43b	*para*-Benzylic	4,4-Difluorocyclohexyl	143 ± 35.7	> 16.7	5.00 ± 3.50	0	0.577	0.200	10.8	30.2
43c	*para*-Benzylic	4-Tetrahydropyranyl	90.3 ± 46.0	> 16.7	92.0 ± 7.00	ND	0.507	0.442	20.4	34.8
43g	*para*-Benzylic	4,4-Dimethylcyclohexyl	661 ± 428	~8.76	0	~0	0.258	0.334	14.9	23.2
44a	*para*-Benzylic	2-Pyridylmethyl	286 ± 263	6.94	ND	16.0 ± 5.00	0.377	0.109	1.77	11.6
44b	*para*-Benzylic	3-Pyridylmethyl	60.5 ± 33.2	~21.4	ND	0	0.355	0.058	1.02	0.423
44c	*para*-Benzylic	4-Pyridylmethyl	134 ± 65.0	~12.2	385 ± 233	ND	0.084	0.059	0.882	4.39
45	*trans*-Allylic	4-Tetrahydropyranylmethyl	24.2 ± 13.7	~19.9	122 ± 29.0	33.5 ± 11.0	3.32	> 20.0	74.5	32.3
46b	*trans*-Allylic	4,4-Difluorocyclohexyl	82.1 ± 74.8	~6.98	49.0 ± 6.00	9.00 ± 6.00	1.45	0.839	31.7	2.12
46c	*trans*-Allylic	4-Tetrahydropyranyl	15.5[b]	~5.61	434 ± 86.5	105 ± 18.0	2.61	7.73	69.9	10.0
46g	*trans*-Allylic	4,4-Dimethylcyclohexyl	64.6 ± 49.5	2.45	ND	ND	0.660	0.990	42.0	7.71
47a	*trans*-Allylic	2-Pyridylmethyl	58.3 ± 38.3	~22.0	50.0 ± 15.0	18.0 ± 2.00	0.709	1.56	31.2	2.98
47b	*trans*-Allylic	3-Pyridylmethyl	92.8 ± 74.2	~35.1	244 ± 125	ND	0.453	1.25	1.84	0.953
47c	*trans*-Allylic	4-Pyridylmethyl	40.7 ± 18.0	> 16.7	212 ± 46.0	78.0 ± 22.0	0.254	0.350	1.51	2.92
48	3,3-Dimethylbutyl	4-Tetrahydropyranylmethyl	43.8 ± 23.3	~13.0	ND	62.0 ± 20.0	1.75	> 20.0	46.1	60.3
49b	3,3-Dimethylbutyl	4,4-Difluorocyclohexyl	168 ± 54.8	~18.8	22.0 ± 3.00	17.5 ± 5.00	2.04	5.58	16.1	16.8
49c	3,3-Dimethylbutyl	4-Tetrahydropyranyl	36.3 ± 13.4	~11.5	57.0 ± 18.0	57.0 ± 16.3	6.21	17.7	21.8	49.8
49g	3,3-Dimethylbutyl	4,4-Dimethylcyclohexyl	541 ± 24.5	~18.8	28.0 ± 9.00	11.0 ± 5.50	2.22	7.83	23.9	51.1

[a]n=2; [b]n=1; ND = not determined; Reported error represents standard deviation.

While compounds **43g**, **44a**, and **49g** demonstrated poor CXCR4 potency (IC_{50} > 250 nM), *trans*-allylic tetrahydropyranyl amine **46c** (IC_{50} = 15.5 nM), *trans*-allylic tetrahydropyranylmethyl amine **45** (IC_{50} = 24.2 ± 13.7 nM), and 3,3-dimethylbutyl tetrahydropyranyl amine **49c** (IC_{50} = 36.3 ± 13.4 nM) retained reasonably good on-target potency

(IC_{50} < 40 nM) relative to the first generation analogs. Additionally, all compounds except for **46b**, **46g**, **46c**, **43g**, and **44a** (2.45 μM ≤ IC_{50} ≤ ~8.76 μM) exhibited minimal off-target activity at mAChR (IC_{50} > 10 μM). Although *trans*-allylic and *para*-benzylic pyridylmethyl amines expectedly demonstrated improved predicted intestinal permeability where measured, *trans*-allylic tetrahydropyranyl and tetrahydropyranylmethyl amines **46c** and **45** performed better than expected in the PAMPA assay. These two analogs (IC_{50} = 2.61 μM and 3.32 μM, respectively), as well as 3,3-dimethylbutyl tetrahydropyranyl derivative **49c** (IC_{50} = 6.21 μM), further exhibited substantially diminished inhibitory activity at the CYP450 2D6 isoform. Each of these three compounds also proved inactive at the other CYP450 isoforms examined, except for the 3A4 isoform, whose activity was only modestly affected by congeners **46c** (IC_{50} = 7.73 μM) and **49c** (IC_{50} = 17.7 μM). Finally, while analog **49c** was poorly stable in human liver microsomes (21.8% remaining after 10 min), the mouse liver microsomal stability was quite robust (49.8% remaining after 10 min) relative to the first generation compounds. Although compounds **46c** and **45** retained comparably good human liver microsomal stability (69.9% and 74.5% remaining after 10 min, respectively), persistence in mouse liver microsomes was more limited (10.0% and 32.3% remaining after 10 min, respectively). Ultimately, derivatives **45**, **46c**, and **49c** demonstrated the most attractive full activity profiles amongst all of the compounds, with **45** and **49c** offering weakened CYP450 2D6 inhibition, improved PAMPA permeability, and elevated mouse liver microsomal stability as compared to TIQ-15.

DISCUSSION & CONCLUSIONS

Of all of the analogs prepared, difluorocyclohexyl amine **13b** (IC_{50} = 3.71 ± 0.620 nM) and *cis*-allylic amine **21** (IC_{50} = 9.26 nM) exhibited the most comparable CXCR4 potency to TIQ-15

(IC_{50} = 6.25 ± 2.05 nM). While 3-pyridylmethyl (**8b**, IC_{50} = 15.3 ± 5.00 nM), 4-pyridylmethyl (**8c**, IC_{50} = 22.7 nM), benzyl (**9**, IC_{50} = 20.1 ± 10.5 nM), piperidinylmethyl (**11d**, IC_{50} = 36.5 nM), sulfonyl (**11f**, IC_{50} = 17.0 ± 13.4 nM), tetrahydropyranyl (**13c**, IC_{50} = 29.4 ± 16.5 nM), and 4,4-dimethylbutyl (**37**, IC_{50} = 24.6 ± 8.46 nM) analogs were the only other first generation compounds to demonstrate < 40 nM CXCR4 antagonist activity, only three of the second generation derivatives, 3,3-dimethylbutyl tetrahydropyranyl (**49c**, IC_{50} = 36.3 ± 13.4 nM), *trans*-allylic tetrahydropyranyl (**46c**, IC_{50} = 15.5 nM), and *trans*-allylic tetrahydropyranylmethyl (**45**, IC_{50} = 24.2 ± 13.7 nM) congeners, achieved this level of potency. Overall, several conclusions can be drawn regarding the intermolecular interactions between CXCR4 and these types of TIQ-15 analogs. First, CXCR4 is unlikely to favorably accommodate two bulky butylamine substituents, as exhibited by disubstituted analogs **7a-d** (471 ± 190 nM ≤ IC_{50} ≤ 16,700 nM). In contrast, there is clearly room for single substitution of the butylamine, as all but two of the mono-functionalized butylamines explored herein maintained < 100 nM CXCR4 antagonist activity. While most of the mono-arylmethyl derivatives retained good on-target potency (15.3 ± 5.00 nM ≤ IC_{50} ≤ 22.7 nM), 2-pyridylmethyl analog **8a** (IC_{50} = 89.5 ± 24.8 nM) exhibited relatively poor activity, potentially due to intramolecular hydrogen bonding between the protonated butylamine and the pyridine nitrogen lone pair. Additionally, the series of heterocyclic compounds containing the methylene spacer potentially benefit from hydrogen bond accepting capacity at the 4-position, as cyclohexylmethyl analog **11a** (IC_{50} = 158 ± 22.8 nM) and 4,4-difluorocyclohexylmethyl analog **11b** (IC_{50} = 168 ± 83.1 nM) were substantially less potent than the others, which are reasonable hydrogen bond acceptors (17.0 ± 13.4 nM ≤ IC_{50} ≤ 69.6 ± 2.78 nM). Furthermore, all saturated carbocyclic and heterocyclic functionalized compounds without a methylene spacer exhibited equipotent activity, with the exception of

difluorocyclohexyl analog **13b** (IC_{50} = 3.71 ± 0.620 nM), which is the most potent compound reported herein. As heteroatom alteration amongst this series had little effect on CXCR4 activity, this impressive potency is likely due to subtle conformational effects[57] and/or dipolar interactions[58] imposed by the difluoromethylene unit. Unfortunately, this remarkable activity did not translate to second generation analogs containing this motif. It can also be concluded that the range of CXCR4 potency varies much more between side chain analogs than between mono-modified butylamine analogs, indicating the relative importance of side chain conformation. The equipotent activity of TIQ-15 (IC_{50} = 6.25 ± 2.05 nM) and *cis*-allylic amine **21** (IC_{50} = 9.26 nM) suggest that the *cis* configuration favorably reinforces the on-target conformation of the TIQ-15 butylamine side chain. Consistent with this assertion is the significantly less potent *trans*-allylic amine **24** (IC_{50} = 129 ± 109 nM), as well as the enhanced potency of *ortho*-benzylic amine **16** (IC_{50} = 50.8 ± 37.1 nM), as compared to *meta*-benzylic (**19a**, IC_{50} = 292 ± 82.9 nM) and *para*-benzylic (**19b**, IC_{50} = 115 ± 85.9 nM) amines. Moreover, *gem*-dimethyl functionalization was tolerated at positions γ and Δ to the central tertiary amine, as highlighted by compounds **34** (IC_{50} = 69.0 ± 54.4 nM) and **37** (IC_{50} = 24.6 ± 8.46 nM), respectively, but this moiety completely abrogated activity when installed at the β position (**30**), suggesting a detrimental steric clash with CXCR4. This steric limitation is also highlighted by the *para*-benzylic amine series (60.5 ± 33.2 nM ≤ IC_{50} ≤ 661 nM), as well as by 3,3-dimethylbutyl dimethylcyclohexyl amine **49g** (IC_{50} = 541 ± 24.5 nM). Despite the relatively poor potency of *trans*-allylic amine **24** (IC_{50} = 129 ± 109 nM) and 3,3-dimethylbutylamine **34** (IC_{50} = 69.0 ± 54.4 nM), second generation *trans*-allylic tetrahydropyranyl (**46c**, IC_{50} = 15.5 nM), *trans*-allylic tetrahydropyranylmethyl (**45**, IC_{50} = 24.2 ± 13.7 nM), and 3,3-dimethylbutyl tetrahydropyranyl (**49c**, IC_{50} = 36.3 ± 13.4 nM) analogs achieved comparatively potent CXCR4 antagonist activity. Interestingly, a series of AMD11070

side chain analogs[32] containing benzimidazoles instead of tetrahydroisoquinolines described similar potency trends between *trans*-allylic, *cis*-allylic, *ortho*-benzylic, *meta*-benzylic, and *para*-benzylic amine side chains, suggesting that these AMD11070 analogs and the corresponding TIQ-15 derivatives adopt analogous binding modes in the same CXCR4 binding pocket.

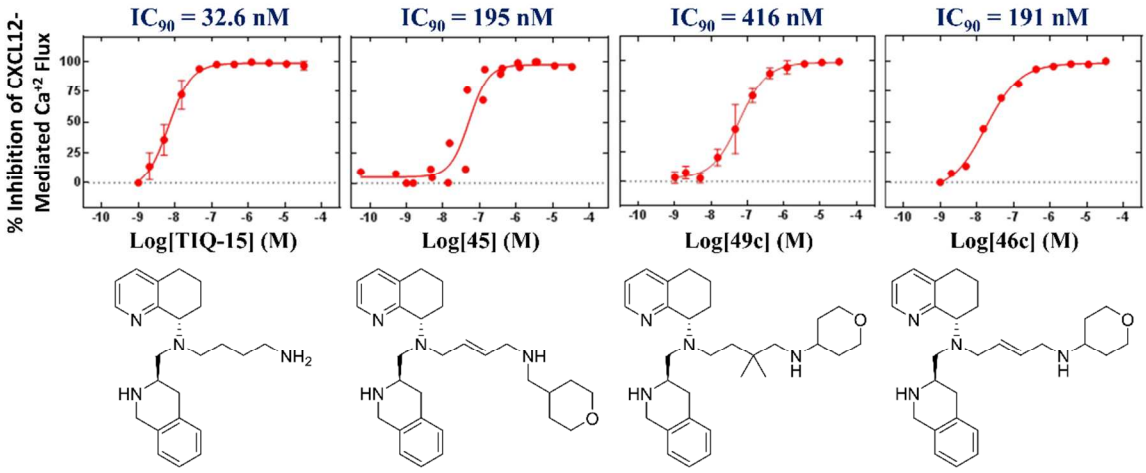
As one of the desirable characteristics of TIQ-15 analogs is oral bioavailability, promising compounds must demonstrate facile intestinal permeability, as estimated using PAMPA, and robust metabolic stability, as measured in human and mouse liver microsomes. The hypothesis that decreasing amine basicity and increasing lipophilicity would improve PAMPA values relative to TIQ-15 was upheld by the compounds described herein. Notably however, reducing amine basicity had a much more profound effect on PAMPA results than did increasing lipophilicity. This is demonstrated by the dramatic permeability enhancement of *cis*- and *trans*-allylic amines **21** and **24**, respectively, juxtaposed to the modest improvement achieved by cyclohexyl and cyclohexylmethyl analogs **13a** and **11a**, respectively. Consistent with this are the generally elevated PAMPA values of allylic and benzylic amines as compared to the butylamines presented in **Table 2**. Two exceptions are 3-pyridylmethyl and 4-pyridylmethyl analogs **8b** and **8c**, respectively, which exhibited negligible PAMPA permeability. Interestingly, the 2-pyridylmethyl congener **8a** proved substantially more permeable, again perhaps suggesting intramolecular hydrogen bonding, which can partially mask the polarity of a protonated amine. Although second generation analogs generally did not perform as robustly as parent *trans*-allylic amine **24**, 3,3-dimethylbutyl (**34**), *para*-benzylic (**19b**), *trans*-allylic tetrahydropyranyl (**46c**), *trans*-allylic tetrahydropyranylmethyl (**45**), and 3,3-dimethylbutyl tetrahydropyranyl (**49c**) amines demonstrated substantially improved PAMPA permeability as compared to TIQ-15. As

for metabolic stability, installation of additional pyridines introduced new metabolic liabilities, ultimately leading to low human and mouse metabolic stability. The series of TIQ-15 analogs mono-substituted with saturated carbocycles and heterocycles generally exhibited moderate metabolic stability in both human and mouse liver microsomes, with piperidinyl (**13d**, 100% remaining after 10 min) and piperidinylmethyl (**11d**, 84.6% remaining after 10 min) analogs demonstrating particularly robust human microsomal stability, and tetrahydropyranyl (**13c**, 55.8% remaining after 10 min), tetrahydropyranylmethyl (**11c**, 48.5% remaining after 10 min), and dimethylcyclohexyl (**13g**, 50.0% remaining after 10 min) amines exhibiting particularly attractive mouse metabolic stability. Interestingly, first generation side chain analogs presented in **Table 3** demonstrated robust human microsomal stability (50.5-100% remaining after 10 min), but poor mouse microsomal stability (1.29-10.7% remaining after 10 min), with the exceptions of 3,3-dimethylbutylamine **34** (51.2% remaining after 10 min), *meta*-benzylic amine **19a** (89.2% remaining after 10 min), and *para*-benzylic amine **19b** (94.2% remaining after 10 min). Despite the high metabolic stability of **19b**, second generation analogs of this compound suffered from substantially increased metabolic lability, resulting in suboptimal metabolic profiles across this series. The only second generation compounds with reasonable metabolic profiles across both species include *trans*-allylic tetrahydropyranylmethyl amine **45**, as well as 3,3-dimethylbutyl dimethylcyclohexyl (**49g**), tetrahydropyranyl (**49c**), and tetrahydropyranylmethyl (**48**) amines.

Another requirement of effective TIQ-15 analogs is limited off-target activity and wide therapeutic window, with an emphasis on clean CYP450 profiles to enable potential utility in combination therapy. Although mAChR antagonist activity was measured as an estimation of compound promiscuity, only *cis*-allylic amine **21** (IC_{50} = 0.605 ± 0.287 μM), *ortho*-benzylic

amine **16** (IC_{50} = 1.20 μM), and *trans*-allylic dimethylcyclohexyl amine **46g** (IC_{50} = 2.45 μM) exhibited antagonist potency < 5 μM, whereas only four other compounds demonstrated 5-10 μM mAChR antagonist activity. In contrast, CYP450 2D6 and 3A4 isoforms are much more significant off-target liabilities, especially for anti-tumor therapies, which commonly involve several different clinical agents in the fight against cancer progression. As clinical candidate AMD11070 and lead compound TIQ-15 potently inhibit the 2D6 isoform, thereby limiting their potential use in combination therapy regimens, it was essential to dial down this activity. Unfortunately, little information could be extracted from the first generation compounds in this regard, except that adding another basic amine reduced activity at 2D6, as demonstrated by piperidinyl (IC_{50} = 2.61 μM) and piperidinylmethyl (IC_{50} = 2.53 μM) analogs **13d** and **11d**, respectively. Of course, incorporation of an additional basic amine would virtually eliminate all hope of achieving passive intestinal permeability, and therefore, these motifs were not pursued further. Although tetrahydropyranylmethyl analog **11c** (IC_{50} = 2.72 μM) was the only other first generation compound to limit 2D6 potency to > 2 μM, five second generation analogs, namely *trans*-allylic tetrahydropyranyl (**46c**, IC_{50} = 2.61 μM) and tetrahydropyranylmethyl (**45**, IC_{50} = 3.32 μM) amines, as well as 3,3-dimethylbutyl difluorocyclohexyl (**49b**, IC_{50} = 2.04 μM), dimethylcyclohexyl (**49g**, IC_{50} = 2.22 μM), and tetrahydropyranyl (**49c**, IC_{50} = 6.21 μM) congeners achieved this substantial reduction in CYP450 inhibition. Each of these five second generation compounds notably inhibited the 2D6 isoform less potently than AMD11070 (IC_{50} = 1.60 μM, unpublished data), and none of them demonstrated antagonist activity at the other CYP450 isoforms < 5 μM. Overall, *trans*-allylic tetrahydropyranyl (**46c**, IC_{50} 2D6:IC_{50} CXCR4 = 168), *trans*-allylic tetrahydropyranylmethyl (**45**, IC_{50} 2D6:IC_{50} CXCR4 = 138), and 3,3-dimethylbutyl tetrahydropyranyl (**49c**, IC_{50} 2D6:IC_{50} CXCR4 = 171) amines achieved

significantly improved 2D6:CXCR4 indexes, as compared to TIQ-15 (IC_{50} 2D6:IC_{50} CXCR4 = 51).



Assay		TIQ-15	45	49c	46c
CXCR4 Ca^{+2} Flux	IC_{50} (nM)	6.25 ± 2.05	24.2 ± 13.7	36.3 ± 13.4	15.5
PAMPA (nm/s)	pH = 7.4	0.00	122 ± 29.0	57.0 ± 18.0	434 ± 86.5
	pH = 5.5	6.00	33.5 ± 11.0	57.0 ± 16.3	105 ± 18.0
CYP450 2D6	IC_{50} (nM)	320	3,330	6,210	2,610
Metabolic Stability (%)	Human	77.0	74.5	21.8	69.9
	Mouse	17.0	32.3	49.8	10.0
2D6:CXCR4 Index	IC_{50} Ratio	51	138	171	168

Figure 4. Activity profile summary of TIQ-15 analogs with improved ADMET properties.

Ultimately, these three compounds exhibited the most attractive full activity profiles, demonstrating enhanced predicted intestinal permeability and elevated 2D6:CXCR4 indexes, therefore representing marked advancements over TIQ-15 (**Figure 4**). These improvements endow *trans*-allylic tetrahydropyranylmethyl amine **45** (mouse CXCR4 IC_{50} = 5.47 ± 1.52 nM, n = 6) and 3,3-dimethylbutyl tetrahydropyranyl amine **49c** (mouse CXCR4 IC_{50} = 20.0 ± 6.01 nM, n = 4) as more appropriate candidates for efficacy studies in preclinical mouse models of cancer

than TIQ-15, whereas *trans*-allylic tetrahydropyranylmethyl amine **45** and *trans*-allylic tetrahydropyranyl amine **46c** (mouse CXCR4 IC_{50} = 9.24 ± 0.45 nM, n = 6) are more suitable clinical candidates than TIQ-15. The discovery of these compounds enables the evaluation of tetrahydroisoquinoline-containing CXCR4 antagonist anti-tumor efficacy as single agent immunomodulatory chemotherapeutics in immunocompetent mouse models,[16] and/or in combination with clinically approved cancer drugs, such as immune checkpoint inhibitors anti-PD-1 or anti-PD-L1.[27] As CXCR4[13] and CXCL12[14] are independent clinical indicators of poor prognosis, these CXCR4 inhibitors have the potential to directly benefit cancer patients with high CXCR4 and/or CXCL12 expression in the primary tumor microenvironment or at secondary metastatic sites. Highlighted not only by the overexpression of CXCR4 in ≥ 48 types of cancer,[11] but also by the upregulation of CXCR4 and CXCL12 in response to tumor hypoxia, a great number of cancer patients could benefit from CXCR4 antagonists. As precision medicine emerges into the clinic, it should be emphasized that the cellular and molecular content of tumor microenvironments vary widely between different tissue types, different stages of tumorigenesis, and different patient populations. Preliminary evaluation of intratumoral expression profiles prior to treatment will enable more targeted therapeutic options, and will specifically facilitate administration of CXCR4 antagonists only to patients with the proper genotype and/or phenotype. Given an appropriate CXCR4 and CXCL12 expression profile, tetrahydroisoquinoline-containing CXCR4 antagonists have the potential to inhibit intratumoral pro-survival signaling, to challenge the initiation of distant metastases, and to improve the leukocyte infiltrate by increasing the ratio of $CD8^+$ cytotoxic T lymphocytes to $FoxP3^+$ regulatory T lymphocytes, thereby sensitizing tumor cells to the host immune system and improving overall clinical outcome.

EXPERIMENTAL

Chemical Methods: Automated flash column chromatography was performed using a Teledyne ISCO CombiFlash Companion system with silica gel-packed columns (SiliCycle Inc.). Analytical thin-layer chromatography (TLC, commercially available from Sigma) was carried out on aluminum-supported silica gel plates (thickness: 200 μm) with fluorescent indicator (F-254). Visualization of compounds on TLC plates was accomplished UV light (254 nm) and/or with phosphomolybdic acid, ninhydrin, or ceric ammonium molybdate. Optical rotation was measured using a Perkin Elmer 341 Polarimeter. NMR spectra (^{1}H, ^{19}F, and ^{13}C) were obtained using either a Varian INOVA 600 MHz spectrometer, a Varian INOVA 500 MHz spectrometer, a Varian INOVA 400 MHz spectrometer, or a Varian VNMR 400 MHz spectrometer. NMR samples were prepared and processed in deuterated chloroform ($CDCl_3$) or deuterated methanol (CD_3OD) using the residual solvent peak ($CDCl_3$: ^{1}H = 7.27 ppm, ^{13}C = 77.23 ppm; CD_3OD: ^{13}C = 49.2 ppm) as an internal reference or using trifluoroacetic acid (TFA) as external reference (TFA: ^{19}F = -76.55 ppm), unless otherwise specified. NMR data are reported to include chemical shifts (δ) reported in ppm, multiplicities indicated as s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet), br (broad), or app (apparent), coupling constants (J) reported in Hz, and integration normalized to 1 atom (H, C, or F). High resolution mass spectrometry (HRMS) was performed by the Emory University Mass Spectrometry Center, directed by Dr. Fred Strobel. Liquid chromatography-mass spectrometry (LC-MS) was performed on an Agilent 1200 HPLC equipped with a 6120 Quadrupole mass spectrometer (ESI-API) eluting at a rate of 1.00 mL/min

with mixtures of HPLC grade MeOH and H_2O or MeCN and H_2O (all spiked with 0.1% formic acid) through an analytical, reverse-phase, Agilent C18 XDB eclipse column (50 mm x 4.6 mm, 3.5 μM). LC-MS samples were prepared in a solution of 75:25 MeOH/H_2O (spiked with 0.1% formic acid), and ultraviolet activity was monitored at 254 nm. Normal phase analytical chiral HPLC was performed on an Agilent 1100 series HPLC equipped with a G1315B diode array detector using mixtures of HPLC grade hexanes/*i*PrOH and a Daicel ChiralPak AD-H column (150 mm x 4.6 mm, 5 μM). Reverse phase HPLC was performed on the same instrument using mixtures of HPLC grade MeCN/H_2O and a Daicel ChiralCel OD-RH column (150 mm x 4.6 mm, 5 μM). X-Ray crystallography was performed by the Emory University X-Ray Crystallography Center, directed by Dr. John Bacsa. Final compound purity was assessed using ^{1}H NMR and LC-MS. All final compounds were determined to be ≥ 95% pure, except for compounds **21** and **49g**, which were determined to be 90-95% pure, as indicated below.

General Reductive Amination Protocol A: A solution of amine (1.00 eq) in DCM (0.10 M) was added to a flask with a stir bar. The aldehyde (2.00 eq) was subsequently added, and the resulting mixture was allowed to stir for 10 min at room temperature under Ar. Added sodium triacetoxyborohydride (3.00 eq), and the resulting reaction mixture was allowed to stir at room temperature under Ar overnight. The next day, LC-MS indicated almost complete conversion of starting material. The reaction mixture was diluted with DCM and quenched with saturated aqueous sodium bicarbonate. The resulting aqueous layer was extracted 3 times with DCM. Combined organic layers were dried over anhydrous sodium sulfate, filtered, and evaporated under reduced pressure.

General Deprotection Protocol: BOC-protected amine (1.00 eq) was added to a flask with a stir bar. Diluted with DCM (0.10 M), and the resulting solution was stirred under Ar and room

temperature for 5 min. Added 2,2,2-trifluoroacetic acid (32.0 eq), and the resulting reaction mixture was allowed to stir at room temperature under Ar overnight. Once TLC indicated complete conversion of starting material, the reaction mixture was quenched with 1 M aqueous sodium hydroxide until the pH reached 13-14. The resulting aqueous layer was extracted 3 times with DCM, and combined organic layers were dried over anhydrous sodium sulfate, filtered, and evaporated under reduced pressure.

N1,N1-bis(pyridin-2-ylmethyl)-N4-(((R)-1,2,3,4-tetrahydroisoquinolin-3-yl)methyl)-N4-((S)-5,6,7,8-tetrahydroquinolin-8-yl)butane-1,4-diamine (**7a**): Synthesis was carried out according to General Reductive Amination Protocol A using amine **6** and 2-pyridinecarboxaldehyde. Purified via column chromatography eluting with 40:1:1 DCM/MeOH/Et_3N to yield a yellow oil (87 mg, 0.134 mmol, 26% yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.52 (d, *J* = 4.5 Hz, 2H), 8.31-8.35 (m, 1H), 7.63 (dt, *J* = 1.0 Hz, *J* = 7.5 Hz, 2H), 7.53-7.54 (m, 2H), 7.23-7.24 (m, 1H), 7.13 (t, *J* = 6.0 Hz, 2H), 7.06-7.10 (m, 2H), 6.94-7.01 (m, 3H), 4.62-4.66 (m, 1.5H), 4.34 (br s, 0.5H), 4.13 (d, *J* = 17.0 Hz, 1H), 3.88 (br s, 1H), 3.79 (s, 4H), 2.74-3.08 (m, 3H), 2.61-2.63 (m, 6H), 2.31 (m, 1H), 1.91-1.99 (m, 2H), 1.58-1.70 (m, 3H) 1.43-1.51 (m, 10H), 1.26-1.35 (m, 2H). HRMS (NSI) *m/z* = 647.40620 (M + H); Theo. for $C_{40}H_{50}O_2N_6$ + H = 647.40680. LC-MS (ESI-API) 75-95% MeOH in H_2O, 5 min, *m/z* = 647.3 (M + H), 324.2 (M/2 + H), t = 0.939 min; 50-95% MeOH in H_2O, 8 min, *m/z* = 647.4 (M + H), 324.2 (M/2 + H), t = 3.917 min. BOC group removal was carried out according to the General Deprotection Protocol. Purified via column chromatography eluting with 40:1:1 DCM/MeOH/Et_3N to yield a yellow oil (59 mg, 0.108 mmol, 80% yield). ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.47 (m, 2H), 8.36 (d, *J* = 3.6 Hz, 1H), 7.60 (dt, *J* = 1.8 Hz, *J* = 7.8 Hz, 2H), 7.48 (d, *J* = 7.8 Hz, 2H), 7.36 (d, *J* = 7.8 Hz, 1H), 7.15-7.16 (m, 2H), 7.05-7.11 (m, 5H), 4.10-4.31 (m, 3H), 3.76 (s, 4H), 2.64-3.09 (m, 9H), 2.46 (app t, *J* = 7.2 Hz, 3H), 2.04-2.06

(m, 1H), 1.93-1.95 (m, 1H), 1.79-1.85 (m, 1H), 1.69-1.75 (m, 1H), 1.47 (m, 2H), 1.31-1.38 (m, 2H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 160.1, 158.7, 149.0 (2C), 146.5 (2C), 137.2, 136.5 (2C), 134.2, 133.6, 129.2, 126.7, 126.6, 126.2, 123.0, 122.9 (2C), 122.0 (2C), 121.8, 62.4, 60.6 (2C), 56.9, 54.4, 53.2, 52.5, 46.8, 29.8, 29.4, 28.4, 27.4, 24.8, 22.0. HRMS (NSI) *m/z* = 547.35399 (M + H); Theo. for $C_{35}H_{42}N_6$ + H = 547.35437. LC-MS (ESI-API) 75-95% MeOH in H_2O, 5 min, *m/z* = 547.3 (M + H), 274.2 (M/2 + H), t = 0.786 min; 25-95% MeOH in H_2O, 8 min, , *m/z* = 547.2 (M + H), 274.2 (M/2 + H), t = 5.464 min.

General Reductive Amination Protocol B: A solution of amine (1.00 eq) in DCM (0.10 M) was added to a flask with a stir bar. The aldehyde (1.00 eq) was subsequently added in dropwise fashion, and the resulting mixture was allowed to stir for 30 min at room temperature under Ar. Added sodium triacetoxyborohydride (3.00 eq), and the resulting reaction mixture was allowed to stir at room temperature overnight. In the morning, TLC indicated complete conversion of starting material. The reaction mixture was diluted with DCM and quenched with saturated aqueous sodium bicarbonate. The resulting aqueous layer was extracted 3 times with DCM. Combined organic layers were dried over anhydrous sodium sulfate, filtered, and evaporated under reduced pressure.

N1,N1-bis(pyridin-3-ylmethyl)-N4-(((R)-1,2,3,4-tetrahydroisoquinolin-3-yl)methyl)-N4-((S)-5,6,7,8-tetrahydroquinolin-8-yl)butane-1,4-diamine (**7b**): Synthesis was carried out according to General Reductive Amination Protocol B using amine **6** and 3-pyridinecarboxyaldehyde. Purified via column chromatography eluting with 9:1 DCM to MeOH to yield a clear oil (222 mg, 0.343 mmol, 64% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.54 (d, *J* = 0.8 Hz, 2H), 8.49 (dd, *J* = 4.8 Hz, *J* = 1.2 Hz, 2H), 8.34-8.35 (m, 1H), 7.67 (d, *J* = 7.2 Hz, 1H), 7.22-7.26 (m, 3H), 7.04-7.12 (m, 2H), 6.95-7.02 (m, 3H), 4.61-4.68 (m, 1.5H), 4.36 (br s, 0.5H), 4.14 (d, *J* = 17.2 Hz,

1H), 3.84 (br s, 1H), 3.55 (s, 4H), 2.90-3.09 (m, 2H), 2.53-2.67 (m, 5H), 2.34-2.37 (m, 3H), 2.00 (br s, 1H), 1.91 (br s, 1H), 1.80 (br s, 1H), 1.64-1.69 (m 2H), 1.47 (m, 10H), 1.26-1.35 (m, 2H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 190.6, 158.0, 154.9, 149.9 (2C), 148.3 (2C), 146.6, 136.2 (2C), 134.8, 133.9, 133.0, 132.9, 129.2, 128.9, 126.2, 125.9, 125.8, 123.2 (2C), 121.2, 79.4, 61.3, 61.0, 55.5 (2C), 54.9, 54.2, 51.3, 49.4, 47.7, 43.2, 30.4, 29.0, 28.5 (3C), 24.6, 21.2. HRMS (NSI) *m/z* = 647.40704 (M + H); Theo. for $C_{40}H_{50}O_2N_6$ + H = 647.40680. LC-MS (ESI-API) 75-95% MeOH in H_2O, 6 min, *m/z* = 647.4 (M + H), 324.2 (M/2 + H), t = 1.426 min; 50-95% MeOH in H_2O, 6 min, *m/z* = 647.4 (M + H), 324.2 (M/2 + H), t = 5.730 min. BOC group removal was carried out according to the General Deprotection Protocol. Purified via column chromatography eluting with 45:3:1 DCM/MeOH/NH_4OH to yield a slightly yellow oil (166 mg, 0.304 mmol, 88% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.55 (d, *J* = 2.0 Hz, 2H), 8.47 (dd, *J* = 1.6 Hz, *J* = 4.8 Hz, 2H), 8.42 (dd, *J* = 1.4 Hz, *J* = 4.6 Hz, 1H), 7.66 (dt, *J* = 1.9 Hz, *J* = 7.7 Hz, 2H), 7.31 (dd, *J* = 7.6 Hz, *J* = 1.2 Hz, 1H), 7.20 (ddd, *J* = 0.7 Hz, *J* = 4.9 Hz, *J* = 7.7 Hz, 2H), 7.07-7.11 (m, 2H), 6.99-7.05 (m, 3H), 4.01-4.04 (m, 2H), 3.86 (d, *J* = 15.2 Hz, 1H), 3.56 (s, 4H), 2.91-3.02 (m, 2H), 2.51-2.79 (m, 6H), 2.41-2.44 (m, 4H), 1.92-2.04 (m, 2H), 1.80-1.89 (m, 1H), 1.66-1.73 (m, 1H), 1.40-1.61 (m, 4H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 158.8, 150.2 (2C), 148.6 (2C), 146.8, 136.6, 136.4 (2C), 135.5, 134.9 (2C), 134.7, 134.0, 129.2, 126.5, 126.0, 125.6, 123.4 (2C), 121.4, 61.4, 57.8, 55.7 (2C), 54.2, 53.7, 52.5, 48.7, 33.8, 29.5, 29.1, 27.5, 24.9, 22.1. HRMS (NSI) *m/z* = 547.35477 (M + H); Theo. for $C_{35}H_{42}N_6$ + H = 547.35437. LC-MS (ESI-API) 50-95% MeOH in H_2O, 6 min, *m/z* = 547.3 (M + H), 274.2 (M/2 + H), t = 0.969 min; 25-95% MeOH in H_2O, 8 min, *m/z* = 547.2 (M + H), 274.2 (M/2 + H), t = 5.530 min.

N1,N1-bis(pyridin-4-ylmethyl)-N4-(((R)-1,2,3,4-tetrahydroisoquinolin-3-yl)methyl)-N4-((S)-5,6,7,8-tetrahydroquinolin-8-yl)butane-1,4-diamine (**7c**): Synthesis was carried out according to

General Reductive Amination Protocol B using amine **6** and 4-pyridinecarboxaldehyde. Purified via column chromatography eluting with 10:1 DCM to MeOH to yield a yellow oil (236 mg, 0.538 mmol, 68% yield). ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.54 (dd, *J* = 4.5 Hz, *J* = 1.5 Hz, 4H), 8.53 (d, *J* = 5.4 Hz, 4H), 8.35 (d, *J* = 16.2 Hz, 1H), 7.29 (br s, 4H), 7.24-7.25 (m, 1H), 7.06-7.11 (m, 2H), 6.96-7.02 (m, 3H), 4.62-4.69 (m, 1.5H), 4.38 (br s, 0.5H), 4.14 (d, *J* = 16.8 Hz, 1H), 3.85 (br s, 1H), 3.55 (s, 4H), 3.01-3.08 (m, 1H), 2.91-2.94 (m, 1H), 2.50-2.61 (m, 5H), 2.35-2.36 (m, 3H), 1.91-1.99 (m, 2H), 1.61-1.66 (m, 2H), 1.47-1.49 (m, 11H), 1.33 (br s, 2H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 191.4, 158.1, 155.0, 151.2, 149.7 (4C), 148.9, 146.7, 136.3, 134.0, 129.3, 126.3 (2C), 125.9, 123.5 (4C), 122.1, 121.3, 79.5, 61.3, 57.5 (2C), 54.9, 54.4, 54.1, 51.4, 49.4, 47.9, 43.4, 30.5, 29.1, 28.6 (3C), 24.8, 21.4. HRMS (NSI) *m/z* = 647.40702 (M + H); Theo. for $C_{40}H_{50}O_2N_6$ + H = 647.40680. LC-MS (ESI-API) 75-95% MeOH in H_2O, 5 min, *m/z* = 647.4 (M + H), 324.2 (M/2 + H), t = 1.000 min; 50-95% MeOH in H_2O, 6 min, *m/z* = 647.3 (M + H), 324.2 (M/2 + H), t = 5.223 min. BOC group removal was carried out according to the General Deprotection Protocol. Purified via column chromatography eluting with 45:3:1 DCM/MeOH/NH_4OH to yield a white foam (38 mg, 0.070 mmol, 78% yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.50 (m, 4H), 8.37 (d, *J* = 6.0 Hz, 1H), 7.32 (d, *J* = 9.5 Hz, 1H), 7.26 (d, *J* = 10.0 Hz, 4H), 7.09-7.11 (m, 2H), 7.00-7.04 (m, 3H), 4.05-4.14 (m, 2H), 3.93-4.08 (m, 1H), 3.53 (s, 4H), 2.97-3.00 (m, 1H), 2.88-2.92 (m, 1H), 2.81 (br s, 1H), 2.69-2.75 (m, 1H), 2.62-2.66 (m, 4H), 2.48-2.53 (m, 1H), 2.38-2.41 (m, 2H), 2.00-2.03 (m, 1H), 1.92-1.95 (m, 1H), 1.78-1.86 (m, 1H), 1.64-1.70 (m, 1H), 1.38-1.55 (m, 4H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 158.7, 150.0 (4C), 148.8 (2C), 146.6, 137.0, 134.2, 134.0, 129.3 (2C), 126.6 (2C), 126.0, 123.6 (4C), 121.7, 62.1, 57.7 (2C), 57.2, 54.1, 53.1, 52.8, 47.4, 32.7, 29.5, 28.6, 27.3, 24.9, 22.1. HRMS (NSI) *m/z* = 547.35464 (M + H); Theo. for $C_{35}H_{42}N_6$ + H = 547.35437. LC-MS (ESI-API) 50-95% MeOH in

H_2O, 6 min, *m/z* = 547.3 (M + H), 274.2 (M/2 + H), t = 1.038 min; 25-95% MeOH in H_2O, 8 min, *m/z* = 547.3 (M + H), 274.2 (M/2 + H), t = 5.477 min.

N1,N1-dibenzyl-N4-(((R)-1,2,3,4-tetrahydroisoquinolin-3-yl)methyl)-N4-((S)-5,6,7,8-tetrahydroquinolin-8-yl)butane-1,4-diamine (**7d**): Synthesis was carried out according to General Reductive Amination Protocol A using amine **6** and benzaldehyde. Purified via column chromatography (CombiFlash, 24 g column, 30 mL/min) eluting with the following gradient to yield a clear oil (235 mg, 0.364 mmol, 68% yield): 0-5 min, 0% MeOH in DCM; 5-15 min, 0-5% MeOH in DCM; 15-25 min, 5% MeOH in DCM; 25-30 min, 5-25% MeOH in DCM. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.36 (d, *J* = 16.0 Hz, 1H), 7.36 (d, *J* = 8.0 Hz, 4H), 7.30 (t, *J* = 7.5 Hz, 4H), 7.21-7.25 (m, 3H), 7.06-7.11 (m, 2H), 6.96-7.02 (m, 3H), 4.64-4.67 (m, 1.5H), 4.36 (br s, 0.5H), 4.14 (d, *J* = 17.0 Hz, 1H), 3.82-3.92 (m, 1H), 3.54 (s, 4H), 2.90-3.10 (m, 2H), 2.50-2.81 (m, 5H), 2.32-2.37 (m, 3H), 1.91-2.00 (m, 2H), 1.62-1.73 (m, 2H), 1.48-1.50 (m, 11H), 1.27-1.35 (m, 2H). HRMS (NSI) *m/z* = 645.41632 (M + H); Theo. for $C_{42}H_{52}O_2N_4$ + H = 645.41630. LC-MS (ESI-API) 75-95% MeOH in H_2O, 5 min, *m/z* = 645.4 (M + H), 323.2 (M/2 + H), t = 1.072 min; 50-95% MeOH in H_2O, 8 min, *m/z* = 645.3 (M + H), 323.2 (M/2 + H), t = 4.371 min. BOC group removal was carried out according to the General Deprotection Protocol. Purified via column chromatography (CombiFlash, 12 g column, 25 mL/min) eluting with the following gradient to yield a yellow oil (133 mg, 0.244 mmol, 67% yield): 0-5 min, 0% 100:10:1 DCM/MeOH/NH_4OH; 5-25 min, 0-100% 100:10:1 DCM/MeOH/NH_4OH; 25-30 min, 100% 100:10:1 DCM/MeOH/NH_4OH. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.44 (d, *J* = 4.5 Hz, 1H), 7.37 (d, *J* = 7.5 Hz, 4H), 7.26-7.32 (m, 5H), 7.19-7.22 (m, 2H), 7.08-7.10 (m, 2H), 7.00-7.04 (m, 3H), 4.00-4.04 (m, 2H), 3.89 (d, *J* = 14.0 Hz, 1H), 3.59 (d, *J* = 13.5 Hz, 2H), 3.51 (d, *J* = 14.0 Hz, 2H), 3.04-3.09 (m, 1H), 2.88 (dd, *J* = 13.0 Hz, *J* = 2.5 Hz, 1H), 2.63-2.78 (m, 4H), 2.53-2.59 (m,

2H), 2.36-2.44 (m, 3H), 2.26-2.31 (app t, *J* = 11.8 Hz, 1H), 2.00-2.04 (m, 1H), 1.94-1.97 (m, 1H), 1.82-1.89 (m, 1H), 1.64-1.73 (m, 1H), 1.47-1.62 (m, 4H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 159.0, 146.8, 140.1 (2C), 136.5, 135.9, 134.9, 134.0, 129.2, 128.9 (4C), 128.2 (4C), 126.8 (2C), 126.6, 126.0, 125.5, 121.4, 61.4, 58.5 (2C), 57.9, 54.5, 53.5, 52.4, 48.9, 34.0, 29.6, 29.5, 27.6, 24.9, 22.1. HRMS (NSI) *m/z* = 545.36387 (M + H); Theo. for $C_{37}H_{44}N_4$ + H = 545.36387. LC-MS (ESI-API) 75-95% MeOH in H_2O, 5 min, *m/z* = 545.3 (M + H), 273.2 (M/2 + H), t = 0.815 min; 25-95% MeOH in H_2O, 8 min, *m/z* = 545.2 (M + H), 273.2 (M/2 + H), t = 6.726 min.

General Reductive Amination Protocol C: A solution of amine (1.00 eq) in DCM (0.10 M) was added to a flask with a stir bar. Added a solution of aldehyde (0.95 eq) in DCM (0.10 M), and the resulting mixture was allowed to stir under Ar at room temperature for 30 min. After this time, sodium triacetoxyborohydride (3.00 eq) was added, and the resulting mixture was allowed to stir at room temperature under Ar. After 5 h, TLC indicated almost complete conversion of starting material. The reaction mixture was diluted with DCM and quenched with saturated aqueous sodium bicarbonate. The resulting aqueous layer was extracted 3 times with DCM. Combined organic layers were dried over anhydrous sodium sulfate, filtered, and evaporated under reduced pressure. The crude material was purified via column chromatography to yield a 2:1 mixture of desired product to double addition product. A solution of this mixture (1.00 eq mono-adduct, 0.50 eq disubstituted adduct) in DCM (0.30 M) was added to a flask with a stir bar. Added *N*-ethyl-*N*-isopropylpropan-2-amine (1.20 eq), and the resulting solution was stirred under Ar at room temperature. Added a solution of di-tert-butyl dicarbonate (1.05 eq) in DCM (0.20 M), and the resulting reaction mixture was stirred vigorously under Ar at room temperature. After one week, TLC indicated mostly starting material. Added 4-dimethylaminopyridine (0.20 eq), and the resulting reaction mixture was allowed to stir under Ar

at room temperature. After 6 h, LC-MS indicated complete conversion of the mono-substituted starting material to the desired BOC-protected derivative. The reaction mixture was poured over saturated aqueous sodium bicarbonate, and the resulting aqueous layer was extracted 3 times with DCM. Combined organic layers were dried over anhydrous sodium sulfate, filtered, and evaporated under reduced pressure.

N1-(pyridin-3-ylmethyl)-N4-(((R)-1,2,3,4-tetrahydroisoquinolin-3-yl)methyl)-N4-((S)-5,6,7,8-tetrahydroquinolin-8-yl)butane-1,4-diamine (**8b**): Reductive amination was carried out according to General Reductive Amination Protocol C using amine **6** and 3-pyridinecarboxaldehyde. Purified via column chromatography (CombiFlash, 12 g column, 30 mL/min) eluting with the following gradient to yield a clear oil (70 mg, 0.107 mmol, 78% yield): 0-5 min, 0% MeOH in DCM; 5-15 min, 0-10% MeOH in DCM; 15-20 min, 10% MeOH in DCM. ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.49-8.54 (m, 2H), 8.36 (d, *J* = 17.4 Hz, 1H), 7.55-7.66 (m, 1H), 7.22-7.25 (m, 2H), 7.07-7.10 (m, 2H), 6.96-7.02 (m, 3H), 4.60-4.68 (m, 2H), 4.36-4.42 (m, 2H), 4.14 (d, *J* = 15.0 Hz, 1H), 3.84-3.89 (m, 1H), 2.94-3.18 (m, 4H), 2.37-2.71 (m, 6H), 1.93-2.00 (m, 2H), 1.59-1.69 (m, 2H), 1.44-1.50 (m, 20H), 1.32-1.36 (m, 2H). HRMS (NSI) *m/z* = 656.41693 (M + H); Theo. for $C_{39}H_{53}O_4N_5$ + H = 656.41703. LC-MS (ESI-API) 75-95% MeOH in H_2O, 5 min, *m/z* = 656.4 (M + H), 328.8 (M/2 + H), t = 3.020 min; 50-95% MeOH in H_2O, 8 min, *m/z* = 656.4 (M + H), 328.6 (M/2 + H), t = 4.528 min. BOC group removal was carried out according to the General Deprotection Protocol. Purified via column chromatography (CombiFlash, 12 g, 25 mL/min) eluting with the following gradient to yield a white oil (19 mg, 0.042 mmol, 24% yield): 0-3 min, 0% 100:10:1 DCM/MeOH/NH_4OH in DCM; 3-15 min, 0-100% 100:10:1 DCM/MeOH/NH_4OH in DCM; 15-30 min, 100% 100:10:1 DCM/MeOH/NH_4OH. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.55 (d, *J* = 2.0 Hz, 1H), 8.48 (dd, *J* = 1.8 Hz, *J* = 4.8 Hz, 1H), 8.42 (dd, *J* = 4.5 Hz, *J* = 1.5 Hz, 1H),

7.66 (dt, *J* = 1.9 Hz, *J* = 7.7 Hz, 1H), 7.32 (d, *J* = 7.0 Hz, 1H), 7.23 (dd, *J* = 5.0 Hz, *J* = 7.5 Hz, 1H), 7.06-7.10 (m, 2H), 6.99-7.04 (m, 3H), 4.06-4.08 (m, 1H), 4.04 (d, *J* = 15.0 Hz, 1H), 3.89 (d, *J* = 15.0 Hz, 1H), 3.79 (s, 2H), 3.01-3.04 (m, 1H), 2.97 (dd, *J* = 3.0 Hz, *J* = 13.5 Hz, 1H), 2.71-2.79 (m, 2H), 2.67 (m, 1H), 2.58-2.64 (m, 5H), 2.34-2.45 (m, 3H), 2.04-2.09 (m, 1H), 1.96-1.99 (m, 1H), 1.87-1.99 (m, 1H), 1.67-1.74 (m, 1H), 1.50-1.58 (m, 4H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 158.9, 149.9, 148.6, 146.9, 136.7, 136.0, 136.0, 135.7, 134.8, 134.1, 129.3, 126.6, 126.1, 125.7, 123.6, 121.6, 61.5, 58.0, 54.5, 52.5, 51.6, 49.6, 48.9, 34.0, 29.6, 29.3, 28.0, 27.8, 22.2. HRMS (NSI) *m/z* = 456.31169 (M + H); Theo. for $C_{29}H_{37}N_5$ + H = 456.31217. LC-MS (ESI-API) 25-95% MeOH in H_2O, 8 min, *m/z* = 456.2 (M + H), 228.6 (M/2 + H), t = 0.734 min; 10-95% MeOH in H_2O, 10 min, *m/z* = 456.2 (M + H), 228.2 (M/2 + H), t = 6.236 min.

N1-(pyridin-4-ylmethyl)-N4-(((R)-1,2,3,4-tetrahydroisoquinolin-3-yl)methyl)-N4-((S)-5,6,7,8-tetrahydroquinolin-8-yl)butane-1,4-diamine (**8c**): Reductive amination was carried out according to General Reductive Amination Protocol C using amine **6** and 4-pyridinecarboxaldehyde. Purified via column chromatography eluting with 10:1 DCM/MeOH. The resulting material was dissolved in diethyl ether, decanted away from a small amount of white residue, and evaporated under reduced pressure to yield a yellow oil (72 mg, 0.110 mmol, 79% yield). ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.55 (br s, 2H), 8.34-8.36 (m, 1H), 7.25 (br s, 1H), 7.08-7.13 (m, 4H), 6.97-7.01 (m, 3H), 4.59-4.69 (m, 2H), 4.38-4.43 (m, 2H), 4.14-4.16 (m, 1H), 3.84 (br s, 1H), 2.91-3.23 (m, 4H), 2.60-2.71 (m, 5H), 2.39 (br s, 1H), 1.93-2.01 (m, 2H), 1.60-1.68 (m, 2H), 1.37-1.50 (m, 22H). HRMS (NSI) *m/z* = 656.41672 (M + H); Theo. for $C_{39}H_{53}O_4N_5$ + H = 656.41703. LC-MS (ESI-API) 50-95% MeOH in H_2O, 8 min, *m/z* = 656.4 (M + H), 328.8 (M/2 + H), t = 4.496 min; 75-95% MeOH in H_2O, 5 min, *m/z* = 656.4 (M + H), 328.8 (M/2 + H), t = 2.137 min. BOC group removal was carried out according to the General Deprotection Protocol. Purified via

column chromatography eluting with 4:1 DCM/MeOH, followed by 40:1:1 DCM/MeOH/Et_3N. The resulting material was dissolved in diethyl ether, decanted away from a small amount remaining residue, and evaporated under reduced pressure to yield a yellow oil (11 mg, 0.024 mmol, 14% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.51 (d, *J* = 4.0 Hz, 2H), 8.44 (d, *J* = 2.4 Hz, 1H), 7.32 (d, *J* = 6.0 Hz, 1H), 7.24 (d, *J* = 4.0 Hz, 2H), 7.06-7.10 (m, 2H), 6.99-7.05 (m, 3H), 4.05-4.09 (m, 1H), 4.03 (d, *J* = 10.0 Hz, 1H), 3.89 (d, *J* = 10.0 Hz, 1H), 3.80 (s, 2H), 3.02-3.08 (m, 1H), 2.97 (dd, *J* = 2.0 Hz, *J* = 8.8 Hz, 1H), 2.57-2.80 (m, 7H), 2.32-2.45 (m, 3H), 2.04-2.10 (m, 2H), 1.94-2.01 (m, 1H), 1.84-1.91 (m, 1H), 1.65-1.75 (m, 1H), 1.50-1.62 (m, 4H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 158.7, 149.8 (2C), 149.7, 146.8, 136.6, 135.6, 134.7, 134.0, 129.2, 126.5, 126.0, 125.6, 123.1 (2C), 121.5, 61.6, 57.9, 54.3, 52.8, 52.4, 49.5, 48.7, 33.8, 29.5, 28.8, 27.9, 27.6, 22.1. HRMS (NSI) *m/z* = 456.31165 (M + H); Theo. for $C_{29}H_{37}N_5$ + H = 456.31217. LC-MS (ESI-API) 50-95% MeOH in H_2O, 6 min, *m/z* = 456.2 (M + H), 228.6 (M/2 + H), t = 0.910 min; 25-95% MeOH in H_2O, 8 min, *m/z* = 456.2 (M + H), 228.6 (M/2 + H), t = 4.689 min.

General Reductive Amination Protocol D: A solution of amine (1.00 eq) in DCM (0.30 M) was added to a flask with a stir bar. Added a solution of aldehyde (1.00 eq) in DCM (0.30 M), and the resulting mixture was stirred under Ar at room temperature for 10 min. Added titanium isopropoxide (1.50 eq) in dropwise fashion, and the resulting mixture was stirred under Ar at room temperature for 2 h. After this time, sodium borohydride (3.00 eq) and MeOH (0.90 M) were added, and the resulting reaction mixture was stirred under Ar at room temperature. After 2 h, TLC indicated almost complete conversion of starting material. The reaction was quenched with 1 M aqueous sodium hydroxide. The organic layer was separated, and MeOH from the aqueous layer was evaporated under reduced pressure. The resulting aqueous layer was extracted

twice with DCM, and combined organic layers were dried over anhydrous sodium sulfate, filtered, and evaporated under reduced pressure.

N1-benzyl-N4-(((R)-1,2,3,4-tetrahydroisoquinolin-3-yl)methyl)-N4-((S)-5,6,7,8-tetrahydroquinolin-8-yl)butane-1,4-diamine (**9**): Reductive amination was carried out according to General Reductive Amination Protocol D using amine **6** and benzaldehyde. Purified via column chromatography (CombiFlash, 12 g column, 25 mL/min) eluting with the following gradient to yield a clear oil (121 mg, 0.218 mmol, 44% yield): 0-5 min, 0% MeOH in DCM; 5-20 min, 0-10% MeOH in DCM; 20-30 min, 10% MeOH in DCM; 30-35 min, 10-50% MeOH in DCM. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.37 (d, *J* = 17.0 Hz, 1H), 7.34 (d, *J* = 5.0 Hz, 4H), 7.24-7.30 (m, 2H), 7.06-7.11 (m, 2H), 6.97-7.02 (m, 3H), 4.64-4.68 (m, 1.5H), 4.38 (br s, 0.5H), 4.15 (d, *J* = 17.0 Hz, 1H), 3.87-3.93 (m, 1H), 3.79 (s, 2H), 2.92-3.11 (m, 2H), 2.67-2.72 (m, 2H), 2.59-2.62 (m, 5H), 2.41 (app p, *J* = 6.9 Hz, 1H), 2.02 (br s, 1H), 1.93 (br s, 2H), 1.71-1.74 (m, 1H), 1.58-1.62 (m, 1H), 1.50 (m, 11H), 1.34-1.44 (m, 2H). HRMS (NSI) *m/z* = 555.36929 (M + H); Theo. for $C_{35}H_{46}O_2N_4$ + H = 555.36935. LC-MS (ESI-API) 75-95% MeOH in H_2O, 5 min, *m/z* = 555.2 (M + H), 278.2 (M/2 + H), t = 1.003 min; 50-95% MeOH in H_2O, 8 min, *m/z* = 555.2 (M + H), 278.2 (M/2 + H), t = 4.157 min. BOC group removal was carried out according to the General Deprotection Protocol. Purified via column chromatography (CombiFlash, 12 g, 25 mL/min) eluting with the following gradient to yield a clear oil (20 mg, 0.044 mmol, 20% yield): 0-3 min, 0% 100:10:1 DCM/MeOH/NH_4OH in DCM; 3-15 min, 0-100% 100:10:1 DCM/MeOH/NH_4OH in DCM; 15-30 min, 100% 100:10:1 DCM/MeOH/NH_4OH. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.44 (dd, *J* = 1.3 Hz, *J* = 4.8 Hz, 1H), 7.29-7.36 (m, 5H), 7.24 (m, 1H), 7.07-7.11 (m, 2H), 6.99-7.05 (m, 3H), 4.07-4.09 (m, 1H), 4.36 (d, *J* = 15.0 Hz, 1H), 3.90 (d, *J* = 15.0 Hz, 1H), 3.79 (s, 2H), 3.04-3.08 (m, 1H), 2.97 (dd, *J* = 3.0 Hz, *J* = 13.3 Hz, 1H), 2.72-2.80 (m,

2H), 2.68 (m, 1H), 2.58-2.65 (m, 5H), 2.42 (dd, *J* = 11.3 Hz, *J* = 15.8 Hz, 1H), 2.35 (dd, *J* = 10.8 Hz, *J* = 13.0 Hz, 1H), 2.05-2.09 (m, 1H), 1.95-2.01 (m, 1H), 1.85-1.93 (m, 1H), 1.66-1.75 (m, 1H), 1.49-1.58 (m, 5H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 159.0, 146.9, 140.7, 136.6, 135.9, 134.9, 134.1, 129.3, 128.6 (2C), 128.3 (2C), 127.1, 126.6, 126.1, 125.6, 121.5, 61.5, 58.0, 54.6, 54.3, 52.6, 49.7, 48.9, 34.0, 29.7, 29.5, 28.1, 27.9, 22.2. HRMS (NSI) *m/z* = 455.31707 (M + H); Theo. for $C_{30}H_{38}N_4$ + H = 455.31692. LC-MS (ESI-API) 50-95% MeOH in H_2O, 6 min, *m/z* = 455.2 (M + H), 228.2 (M/2 + H), t = 0.891 min; 10-95% MeOH in H_2O, 10 min, *m/z* = 455.2 (M + H), 228.2 (M/2 + H), t = 7.333 min.

N1-(cyclohexylmethyl)-N4-(((R)-1,2,3,4-tetrahydroisoquinolin-3-yl)methyl)-N4-((S)-5,6,7,8-tetrahydroquinolin-8-yl)butane-1,4-diamine (**11a**): Synthesis was carried out according to General Reductive Amination Protocol D using amine **6** and aldehyde **10a**. Purified via column chromatography (CombiFlash, 24 g column, 30 mL/min) eluting with the following gradient to yield a clear oil (82 mg, 0.146 mmol, 27% yield): 0-3 min, 0% 100:10:1 DCM/MeOH/NH_4OH in DCM; 3-18 min, 0-100% 100:10:1 DCM/MeOH/NH_4OH in DCM; 18-30 min, 100% 100:10:1 DCM/MeOH/NH_4OH. ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.37 (d, *J* = 21.6 Hz, 1H), 7.24 (d, *J* = 7.2 Hz, 1H), 7.07-7.11 (m, 2H), 6.96-7.03 (m, 3H), 4.63-4.67 (m, 1.5H), 4.38 (br s, 0.5H), 4.13 (d, *J* = 18.0 Hz, 1H), 3.86-3.92 (m, 1H), 2.92-3.10 (m, 2H), 2.69 (m, 2H), 2.52-2.62 (m, 5H), 2.40-2.42 (m, 3H), 2.02 (m, 1H), 1.93 (m, 1H), 1.70-1.75 (m, 4H), 1.60-1.67 (m, 2H), 1.50 (s, 9H), 1.34-1.46 (m, 5H), 1.20-1.28 (m, 2H), 1.12-1.19 (m, 1H), 0.89 (ddd, *J* = 2.4 Hz, *J* = 12.2 Hz, *J* = 24.6 Hz, 2H). HRMS (NSI) *m/z* = 561.41620 (M + H); Theo. for $C_{35}H_{52}N_4O_2$ + H = 561.41630. LC-MS (ESI-API) 75-95% MeOH in H_2O, 5 min, *m/z* = 561.8 (M + H), 281.5 (M/2 + H), t = 0.831 min; 50-95% MeOH in H_2O, 8 min, *m/z* = 561.8 (M + H), 281.5 (M/2 + H), t = 4.605 min. BOC group removal was carried out according to the General Deprotection Protocol. Purified via

column chromatography (CombiFlash, 12 g column, 25 mL/min) eluting with 100:10:1 DCM/MeOH/NH_4OH to yield a clear oil (28 mg, 0.061 mmol, 42% yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.44 (d, *J* = 4.0 Hz, 1H), 7.31 (d, *J* = 7.5 Hz, 1H), 7.06-7.09 (m, 2H), 7.00-7.04 (m, 3H), 4.03-4.09 (m, 2H), 3.90 (d, *J* = 15.0 Hz, 1H), 3.04-3.09 (m, 1H), 2.96 (dd, *J* = 3.0 Hz, *J* = 13.5 Hz, 1H), 2.73-2.80 (m, 2H), 2.67 (m, 1H), 2.61-2.64 (m, 2H), 2.57-2.59 (m, 3H), 2.39-2.47 (m, 3H), 2.34 (dd, *J* = 10.3 Hz, *J* = 13.3 Hz, 1H), 2.06-2.10 (m, 1H), 1.94-2.00 (m, 1H), 1.86-1.93 (m, 1H), 1.56-1.74 (m, 6H), 1.40-1.56 (m, 6H), 1.10-1.28 (m, 3H), 0.85-0.93 (m, 2H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 158.8, 146.8, 136.5, 135.8, 134.8, 134.0, 129.2, 126.5, 125.9, 125.5, 121.4, 61.4, 57.9, 57.0, 54.5, 52.4, 50.3, 48.8, 38.0, 33.9, 31.6 (2C), 29.6, 29.2, 28.0, 27.8, 26.8, 26.2 (2C), 22.1. HRMS (NSI) *m/z* = 461.36405 (M + H); Theo. for $C_{30}H_{44}N_4$ + H = 461.36387. LC-MS (ESI-API) 25-95% MeOH in H_2O, 8 min, *m/z* = 461.4 (M + H), 231.4 (M/2 + H), t = 5.589 min; 10-95% MeOH in H_2O, 10 min, *m/z* = 461.5 (M + H), 231.4 (M/2 + H), t = 7.668 min.

N1-((4,4-difluorocyclohexyl)methyl)-N4-(((R)-1,2,3,4-tetrahydroisoquinolin-3-yl)methyl)-N4-((S)-5,6,7,8-tetrahydroquinolin-8-yl)butane-1,4-diamine (**11b**): Synthesis was carried out according to General Reductive Amination Protocol D using amine **6** and aldehyde **10b**. Purified via column chromatography (CombiFlash, 24 g column, 30 mL/min) eluting with the following gradient to yield a clear oil (198 mg, 0.332 mmol, 62% yield): 0-3 min, 0% 100:10:1 DCM/MeOH/NH_4OH in DCM; 3-18 min, 0-100% 100:10:1 DCM/MeOH/NH_4OH in DCM; 18-30 min, 100% 100:10:1 DCM/MeOH/NH_4OH. ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.36 (d, *J* = 23.4 Hz, 1H), 7.25 (d, *J* = 6.0 Hz, 1H), 7.07-7.12 (m, 2H), 6.98-7.03 (m, 3H), 4.63-4.69 (m, 1.5H), 4.39 (br s, 0.5H), 4.14 (d, *J* = 17.4 Hz, 1H), 3.89 (m, 1H), 3.02-3.09 (m, 1H), 2.93-2.95 (m, 1H), 2.69 (m, 2H), 2.54-2.62 (m, 5H), 2.48 (d, *J* = 7.2 Hz, 2H), 2.39-2.44 (m, 1H), 2.06-2.11 (m, 2H),

2.02 (m, 1H), 1.93 (m, 1H), 1.82 (d, *J* = 13.8 Hz, 2H), 1.69-1.76 (m, 3H), 1.59-1.68 (m, 2H), 1.50 (m, 10H), 1.40-1.47 (m, 4H), 1.23-1.30 (m, 2H). HRMS (NSI) *m/z* = 597.39766 (M + H); Theo. for $C_{35}H_{50}N_4O_2F_2$ + H = 597.39746. LC-MS (ESI-API) 75-95% MeOH in H_2O, 5 min, *m/z* = 598.0 (M + H), 299.6 (M/2 + H), t = 1.002 min; 50-95% MeOH in H_2O, 8 min, *m/z* = 598.0 (M + H), 299.6 (M/2 + H), t = 4.373 min. BOC group removal was carried out according to the General Deprotection Protocol. Purified via column chromatography (CombiFlash, 12 g column, 25 mL/min) eluting with 100:10:1 DCM/MeOH/NH_4OH to yield a clear oil (161 mg, 0.324 mmol, 98% yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.44 (dd, *J* = 1.5 Hz, *J* = 4.5 Hz, 1H), 7.32 (dt, *J* = 0.7 Hz, *J* = 7.5 Hz, 1H), 7.06-7.10 (m, 2H), 6.99-7.06 (m, 3H), 4.02-4.09 (m, 2H), 3.91 (d, *J* = 15.0 Hz, 1H), 3.04-3.09 (m, 1H), 2.97 (dd, *J* = 3.3 Hz, *J* = 13.3 Hz, 1H), 2.71-2.80 (m, 2H), 2.68 (m, 1H), 2.58-2.65 (m, 5H), 2.49 (d, *J* = 6.5 Hz, 2H), 2.41 (dd, *J* = 11.3 Hz, *J* = 15.8 Hz, 1H), 2.35 (dd, *J* = 10.0 Hz, *J* = 13.0 Hz, 1H), 2.05-2.11 (m, 3H), 1.95-2.01 (m, 1H), 1.86-1.94 (m, 1H), 1.80-1.83 (m, 2H), 1.62-1.76 (m, 3H), 1.45-1.60 (m, 5H), 1.25 (ddd, *J* = 3.3 Hz, *J* = 12.8 Hz, *J* = 25.0 Hz, 2H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 158.7, 146.8, 136.6, 135.7, 134.7, 134.0, 129.1, 126.5, 125.9, 125.5, 123.8 (t, *J* = 238.8 Hz), 121.4, 61.4, 57.9, 55.3, 54.4, 52.4, 50.3, 48.8, 36.3, 33.9, 33.4 (dd, *J* = 22.5 Hz, *J* = 25.0 Hz, 2C), 29.5, 29.0, 28.0, 27.7, 27.4, 27.3, 22.0. ^{19}F NMR (376 MHz, $CDCl_3$, TFA standard) δ -91.3 (d, *J* = 234.2 Hz), -102.0 (app dt, *J* = 33.2 Hz, *J* = 233.1 Hz). HRMS (NSI) *m/z* = 497.34518 (M + H); Theo. for $C_{30}H_{42}N_4F_2$ + H = 497.34503. LC-MS (ESI-API) 50-95% MeOH in H_2O, 6 min, *m/z* = 497.6 (M + H), 249.4 (M/2 + H), t = 0.887 min; 10-95% MeOH in H_2O, 10 min, *m/z* = 497.8 (M + H), 249.4 (M/2 + H), t = 7.390 min.

N1-((tetrahydro-2H-pyran-4-yl)methyl)-N4-(((R)-1,2,3,4-tetrahydroisoquinolin-3-yl)methyl)-N4-((S)-5,6,7,8-tetrahydroquinolin-8-yl)butane-1,4-diamine (**11c**): Synthesis was carried out

according to General Reductive Amination Protocol D using amine **6** and aldehyde **10c**. Purified via column chromatography (CombiFlash, 12 g, 25 mL/min) eluting with the following gradient to yield a clear oil (170 mg, 0.302 mmol, 61% yield): 0-5 min, 0% MeOH in DCM; 5-20 min, 0-10% MeOH in DCM; 20-30 min (10% MeOH in DCM); 30-35 min, 10-50% MeOH in DCM. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.37 (d, *J* = 16.5 Hz, 1H), 7.25 (d, *J* = 7.5 Hz, 1H), 7.07-7.12 (m, 2H), 6.97-7.02 (m, 3H), 4.62-4.68 (m, 1.5H), 4.38 (br s, 0.5H), 4.14 (d, *J* = 17.0 Hz, 1H), 3.97 (dd, *J* = 3.8 Hz, *J* = 11.3 Hz, 2H), 3.88-3.89 (m, 1H), 3.38 (dt, *J* = 11.8, *J* = 1.5 Hz, 2H), 3.01-3.09 (m, 1H), 2.92-2.95 (m, 1H), 2.67-2.69 (m, 2H), 2.55-2.62 (m, 5H), 2.49 (d, *J* = 6.5 Hz, 2H), 2.38-2.44 (m, 1H), 1.92-2.02 (m, 2H), 1.72 (m, 2H), 1.62-1.65 (m, 4H), 1.50 (s, 9H), 1.37-1.46 (m, 4H), 1.29 (ddd, *J* = 4.1 Hz, *J* = 12.4 Hz, *J* = 24.5 Hz, 2H). HRMS (NSI) *m/z* = 563.39569 (M + H); Theo. for $C_{34}H_{50}O_3N_4$ + H = 563.39557. LC-MS (ESI-API) 75-95% MeOH in H_2O, 5 min, *m/z* = 563.3 (M + H), 282.2 (M/2 + H), t = 0.865 min; 50-95% MeOH in H_2O, 8 min, *m/z* = 563.4 (M + H), 282.2 (M/2 + H), t = 4.023 min. BOC group removal was carried out according to the General Deprotection Protocol. Purified via column chromatography (CombiFlash, 12 g column, 25 mL/min) eluting with the following gradient to yield a clear oil (76 mg, 0.164 mmol, 54% yield): 0-3 min, 0% 100:10:1 DCM/MeOH/NH_4OH in DCM; 3-15 min, 0-100% 100:10:1 DCM/MeOH/NH_4OH in DCM; 15-30 min, 100% 100:10:1 DCM/MeOH/NH_4OH. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.44 (dd, *J* = 4.5 Hz, *J* = 1.0 Hz, 1H), 7.32 (dd, *J* = 0.5 Hz, *J* = 7.5 Hz, 1H), 7.06-7.10 (m, 2H), 6.99-7.05 (m, 3H), 4.07-4.09 (m, 1H), 4.04 (d, *J* = 15.0 Hz, 1H), 3.96 (dd, *J* = 4.0 Hz, *J* = 11.0 Hz, 2H), 3.90 (d, *J* = 15.0 Hz, 1H), 3.38 (dt, *J* = 1.8 Hz, *J* = 11.8 Hz, 2H), 3.04 (m, 1H), 2.97 (dd, *J* = 3.3 Hz, *J* = 13.3 Hz, 1H), 2.71-2.80 (m, 2H), 2.68 (m, 1H), 2.58-2.65 (m, 5H), 2.49 (d, *J* = 7.0 Hz, 2H), 2.42 (dd, *J* = 11.3 Hz, *J* = 16.0 Hz, 1H), 2.36 (dd, *J* = 10.3 Hz, *J* = 13.3 Hz, 1H), 2.05-2.10 (m, 1H), 1.96-2.00 (m, 1H), 1.88-1.93 (m, 1H), 1.65-1.75 (m, 2H), 1.61-

1.64 (m, 2H), 1.47-1.59 (m, 4H), 1.28 (ddd, *J* = 4.5 Hz, *J* = 12.3 Hz, *J* = 24.8 Hz, 2H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 158.8, 146.8, 136.6, 135.7, 134.8, 134.1, 129.2, 126.5, 126.0, 125.6, 121.5, 68.0 (2C), 61.5, 57.9, 56.4, 54.5, 52.5, 50.3, 48.9, 35.5, 33.9, 31.5 (2C), 29.6, 29.2, 28.1, 27.8, 22.1. HRMS (NSI) *m/z* = 463.34319 (M + H); Theo. for $C_{29}H_{42}ON_4$ + H = 463.34314. LC-MS (ESI-API) 25-95% MeOH in H_2O, 8 min, *m/z* = 463.2 (M + H), 232.2 (M/2 + H), t = 0.734 min; 10-95% MeOH in H_2O, 10 min, *m/z* = 463.2 (M + H), 232.2 (M/2 + H), t = 6.799 min.

N1-(piperidin-4-ylmethyl)-N4-(((R)-1,2,3,4-tetrahydroisoquinolin-3-yl)methyl)-N4-((S)-5,6,7,8-tetrahydroquinolin-8-yl)butane-1,4-diamine (**11d**): Synthesis was carried out according to General Reductive Amination Protocol D using amine **6** and aldehyde **10d**. Purified via column chromatography (CombiFlash, 24 g, 30 mL/min) eluting with the following gradient to yield a white foam (177 mg, 0.267 mmol, 53% yield): 0-3 min, 0% 100:10:1 DCM/MeOH/NH_4OH in DCM; 3-18 min, 0-100% 100:10:1 DCM/MeOH/NH_4OH in DCM; 18-30 min, 100% 100:10:1 DCM/MeOH/NH_4OH in DCM. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.37 (d, *J* = 17.5 Hz, 1H), 7.25 (d, *J* = 7.0 Hz, 1H), 7.07-7.12 (m, 2H), 6.97-7.02 (m, 3H), 4.62-4.69 (m, 1.5H), 4.38 (br s, 0.5H), 4.08-4.16 (m, 3H), 3.89 (br s, 1H), 3.02-3.10 (m, 1H), 2.92-2.95 (m, 1H), 2.69 (m, 3H), 2.59-2.63 (m, 4H), 2.53 (br s, 2H), 2.47 (d, *J* = 6.5 Hz, 2H), 2.41 (app p, *J* = 6.8 Hz, 1H), 2.01-2.05 (m, 1H), 1.93 (br s, 1H), 1.68-1.70 (m, 4H), 1.58-1.70 (m, 2H), 1.50 (s, 9H), 1.33-1.46 (m, 13H), 1.10 (ddd, *J* = 4.1 Hz, *J* = 12.4 Hz, *J* = 24.5 Hz, 2H). HRMS (NSI) *m/z* = 662.46344 (M + H); Theo. for $C_{39}H_{59}O_4N_5$ + H = 662.46398. LC-MS (ESI-API) 75-95% MeOH in H_2O, 5 min, *m/z* = 662.4 (M + H), 562.4 (M + H – BOC), 303.8 ((M - BOC)/2 + Na), t = 1.135 min; 50-95% MeOH in H_2O, 8 min, *m/z* = 662.4 (M + H), 562.4 (M + H – BOC), 303.8 ((M - BOC)/2 + Na), t = 4.329 min. BOC group removal was carried out according to the General Deprotection Protocol. Purified via column chromatography (CombiFlash, 12 g column,

25 mL/min) eluting with 80:20:3 DCM/MeOH/NH_4OH to yield a yellow foam (123 mg, 0.267 mmol, 74% yield. ^1H NMR (500 MHz, $CDCl_3$) δ 8.44 (d, *J* = 3.5 Hz, 1H), 7.33 (d, *J* = 7.5 Hz, 1H), 7.07-7.11 (m, 2H), 7.01-7.06 (m, 3H), 4.06-4.11 (m, 2H), 3.92 (d, *J* = 15.0 Hz, 1H), 3.25-3.41 (br s, 3H), 3.13 (app d, *J* = 12.5 Hz, 2H), 2.95-3.04 (m, 3H), 2.73-2.80 (m, 2H), 2.54-2.68 (m, 7H), 2.42-2.51 (m, 3H), 2.08-2.10 (m, 1H), 1.96-1.99 (m, 1H), 1.85-1.93 (m, 1H), 1.67-1.77 (m, 3H), 1.49-1.66 (m, 4H), 1.18-1.25 (m, 3H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 158.7, 146.8, 136.7, 135.3, 134.6, 134.1, 129.2, 126.5, 126.1, 125.6, 121.5, 61.6, 57.8, 56.0, 54.3, 52.5, 52.2, 50.1, 48.6, 45.7, 35.9, 33.7, 30.6, 30.5, 29.5, 28.8, 27.7 (2C), 22.1. HRMS (NSI) *m/z* = 462.35969 (M + H); Theo. for $C_{29}H_{43}N_5$ + H = 462.35912. LC-MS (ESI-API) 25-95% MeOH in H_2O, 8 min, *m/z* = 462.4 (M + H), 231.8 (M/2 + H), t = 0.738 min; 10-95% MeOH in H_2O, 10 min, *m/z* = 462.4 (M + H), 231.8 (M/2 + H), t = 0.623 min.

N1-((tetrahydro-2H-thiopyran-4-yl)methyl)-N4-(((R)-1,2,3,4-tetrahydroisoquinolin-3-yl)methyl)-N4-((S)-5,6,7,8-tetrahydroquinolin-8-yl)butane-1,4-diamine (**11e**): Synthesis was carried out according to General Reductive Amination Protocol D using amine **6** and aldehyde **10e**. Purified via column chromatography (CombiFlash, 24 g column, 30 mL/min) eluting with the following gradient to yield a clear oil (179 mg, 0.309 mmol, 58% yield): 0-3 min, 0% 100:10:1 DCM/MeOH/NH_4OH in DCM; 3-18 min, 0-100% 100:10:1 DCM/MeOH/NH_4OH in DCM; 18-30 min, 100% 100:10:1 DCM/MeOH/NH_4OH. ^1H NMR (600 MHz, $CDCl_3$) δ 8.36 (d, *J* = 22.8 Hz, 1H), 7.24 (d, *J* = 6.0 Hz, 1H), 7.07-7.11 (m, 2H), 6.97-7.02 (m, 3H), 4.62-4.68 (m, 1.5H), 4.38 (br s, 0.5H), 4.13 (d, *J* = 16.8 Hz, 1H), 3.88 (m, 1H), 3.01-3.09 (m, 1H), 2.92-2.94 (m, 1H), 2.66-2.70 (m, 4H), 2.58-2.61 (m, 5H), 2.52 (m, 2H), 2.44 (d, *J* = 6.6 Hz, 2H), 2.38-2.43 (m, 1H), 2.04-2.06 (m, 3H), 1.92 (m, 1H), 1.73 (m, 1H), 1.59-1.64 (m, 1H), 1.50 (s, 9H), 1.43-1.46 (m, 5H), 1.35 (ddd, *J* = 2.6 Hz, *J* = 12.2 Hz, *J* = 24.5 Hz, 2H). HRMS (NSI) *m/z* =

579.37342 (M + H); Theo. for $C_{34}H_{50}N_4O_2S$ + H = 579.37272. LC-MS (ESI-API) 75-95% MeOH in H_2O, 5 min,*m/z* = 579.6 (M + H), 290.4 (M/2 + H), t = 0.852 min; 50-95% MeOH in H_2O, 8 min, *m/z* = 579.6 (M + H), 290.4 (M/2 + H), t = 4.044 min. BOC group removal was carried out according to the General Deprotection Protocol. Purified via column chromatography (CombiFlash, 12 g column, 25 mL/min) eluting with 100:10:1 DCM/MeOH/NH_4OH to yield a clear oil (127 mg, 0.265 mmol, 86% yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.44 (dd, *J* = 1.5 Hz, *J* = 4.5 Hz, 1H), 7.32 (dd, *J* = 0.5 Hz, *J* = 7.5 Hz, 1H), 7.06-7.10 (m, 2H), 7.00-7.05 (m, 3H), 4.02-4.09 (m, 2H), 3.90 (d, *J* = 15.0 Hz, 1H), 3.03-3.08 (m, 1H), 2.96 (dd, *J* = 3.3 Hz, *J* = 13.3 Hz, 1H), 2.71-2.80 (m, 3H), 2.67-2.70 (m, 2H), 2.63-2.64 (m, 1H), 2.56-2.62 (m, 6H), 2.45 (d, *J* = 7.0 Hz, 1H), 2.41 (dd, *J* = 11.8 Hz, *J* = 16.3 Hz, 1H), 2.34 (dd, *J* = 10.0 Hz, *J* = 13.0 Hz, 1H), 2.02-2.10 (m, 3H), 1.95-2.00 (m, 1H), 1.86-1.93 (m, 1H), 1.66-1.74 (m, 1H), 1.43-1.59 (m, 6H), 1.34 (ddd, *J* = 3.5 Hz, *J* = 11.8 Hz, *J* = 24.8 Hz, 2H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 158.7, 146.7, 136.5, 135.6, 134.7, 134.0, 129.1, 126.5, 125.9, 125.5, 121.4, 61.4, 57.9, 56.6, 54.4, 52.4, 50.2, 48.7, 37.7, 33.8, 32.5 (2C), 29.5, 29.0, 28.6 (2C), 27.9, 27.7, 22.0. HRMS (NSI) *m/z* = 479.32040 (M + H); Theo. for $C_{29}H_{42}N_4S$ + H = 479.32029. LC-MS (ESI-API) 50-95% MeOH in H_2O, 6 min, *m/z* = 479.6 (M + H), 240.4 (M/2 + H), t = 0.900 min; 10-95% MeOH in H_2O, 10 min, *m/z* = 479.6 (M + H), 240.4 (M/2 + H), t = 7.311 min.

4-(((4-((((R)-1,2,3,4-tetrahydroisoquinolin-3-yl)methyl)((S)-5,6,7,8-tetrahydroquinolin-8-yl)amino)butyl)amino)methyl)tetrahydro-2H-thiopyran 1,1-dioxide (**11f**): Synthesis was carried out according to General Reductive Amination Protocol D using amine **6** and aldehyde **10f**. Purified via column chromatography (CombiFlash, 24 g column, 30 mL/min) eluting with the following gradient to yield a white foam (100 mg, 0.164 mmol, 33% yield): 0-3 min, 0% 100:10:1 DCM/MeOH/NH_4OH in DCM; 3-18 min, 0-100% 100:10:1 DCM/MeOH/NH_4OH in

DCM; 18-30 min, 100% 100:10:1 DCM/MeOH/NH_4OH. ^1H NMR (500 MHz, $CDCl_3$) δ 8.37 (d, *J* = 18.0 Hz, 1H), 7.25 (m, 1H), 7.08-7.13 (m, 2H), 6.98-7.06 (m, 3H), 4.59-4.71 (m, 1.5H), 4.39 (br s, 0.5H), 4.14 (d, *J* = 17.0 Hz, 1H), 3.89 (dd, *J* = 6.0 Hz, *J* = 9.0 Hz, 1H), 3.05-3.08 (m, 3H), 2.92-2.99 (m, 3H), 2.53-2.73 (m, 9H), 2.40-2.42 (m, 1H), 2.17-2.20 (m, 2H), 2.01-2.05 (m, 1H), 1.92-1.94 (m, 1H), 1.80-1.88 (m, 2H), 1.71-1.75 (m, 1H), 1.56-1.67 (m, 2H), 1.50 (s, 9H), 1.37-1.44 (m, 5H). HRMS (NSI) *m/z* = 611.36295 (M + H); Theo. for $C_{34}H_{50}N_4O_4S$ + H = 611.36255. LC-MS (ESI-API) 75-95% MeOH in H_2O, 5 min, *m/z* = 611.2 (M + H), 511.3 (M – BOC + H), 306.2 (M/2 + H), t = 0.875 min; 50-95% MeOH in H_2O, 8 min, *m/z* = 611.3 (M + H), 511.2 (M – BOC + H), 306.2 (M/2 + H), t = 3.982 min. BOC group removal was carried out according to the General Deprotection Protocol. Purified via column chromatography (CombiFlash, 12 g column, 25 mL/min) eluting with 100:10:1 DCM/MeOH/NH_4OH to yield a white foam (77 mg, 0.164 mmol, 92% yield). ^1H NMR (500 MHz, $CDCl_3$) δ 8.44 (dd, *J* = 1.5 Hz, *J* = 4.5 Hz, 1H), 7.33 (dt, *J* = 0.8 Hz, *J* = 7.7 Hz, 1H), 7.07-7.11 (m, 2H), 7.00-7.06 (m, 3H), 4.02-4.09 (m, 2H), 3.89 (d, *J* = 15.0 Hz, 1H), 3.01-3.07 (m, 3H), 2.90-3.00 (m, 3H), 2.71-2.80 (m, 2H), 2.69-2.71 (m, 1H), 2.58-2.66 (m, 5H), 2.53 (d, *J* = 7.0 Hz, 2H), 2.41 (dd, *J* = 11.5 Hz, *J* = 16.0 Hz, 1H), 2.35 (dd, *J* = 10.8 Hz, *J* = 12.8 Hz, 1H), 2.15-2.19 (m, 2H), 2.05-2.10 (m, 1H), 1.96-2.01 (m, 1H), 1.79-1.94 (m, 3H), 1.66-1.75 (m, 2H), 1.46-1.65 (m, 5H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 158.8, 146.8, 136.7, 135.7, 134.8, 134.1, 129.2, 126.5, 126.1, 125.6, 121.5, 61.6, 57.9, 54.5, 54.4, 52.5, 51.0 (2C), 50.2, 48.8, 36.1, 33.9, 29.6, 28.9, 28.4 (2C), 28.0, 27.7, 22.1. HRMS (NSI) *m/z* = 511.31035 (M + H); Theo. for $C_{29}H_{42}N_4O_2S$ + H = 511.31012. LC-MS (ESI-API) 25-95% MeOH in H_2O, 8 min, *m/z* = 511.2 (M + H), 256.2 (M/2 + H), t = 0.732 min; 10-95% MeOH in H_2O, 10 min, *m/z* = 511.2 (M + H), 256.2 (M/2 + H), t = 6.176 min.

General Reductive Amination Protocol E: A solution of amine (1.00 eq) in DCM (0.12 M) was added to a flask with a stir bar. Added a solution (0.54 M) of ketone (1.00 eq) in DCM, and the resulting reaction mixture was stirred at room temperature under Ar for 5 min. Added acetic acid (0.031 mL, 0.538 mmol, 1.00 eq), and the resulting mixture was stirred at room temperature under Ar for 2.5 h. Added sodium triacetoxyborohydride (0.342 g, 1.61 mmol, 3.00 eq), and the resulting reaction mixture was stirred under Ar at room temperature overnight. Once LC-MS indicated disappearance of starting material, the reaction mixture was diluted with DCM and washed once with 1 M aqueous sodium hydroxide. The resulting aqueous layer was extracted three times with DCM. Combined organic layers were dried over anhydrous sodium sulfate, filtered, and evaporated under reduced pressure.

N1-cyclohexyl-N4-(((R)-1,2,3,4-tetrahydroisoquinolin-3-yl)methyl)-N4-((S)-5,6,7,8-tetrahydroquinolin-8-yl)butane-1,4-diamine (**13a**): Synthesis was carried out according to General Reductive Amination Protocol E using amine **6** and ketone **12a**. Purified via column chromatography (CombiFlash, 12 g column, 25 mL/min) eluting with the following gradient to yield a clear oil (162 mg, 0.296 mmol, 55% yield): 0-5 min, 0% MeOH in DCM; 5-15 min, 0-10% MeOH in DCM; 15-25 min, 10% MeOH in DCM; 25-30 min, 10-50% MeOH in DCM. ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.37 (d, J = 23.4 Hz, 1H), 7.25 (d, J = 7.2 Hz, 1H), 6.97-7.12 (m, 5H), 4.63-4.68 (m, 1.5H), 4.38 (br s, 0.5H), 4.14 (d, J = 16.8 Hz, 1H), 3.86-3.92 (m, 1H), 3.02-3.11 (m, 1H), 2.92-2.94 (m, 1H), 2.68-2.72 (m, 2H), 2.57-2.62 (m, 5H), 2.40 (br s, 2H), 2.02 (br s, 1H), 1.93-1.95 (m, 1H), 1.87 (d, J = 11.4 Hz, 2H), 1.72-1.74 (m, 4H), 1.61-1.63 (m, 2H), 1.50 (s, 9H), 1.36-1.43 (m, 4H), 1.25 (ddt, J = 25.5 Hz, J = 12.8 Hz, J = 3.2 Hz, 2H), 1.16 (ddt, J = 24.9 Hz, J = 12.3 Hz, J = 3.4 Hz, 1H), 1.00-1.09 (m, 2H). HRMS (NSI) *m/z* = 547.40106 (M + H); Theo. for $C_{34}H_{50}O_2N_4$ + H = 547.40065. LC-MS (ESI-API) 75-95% MeOH in H_2O, 5 min,

m/z = 547.4 (M + H), 274.2 (M/2 + H), t = 0.826 min; 50-95% MeOH in H_2O, 8 min, *m/z* = 547.4 (M + H), 274.2 (M/2 + H), t = 4.049 min. BOC group removal was carried out according to the General Deprotection Protocol. Purified via column chromatography (CombiFlash, 12 g column, 25 mL/min) eluting with the following gradient to yield a white foam (130 mg, 0.291 mmol, 98% yield): 0-5 min, 0% 100:10:1 DCM/MeOH/NH_4OH in DCM; 5-15 min, 0-100% 100:10:1 DCM/MeOH/NH_4OH in DCM; 15-25 min, 100% 100:10:1 DCM/MeOH/NH_4OH. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.43 (d, *J* = 5.0 Hz, 1H), 7.32 (d, *J* = 12.5 Hz, 1H), 7.06-7.09 (m, 2H), 7.00-7.05 (m, 3H), 4.07-4.09 (dd, *J* = 6.5 Hz, *J* = 10.0 Hz, 1H), 4.05 (d, *J* = 15.0 Hz, 1H), 3.91 (d, *J* = 15.0 Hz, 1H), 3.03-3.08 (app p, *J* = 6.1 Hz, 1H), 2.96-2.99 (dd, *J* = 2.8 Hz, *J* = 13.3 Hz, 1H), 2.73-2.80 (m, 2H), 2.58-2.67 (m, 6H), 2.33-2.44 (m, 3H), 2.07-2.09 (m, 1H), 1.96-1.99 (m, 1H), 1.88-1.90 (m, 3H), 1.70-1.74 (m, 3H), 1.46-1.60 (m, 5H), 1.02-1.28 (m, 6H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 158.8, 146.7, 136.4, 135.7, 134.7, 133.9, 129.0, 126.4, 125.8, 125.4, 121.3, 61.3, 57.8, 56.9, 54.4, 52.3, 48.7, 46.9, 33.8, 33.5, 33.5, 29.4, 29.2, 28.2, 27.8, 26.2, 25.1 (2C), 22.0. HRMS (NSI) *m/z* = 447.34800 (M + H); Theo. for $C_{29}H_{42}N_4$ + H = 447.34822. LC-MS (ESI-API) 50-95% MeOH in H_2O, 8 min, *m/z* = 447.2 (M + H), 224.2 (M/2 + H), t = 0.859 min; 25-95% MeOH in H_2O, 8 min, *m/z* = 447.2 (M + H), 224.2 (M/2 + H), t = 5.457 min.

N1-(4,4-difluorocyclohexyl)-N4-(((R)-1,2,3,4-tetrahydroisoquinolin-3-yl)methyl)-N4-((S)-5,6,7,8-tetrahydroquinolin-8-yl)butane-1,4-diamine (**13b**): tert-butyl (*R*)-3-(((4-aminobutyl)((*S*)-5,6,7,8-tetrahydroquinolin-8-yl)amino)methyl)-3,4-dihydroisoquinoline-2(1H)-carboxylate (**6**, 0.180 g, 0.387 mmol, 1.00 eq), 4,4-difluorocyclohexanone (**12b**, 0.073 g, 0.542 mmol, 1.40 eq) and acetic acid (0.022 mL, 0.387 mmol, 1.00 eq) were added to a dry 15 mL flask and diluted with DCE (3.87 mL). Sodium triacetoxyborohydride (0.197 g, 0.930 mmol, 2.40 eq) was then added, and the resulting reaction mixture was stirred at room temperature for 2 h. After this time,

LC-MS indicated complete conversion of starting material to desired product. The reaction mixture was diluted with saturated aqueous sodium bicarbonate until the aqueous layer remained basic. The aqueous layer was then extracted with DCM (2 x 30 mL), and combined organic layers were dried over anhydrous sodium sulfate, filtered, and evaporated under reduced pressure. The crude material was purified via column chromatography eluting with 95:5:0.5 DCM/MeOH/NH_4OH to yield the desired product (0.120 g, 0.206 mmol, 53% yield). ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.36 (d, *J* = 23.4 Hz, 1H), 7.24 (m, 1H), 7.07-7.11 (m, 2H), 6.96-7.02 (m, 3H), 4.60-4.68 (m, 1.5H), 4.38 (br s, 0.5H), 4.14 (d, *J* = 16.8 Hz, 1H), 3.89 (m, 1H), 3.01-3.09 (m, 1H), 2.92-2.94 (m, 1H), 2.59-2.68 (m, 8H), 2.39-2.43 (m, 1H), 2.05-2.12 (m, 2H), 2.02 (br s, 1H), 1.92 (br s, 1H), 1.86-1.88 (m, 2H), 1.69-1.80 (m, 3H), 1.60-1.63 (m, 1H), 1.49 (s, 9H), 1.39-1.47 (m, 6H). HRMS (APCI) *m/z* = 583.38181 (M + H); Theo. for $C_{34}H_{48}F_2N_4O_2$ + H = 583.38181. BOC group removal was carried out according to the General Deprotection Protocol. Purified via column chromatography eluting with 98:2:0.5 DCM/MeOH/NH_4OH, followed by 95:5:0.5 DCM/MeOH/NH_4OH, followed by 90:10:0.5 DCM/MeOH/NH_4OH to yield the desired product (0.045 g, 0.093 mmol, 45% yield). ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.43 (d, *J* = 3.6 Hz, 1H), 7.33 (d, *J* = 7.8 Hz, 1H), 7.08-7.11 (m, 2H), 7.01-7.06 (m, 3H), 4.06-4.09 (m, 2H), 3.91 (d, *J* = 15.0 Hz, 1H), 2.98-3.02 (m, 2H), 2.74-2.79 (m, 2H), 2.57-2.68 (m, 7H), 2.41-2.49 (m, 3H), 2.08-2.12 (m, 3H), 1.97-1.99 (m, 1H), 1.87-1.93 (m, 3H), 1.70-1.79 (m, 3H), 1.43-1.57 (m, 6H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 158.9, 146.9, 136.8, 135.3, 134.7, 134.2, 129.3, 126.7, 126.2, 125.8, 123.6 (t, *J* = 239.6 Hz), 121.7, 61.8, 57.8, 54.5, 54.4, 52.6, 48.6, 47.6, 33.7, 32.0 (t, *J* = 24.2 Hz, 2C), 29.7, 29.1, 29.0 (2C), 28.4, 27.9, 22.2. ^{19}F NMR (376 MHz, $CDCl_3$, TFA standard) δ -96.0 (app d, *J* = 233.5 Hz), -100.4 (app d, *J* = 232.0 Hz). HRMS (ESI) *m/z* = 483.32994 (M + H); Theo. for $C_{29}H_{40}N_4F_2$ + H = 483.32938. LC-MS (ESI-API) 85% MeOH in

H_2O isocratic, 3 min, *m/z* = 483.2 (M + H), 242.2 (M/2 + H), t = 0.519 min; 85% MeCN in H_2O isocratic, 3 min, *m/z* = 483.2 (M + H), 242.2 (M/2 + H), t = 0.560 min.

N1-(tetrahydro-2H-pyran-4-yl)-N4-(((R)-1,2,3,4-tetrahydroisoquinolin-3-yl)methyl)-N4-((S)-5,6,7,8-tetrahydroquinolin-8-yl)butane-1,4-diamine (**13c**): Synthesis was carried out according to General Reductive Amination Protocol E using amine **6** and ketone **12c**. Purified via column chromatography (CombiFlash, 24 g column, 30 mL/min) eluting with the following gradient to yield a white foam (118 mg, 0.215 mmol, 40% yield): 0-5 min, 0% MeOH in DCM; 5-20 min, 0-10% MeOH in DCM; 20-25 min, 10% MeOH in DCM; 25-30 min, 10-50% MeOH in DCM. ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.37 (d, *J* = 22.2 Hz, 1H), 7.25-7.26 (m, 1H), 7.07-7.12 (m, 2H), 6.98-7.03 (m, 3H), 4.60-4.69 (m, 1.5H), 4.38 (br s, 0.5H), 4.15 (d, *J* = 16.8 Hz, 1H), 3.98 (d, *J* = 11.4 Hz, 2H), 3.88-3.89 (m, 1H), 3.40 (t, *J* = 11.4 Hz, 2H), 3.01-3.09 (m, 1H), 2.92-2.95 (m, 1H), 2.59-2.67 (m, 8H), 2.42 (app p, *J* = 6.6 Hz, 1H), 2.02 (br s, 1H), 1.93 (br s, 1H), 1.82 (d, *J* = 10.2 Hz, 2H), 1.72-1.75 (m, 1H), 1.60-1.65 (m, 2H), 1.50 (s, 9H), 1.39-1.47 (m, 7H). HRMS (NSI) *m/z* = 549.37972 (M + H); Theo. for $C_{33}H_{48}O_3N_4$ + H = 549.37992. LC-MS (ESI-API) 75-95% MeOH in H_2O, 5 min, *m/z* = 549.3 (M + H), 275.2 (M/2 + H), t = 1.098 min; 50-95% MeOH in H_2O, 8 min, *m/z* = 549.3 (M + H), 275.2 (M/2 + H), t = 4.003 min. BOC group removal was carried out according to the General Deprotection Protocol. Purified via column chromatography (CombiFlash, 12 g column, 25 mL/min) eluting with the following gradient to yield a clear oil (31 mg, 0.069 mmol, 32% yield): 0-2 min, 0% 100:10:1 DCM/MeOH/NH_4OH in DCM; 2-10 min, 0-100% 100:10:1 DCM/MeOH/NH_4OH in DCM; 10-30 min, 100% 100:10:1 DCM/MeOH/NH_4OH. ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.44 (d, *J* = 4.2 Hz, 1H), 7.33 (d, *J* = 7.2 Hz, 1H), 7.07-7.10 (m, 2H), 7.00-7.05 (m, 3H), 4.08 (dd, *J* = 6.3 Hz, *J* = 9.9 Hz, 1H), 4.04 (d, *J* = 15.0 Hz, 1H), 3.97 (app d, *J* = 12.6 Hz, 2H), 3.90 (d, *J* = 15.0 Hz, 1H), 3.37-3.41 (dt, *J* = 1.4

Hz, *J* = 11.7 Hz, 2H), 3.01-3.04 (m, 1H), 2.99 (dd, *J* = 3.0 Hz, *J* = 13.2 Hz, 1H), 2.68-2.77 (m, 2H), 2.59-2.67 (m, 7H), 2.43 (dd, *J* = 11.1 Hz, *J* = 15.9 Hz, 1H), 2.37 (dd, *J* = 9.9 Hz, *J* = 13.2 Hz, 1H), 2.35 (br s, 1H), 2.06-2.09 (m 1H), 1.96-2.00 (m, 1H), 1.89-1.93 (ddd, *J* = 2.7 Hz, *J* = 12.6 Hz, *J* = 22.8 Hz, 1H), 1.82 (dt, *J* = 12.8 Hz, *J* = 1.7 Hz, 2H), 1.67-1.74 (m, 1H), 1.47-1.59 (m, 4H), 1.39 (ddd, *J* = 3.9 Hz, *J* = 11.7 Hz, *J* = 23.7 Hz, 2H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 158.9, 146.9, 136.7, 135.8, 134.8, 134.1, 129.3, 126.6, 126.1, 125.6, 121.6, 67.1 (2C), 61.5, 58.0, 54.6, 54.3, 52.5, 48.9, 46.7, 34.0, 34.0, 34.0, 29.6, 29.2, 28.4, 27.9, 22.2. HRMS (NSI) *m/z* = 449.32727 (M + H); Theo. for $C_{28}H_{40}ON_4$ + H = 449.32749. LC-MS (ESI-API) 25-95% MeOH in H_2O, 8 min, *m/z* = 449.2 (M + H), 225.2 (M/2 + H), t = 0.732 min; 10-95% MeOH in H_2O, 6 min, *m/z* = 449.4 (M + H), 225.2 (M/2 + H), t = 4.521 min.

N1-(piperidin-4-yl)-N4-(((R)-1,2,3,4-tetrahydroisoquinolin-3-yl)methyl)-N4-((S)-5,6,7,8-tetrahydroquinolin-8-yl)butane-1,4-diamine (**13d**): Synthesis was carried out according to General Reductive Amination Protocol E using amine **6** and ketone **12d**. Purified via column chromatography (CombiFlash, 24 g column, 30 mL/min) eluting with the following gradient to yield a white foam (186 mg, 0.287 mmol, 53% yield): 0-5 min, 0% MeOH in DCM; 5-20 min, 0-10% MeOH in DCM; 20-25 min, 10% MeOH in DCM; 25-30 min, 10-50% MeOH in DCM. ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.36 (d, *J* = 24.6 Hz, 1H), 7.24 (br s, 1H), 7.09-7.11 (m, 2H), 6.97-7.02 (m, 3H), 4.61-4.68 (m, 1.5H), 4.38 (br s, 0.5H), 4.14 (d, *J* = 17.4 Hz, 1H), 4.01-4.06 (m, 2H), 3.88 (br s, 1H), 3.02-3.09 (m, 1H), 2.92-2.94 (m, 1H), 2.78 (br s, 2H), 2.68 (br s, 2H), 2.58-2.62 (m, 6H), 2.38-2.43 (m, 1H), 2.02 (br s, 1H), 1.93 (br s, 1H), 1.82 (d, *J* = 9.6 Hz, 2H), 1.73-1.74 (m, 1H), 1.60-1.63 (m, 1H), 1.49 (s, 9H), 1.45 (s, 9H), 1.35-1.43 (m, 4H), 1.22-1.25 (m, 3H). HRMS (NSI) *m/z* = 648.44846 (M + H); Theo. for $C_{38}H_{57}O_4N_5$ + H = 648.44833. LC-MS (ESI-API) 75-95% MeOH in H_2O, 5 min, *m/z* = 648.4 (M + H), 548.4 (M + H - BOC), 296.8 ((M

– BOC)/2 + Na), 274.9 ((M - BOC)/2 + H), t = 0.839 min; 50-95% MeOH in H_2O, 8 min, *m/z* = 648.4 (M + H), 548.4 (M + H - BOC), 296.8 ((M – BOC)/2 + Na), t = 4.231 min. BOC group removal was carried out according to the General Deprotection Protocol. Purified via column chromatography (CombiFlash, 12 g column, 25 mL/min) eluting with the following gradient to yield a white foam (97 mg, 0.217 mmol, 75% yield): 0-3 min, 0% 100:10:1 DCM/MeOH/NH_4OH in DCM; 3-13 min, 0-100% 100:10:1 DCM/MeOH/NH_4OH in DCM; 13-25 min, 100% 100:10:1 DCM/MeOH/NH_4OH; 25-42 min, 100% 80:20:3 DCM/MeOH/NH_4OH. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.44 (dd, *J* = 1.0 Hz, *J* = 4.5 Hz, 1H), 7.33 (d, *J* = 7.5 Hz, 1H), 7.07-7.11 (m, 2H), 6.99-7.05 (m, 3H), 4.07 (m, 1H), 4.06 (d, *J* = 15.0 Hz, 1H), 3.89 (d, *J* = 15.0 Hz, 1H), 3.08-3.10 (m, 2H), 3.02 (dd, *J* = 2.8 Hz, *J* = 13.3 Hz, 1H), 2.93-2.99 (m, 1H), 2.73-2.80 (m, 2H), 2.54-2.68 (m, 10H), 2.41-2.50 (m, 3H), 2.07-2.11 (m, 1H), 1.97-1.99 (m, 1H), 1.85-1.93 (m, 3H), 1.67-1.74 (m, 1H), 1.46-1.60 (m, 4H), 1.23-1.31 (m, 2H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 158.6, 146.8, 136.7, 135.4, 134.6, 134.0, 129.1, 126.5, 126.0, 125.6, 121.5, 61.6, 57.8, 55.0, 54.2, 52.4, 48.5, 46.4, 45.1 (2C), 33.7, 33.3, 29.5, 28.5, 27.9, 27.7, 22.0 (2C). HRMS (NSI) *m/z* = 448.34330 (M + H); Theo. for $C_{28}H_{41}N_5$ + H = 448.34347. LC-MS (ESI-API) 25-95% MeOH in H_2O, 8 min, *m/z* = 448.2 (M + H), 224.8 (M/2 + H), t = 0.731 min; 10-95% MeOH in H_2O, 6 min, *m/z* = 448.4 (M + H), 224.6 (M/2 + H), t = 0.617 min.

N1-(tetrahydro-2H-thiopyran-4-yl)-N4-(((R)-1,2,3,4-tetrahydroisoquinolin-3-yl)methyl)-N4-((S)-5,6,7,8-tetrahydroquinolin-8-yl)butane-1,4-diamine (**13e**): Synthesis was carried out according to General Reductive Amination Protocol E using amine **6** and ketone **12e**. Purified via column chromatography (CombiFlash, 24 g column, 30 mL/min) eluting with the following gradient to yield a clear oil (158 mg, 0.280 mmol, 52% yield): 0-2 min, 0% MeOH in DCM; 2-13 min, 0-10% MeOH in DCM; 13-23 min, 10% MeOH in DCM; 23-28 min, 10-50% MeOH in

DCM; 28-33 min, 50% MeOH in DCM. ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.36 (d, *J* = 22.8 Hz, 1H), 7.23 (br s, 1H), 7.08-7.10 (m, 2H), 6.96-7.01 (m, 3H), 4.59-4.67 (m, 1.5H), 4.37 (br s, 0.5H), 4.13 (d, *J* = 16.8 Hz, 1H), 3.87 (br s, 1H), 3.00-3.08 (m, 1H), 2.91-2.94 (m, 1H), 2.64-2.68 (m, 5H), 2.58-2.62 (m, 6H), 2.39-2.42 (m, 2H), 2.15 (d, *J* = 10.2 Hz, 2H), 2.01 (br s, 1H), 1.92 (br s, 1H), 1.72 (br s, 1H), 1.59-1.62 (m, 1H), 1.49 (m, 11H), 1.36-1.45 (m, 5H). HRMS (NSI) *m/z* = 565.35685 (M + H); Theo. for $C_{33}H_{48}O_2N_4S$ + H = 565.35707. LC-MS (ESI-API) 75-95% MeOH in H_2O, 5 min, *m/z* = 565.2 (M + H), 283.2 (M/2 + H), t = 1.147 min; 50-95% MeOH in H_2O, 8 min, *m/z* = 565.2 (M + H), 283.2 (M/2 + H), t = 4.473 min. BOC group removal was carried out according to the General Deprotection Protocol. Purified via column chromatography (CombiFlash, 12 g column, 25 mL/min) eluting with the following gradient to yield a clear oil (53 mg, 0.114 mmol, 41% yield): 0-3 min, 0% 100:10:1 DCM/MeOH/NH_4OH in DCM; 3-10 min, 0-100% 100:10:1 DCM/MeOH/NH_4OH in DCM; 10-30 min, 100% 100:10:1 DCM/MeOH/NH_4OH. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.45 (d, *J* = 4.0 Hz, 1H), 7.33 (d, *J* = 8.5 Hz, 1H), 7.07-7.10 (m, 2H), 7.00-7.06 (m, 3H), 4.07-4.09 (m, 1H), 4.05 (d, *J* = 15.0 Hz, 1H), 3.91 (d, *J* = 15.5 Hz, 1H), 3.03-3.09 (m, 1H), 2.96-2.99 (dd, *J* = 3.0 Hz, *J* = 13.0 Hz, 1H), 2.71-2.80 (m, 2H), 2.58-2.69 (m, 9H), 2.39-2.46 (m, 2H), 2.35 (dd, *J* = 10.0 Hz, *J* = 13.0 Hz, 1H), 2.15-2.18 (m, 2H), 2.05-2.10 (m, 1H), 1.95-2.01 (m, 1H), 1.69-1.75 (m, 2H), 1.44-1.60 (m, 7H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 158.9, 146.9, 136.6, 135.8, 134.8, 134.1, 129.2, 126.6, 126.0, 125.6, 121.5, 61.5, 58.0, 56.3, 54.6, 52.5, 48.9, 46.9, 34.8, 34.8, 34.0, 29.6, 29.3, 28.4, 27.9, 27.8 (2C), 22.1. HRMS (NSI) *m/z* = 465.30452 (M + H); Theo. for $C_{28}H_{40}N_4S$ + H = 465.30464. LC-MS (ESI-API) 50-95% MeOH in H_2O, 8 min, *m/z* = 465.2 (M + H), 233.2 (M/2 + H), t = 1.020 min; 25-95% MeOH in H_2O, 8 min, *m/z* = 465.2 (M + H), 233.3 (M/2 + H), t = 5.190 min.

4-((4-((((R)-1,2,3,4-tetrahydroisoquinolin-3-yl)methyl)((S)-5,6,7,8-tetrahydroquinolin-8-yl)amino)butyl)amino)tetrahydro-2H-thiopyran 1,1-dioxide (**13f**): Synthesis was carried out according to General Reductive Amination Protocol E using amine **6** and ketone **12f**. Purified via column chromatography (CombiFlash, 24 g column, 30 mL/min) eluting with the following gradient to yield a white foam (164 mg, 0.275 mmol, 51% yield): 0-2 min, 0% MeOH in DCM; 2-13 min, 0-10% MeOH in DCM; 13-23 min, 10% MeOH in DCM; 23-28 min, 10-50% MeOH in DCM; 28-33 min, 50% MeOH in DCM. ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.37 (d, J = 23.4 Hz, 1H), 7.24-7.27 (m, 1H), 7.10 (app p, J = 6.9 Hz, 2H), 6.99-7.03 (m, 3H), 4.62-4.71 (m, 1H), 4.58 (br s, 0.5H), 4.40 (br s, 0.5H), 4.15 (d, J = 16.8 Hz, 1H), 3.90 (br s, 1H), 3.30 (br s, 2H), 3.04 (br s, 1H), 2.94 (dd, J = 15.6 Hz, J = 5.4 Hz, 1H), 2.86 (br s, 3H), 2.50-2.66 (m, 7H), 2.43 (br s, 1H), 2.22 (dd, J = 11.1 Hz, J = 14.1 Hz, 2H), 2.04 (br s, 3H), 1.93 (br s, 1H), 1.71-1.73 (m, 1H), 1.60-1.66 (m, 1H), 1.50 (s, 9H), 1.37-1.53 (m, 5H). HRMS (NSI) *m/z* = 597.34651 (M + H); Theo. for $C_{33}H_{48}O_4N_4S$ + H = 597.34690. LC-MS (ESI-API) 75-95% MeOH in H_2O, 5 min, *m/z* = 597.3 (M + H), 497.3 (M – BOC + H), 299.2 (M/2 + H), t = 0.887 min; 50-95% MeOH in H_2O, 8 min, *m/z* = 597.3 (M + H), 497.3 (M – BOC + H), 299.2 (M/2 + H), t = 4.257 min. BOC group removal was carried out according to the General Deprotection Protocol. Purified via column chromatography (CombiFlash, 12 g column, 25 mL/min) eluting with the following gradient to yield a white foam (56 mg, 0.113 mmol, 41% yield): 0-3 min, 0% 100:10:1 DCM/MeOH/NH_4OH in DCM; 3-10 min, 0-100% 100:10:1 DCM/MeOH/NH_4OH in DCM; 10-30 min, 100% 100:10:1 DCM/MeOH/NH_4OH. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.45 (dd, J = 1.5 Hz, J = 4.5 Hz, 1H), 7.33 (dt, J = 7.7 Hz, J = 0.9 Hz, 1H), 7.06-7.11 (m, 2H), 7.00-7.05 (m, 3H), 4.07-4.09 (m, 1H), 4.04 (d, J = 15.0 Hz, 1H), 3.88 (d, J = 14.5 Hz, 1H), 3.27-3.32 (m, 2H), 2.98-3.04 (m, 2H), 2.80-2.88 (m, 3H), 2.56-2.78 (m, 8H), 2.43 (dd, J = 10.3 Hz, J = 15.3 Hz, 1H),

2.38 (dd, *J* = 10.3 Hz, *J* = 12.8 Hz, 1H), 2.19-2.24 (m, 2H), 1.97-2.10 (m, 5H), 1.87-1.94 (m, 1H), 1.67-1.76 (m, 1H), 1.45-1.62 (m, 4H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 158.8, 146.9, 136.7, 135.7, 134.7, 134.1, 129.2, 126.6, 126.1, 125.7, 121.6, 61.6, 57.9, 54.5, 52.5, 51.0, 48.8, 47.8 (2C), 47.3, 33.9, 29.6, 29.4, 29.4, 28.9, 28.2, 27.8, 22.1. HRMS (NSI) *m/z* = 497.29454 (M + H); Theo. for $C_{28}H_{40}O_2N_4S$ + H = 497.29447. LC-MS (ESI-API) 25-95% MeOH in H_2O, 8 min, *m/z* = 497.2 (M + H), 249.2 (M/2 + H), t = 0.725 min; 10-95% MeOH in H_2O, 6 min, *m/z* = 497.2 (M + H), 249.3 (M/2 + H), t = 4.274 min.

N1-(4,4-dimethylcyclohexyl)-N4-(((R)-1,2,3,4-tetrahydroisoquinolin-3-yl)methyl)-N4-((S)-5,6,7,8-tetrahydroquinolin-8-yl)butane-1,4-diamine (**13g**): To solution of (*R*)-tert-butyl 3-(((4-aminobutyl)((*S*)-5,6,7,8-tetrahydroquinolin-8-yl)amino)methyl)-3,4-dihydroisoquinoline-2(1H)-carboxylate (**6**, 0.260 g, 0.560 mmol, 1.00 eq) in DCE (5.60 mL) was added 4,4-dimethylcyclohexanone (**12g**, 0.078 g, 0.616 mmol, 1.10 eq). Acetic acid (0.032 mL, 0.560 mmol, 1.00 eq) was then added, and the resulting mixture was allowed to stir for 20 min. After this time, sodium triacetoxyborohydride (0.296 g, 1.40 mmol, 2.50 eq) was added, and the resulting reaction mixture was allowed to stir overnight. The reaction mixture was diluted with saturated aqueous sodium bicarbonate until the aqueous layer remained basic. The aqueous layer was then extracted with DCM (2 x 30 mL), and combined organic layers were dried over anhydrous sodium sulfate, filtered, and evaporated under reduced pressure. The crude material was purified via column chromatography eluting with 100 mL of 90:10:0.5 DCM/MeOH/NH_4OH, followed by 100 mL of 85:15:0.5 DCM/MeOH/NH_4OH, followed by 80:20:0.5 DCM/MeOH/NH_4OH to yield the desired product (0.300 g, 0.522 mmol, 49% yield). ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.35 (d, *J* = 20.4 Hz, 1H), 7.22 (d, *J* = 6.6 Hz, 1H), 7.05-7.09 (m, 2H), 6.94-7.02 (m, 3H), 4.61-4.66 (m, 1.5H), 4.36 (br s, 0.5H), 4.12 (d, *J* = 16.8 Hz, 1H), 3.85-

3.90 (m, 1H), 2.90-3.09 (m, 1H), 2.90-2.93 (m, 1H), 2.57-2.68 (m, 7H), 2.37-2.41 (m, 2H), 2.00 (br s, 1H), 1.91 (br s, 1H), 1.56-1.69 (m, 4H), 1.48 (s, 9H), 1.24-1.45 (m, 8H), 1.78 (dt, *J* = 3.0 Hz, *J* = 12.9 Hz, 2H), 0.89 (s, 6H). HRMS (ESI) *m/z* = 575.43097 (M + H); Theo. for $C_{36}H_{54}N_4O_2$ + H = 575.43195. LC-MS (ESI-API) 75-95% MeOH in H_2O, 3 min, *m/z* = 575.4 (M + H), 287.2 (M/2 + H), t = 0.773 min; 50-95% MeOH in H_2O, 3 min, *m/z* = 575.4 (M + H), 288.2 (M/2 + H), t = 2.937 min. BOC group removal was carried out according to the General Deprotection Protocol. Purified via column chromatography eluting with 100 mL of 95:5:0.5 DCM/MeOH/NH_3 in MeOH (7.0 M), followed by 100 mL of 90:10:0.5 DCM/MeOH/NH_3 in MeOH (7.0 M), followed by 85:15:0.5 DCM/MeOH/NH_3 in MeOH (7.0 M) to yield the desired product (0.048 g, 0.101 mmol, 19% yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.44 (dd, *J* = 1.8 Hz, *J* = 4.8 Hz, 1H), 7.33 (dt, *J* = 0.8 Hz, *J* = 7.5 Hz, 1H), 7.07-7.10 (m, 2H), 7.00-7.07 (m, 3H), 4.07-4.09 (m, 1H), 4.05 (d, *J* = 12.5 Hz, 1H), 3.92 (d, *J* = 15.0 Hz, 1H), 3.04-3.08 (m, 1H), 2.98 (dd, *J* = 3.0 Hz, *J* = 13.0 Hz, 1H), 2.68-2.80 (m, 3H), 2.58-2.67 (m, 5H), 2.42 (dd, *J* = 11.0 Hz, *J* = 16.0 Hz, 1H), 2.33-2.39 (m, 2H), 2.05-2.11 (m, 1H), 1.95-2.01 (m, 1H), 1.86-1.93 (m, 1H), 1.44-1.75 (m, 7H), 1.36-1.40 (m, 2H), 1.16-1.31 (m, 5H), 0.90 (s, 3H), 0.86 (s, 3H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 158.7, 146.8, 136.6, 135.5, 134.6, 134.0, 129.1, 126.5, 126.0, 125.5, 121.5, 61.5, 57.8, 57.3, 54.3, 52.4, 48.6, 47.0, 37.9, 33.8, 32.4, 30.1, 29.8, 29.5, 29.0, 29.0, 28.9, 28.0, 27.7, 24.7, 22.0. HRMS (ESI) *m/z* = 475.37893 (M + H); Theo. for $C_{31}H_{46}N_4$ + H = 475.37952. LC-MS (ESI-API) 75-95% MeOH in H_2O, 3 min, *m/z* = 475.3 (M + H), 238.2 (M/2 + H), t = 1.158 min.

tert-butyl (R)-3-(((2-cyanobenzyl)((S)-5,6,7,8-tetrahydroquinolin-8-yl)amino)methyl)-3,4-dihydroisoquinoline-2(1H)-carboxylate (1**5**): To a solution of secondary amine **14** (0.298 g, 0.757 mmol, 1.00 eq) in DCM (6.00 mL) was added 2-cyanobenzaldehyde (0.099 g, 0.757 mmol, 1.00 eq) immediately followed by sodium triacetoxyborohydride (0.241 g, 1.14 mmol,

1.50 eq). The resulting reaction mixture was allowed to stir at room temperature for 4 h. The reaction was quenched with saturated aqueous sodium bicarbonate and extracted with DCM. The organic layer was washed with brine, dried over anhydrous sodium sulfate, filtered, and concentrated to yield an off-white foam (0.360 g). The crude material was purified by column chromatography (CombiFlash, 24 g column, 30 mL/min) eluting with the following gradient to yield an off-white foam (0.246 g, 0.484 mmol, 64% yield): 0-15 min, 0-25% EtOAc in hexanes. HRMS (NSI) *m/z* = 509.29073 (M + H); Theo. for $C_{32}H_{36}N_4O_2$ + H = 509.29110.

(S)-N-(2-(aminomethyl)benzyl)-N-(((R)-1,2,3,4-tetrahydroisoquinolin-3-yl)methyl)-5,6,7,8-tetrahydroquinolin-8-amine (**16**): To a solution of tert-butyl (*R*)-3-(((2-cyanobenzyl)((*S*)-5,6,7,8-tetrahydroquinolin-8-yl)amino)methyl)-3,4-dihydroisoquinoline-2(1H)-carboxylate (**15**, 0.224 g, 0.440 mmol, 1.00 eq) in dry MeOH (4.40 mL) chilled to 0˚C was added di-tert-butyl dicarbonate (0.210 mL, 0.880 mmol, 2.00 eq) and nickel(II) chloride hexahydrate (10.5 mg, 0.044 mmol, 0.100 eq), and the resulting mixture was stirred under Ar. Sodium borohydride (0.351 g, 9.27 mmol, 21.1 eq) was added in portionwise fashion, and the resulting reaction mixture was slowly allowed to warm to room temperature and stir for 72 h. After this time, diethylenetriamine (0.048 mL, 0.440 mmol, 1.00 eq) and a small volume of MeOH were added, and the resulting mixture was stirred for 24 h at room temperature before concentrating under reduced pressure. The resulting light grey oil was diluted with EtOAc and washed with saturated aqueous sodium bicarbonate twice. The organic layer was washed once with brine, dried over anhydrous sodium sulfate, and concentrated to an off-white foam (0.292 g). The crude material was purified by column chromatography (CombiFlash, 24 g column, 30 mL/min) eluting with the following gradient to yield a clear oil (0.069 g, 0.113 mmol, 26% yield) and starting material (0.079 g, 0.155 mmol, 35% recovered): 0-15 min, 0-25% EtOAc in hexanes. LC-MS (ESI-API) 95%

MeOH in H_2O isocratic, 3 min, *m/z* = 613.3 (M + H), t = 1.153 min. Amine deprotection was carried out according to the General Deprotection Protocol, yielding a yellow oil (29.0 mg, 0.070 mmol, 62% yield). ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.51 (dd, *J* = 1.6 Hz, *J* = 4.8 Hz, 1H), 7.46 (dd, *J* = 1.6 Hz, *J* = 7.2 Hz, 1H), 7.38 (dd, *J* = 1.8 Hz, *J* = 7.4 Hz, 1H), 7.31-7.34 (m, 1H), 7.21-7.29 (m, 2H), 7.04-7.09 (m, 3H), 6.96-7.02 (m, 2H), 4.47 (d, *J* = 13.2 Hz, 1H), 3.77-4.10 (m, 6H), 3.36-3.69 (m, 3H), 2.73-2.84 (m, 2H), 2.53-2.66 (m, 3H), 2.45 (dd, *J* = 10.2 Hz, *J* = 13.4 Hz, 1H), 2.35 (dd, *J* = 11.0 Hz, *J* = 16.2 Hz, 1H), 2.11-2.15 (m, 1H), 1.93-2.03 (m, 2H), 1.57-1.69 (m, 1H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 162.8, 149.6, 149.4, 141.0, 139.8, 137.8, 136.9, 134.0, 133.4, 131.7, 130.7, 130.1, 129.6, 128.8, 128.6, 128.4, 124.6, 60.3, 59.5, 54.5, 49.8, 44.7, 36.0, 32.3, 32.0, 24.6, 24.3. HRMS (NSI) *m/z* = 413.26985 (M + H); Theo. for $C_{27}H_{32}N_4$ + H = 413.26997. LC-MS (ESI-API) 75-95% MeOH in H_2O, 3 min, *m/z* = 413.2 (M + H), 207.2 (M/2 + H), t = 0.836 min; 25-95% MeOH in H_2O, 8 min, *m/z* = 413.2 (M + H), 207.2 (M/2 + H), t = 1.766 min; 10-95% MeOH in H_2O, 10 min, *m/z* = 413.2 (M + H), 207.2 (M/2 + H), t = 4.292 min.

General Reduction Protocol: Carboxylic acid (1.00 eq) was added to a flame-dried flask with a stir bar. Diluted with THF (0.10 M), and the resulting solution was stirred vigorously under Ar at room temperature. Added borane-dimethyl sulfide complex (3.50 eq) dropwise via syringe pump at a rate of 6.0 mL/hr, and the resulting reaction mixture was allowed to stir overnight at room temperature under Ar. In the morning, the reaction was quenched dropwise with MeOH at room temperature, ensuring that the ensuing gas expulsion did not become too vigorous. 1 M Aqueous sodium hydroxide was added, and the resulting aqueous layer was extracted 3 times with EtOAc. Combined organic layers were washed once with 1 M aqueous sodium hydroxide,

washed once with brine, dried over anhydrous sodium sulfate, filtered, and evaporated under reduced pressure.

tert-butyl (3-(hydroxymethyl)benzyl)carbamate (**17a**): Synthesis was carried out according to the General Reduction Protocol starting with 3-(((tert-butoxycarbonyl)amino)methyl)benzoic acid. Purified via column chromatography (CombiFlash, 24 g column, 30 mL/min) eluting with the following gradient to yield a slightly yellow oil (700 mg, 2.95 mmol, 74% yield): 0-5 min, 0% MeOH in DCM; 5-10 min, 0-5% MeOH in DCM; 10-15 min, 5% MeOH in DCM; 15-20 min, 5-10% MeOH in DCM; 20-30 min, 10% MeOH in DCM; 30-35 min, 10-50% MeOH in DCM. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.32 (app t, J = 7.5 Hz, 1H), 7.28 (m, 1H), 7.25-7.27 (m, 1H), 7.21 (d, J = 7.5 Hz, 1H), 4.92 (br s, 1H), 4.67 (s, 2H), 4.31 (d, J = 6.0 Hz, 2H), 2.11 (br s, 1H), 1.46 (s, 9H). HRMS (NSI) *m/z* = 238.14399 (M + H); Theo. for $C_{13}H_{19}O_3N$ + H = 238.14377. LC-MS (ESI-API) 75-95% MeOH in H_2O, 5 min, *m/z* = 260.2 (M + Na), 196.2 (M – *t*BuO + MeOH), 164.2 (M – *t*BuO), 138.2 (M + H – BOC), t = 1.362 min.

tert-butyl (4-(hydroxymethyl)benzyl)carbamate (**17b**): Synthesis was carried out according to the General Reduction Protocol starting with 4-(((tert-butoxycarbonyl)amino)methyl)benzoic acid. Purified. Purified via column chromatography (CombiFlash, 24 g column, 30 mL/min) eluting with the following gradient to yield a white solid (462 mg, 1.95 mmol, 49% yield): 0-5 min, 5% MeOH in DCM; 5-10 min, 5-10% MeOH in DCM; 10-20 min, 10% MeOH in DCM; 20-25 min, 10-50% MeOH in DCM. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.33 (d, J = 8.0 Hz, 2H), 7.26-7.28 (m, 2H), 4.87 (br s, 1H), 4.68 (s, 2H), 4.31 (d, J = 5.5 Hz, 2H), 1.87 (br s, 1H), 1.46 (s, 9H). HRMS (NSI) *m/z* = 238.14408 (M + H); Theo. for $C_{13}H_{19}O_3N$ + H = 238.14377. LC-MS (ESI-API) 75-95% MeOH in H_2O, 5 min, *m/z* = 260.2 (M + Na), 164.2 (M – *t*BuO), t = 1.862 min.

General Reductive Amination Protocol F: Dess-Martin periodinane (1.25 eq) was added to a flask with a stir bar. Diluted with DCM (0.25 M), and the resulting slurry was stirred vigorously at room temperature. Added a solution of alcohol (1.00 eq) in DCM (0.20 M) in dropwise fashion, and the resulting reaction mixture was stirred vigorously under Ar at room temperature. After 2.5 h, TLC indicated complete conversion of starting material. The reaction mixture was poured over a 1:1 mixture of saturated aqueous $NaHCO_3$ and saturated aqueous $Na_2S_2O_3$ (55 mL per mmol alcohol). The resulting organic layer was dried over anhydrous sodium sulfate, filtered, and evaporated under reduced pressure. A solution of amine (1.00 eq) in DCM (0.12 M) was added to a flask with a stir bar. Added a solution of aldehyde (1.05 eq) in DCM (0.53 M), and the resulting mixture was stirred under Ar at room temperature for 5 min. After this time, acetic acid (1.00 eq) was added, and the resulting mixture was stirred under Ar at room temperature for 15 min. After this time, sodium triacetoxyborohydride (3.00 eq) was added, and the resulting reaction mixture was allowed to stir overnight at room temperature under Ar. In the morning, the reaction mixture was diluted with DCM and washed once with 1 M aqueous sodium hydroxide. The resulting aqueous layer was extracted 3 times with DCM. Combined organic layers were dried over anhydrous sodium sulfate, filtered, and evaporated under reduced pressure. The crude material was taken up in DCM (40 mL per mmol amine), filtered, and evaporated under reduced pressure.

(S)-N-(3-(aminomethyl)benzyl)-N-(((R)-1,2,3,4-tetrahydroisoquinolin-3-yl)methyl)-5,6,7,8-tetrahydroquinolin-8-amine (**19a**): Synthesis was carried out according to General Reductive Amination Protocol F using alcohol **17a** and amine **14**. Purified via column chromatography (CombiFlash, 24 g column, 30 mL/min) eluting with the following gradient to yield a white foam (298 mg, 0.486 mmol, 55% yield): 0-3 min, 0% MeOH in DCM; 3-15 min, 0-5% MeOH in

DCM; 15-20 min, 5% MeOH in DCM; 20-25 min, 5-25% MeOH in DCM.^{1}H NMR (600 MHz, $CDCl_3$) δ 8.45 (d, *J* = 9.0 Hz, 1H), 7.25-7.29 (m, 2H), 7.17-7.24 (m, 2H), 7.14 (d, *J* = 6.0 Hz, 1H), 7.04-7.09 (m, 2H), 6.84-7.01 (m, 3H), 5.34 (br s, 0.5H), 4.96 (br s, 0.5H), 4.62 (d, *J* = 16.8 Hz, 1H), 4.49 (d, *J* = 16.8 Hz, 1H), 4.24-4.35 (m, 2H), 3.99-4.03 (m, 1H), 3.55-3.78 (m, 3H), 2.88-3.00 (m, 3H), 2.60-2.71 (m, 3H), 2.21 (br s, 1H), 1.97 (br s, 1H), 1.79-1.83 (m, 2H), 1.64-1.71 (m, 1H), 1.47-1.52 (m, 18H). HRMS (NSI) *m/z* = 613.37482 (M + H); Theo. for $C_{37}H_{48}O_4N_4$ + H = 613.37483. LC-MS (ESI-API) 75-95% MeOH in H_2O, 8 min, *m/z* = 613.3 (M + H), t = 5.047 min; 50-95% MeOH in H_2O, 8 min, *m/z* = 613.2 (M + H), t = 4.848 min. BOC group removal was carried out according to the General Deprotection Protocol. Purified via column chromatography (CombiFlash, 12 g column, 25 mL/min) eluting with the following gradient to yield a white foam (121 mg, 0.293 mmol, 65% yield): 0-3 min, 0% 100:10:1 DCM/MeOH/NH_4OH in DCM; 3-15 min, 0-100% 100:10:1 DCM/MeOH/NH_4OH in DCM; 15-25 min, 100% 100:10:1 DCM/MeOH/NH_4OH. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.47-8.49 (m, 1H), 7.23-7.39 (m, 4H), 7.14 (d, *J* = 7.5 Hz, 1H), 7.01-7.05 (m, 3H), 6.93-6.98 (m, 2H), 4.34-4.43 (m, 1H), 4.08 (dd, *J* = 6.5 Hz, *J* = 10.5 Hz, 1H), 3.94 (d, *J* = 15.0 Hz, 1H), 3.86 (d, *J* = 14.5 Hz, 1H), 3.84 (s, 2H), 3.62-3.68 (m, 1H), 2.89 (m, 1H), 2.71-2.77 (m, 1H), 2.60-2.65 (m, 2H), 2.53 (dd, *J* = 3.5 Hz, *J* = 16.0 Hz, 1H), 2.32-2.44 (m, 2H), 2.09-2.15 (m, 2H), 1.89-1.97 (m, 3H), 1.59-1.68 (m, 2H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 158.9, 146.9, 143.3, 142.4, 136.5, 135.8, 134.7, 134.2, 129.1, 128.5, 127.2, 126.9, 126.5, 125.9, 125.5, 125.5, 121.5, 62.3, 59.5, 58.2, 52.1, 48.7, 46.7, 33.9, 29.5, 29.5, 22.2. HRMS (NSI) *m/z* = 413.26977 (M + H); Theo. for $C_{27}H_{32}N_4$ + H = 413.26997. LC-MS (ESI-API) 50-95% MeOH in H_2O, 8 min, *m/z* = 413.2 (M + H), 207.2 (M/2 + H), t = 0.885 min; 10-95% MeOH in H_2O, 10 min, *m/z* = 413.2 (M + H), 207.2 (M/2 + H), t = 6.694 min.

(S)-N-(4-(aminomethyl)benzyl)-N-(((R)-1,2,3,4-tetrahydroisoquinolin-3-yl)methyl)-5,6,7,8-tetrahydroquinolin-8-amine (**19b**): Synthesis was carried out according to General Reductive Amination Protocol F using alcohol **17b** and amine **14**. Purified via column chromatography (CombiFlash, 24 g column, 30 mL/min) eluting with 40:1 DCM/MeOH to yield a white foam (275 mg, 0.449 mmol, 51% yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.44 (d, *J* = 12.5 Hz, 1H), 7.24-7.32 (m, 3H), 7.16 (br s, 2H), 7.06 (m, 2H), 6.83-7.00 (m, 3H), 4.65-4.80 (m, 2H), 4.44-4.51 (m, 1H), 4.29 (s, 2H), 3.86-4.09 (m, 2H), 3.55-3.71 (m, 2H), 2.85-3.05 (m, 2H), 2.51-2.69 (m, 4H), 2.10-2.22 (m, 1H), 1.95 (br s, 1H), 1.66-1.82 (m, 2H), 1.45-1.53 (m, 18H). HRMS (NSI) *m/z* = 613.37490 (M + H); Theo. for $C_{37}H_{48}O_4N_4$ + H = 613.37483. LC-MS (ESI-API) 75-95% MeOH in H_2O, 5 min, *m/z* = 613.3 (M + H), t = 4.666 min; 50-95% MeOH in H_2O, 8 min, *m/z* = 613.2 (M + H), t = 6.799 min. Synthesis was carried out according to the General Deprotection Protocol. Purified via column chromatography (CombiFlash, 12 g column, 25 mL/min) eluting with the following gradient to yield a clear oil (82 mg, 0.199 mmol, 49% yield): 0-3 min, 0% 100:10:1 DCM/MeOH/NH_4OH in DCM; 3-15 min, 0-100% 100:10:1 DCM/MeOH/NH_4OH in DCM; 15-25 min, 100% 100:10:1 DCM/MeOH/NH_4OH. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.51 (dd, *J* = 1.5 Hz, *J* = 4.5 Hz, 1H), 7.43 (d, *J* = 8.0 Hz, 2H), 7.34 (dt, *J* = 0.9 Hz, *J* = 7.8 Hz, 1H), 7.26 (d, *J* = 8.0 Hz, 2H), 7.03-7.07 (m, 3H), 6.96-7.02 (m, 2H), 4.37 (d, *J* = 14.0 Hz, 1H), 4.11 (dd, *J* = 6.5 Hz, *J* = 10.0 Hz, 1H), 3.97 (d, *J* = 15.0 Hz, 1H), 3.90 (d, *J* = 14.5 Hz, 1H), 3.84 (s, 2H), 3.68 (d, *J* = 15.0 Hz, 1H), 2.91 (dd, *J* = 2.8 Hz, *J* = 13.3 Hz, 1H), 2.74-2.80 (m, 1H), 2.64-2.70 (m, 2H), 2.55 (dd, *J* = 3.5 Hz, *J* = 16.0 Hz, 1H), 2.36-2.46 (m, 3H), 2.11-2.16 (m, 1H), 1.91-2.00 (m, 3H), 1.62-1.88 (m, 2H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 158.9, 146.9, 141.8, 140.5, 136.5, 135.7, 134.7, 134.2, 129.1, 128.6 (2C), 127.1 (2C), 126.5, 125.9, 125.5, 121.5, 62.1, 59.2, 58.0, 52.1, 48.6, 46.4, 33.8, 29.6, 29.5, 22.2. HRMS (NSI) *m/z* =

413.26930 (M + H); Theo. for $C_{27}H_{32}N_4$ + H = 413.26997. LC-MS (ESI-API) 50-95% MeOH in H_2O, 8 min, *m/z* = 413.3 (M + H), 207.2 (M/2 + H), t = 0.844 min; 10-95% MeOH in H_2O, 10 min, *m/z* = 413.3 (M + H), 207.2 (M/2 + H), t = 6.354 min.

(Z)-N1-(((R)-1,2,3,4-tetrahydroisoquinolin-3-yl)methyl)-N1-((S)-5,6,7,8-tetrahydroquinolin-8-yl)but-2-ene-1,4-diamine (**21**): To a solution of tert-butyl (*R*)-3-((((*S*)-5,6,7,8-tetrahydroquinolin-8-yl)amino)methyl)-3,4-dihydroisoquinoline-2(1H)-carboxylate (**14**, 0.298 g, 0.757 mmol, 1.00 eq) in MeCN (7.00 mL) was added *N*,*N*-diisopropylethylamine (0.198 mL, 1.14 mmol, 1.50 eq) and potassium iodide (0.013 g, 0.076 mmol, 0.100 eq), and the resulting mixture was stirred vigorously at room temperature under Ar. To the stirring mixture was added tert-butyl (*Z*)-(4-chlorobut-2-en-1-yl)carbamate (**20**, 0.187 g, 0.909 mmol, 1.20 eq), and the resulting reaction mixture was warmed to 50°C and stirred for 72 h under Ar. After this time, the reaction was quenched with saturated aqueous sodium bicarbonate. The resulting aqueous layer was extracted three times with DCM, and combined organic layers were washed once with brine, dried over anhydrous sodium sulfate, filtered, and concentrated to an off-white foam (0.430 g). The crude material was purified by column chromatography (CombiFlash, 12 g column, 25 mL/min) eluting with the following gradient to yield an off-white foam (0.293 g, 0.521 mmol, 69% yield): 0-15 min, 0-25% EtOAc in hexanes. HRMS (NSI) *m/z* = 563.35924 (M + H); Theo. for $C_{33}H_{46}O_4N_4$ + H = 563.35918. LC-MS (ESI-API) 75-95% MeOH in H_2O, 3 min, *m/z* = 563.3 (M + H), t = 1.500 min. BOC group removal was carried out according to the General Deprotection Protocol. The crude material was purified via column chromatography eluting with 9:1:1 DCM/MeOH/NH_4OH) to yield a light brown oil (76.1 mg, 0.210 mmol, 59% yield, 90-95% purity). ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.45 (d, *J* = 4.5 Hz, 1H), 7.32 (d, *J* = 8.0 Hz, 1H), 7.11-6.99 (m, 5H), 5.60-5.73 (m, 2H), 4.10 (dd, *J* = 10.0 Hz, *J* = 6.5 Hz, 1H), 4.03 (d, *J* = 15.0 Hz,

1H), 3.82-3.90 (m, 2H), 3.42 (dd, *J* = 6.6 Hz, *J* = 14.5 Hz, 1H), 3.28-3.33 (m, 2H), 2.75-2.86 (m, 3H), 2.64-2.74 (m, 1H), 2.57 (dd, *J* = 3.3 Hz, *J* = 16.3 Hz, 1H), 2.36-2.43 (m, 2H), 2.04-2.24 (m, 4H), 1.95-2.00 (m, 1H), 1.87-1.92 (m, 1H), 1.65-1.74 (m, 1H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 158.6, 146.9, 136.6, 135.7, 134.6, 134.1, 132.9, 130.3, 129.2, 126.5, 126.0, 125.6, 121.5, 60.6, 57.3, 51.9, 50.9, 48.6, 38.9, 33.9, 29.5, 28.5, 22.1. HRMS (NSI) *m/z* = 363.25433 (M + H); Theo. for $C_{23}H_{30}N_4$ + H = 363.25432. LC-MS (ESI-API) 25-95% MeOH in H_2O, 3 min, *m/z* = 363.2 (M + H), 182.2 (M/2 + H), t = 0.588 min; 10-95% MeOH in H_2O, 3 min, *m/z* = 363.2 (M + H), 182.3 (M/2 + H), t = 0.909 min; 0-95% MeCN in H_2O, 8 min, *m/z* = 363.2 (M + H), 182.2 (M/2 + H), t = 3.731 min.

tert-butyl (R)-3-((((E)-4-aminobut-2-en-1-yl)((S)-5,6,7,8-tetrahydroquinolin-8-yl)amino)methyl)-3,4-dihydroisoquinoline-2(1H)-carboxylate (**23**): *(R)*-tert-Butyl 3-(((*(S)*-5,6,7,8-tetrahydroquinolin-8-yl)amino)methyl)-3,4-dihydroisoquinoline-2(1H)-carboxylate (**14**, 4.96 g, 12.6 mmol, 1.00 eq) was added to a 250 mL flask equipped with a stir bar and a reflux condenser. Diluted with acetonitrile (126 mL), and the resulting solution was stirred vigorously at room temperature under Ar. Added (*E*)-2-(4-bromobut-2-en-1-yl)isoindoline-1,3-dione (**22**, 4.24 g, 15.1 mmol, 1.20 eq), potassium iodide (0.209 g, 1.26 mmol, 0.100 eq), and diisopropylethylamine (4.39 mL, 25.2 mmol, 2.00 eq), warmed to 50°C, and the resulting reaction mixture was stirred under Ar overnight. After 19 h, TLC indicated complete conversion of starting material to one major spot. The reaction mixture was washed once with brine, and the resulting aqueous layer was extracted 3 times with DCM. Combined organic layers were washed once with brine, dried over anhydrous sodium sulfate, filtered, and evaporated under reduced pressure to yield a brown oil. Purified via column chromatography (CombiFlash, 120 g column, 75 mL/min) eluting with the following gradient to yield a yellow foam (3.78 g, 6.38 mmol, 51%

yield): 0-3 min, 0% 100:10:1 DCM/MeOH/NH_4OH in DCM; 3-18 min, 0-100% 100:10:1 DCM/MeOH/NH_4OH in DCM; 18-30 min, 100% 100:10:1 DCM/MeOH/NH_4OH in DCM. ^1H NMR (600 MHz, $CDCl_3$) δ 8.34 (br s, 1H), 7.83-7.88 (m, 2H), 7.71-7.78 (m, 2H), 7.19 (d, *J* = 7.2 Hz, 1H), 7.07-7.08 (m, 2H), 6.98-7.03 (m, 2H), 6.92 (dd, *J* = 4.8 Hz, *J* = 7.8 Hz, 1H), 5.75-5.76 (m, 1H), 5.57 (dt, *J* = 6.0 Hz, *J* = 15.6 Hz, 1H), 4.61-4.67 (m, 1.5H), 4.40 (br s, 0.5H), 4.18 (d, *J* = 6.0 Hz, 2H), 4.06-4.10 (m, 1H), 3.91 (m, 1H), 3.33-3.40 (m, 1H), 3.14-3.15 (m, 1H), 2.89-3.00 (m, 2H), 2.60-2.67 (m, 2H), 2.44-2.57 (m, 2H), 1.91-1.98 (m, 2H), 1.57-1.71 (m, 2H), 1.47 (s, 9H). LC-MS (ESI-API) 75-95% MeOH in H_2O, 5 min, *m/z* = 593.2 (M + H), t = 0.709 min; 50-95% MeOH in H_2O, 8 min, *m/z* = 593.2 (M + H), t = 2.762 min. The purified product (3.76 g, 6.34 mmol, 1.00 eq) was added to a 500 mL flask with a stir bar. The starting material was diluted with MeOH (63.4 mL), and the resulting solution was stirred vigorously at room temperature under Ar. Added a solution of hydrazine (6.78 mL, 50.7 mmol, 8.00 eq) in water (24% by wt.) in dropwise fashion via syringe pump at a rate of 6 mL/hr, and the resulting reaction mixture was stirred vigorously at room temperature under Ar overnight. After 23 h, the reaction mixture was partitioned between DCM and 1 M aqueous sodium hydroxide. The resulting aqueous layer was extracted 3 times with DCM, and combined organic layers were dried over anhydrous sodium sulfate, filtered, and evaporated under reduced pressure to yield a white foam (2.64 g). The crude material was purified via column chromatography (CombiFlash, 80 g column, 50 mL/min) eluting with the following gradient to yield a white foam (2.23 g, 4.81 mmol, 76% yield): 0-3 min, 0% 100:10:1 DCM/MeOH/NH_4OH in DCM; 3-18 min, 0-100% 100:10:1 DCM/MeOH/NH_4OH in DCM; 18-40 min, 100% 100:10:1 DCM/MeOH/NH_4OH in DCM. ^1H NMR (500 MHz, $CDCl_3$) δ 8.39 (d, *J* = 17.5 Hz, 1H), 7.25 (d, *J* = 6.5 Hz, 1H), 7.06-7.11 (m, 2H), 6.98-7.03 (m, 3H), 5.55-5.64 (m, 2H), 4.68 (dd, *J* = 17.8 Hz, *J* = 26.8 Hz, 1.5H),

4.47 (br s, 0.5H), 4.10 (app t, *J* = 15.3 Hz, 1H), 3.98 (dd, *J* = 6.0 Hz, *J* = 8.5 Hz, 1H), 3.27-3.35 (m, 1H), 3.18 (d, *J* = 4.5 Hz, 2H), 3.09 (dd, *J* = 4.5 Hz, *J* = 14.0 Hz, 1H), 2.92-3.03 (m, 2H), 2.63-2.72 (m, 2H), 2.51-2.59 (m, 2H), 1.92-2.02 (m, 2H), 1.74 (dd, *J* = 10.5 Hz, *J* = 21.0 Hz, 1H), 1.58-1.66 (m, 1H), 1.50 (s, 9H), 1.26-1.42 (m, 2H). HRMS (NSI) *m/z* = 463.30612 (M + H); Theo. for $C_{28}H_{38}N_4O_2$ + H = 463.30675. LC-MS (ESI-API) 75-95% MeOH in H_2O, 5 min, *m/z* = 463.2 (M + H), t = 0.558 min; 50-95% MeOH in H_2O, 8 min, *m/z* = 463.2 (M + H), 363.2 (M – BOC + H), 232.2 (M/2 + H), t = 0.719 min.

(E)-N1-(((R)-1,2,3,4-tetrahydroisoquinolin-3-yl)methyl)-N1-((S)-5,6,7,8-tetrahydroquinolin-8-yl)but-2-ene-1,4-diamine (**24**): The BOC protecting group of amine **23** was removed according to the General Deprotection Protocol. The crude material was purified via column chromatography (CombiFlash) eluting with following gradient to yield the desired product (0.013 g, 0.036 mmol, 25% yield): 0-3 min, 0% 80:20:3 DCM/MeOH/NH_4OH in DCM; 3-8 min, 10% 80:20:3 DCM/MeOH/NH_4OH in DCM; 8-17 min, 50% 80:20:3 DCM/MeOH/NH_4OH in DCM. ^{1}H NMR (500Hz, $CDCl_3$) δ 8.45 (dd, *J* = 1.0 Hz, *J* = 4.5 Hz, 1H), 7.32 (dd, *J* = 0.5 Hz, *J* = 7.5 Hz, 1H), 6.99-7.10 (m, 5H), 5.65-5.77 (m, 2H), 4.09-4.14 (m, 1H), 4.02 (d, *J* = 15.0 Hz, 1H), 3.83 (d, *J* = 15.0 Hz, 1H), 3.60 (dd, *J* = 6.3 Hz, *J* = 13.8 Hz, 1H), 3.22-3.33 (m, 3H), 2.90 (dd, *J* = 3.3 Hz, *J* = 13.3 Hz, 1H), 2.68-2.84 (m, 3H), 2.59-2.65 (m, 2H), 2.41-2.47 (m, 4H), 2.04-2.09 (m, 1H), 1.87-2.01 (m, 2H), 1.66-1.74 (m, 1H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 158.8, 147.0, 136.7, 135.6, 134.8, 134.1, 133.2, 129.9, 129.3, 126.6, 126.1, 125.7, 121.6, 61.4, 57.2, 56.3, 52.2, 48.6, 43.9, 33.8, 29.6, 28.1, 22.0. HRMS (NSI) *m/z* = 363.25441 (M + H); Theo. for $C_{23}H_{30}N_4$ + H = 363.25432. LC-MS (ESI-API) 75-95% MeOH in H_2O, 3 min, *m/z* = 363.2 (M + H), 182.3 (M/2 + H), t = 0.645 min; 50-95% MeOH in H_2O, 3 min, *m/z* = 363.2 (M + H), 182.3 (M/2 + H), t = 0.617 min; 25-95% MeOH in H_2O, 3 min, *m/z* = 363.2 (M + H), 182.2 (M/2 + H),

t = 0.525 min; 10-95% MeOH in H_2O, 3 min, *m/z* = 363.2 (M + H), 182.2 (M/2 + H), t = 0.868 min.

S-octyl 4-(1,3-dioxoisoindolin-2-yl)-2,2-dimethylbutanethioate (**26**): A 250 mL flask equipped with a stir bar was set under Ar atmosphere and charged with a solution of trimethyl aluminum (2.0 M in hexanes, 19.7 mL, 39.4 mmol, 3.00 eq). The solution was cooled to 0˚C, and 1-octane thiol (6.84 mL, 39.4 mmol, 3.00 eq) was added dropwise. After stirring at room temperature for 20 min, a solution of 3,3-dimethyldihydrofuran-2(3H)-one (**25**, 1.50 g, 13.1 mmol, 1.00 eq) in DCM (30.6 mL) was added dropwise, and the resulting reaction mixture was stirred at room temperature for 12 h. After this time, the reaction was quenched by addition of diethyl ether (77.0 mL) and 1 N aqueous HCl (116 mL). The resulting aqueous layer was extracted three times with diethyl ether, and combined organic layers were washed once with 1 N aqueous HCl, washed once with saturated aqueous sodium bicarbonate, dried over anhydrous sodium sulfate, filtered, and evaporated under reduced pressure. The crude product was purified via silica gel column, eluting with a gradient of 5-25% EtOAc in hexanes to afford a clear liquid (2.26 g, 8.68 mmol, 66% yield). A portion of the purified alcohol (0.429 g, 1.65 mmol, 1.00 eq), phthalimide (0.279 g, 1.89 mmol, 1.15 eq), triphenylphosphine (0.475 g, 1.81 mmol, 1.10 eq), and THF (8.20 mL) were added to a 50 mL Schlenk tube equipped with a stir bar. Then DIAD (0.368 mL, 1.89 mmol, 1.15 eq) was added, and the resulting reaction mixture was stirred for 30 min at room temperature. After this time, the reaction was quenched by addition of water. The resulting aqueous layer was extracted three times with diethyl ether, and combined organic layers were dried over anhydrous sodium sulfate, filtered, and evaporated under reduced pressure. The crude material was purified via column chromatography eluting with a gradient of 10-20% EtOAc in hexanes to yield the desired product as a clear oil (0.549 g, 1.41 mmol, 86% yield). ^{1}H NMR

(400 MHz, $CDCl_3$) δ 7.79 (dd, *J* = 5.4 Hz, *J* = 3.1 Hz, 2H), 7.67 (dd, *J* = 5.5 Hz, *J* = 3.0 Hz, 2H), 3.63-3.68 (m, 2H), 2.70 (t, *J* = 7.4 Hz, 2H), 1.92-1.97 (m, 2H), 1.45 (p, *J* = 7.5 Hz, 2H), 1.28 (s, 6H), 1.18-1.34 (m, 10H), 0.83 (t, *J* = 6.8 Hz, 3H). LC-MS (ESI-API) 95% MeOH in H_2O isocratic, 3 min, *m/z* = 412.0 (M + Na), t = 1.407 min.

(S)-2-(3,3-dimethyl-4-((5,6,7,8-tetrahydroquinolin-8-yl)amino)butyl)isoindoline-1,3-dione (**28**): A 20 mL vial equipped with a stir bar was charged with the thioester **26** (0.594 g, 1.53 mmol, 1.00 eq), palladium(II) chloride (0.027 g, 0.152 mmol, 0.100 eq), triethylamine (0.043 mL, 0.305 mmol, 0.200 eq) and DCM (3.05 mL). Then triethylsilane (0.731 mL, 4.57 mmol, 3.00 eq) was added in dropwise fashion, turning the reaction mixture into a black suspension. After stirring at room temperature for 20 min, the reaction was quenched by addition of 10% aqueous citric acid, and the resulting mixture was filtered through a plug of celite. The aqueous layer was extracted twice with DCM, and combined organic layers were dried over anhydrous sodium sulfate, filtered, and evaporated under reduced pressure. The crude product was purified via silica gel column eluting with a gradient of 10-30% EtOAc in hexanes to afford the desired product as a clear oil (0.321 g, 1.31 mmol, 86% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.47 (s, 1H), 7.84 (dd, *J* = 5.4 Hz, *J* = 3.1 Hz, 2H), 7.71 (dd, *J* = 5.5 Hz, *J* = 3.0 Hz, 2H), 3.61-3.70 (m, 2H), 1.84-1.93 (m, 2H), 1.16 (s, 6H). LC-MS (ESI-API) 95% MeOH in H_2O isocratic, 3 min, *m/z* = 246.0 (M + H), t = 0.580 min. The purified material (0.321 g, 1.31 mmol, 1.00 eq) was transferred to a 20 mL vial equipped with a stir bar, and (*S*)-5,6,7,8-tetrahydroquinolin-8-amine (**27**, 0.291 g, 1.96 mmol, 1.50 eq), sodium triacetoxyborohydride (0.444 g, 2.09 mmol, 1.60 eq), and DCE (8.72 mL) were added. The resulting reaction mixture was stirred at room temperature for 1 h. After this time, the reaction was quenched with saturated aqueous sodium bicarbonate, and the resulting aqueous layer was extracted three time with DCM. Combined organic layers

were dried over anhydrous sodium sulfate, filtered, and evaporated under reduced pressure. The crude product was purified via silica gel column eluting with EtOAc to afford the desired product as a white solid (0.492 g, 1.30 mmol, 100% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.38 (d, *J* = 4.7 Hz, 1H), 7.82 (dd, *J* = 5.5 Hz, *J* = 3.0 Hz, 2H), 7.68 (dd, *J* = 5.5 Hz, *J* = 3.0 Hz, 2H), 7.35 (d, *J* = 7.7 Hz, 1H), 7.05 (dd, *J* = 7.6 Hz, *J* = 4.7 Hz, 2H), 3.69-3.75 (m, 3H), 2.81 (dt, *J* = 13.6 Hz, *J* = 6.6 Hz, 1H), 2.72 (dt, *J* = 16.8 Hz, *J* = 5.6 Hz, 1H), 2.61 (d, *J* = 11.3 Hz, 1H), 2.55 (d, *J* = 11.4 Hz, 1H), 1.98-2.14 (m, 2H), 1.62-1.82 (m, 4H), 1.04 (s, 3H), 1.03 (s, 3H). LC-MS (ESI-API) 95% MeOH in H_2O isocratic, 3 min, *m/z* = 378.2 (M/2 + H), t = 0.469 min.

2,2-dimethyl-N1-(((R)-1,2,3,4-tetrahydroisoquinolin-3-yl)methyl)-N1-((S)-5,6,7,8-tetrahydroquinolin-8-yl)butane-1,4-diamine (**30**): A 50 mL Schlenk tube equipped with a stir bar was charged with amine **28** (0.223 g, 0.591 mmol, 1.00 eq), aldehyde **29** (0.170 g, 0.650 mmol, 1.10 eq), and DCM (5.91 mL). Then titanium isopropoxide (0.190 mL, 0.650 mmol, 1.10 eq) was added, and the resulting mixture was stirred at room temperature for 1 h. After this time, sodium triacetoxyborohydride (0.250 g, 1.18 mmol, 2.00 eq) was added, and the resulting reaction mixture was stirred at room temperature for 12 h. After this time, the reaction was quenched by addition of saturated aqueous sodium bicarbonate, and the resulting aqueous layer was extracted three times with DCM. Combined organic layers were washed once with brine, dried over anhydrous sodium sulfate, filtered, and evaporated under reduced pressure. The crude product was purified via silica gel column, eluting with a gradient of 0-40% EtOAc in hexanes to afford the desired product as a slightly yellow powder (0.207 g, 0.332 mmol, 56% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.38 (br s, 0.7H), 8.33 (br s, 0.3H), 7.82 (dd, *J* = 5.5 Hz, *J* = 3.0 Hz, 2H), 7.70 (dd, *J* = 5.5 Hz, *J* = 3.1 Hz, 2H), 7.10-7.21 (m, 1H), 6.92-7.08 (m, 4H), 6.88 (dd, *J* = 7.7 Hz, *J* = 4.6 Hz, 1H), 4.71 (br s, 0.3H), 4.71 (d, *J* = 16.8 Hz, 0.7H), 4.67 (d, *J* = 16.8 Hz,

0.3H), 4.44 (br s, 0.7H), 4.27 (d, *J* = 17.4 Hz, 0.3H), 4.15 (d, *J* = 17.2 Hz, 0.7H), 4.05 (br s, 0.3H), 3.94 (br s, 0.7H), 3.54-3.69 (m, 2H), 3.30 (d, *J* = 16.1 Hz, 0.7H), 3.15 (d, *J* = 16.2 Hz, 0.3H), 2.82-3.02 (m, 2H), 2.08-2.68 (m, 7H), 1.87-1.94 (m, 1H), 1.31-1.80 (m, 3H), 1.49 (s, 6.3H), 1.46 (s, 2.7H), 0.98 (s, 2.1H), 0.91 (s, 2.1H), 0.87 (s, 0.9H), 0.84 (s, 0.9H). LC-MS (ESI-API) 75-95% MeOH in H_2O, 6 min, *m/z* = 623.2 (M + H), t = 0.946 min. The purified material (0.129 g, 0.207 mmol, 1.00 eq) was added to a 20 mL vial equipped with a stir bar, and diluted with MeOH (1.04 mL). Then a solution of hydrazine (0.217 mL, 1.66 mmol, 8.00 eq) in water (24% by wt.) was added, and the resulting reaction mixture was stirred at room temperature for 12 h. After this time, the reaction was quenched by addition of saturated aqueous sodium bicarbonate, and the resulting aqueous layer was extracted three times with DCM. Combined organic layers were washed once with brine, dried over anhydrous sodium sulfate, filtered, and evaporated under reduced pressure. The crude material was carried forward without further purification. The remaining BOC group was removed according to the General Deprotection Protocol. The crude material was purified via silica gel column eluting with a gradient of 0-60% 70:30:3 DCM/MeOH/NH_4OH in DCM to afford the desired product as a slightly yellow powder (0.069 g, 0.176 mmol, 85% yield over 2 steps). ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.44 (d, *J* = 4.8 Hz, 1H), 7.31 (d, *J* = 7.8 Hz, 1H), 7.04-7.08 (m, 3H), 7.00-7.01 (m, 2H), 4.00-4.05 (m, 2H), 3.80 (d, *J* = 15.0 Hz, 1H), 3.55 (d, *J* = 13.8 Hz, 1H), 2.85 (d, *J* = 12.6 Hz, 1H), 2.73-2.78 (m, 3H), 2.60-2.67 (m, 2H), 2.50 (dd, *J* = 15.9 Hz, *J* = 3.3 Hz, 1H), 2.45 (d, *J* = 14.4 Hz, 1H), 2.35 (dd, *J* = 15.9 Hz, *J* = 11.7 Hz, 1H), 2.21-2.23 (m, 1H), 2.15 (app t, *J* = 12.0 Hz, 1H), 1.90-1.99 (m, 3H), 1.67-1.73 (m, 3H), 1.43-1.56 (m, 2H), 0.98 (s, 3H), 0.96 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 158.9, 146.4, 136.3, 135.5, 134.6, 133.6, 128.9, 126.4, 125.7, 125.3, 121.3, 68.9, 64.3, 59.6, 52.1, 48.4, 44.7, 37.8, 35.4, 33.6, 29.9, 29.5, 25.8, 25.3, 22.5. HRMS (NSI) *m/z* = 393.30915 (M + H);

Theo. for $C_{25}H_{36}N_4$ + H = 393.30127. LC-MS (ESI-API) 75-95% MeOH in H_2O, 3 min, *m/z* = 393.2 (M + H), 197.2 (M/2 + H), t = 0.855 min; 25-95% MeOH in H_2O, 8 min, *m/z* = 393.2 (M + H), 197.2 (M/2 + H), t = 0.790 min; 10-95% MeOH in H_2O, 10 min, *m/z* = 393.2 (M + H), 197.2 (M/2 + H), t = 3.284 min.

2-(4-hydroxy-2,2-dimethylbutyl)isoindoline-1,3-dione (**32**): A 100 mL flask equipped with a stir bar was charged with 2,2-dimethylbutane-1,4-diol (**31**, 1.43 g, 12.1 mmol, 1.00 eq), imidazole (1.24 g, 18.2 mmol, 1.50 eq) and DMF (30.0 mL). Then TBSCl (1.82 g, 12.1 mmol, 1.00 eq) was added at 0°C, and the resulting reaction mixture was stirred at room temperature for 1 h. After this time, the reaction was quenched by addition of saturated aqueous ammonium chloride, and the resulting aqueous layer was extracted three times with diethyl ether. Combined organic layers were washed once with brine, dried over anhydrous magnesium sulfate, filtered, and evaporated under reduced pressure. The crude product was purified via silica gel column eluting with a gradient of 10-30% EtOAc in hexanes to afford the desired product as a clear oil (1.88 g, 8.08 mmol, 67% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.70 (dd, *J* = 5.0 Hz, *J* = 4.0 Hz, 2H), 3.59 (t, *J* = 7.0 Hz, 1H), 3.28 (d, *J* = 7.0 Hz, 2H), 1.50 (dd, *J* = 5.0 Hz, *J* = 4.0 Hz, 2H), 0.91 (s, 9H), 0.90 (s, 6H), 0.09 (s, 6H). A 100 mL flask equipped with a stir bar was charged with the purified alcohol (1.88 g, 8.08 mmol, 1.00 eq), phthalimide (1.43 g, 9.79 mmol, 1.20 eq), triphenylphosphine (2.44 g, 9.30 mmol, 1.15 eq) and THF (40.4 mL). Then DIAD (1.90 mL, 9.79 mmol, 1.21 eq) was added, and the resulting reaction mixture was stirred at room temperature for 24 h. After this time, phthalimide (0.715 g, 4.86 mmol, 0.600 eq), triphenylphosphine (1.22 g, 4.65 mmol, 0.580 eq), and DIAD (0.950 mL, 4.90 mmol, 0.605 eq) were added, and stirring was continued at room temperature for 12 h. After this time, the reaction was quenched by addition of water, and the resulting aqueous layer was extracted twice with

DCM. Combined organic layers were washed once with water, washed once with brine, dried over anhydrous sodium sulfate, filtered, and evaporated under reduced pressure. The crude product was filtered through a silica gel plug eluting with 10% EtOAc in hexanes to afford the desired product as a clear oil (3.03 g), which was carried forward without further purification. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.85 (dd, *J* = 5.4 Hz, *J* = 3.1 Hz, 2H), 7.72 (dd, *J* = 5.4 Hz, *J* = 3.1 Hz, 2H), 3.77 (t, *J* = 7.0 Hz, 2H), 3.55 (s, 2H), 1.57 (t, *J* = 7.0 Hz, 2H), 0.97 (s, 6H), 0.89 (s, 9H), 0.06 (s, 6H). A 25 mL flask equipped with a stir bar was charged with the crude material (2.92 g, 8.08 mmol, 1.00 eq), 2 M aqueous HCl (8.08 mL, 16.2 mmol, 2.00 eq), and THF (16.0 mL), and the resulting reaction mixture was stirred at room temperature for 2 h. After this time, the reaction was quenched by addition of saturated aqueous sodium bicarbonate, and the resulting aqueous layer was extracted three times with DCM. Combined organic layers were dried over anhydrous sodium sulfate, filtered, and evaporated under reduced pressure. The crude product was purified via silica gel column eluting with a gradient of 10-40% EtOAc in hexanes to afford the desired product as a white solid (1.67 g, 6.75 mmol, 84% yield over 2 steps). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.72-7.77 (m, 2H), 7.61-7.67 (m, 2H), 3.74 (t, *J* = 7.1 Hz, 2H), 3.51 (s, 2H), 2.77 (s, 1H), 1.49 (t, *J* = 7.1 Hz, 2H), 0.91 (s, 6H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 169.0 (2C), 133.8 (2C), 131.7 (2C), 123.0 (2C), 59.2, 48.0, 42.0, 35.4, 25.8 (2C). LC-MS (ESI-API) 95% MeOH in H_2O, 3 min, *m/z* = 248.0 (M + H), t = 0.562 min.

tert-butyl (R)-3-(((4-amino-3,3-dimethylbutyl)((S)-5,6,7,8-tetrahydroquinolin-8-yl)amino)methyl)-3,4-dihydroisoquinoline-2(1H)-carboxylate (**33**): A 50 mL Schlenk flask equipped with a stir bar was charged with the alcohol **32** (0.438 g, 1.77 mmol, 1.00 eq), triethylamine (1.31 mL, 9.39 mmol, 5.30 eq), and DCM (5.40 mL). After the mixture was cooled to 0°C, a solution of sulfur trioxide-pyridine complex (1.13 g, 7.08 mmol, 4.00 eq) in DMSO

(5.40 mL) was added dropwise, and the resulting reaction mixture was stirred at 0 °C for 3 h. After this time, the reaction mixture was quenched by addition of saturated aqueous sodium bicarbonate, and the resulting aqueous layer was extracted three times with diethyl ether. Combined organic layers were dried over anhydrous sodium sulfate, filtered, and evaporated under reduced pressure. The crude product was purified via silica gel column eluting with a gradient of 0-50% EtOAc in hexanes to afford the desired product as a white solid (0.334 g, 1.36 mmol, 77% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.82 (t, *J* = 2.4 Hz, 1H), 7.78 (dd, *J* = 5.5 Hz, *J* = 3.0 Hz, 2H), 7.68 (dd, *J* = 5.4 Hz, *J* = 3.1 Hz, 2H), 3.58 (s, 2H), 2.31 (d, *J* = 2.5 Hz, 2H), 1.07 (s, 6H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 201.9, 168.7 (2C), 134.0 (2C), 131.7 (2C), 123.2 (2C), 52.6, 47.7, 35.8, 25.9 (2C). LC-MS (ESI-API) 95% MeOH in H_2O isocratic, 3 min, *m/z* = 268.0 (M + Na), t = 0.572 min. To a 20 mL vial equipped with a stir bar was added the purified aldehyde (0.270 g, 1.10 mmol, 1.20 eq), amine **14** (0.361 g, 0.917 mmol, 1.00 eq), acetic acid (0.074 mL, 1.28 mmol, 1.40 eq), sodium triacetoxyborohydride (0.253 g, 1.19 mmol, 1.30 eq), and DCE (9.17 mL). After stirring at room temperature for 12 h, the reaction was quenched by addition of saturated aqueous sodium bicarbonate, and the resulting aqueous layer was extracted three times with diethyl ether. Combined organic layers were washed once with saturated aqueous sodium bicarbonate, washed once with brine, dried over anhydrous sodium sulfate, filtered, and evaporated under reduced pressure. The crude product was purified via silica gel column eluting with a gradient of 0-40% EtOAc in hexanes to afford the desired product as a clear oil (0.463 g, 0.743 mmol, 81% yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.34 (br s, 1H), 7.83-7.89 (m, 2H), 7.71-7.75 (m, 2H), 7.21 (d, *J* = 9.0 Hz, 1H), 7.04-7.09 (m, 3H), 6.96-6.98 (m, 1H), 6.93 (dd, *J* = 4.5 Hz, *J* = 7.5 Hz, 1H), 4.66 (d, *J* = 16.5 Hz, 1.5H), 4.42 (br s, 0.5H), 4.08 (d, *J* = 17.0 Hz, 1H), 3.82-3.96 (m, 1H), 3.42 (s, 2H), 2.93-3.14 (m, 2H), 2.73-2.79 (m, 1.5H), 2.58-2.69

(m, 4H), 2.49 (app t, *J* = 11.0 Hz, 0.5H), 1.94-1.98 (m, 2H), 1.73-1.79 (m, 1H), 1.62-1.68 (m, 1H), 1.44-1.54 (m, 10H), 1.25-1.37 (m, 1H), 1.01 (s, 0.5H), 0.86 (s, 3H), 0.81 (s, 2.5H). HRMS (NSI) *m/z* = 623.36086 (M + H); Theo. for $C_{38}H_{46}N_4O_4$ + H = 623.35918. LC-MS (ESI-API) 75-95% MeOH in H_2O, 6 min, *m/z* = 623.3 (M + H), t = 1.176 min; 50-95% MeOH in H_2O, 8 min, *m/z* = 623.4 (M + H), t = 4.922 min. A 20 mL vial equipped with a stir bar was charged with a solution of purified amine (0.177 g, 0.284 mmol, 1.00 eq) in MeOH (1.42 mL). Then a solution of hydrazine (0.301 mL, 2.27 mmol, 8.00 eq) in water (24% by wt.) was added, and the resulting reaction mixture was stirred at room temperature for 24 h. After this time, the reaction was quenched by addition of saturated aqueous sodium carbonate, and the resulting aqueous layer was extracted three times with DCM. Combined organic layers were dried over anhydrous sodium sulfate, filtered, and evaporated under reduced pressure. The crude material was carried forward without further purification. ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.38 (d, *J* = 10.2 Hz, 1H), 7.26 (d, *J* = 7.2 Hz, 1H), 7.08-7.11 (m, 2H), 7.04-7.06 (m, 1H), 6.98 (m, 2H), 4.62-4.68 (m, 1.5H), 4.39 (br s, 0.5H), 4.07-4.13 (m, 1H), 3.92 (m, 1H), 2.89-3.12 (m, 2H), 2.71 (m, 1H), 2.56-2.66 (m, 4H), 2.42-2.46 (m, 2H), 2.32-2.38 (m, 2H), 1.96-2.03 (m, 2H), 1.74 (m, 1H), 1.61-1.63 (m, 2H), 1.50 (s, 9H), 1.21-1.42 (m, 2H), 0.85 (s, 1H), 0.77 (s, 5H). HRMS (NSI) *m/z* = 493.35435 (M + H); Theo. for $C_{30}H_{44}N_4O_2$ + H = 493.35370. LC-MS (ESI-API) 75-95% MeOH in H_2O, 5 min, *m/z* = 493.2 (M + H), 247.2 (M/2 + H), t = 0.519 min; 50-95% MeOH in H_2O, 8 min, *m/z* = 493.2 (M + H), 393.2 (M – BOC + H), 247.2 (M/2 + H), t = 0.921 min.

3,3-dimethyl-N1-(((R)-1,2,3,4-tetrahydroisoquinolin-3-yl)methyl)-N1-((S)-5,6,7,8-tetrahydroquinolin-8-yl)butane-1,4-diamine (**34**): The BOC protecting group of amine **33** was removed according to the General Deprotection Protocol. The crude material was purified via silica gel column eluting with a gradient of 0-20% MeOH in DCM with 1% NH_4OH to afford the

desired product as a slightly yellow solid (0.102 g, 0.260 mmol, 91% yield). ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.45 (d, *J* = 4.7 Hz, 1H), 7.32 (d, *J* = 7.7 Hz, 1H), 6.99-7.10 (m, 5H), 4.07 (dd, *J* = 9.9 Hz, *J* = 6.3 Hz, 1H), 4.03 (d, *J* = 15.1 Hz, 1H), 3.87 (d, *J* = 15.1 Hz, 1H), 2.99 (dt, *J* = 11.6 Hz, *J* = 5.8 Hz, 1H), 2.94 (dd, *J* = 13.1 Hz, *J* = 3.2 Hz, 1H), 2.57-2.80 (m, 5H), 2.39-2.46 (m, 3H), 2.35 (dd, *J* = 13.2 Hz, *J* = 10.1 Hz, 1H), 2.03-2.10 (m, 1H), 1.94-2.02 (m, 1H), 1.84-1.94 (m, 1H), 1.67-1.75 (m, 1H), 1.60 (ddd, *J* = 13.4 Hz, *J* = 11.4 Hz, *J* = 4.9 Hz, 1H), 1.37 (ddd, *J* = 13.3 Hz, *J* = 11.1 Hz, *J* = 5.0 Hz, 1H), 0.84 (s, 6H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 158.5, 146.6, 136.2, 135.6, 134.6, 133.7, 128.9, 126.2, 125.6, 125.2, 121.2, 61.0, 57.8, 52.7, 52.1, 49.6, 48.6, 38.8, 34.0, 33.7, 29.2, 28.7, 24.9, 24.7, 21.7. HRMS (NSI) *m/z* = 393.30074 (M + H); Theo. for $C_{25}H_{36}N_4$ + H = 393.30127. LC-MS (ESI-API) 65% MeOH in H_2O isocratic, 3 min, *m/z* = 393.2 (M + H), 197.2 (M/2 + H), t = 0.875 min; 75-95% MeOH in H_2O, 3 min, *m/z* = 393.2 (M + H), 197.2 (M/2 + H), t = 0.773 min; 55% MeOH in H_2O isocratic, 3 min, *m/z* = 393.2 (M + H), 197.2 (M/2 + H), t = 0.890 min.

tert-butyl 2,2-dimethyl-5-oxopyrrolidine-1-carboxylate (**36**): To a solution of methyl 4-methyl-4-nitropentanoate (**35**, 15.0 g, 86.0 mmol, 1.00 eq) in EtOH (100 mL) was added a catalytic amount of Raney nickel. The resulting heterogeneous mixture was stirred vigorously, degassed using weak vacuum, and subsequently flushed with Ar. This degas cycle was repeated twice. Weak vacuum was applied to the flask once more, and the flask was finally flushed with hydrogen gas (40 psi). The resulting reaction mixture was shaken vigorously at room temperature for 18 h using a Parr hydrogenator. After this time, the reaction mixture was filtered over a plug of celite, and the resulting mother liquor was stirred at reflux for an additional 18 h. After this time, solvent was evaporated under reduced pressure to yield the desired product (8.50 g, 75.0 mmol, 88% yield). To a solution of the crude material (4.00 g, 35.3 mmol, 1.00 eq) in

DCM (70.7 mL) was added di-tert-butyl dicarbonate (12.3 g, 56.4 mmol, 2.90 eq), triethylamine (0.493 mL, 3.53 mmol, 0.100 eq), and 4-dimethylaminopyridine (0.432 g, 3.53 mmol, 0.100 eq). The resulting reaction mixture was warmed to reflux and stirred for 18 h. After this time, the reaction mixture was quenched with saturated aqueous ammonium chloride. The resulting organic layer was washed with saturated aqueous ammonium chloride (2 x 70 mL), and combined aqueous layers were extracted with DCM (3 x 50 mL). Combined organic layers were washed once with brine, dried over anhydrous sodium sulfate, filtered, and evaporated under reduced pressure. The crude material was purified via column chromatography eluting with 10% EtOAc in hexanes, followed by 20% EtOAc in hexanes, followed by DCM to yield the desired product as a yellow crystalline solid (6.15 g, 28.8 mmol, 82% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.47 (t, *J* = 7.8 Hz, 2H), 1.84 (t, *J* = 8.2 Hz, 2H), 1.53 (s, 9H), 1.45 (s, 6H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 174.6, 150.3, 82.7, 62.1, 34.3, 30.8, 28.1 (3C), 26.9 (2C). HRMS (ESI) *m/z* = 236.12545 (M + Na); Theo. for $C_{11}H_{19}O_3N$ + Na = 236.12571. LC-MS (ESI-API) 75-95% MeOH in H_2O, 5 min, *m/z* = 236.2 (M + Na), 449.2 (2M + Na), t = 2.511 min.

4-methyl-N1-(((R)-1,2,3,4-tetrahydroisoquinolin-3-yl)methyl)-N1-((S)-5,6,7,8-tetrahydroquinolin-8-yl)pentane-1,4-diamine (**37**): A solution of lactam **36** (3.10 g, 14.5 mmol, 1.00 eq) in dry diethyl ether (29.1 mL) was cooled to -78˚C and stirred under Ar. A solution of diisobutylaluminum hydride (17.4 mL, 17.4 mmol, 1.2 eq) in toluene (1.0 M) was slowly added, and the resulting reaction mixture was stirred at -78˚C under Ar for 4 h. Then the reaction mixture was slowly allowed to warm to room temperature and stirred for another 2 h. After this time, the reaction was quenched with water at 0˚C, and the resulting aqueous layer was extracted with EtOAc (4 x 40 mL). Combined organic layers were washed with water (3 x 100 mL), washed once with brine, dried over anhydrous sodium sulfate, filtered, and evaporated under

reduced pressure. The crude material was purified via column chromatography eluting with 10% EtOAc in hexanes, following by DCM to yield the desired product as a white solid (2.23 g, 10.4 mmol, 71% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.36-5.47 (m, 1H), 3.72 (s, 0.7H), 2.89 (s, 0.3H), 1.91-2.12 (m, 2H), 1.70-1.82 (m, 2H), 1.43-1.50 (m, 12H), 1.29 (s, 0.9H), 1.25 (s, 2.1H). The purified material (1.09 g, 5.08 mmol, 2.00 eq) and acetic acid (0.335 mL, 5.84 mmol, 2.3 eq) were added to a solution of secondary amine **14** (1.00 g, 2.54 mmol, 1.00 eq) in DCM (25.4 mL), and the resulting mixture was stirred at room temperature for 2 h. Then sodium triacetoxyborohydride (1.08 g, 5.08 mmol, 2.00 eq) was added, and the resulting reaction mixture was stirred at room temperature for 58 h. After this time, the reaction mixture was quenched with 1 M aqueous potassium carbonate, the resulting organic layer was washed with 1 M aqueous potassium carbonate (3 x 70 mL), and combined aqueous layers were extracted with DCM (2 x 30 mL). Combined organic layers were washed once with brine, dried over anhydrous sodium sulfate, filtered, and evaporated under reduced pressure. The crude material was purified via column chromatography eluting with DCM, followed by 1% MeOH in DCM to yield the desired product as a yellow oil (0.450 g, 0.759 mmol, 30% yield). BOC group removal was carried out according to the General Deprotection Protocol. The crude material was purified via column chromatography eluting with 90:10 DCM/MeOH, followed by 80:20:2 DCM/MeOH/NH_4OH to yield the desired product as a viscous yellow oil (0.094 g, 0.240 mmol, 36% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.49 (d, J = 3.2 Hz, 1H), 7.33 (d, J = 7.2 Hz, 1H), 6.99-7.09 (m, 5H), 3.77-4.08 (m, 4H), 2.93-2.99 (m, 2H), 2.41-2.80 (m, 8H), 2.09-2.12 (m, 1H), 1.84-2.01 (m, 2H), 1.69-1.72 (m, 1H), 1.25-1.56 (m, 5H), 1.17 (s, 3H), 1.16 (s, 3H). HRMS (ESI) *m/z* = 393.30118 (M + H); Theo. for $C_{25}H_{36}N_4$ + H = 393.30127. LC-MS (ESI-API) 50-95% MeOH in H_2O, 5 min, *m/z* = 393.4 (M + H), 376.2 (M – NH_3 + H), 197.3 (M/2 + H), t =

1.350 min; 75-95% MeOH in H_2O, 5 min, *m/z* = 393.3 (M + H), 376.4 (M – NH_3 + H), 197.3 (M/2 + H), t = 1.277 min.

4-((1,3-dioxoisoindolin-2-yl)methyl)benzaldehyde (**40**): (4-(Aminomethyl)phenyl)methanol (**38**, 5.00 g, 36.4 mmol, 1.00 eq) was added to a 500 mL flask with a stir bar. Diluted with THF (121 mL), added triethylamine (5.08 mL, 36.4 mmol, 1.00 eq) and ethyl 1,3-dioxoisoindoline-2-carboxylate (**39**, 7.99 g, 36.4 mmol, 1.00 eq), and the resulting reaction mixture was stirred vigorously at room temperature under Ar overnight. After 17 h, TLC indicated complete conversion of starting material to one major spot. Solvent was evaporated under reduced pressure to yield a yellow paste, which was carried forward without further purification. Dess-Martin periodinane (17.0 g, 40.1 mmol, 1.10 eq) was added to a 500 mL flask with a stir bar. The white solid was diluted with DCM (121 mL), and the resulting slurry was stirred vigorously at room temperature. Added a solution of the crude alcohol (9.74 g, 36.4 mmol, 1.00 eq) in DCM (121 mL) in dropwise fashion via addition funnel, and the resulting reaction mixture was stirred vigorously at room temperature under Ar. After 3 h, TLC indicated complete consumption of starting material. The reaction mixture was poured over 750 mL of a 1:1 mixture of saturated aqueous sodium bicarbonate and saturated aqueous sodium thiosulfate. The resulting organic layer was washed once with 150 mL of a 1:1 mixture of saturated aqueous sodium bicarbonate and saturated aqueous sodium thiosulfate, washed once with brine, dried over anhydrous sodium sulfate, filtered, and evaporated under reduced pressure to yield an orange oil. Half of the crude material was immediately carried forward without further purification. ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.99 (s, 1H), 7.84-7.89 (m, 4H), 7.71-7.76 (m, 2H), 7.58 (d, *J* = 8.0 Hz, 2H), 4.93 (s, 2H).

tert-butyl (R)-3-(((4-(aminomethyl)benzyl)((S)-5,6,7,8-tetrahydroquinolin-8-yl)amino)methyl)-3,4-dihydroisoquinoline-2(1H)-carboxylate (**41**): Sodium triacetoxyborohydride (11.1 g, 52.1 mmol, 3.00 eq) was added to a 500 mL flask with a stir bar. Diluted with DCM (87.0 mL), and the resulting slurry was stirred vigorously at room temperature under Ar. Added a solution of secondary amine **14** (6.84 g, 17.4 mmol, 1.00 eq) in DCM (43.4 mL), followed by a solution of aldehyde **40** (4.84 g, 18.3 mmol, 1.05 eq) in DCM (43.4 mL), and the resulting reaction mixture was stirred vigorously at room temperature under Ar overnight. After 15 h, TLC indicated complete consumption of starting material. The reaction mixture was diluted with DCM and washed three times with 1 M aqueous sodium hydroxide. The resulting aqueous layer was extracted twice with DCM. Combined organic layers were washed once with brine, dried over anhydrous sodium sulfate, filtered, and evaporated under reduced pressure to yield a yellow foam (11.0 g). The crude material was purified via column chromatography (CombiFlash, 120 g column, 75 mL/min) eluting with the following gradient to yield a white foam (7.30 g, 11.4 mmol, 65% yield): 0-3 min, 0% MeOH in DCM; 3-8 min, 0-1% MeOH in DCM; 8-13 min, 1% MeOH in DCM; 13-23 min, 1-2% MeOH in DCM; 23-30 min, 2% MeOH in DCM; 30-40 min, 2-20% MeOH in DCM. ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.40 (d, J = 14.4 Hz, 1H), 7.85-7.87 (m, 2H), 7.72 (dd, J = 3.0 Hz, J = 5.4 Hz, 2H), 7.29-7.30 (m, 3H), 7.19-7.26 (m, 2H), 7.03 (app t, J = 6.6 Hz, 1.5H), 6.93-6.97 (m, 3H), 6.74 (d, J = 6.6 Hz, 0.5H), 4.83-4.88 (m, 2H), 4.61-4.76 (m, 1H), 4.36-4.39 (m, 1H), 4.01-4.10 (m, 1H), 3.85-3.88 (m, 1H), 3.60 (app t, J = 16.2 Hz, 1H), 3.50 (d, J = 14.4 Hz, 0.5H), 3.36 (d, J = 17.4 Hz, 0.5H), 2.89-3.09 (m, 2H), 2.80 (d, J = 16.2 Hz, 0.5H), 2.62-2.66 (m, 3H), 2.48 (dd, J = 9.6 Hz, J = 12.0 Hz, 0.5H), 2.25-2.27 (m, 0.5H), 2.08 (m, 0.5H), 1.93-1.95 (m, 1H), 1.75 (dd, J = 11.7 Hz, J = 23.1 Hz, 1H), 1.59-1.69 (m, 1H), 1.48 (s, 4.5H), 1.42 (s, 4.5H). HRMS (NSI) *m/z* = 643.32782 (M + H); Theo. for $C_{40}H_{42}N_4O_4$ + H =

643.32788. LC-MS (ESI-API) 75-95% MeOH in H_2O, 5 min, *m/z* = 643.2 (M + H), t = 1.137 min; 50-95% MeOH in H_2O, 8 min, *m/z* = 643.2 (M + H), t = 3.216 min. The purified material (0.769 g, 1.20 mmol, 1.00 eq) was added to a 50 mL flask with a stir bar. Diluted with MeOH (12.0 mL), added a small volume of DCM to coerce all of the starting material into solution, and the resulting solution was stirred vigorously at room temperature under Ar. Added a solution of hydrazine (1.29 mL, 9.57 mmol, 8.00 eq) in water (24% by wt.) in dropwise fashion via syringe pump at a rate of 4 mL/hr, and the resulting reaction mixture was stirred vigorously at room temperature under Ar overnight. After 17 h, TLC indicated almost complete conversion of starting material to one major product spot. The reaction mixture was partitioned between DCM and 1 M aqueous sodium hydroxide. The resulting aqueous layer was extracted 3 times with DCM, and combined organic layers were dried over anhydrous sodium sulfate, filtered, and evaporated under reduced pressure to yield a yellow foam (617 mg). The crude material was purified via column chromatography (CombiFlash, 24 g column, 30 mL/min) eluting with the following gradient to yield a white foam (474 mg, 0.925 mmol, 77% yield): 0-3 min, 0% 100:10:1 DCM/MeOH/NH_4OH in DCM; 3-18 min, 0-100% 100:10:1 DCM/MeOH/NH_4OH in DCM; 18-30 min, 100% 100:10:1 DCM/MeOH/NH_4OH. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.43 (d, *J* = 10.0 Hz, 1H), 7.29-7.34 (m, 2H), 7.25 (d, *J* = 7.5 Hz, 1H), 7.19-7.20 (m, 2H), 7.04-7.09 (m, 2H), 6.82-7.00 (m, 3H), 4.65-4.78 (m, 1H), 4.45-4.52 (m, 1H), 3.89-4.10 (m, 2H), 3.85 (s, 2H), 3.57-3.81 (m, 2H), 2.86-3.05 (m, 2H), 2.52-2.75 (m, 3H), 2.10-2.24 (m, 1H), 1.94-1.95 (m, 1H), 1.79 (app p, *J* = 11.0 Hz, 1H), 1.42-1.66 (m, 12H). HRMS (NSI) *m/z* = 513.32201 (M + H); Theo. for $C_{32}H_{40}N_4O_2$ + H = 513.32240. LC-MS (ESI-API) 50-95% MeOH in H_2O, 6 min, *m/z* = 513.2 (M + H), 457.2 (M – *t*Bu + H), 413.2 (M – BOC + H), 257.1 (M/2 + H), t = 0.854 min; 25-

95% MeOH in H_2O, 8 min, *m/z* = 513.2 (M + H), 457.2 (M – *t*Bu + H), 413.2 (M – BOC + H), 257.1 (M/2 + H), t = 4.136 min.

(S)-N-(4-((((tetrahydro-2H-pyran-4-yl)methyl)amino)methyl)benzyl)-N-(((R)-1,2,3,4-tetrahydroisoquinolin-3-yl)methyl)-5,6,7,8-tetrahydroquinolin-8-amine (**42**): Reductive amination was carried out according to General Reductive Amination Protocol D using amine **41** and aldehyde **10c**. Purified via column chromatography (CombiFlash, 24 g column, 30 mL/min) eluting with the following gradient to yield a clear oil (110 mg, 0.180 mmol, 37% yield): 0-3 min, 0% 100:10:1 DCM/MeOH/NH_4OH in DCM; 3-18 min, 0-100% 100:10:1 DCM/MeOH/NH_4OH in DCM; 18-30 min, 100% 100:10:1 DCM/MeOH/NH_4OH. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.43 (d, *J* = 10.0 Hz, 1H), 7.29-7.32 (m, 2H), 7.24 (d, *J* = 7.0 Hz, 1H), 7.19 (br s, 2H), 7.04-7.07 (m, 2H), 6.81-7.00 (m, 3H), 4.65-4.78 (m, 1H), 4.45-4.50 (m, 1H), 3.89-4.09 (m, 4H), 3.57-3.81 (m, 4H), 3.40 (dt, *J* = 1.8 Hz, *J* = 11.8 Hz, 2H), 2.85-3.03 (m, 3H), 2.57-2.75 (m, 3H), 2.54 (d, *J* = 7.0 Hz, 2H), 2.10-2.24 (m, 1H), 1.93-1.95 (m, 1H), 1.71-1.78 (m, 2H), 1.66-1.67 (m, 3H), 1.46-1.58 (m, 10H), 1.26-1.36 (m, 2H). HRMS (NSI) *m/z* = 611.39646 (M + H); Theo. for $C_{38}H_{50}N_4O_3$ + H = 611.39557. LC-MS (ESI-API) 50-95% MeOH in H_2O, 6 min, *m/z* = 611.2 (M + H), 306.2 (M/2 + H), t = 1.126 min; 25-95% MeOH in H_2O, 8 min, *m/z* = 611.2 (M + H), 306.2 (M/2 + H), t = 4.534 min. BOC group removal was carried out according to the General Deprotection Protocol. Purified via column chromatography (CombiFlash, 12 g column, 25 mL/min) eluting with the following gradient to yield a clear oil (81.0 mg, 0.159 mmol, 88% yield): 0-3 min, 0% 100:10:1 DCM/MeOH/NH_4OH in DCM; 3-18 min, 0-100% 100:10:1 DCM/MeOH/NH_4OH in DCM; 18-30 min, 100% 100:10:1 DCM/MeOH/NH_4OH. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.51 (d, *J* = 4.0 Hz, 1H), 7.42 (d, *J* = 7.5 Hz, 2H), 7.33 (d, *J* = 7.5 Hz, 1H), 7.25 (d, *J* = 8.0 Hz, 2H), 7.04-7.08 (m, 3H), 6.95-7.01 (m, 2H), 4.38 (d, *J* = 14.0 Hz, 1H), 4.11 (dd, *J*

= 6.5 Hz, *J* = 9.5 Hz, 1H), 3.90-3.98 (m, 4H), 3.76 (s, 2H), 3.67 (d, *J* = 15.0 Hz, 1H), 3.38 (dt, *J* = 1.3 Hz, *J* = 11.8 Hz, 2H), 2.90 (dd, *J* = 2.8 Hz, *J* = 13.3 Hz, 1H), 2.73-2.80 (m, 1H), 2.63-2.68 (m, 2H), 2.55 (dd, *J* = 3.5 Hz, *J* = 16.0 Hz, 1H), 2.51 (d, *J* = 7.0 Hz, 2H), 2.35-2.48 (m, 2H), 2.12-2.16 (m, 1H), 1.91-1.99 (m, 3H), 1.66-1.76 (m, 2H), 1.62-1.65 (m, 2H), 1.25-1.33 (m, 3H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 159.0, 146.9, 140.8, 139.0, 136.6, 135.7, 134.7, 134.2, 129.2, 128.5 (2C), 128.1 (2C), 126.5, 126.0, 125.5, 121.5, 68.0 (2C), 62.4, 59.4, 58.1, 55.7, 54.0, 52.1, 48.7, 35.6, 33.9, 31.5 (2C), 29.7, 29.6, 22.2. HRMS (NSI) *m/z* = 511.34459 (M + H); Theo. for $C_{33}H_{42}N_4O$ + H = 511.34314. LC-MS (ESI-API) 25-95% MeOH in H_2O, 8 min, *m/z* = 511.2 (M + H), 256.2 (M/2 + H), t = 0.722 min; 10-95% MeOH in H_2O, 10 min, *m/z* = 511.2 (M + H), 256.2 (M/2 + H), t = 3.070 min.

(S)-N-(4-(((4,4-difluorocyclohexyl)amino)methyl)benzyl)-N-(((R)-1,2,3,4-tetrahydroisoquinolin-3-yl)methyl)-5,6,7,8-tetrahydroquinolin-8-amine (**43b**): Reductive amination was carried out according to General Reductive Amination Protocol E using amine **41** and ketone **12b**. Purified via column chromatography (CombiFlash, 24 g column, 30 mL/min) eluting with the following gradient to yield a clear oil (238 mg, 0.377 mmol, 84% yield): 0-3 min, 0% 100:10:1 DCM/MeOH/NH_4OH in DCM; 3-18 min, 0-100% 100:10:1 DCM/MeOH/NH_4OH in DCM; 18-30 min, 100% 100:10:1 DCM/MeOH/NH_4OH. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.43 (d, *J* = 9.5 Hz, 1H), 7.30-7.32 (m, 2H), 7.24 (d, *J* = 7.5 Hz, 1H), 7.19 (m, 2H), 7.04-7.07 (m, 2H), 6.82-7.00 (m, 3H), 4.65-4.77 (m, 1H), 4.45-4.52 (m, 1H), 3.90-4.08 (m, 2H), 3.59-3.82 (m, 4H), 2.87-3.03 (m, 2H), 2.53-2.75 (m, 4H), 2.10-2.24 (m, 3H), 1.90-1.94 (m, 3H), 1.71-1.87 (m, 4H), 1.64-1.66 (m, 1H), 1.46-1.60 (m, 12H). HRMS (NSI) *m/z* = 631.38184 (M + H); Theo. for $C_{38}H_{48}F_2N_4O_2$ + H = 631.38181. LC-MS (ESI-API) 50-95% MeOH in H_2O, 8 min, *m/z* = 631.2 (M + H), 316.2 (M/2 + H), t = 1.620 min; 25-95% MeOH in H_2O, 8 min, *m/z*

= 631.2 (M + H), 316.2 (M/2 + H), t = 4.864 min. BOC group removal was carried out according to the General Deprotection Protocol. Purified via column chromatography (CombiFlash, 12 g column, 25 mL/min) eluting with the following gradient to yield a white foam (147 mg, 0.277 mmol, 73% yield): 0-3 min, 0% 100:10:1 DCM/MeOH/NH_4OH in DCM; 3-18 min, 0-100% 100:10:1 DCM/MeOH/NH_4OH in DCM; 18-30 min, 100% 100:10:1 DCM/MeOH/NH_4OH. ^1H NMR (500 MHz, $CDCl_3$) δ 8.51 (dd, *J* = 1.5 Hz, *J* = 5.0 Hz, 1H), 7.43 (d, *J* = 8.0 Hz, 2H), 7.33 (dt, *J* = 0.8 Hz, *J* = 7.5 Hz, 1H), 7.26 (d, *J* = 8.0 Hz, 2H), 7.04-7.08 (m, 3H), 6.95-7.02 (m, 2H), 4.39 (d, *J* = 14.5 Hz, 1H), 4.10 (dd, *J* = 6.8 Hz, *J* = 10.3 Hz, 1H), 3.94 (dd, *J* = 14.8 Hz, *J* = 24.8 Hz, 2H), 3.78 (s, 2H), 3.68 (d, *J* = 15.0 Hz, 1H), 2.90 (dd, *J* = 3.0 Hz, *J* = 13.0 Hz, 1H), 2.74-2.80 (m, 2H), 2.63-2.70 (m, 3H), 2.55 (dd, *J* = 3.5 Hz, *J* = 16.0 Hz, 1H), 2.35-2.45 (m, 2H), 2.07-2.16 (m, 3H), 1.88-1.99 (m, 4H), 1.65-1.80 (m, 4H), 1.50-1.58 (m, 2H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 158.9, 146.9, 140.9, 138.9, 136.5, 135.7, 134.7, 134.2, 129.1, 128.6 (2C), 128.0 (2C), 126.5, 125.9, 125.5, 123.5 (t, *J* = 239.4 Hz), 121.5, 62.3, 59.4, 58.0, 53.2, 52.1, 51.2, 48.7, 33.9, 31.5 (t, *J* = 23.8 Hz, 2C), 29.6, 29.5, 28.8 (t, *J* = 4.4 Hz, 2C), 22.2. ^{19}F NMR (376 MHz, $CDCl_3$, TFA standard) δ -95.7 (app d, *J* = 236.9 Hz), -98.6 (app d, *J* = 229.4 Hz). HRMS (NSI) *m/z* = 531.32872 (M + H); Theo. for $C_{33}H_{40}F_2N_4$ + H = 531.32938. LC-MS (ESI-API) 25-95% MeOH in H_2O, 8 min, *m/z* = 531.2 (M + H), 266.2 (M/2 + H), t = 1.124 min; 10-95% MeOH in H_2O, 10 min, *m/z* = 531.2 (M + H), 266.2 (M/2 + H), t = 3.512 min.

(S)-N-(4-(((tetrahydro-2H-pyran-4-yl)amino)methyl)benzyl)-N-(((R)-1,2,3,4-tetrahydroisoquinolin-3-yl)methyl)-5,6,7,8-tetrahydroquinolin-8-amine (**43c**): Reductive amination was carried out according to General Reductive Amination Protocol E using amine **41** and ketone **12c**. Purified via column chromatography (CombiFlash, 24 g column, 30 mL/min) eluting with the following gradient to yield a white foam (220 mg, 0.369 mmol, 76% yield): 0-3

min, 0% 100:10:1 DCM/MeOH/NH_4OH in DCM; 3-18 min, 0-100% 100:10:1 DCM/MeOH/NH_4OH in DCM; 18-30 min, 100% 100:10:1 DCM/MeOH/NH_4OH. ^1H NMR (500 MHz, $CDCl_3$) δ 8.43 (d, *J* = 9.0 Hz, 1H), 7.29-7.32 (m, 2H), 7.24 (d, *J* = 7.5 Hz, 1H), 7.20 (br s, 2H), 7.04-7.07 (m, 2H), 6.83-7.00 (m, 3H), 4.66-4.77 (m, 1H), 4.45-4.52 (m, 1H), 3.90-4.08 (m, 4H), 3.59-3.85 (m, 4H), 3.41 (dt, *J* = 2.0 Hz, *J* = 11.8 Hz, 2H), 2.86-3.03 (m, 3H), 2.53-2.78 (m, 4H), 2.11-2.22 (m, 1H), 1.93 (m, 1H), 1.87 (dd, *J* = 1.5 Hz, *J* = 12.5 Hz, 2H), 1.78 (app p, *J* = 10.3 Hz, 1H), 1.64-1.65 (m, 1H), 1.43-1.58 (m, 12H). HRMS (NSI) *m/z* = 597.38109 (M + H); Theo. for $C_{37}H_{48}N_4O_3$ + H = 597.37992. LC-MS (ESI-API) 50-95% MeOH in H_2O, 6 min, *m/z* = 597.2 (M + H), 299.2 (M/2 + H), t = 0.906 min; 25-95% MeOH in H_2O, 8 min, *m/z* = 597.2 (M + H), 299.2 (M/2 + H), t = 4.219 min. BOC group removal was carried out according to the General Deprotection Protocol. Purified via column chromatography (CombiFlash, 12 g column, 25 mL/min) eluting with the following gradient to yield a white foam (159 mg, 0.320 mmol, 87% yield). 0-3 min, 0% 100:10:1 DCM/MeOH/NH_4OH in DCM; 3-18 min, 0-100% 100:10:1 DCM/MeOH/NH_4OH in DCM; 18-30 min, 100% 100:10:1 DCM/MeOH/NH_4OH. ^1H NMR (500 MHz, $CDCl_3$) δ 8.51 (d, *J* = 3.5 Hz, 1H), 7.43 (d, *J* = 8.0 Hz, 2H), 7.33 (dd, *J* = 0.5 Hz, *J* = 7.5 Hz, 1H), 7.26 (d, *J* = 7.0 Hz, 2H), 7.04-7.07 (m, 3H), 6.95-7.01 (m, 2H), 4.39 (d, *J* = 14.0 Hz, 1H), 4.10 (dd, *J* = 6.5 Hz, *J* = 10.0 Hz, 1H), 3.90-3.98 (m, 4H), 3.81 (s, 2H), 3.68 (d, *J* = 15.0 Hz, 1H), 3.70 (dt, *J* = 2.0 Hz, *J* = 11.8 Hz, 2H), 2.90 (dd, *J* = 2.5 Hz, *J* = 8.3 Hz, 1H), 2.64-2.80 (m, 5H), 2.55 (dd, *J* = 3.5 Hz, *J* = 16.0 Hz, 1H), 2.35-2.45 (m, 2H), 2.11-2.15 (m, 1H), 1.91-1.99 (m, 2H), 1.83-1.86 (m, 2H), 1.62-1.70 (m, 1H), 1.26-1.49 (m, 3H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 158.9, 146.9, 140.7, 138.9, 136.5, 135.7, 134.7, 134.1, 129.1, 128.5 (2C), 128.0 (2C), 126.4, 125.9, 125.5, 121.4, 66.8 (2C), 62.2, 59.3, 58.0, 53.2, 52.1, 50.3, 48.7, 33.9, 33.8, 33.8, 29.6, 29.5, 22.2. HRMS (NSI) *m/z* = 497.32792 (M + H); Theo. for $C_{32}H_{40}N_4O$ + H = 497.32749. LC-

MS (ESI-API) 25-95% MeOH in H_2O, 8 min, *m/z* = 497.2(M + H), 249.2 (M/2 + H), t = 0.631 min; 10-95% MeOH in H_2O, 10 min, *m/z* = 497.2 (M + H), 249.2 (M/2 + H), t = 2.688 min.

(S)-N-(4-(((4,4-dimethylcyclohexyl)amino)methyl)benzyl)-N-(((R)-1,2,3,4-tetrahydroisoquinolin-3-yl)methyl)-5,6,7,8-tetrahydroquinolin-8-amine (**43g**): Reductive amination was carried out according to General Reductive Amination Protocol E using amine **41** and ketone **12g**. Purified via column chromatography (CombiFlash, 24 g column, 30 mL/min) eluting with the following gradient to yield a white foam (166 mg, 0.267 mmol, 59% yield): 0-3 min, 0% 100:10:1 DCM/MeOH/NH_4OH in DCM; 3-18 min, 0-100% 100:10:1 DCM/MeOH/NH_4OH in DCM; 18-30 min, 100% 100:10:1 DCM/MeOH/NH_4OH. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.43 (d, *J* = 9.0 Hz, 1H), 7.23-7.31 (m, 3H), 7.19 (br s, 2H), 7.05-7.07 (m, 2H), 6.82-7.00 (m, 3H), 4.65-4.79 (m, 1H), 4.44-4.51 (m, 1H), 4.00-4.11 (m, 1H), 3.89-3.92 (m, 1H), 3.79 (s, 2H), 3.56-3.75 (m, 2H), 3.02-3.05 (m, 1H), 2.71-2.99 (m, 2H), 2.52-2.74 (m, 3H), 2.46 (m, 1H), 2.10-2.24 (m, 1H), 1.94-1.95 (m, 1H), 1.63-1.77 (m, 5H), 1.46-1.58 (m, 9H), 1.32-1.43 (m, 4H), 1.18-1.24 (m, 2H), 0.92 (s, 3H), 0.91 (s, 3H). HRMS (NSI) *m/z* = 623.43140 (M + H); Theo. for $C_{40}H_{54}N_4O_2$ + H = 623.43195. LC-MS (ESI-API) 50-95% MeOH in H_2O, 8 min, *m/z* = 623.3 (M + H), 312.2 (M/2 + H), t = 2.592 min; 25-95% MeOH in H_2O, 8 min, *m/z* = 623.2 (M + H), 312.2 (M/2 + H), t = 5.806 min. BOC group removal was carried out according to the General Deprotection Protocol. Purified via column chromatography (CombiFlash, 12 g column, 25 mL/min) eluting with the following gradient to yield a white foam (99.0 mg, 0.189 mmol, 71% yield): 0-3 min, 0% 100:10:1 DCM/MeOH/NH_4OH in DCM; 3-18 min, 0-100% 100:10:1 DCM/MeOH/NH_4OH in DCM; 18-30 min, 100% 100:10:1 DCM/MeOH/NH_4OH. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.51 (dd, *J* = 1.5 Hz, *J* = 5.0 Hz, 1H), 7.41 (d, *J* = 8.0 Hz, 2H), 7.33 (dt, *J* = 0.8 Hz, *J* = 7.8 Hz, 1H), 7.26 (d, *J* = 8.0 Hz, 2H), 7.04-7.08 (m, 3H), 6.95-7.02 (m, 2H), 4.40 (d, *J* =

14.0 Hz, 1H), 4.10 (dd, *J* = 6.5 Hz, *J* = 10.0 Hz, 1H), 3.94 (dd, *J* = 14.3 Hz, *J* = 26.3 Hz, 2H), 3.79 (s, 2H), 3.69 (d, *J* = 15.0 Hz, 1H), 2.89 (dd, *J* = 2.8 Hz, *J* = 13.3 Hz, 1H), 2.63-2.79 (m, 4H), 2.55 (dd, *J* = 3.5 Hz, *J* = 16.0 Hz, 1H), 2.35-2.47 (m, 3H), 2.10-2.15 (m, 1H), 1.90-1.99 (m, 2H), 1.70-1.75 (m, 2H), 1.61-1.69 (m, 1H), 1.37-1.40 (m, 2H), 1.27-1.34 (m, 3H), 1.19 (dt, *J* = 3.5 Hz, *J* = 12.8 Hz, 2H), 0.91 (s, 3H), 0.90 (s, 3H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 159.0, 146.9, 140.6, 139.4, 136.5, 135.8, 134.8, 134.2 129.2, 128.5 (2C), 128.1 (2C), 126.5, 125.9, 125.5, 121.5, 62.2, 59.4, 58.0, 56.5, 52.1, 51.1, 48.7, 37.9, 33.9, 32.3, 30.3, 29.8, 29.5, 29.4, 25.0, 22.2. HRMS (NSI) *m/z* = 523.37923 (M + H); Theo. for $C_{35}H_{46}N_4$ + H = 523.37952. LC-MS (ESI-API) 25-95% MeOH in H_2O, 8 min, *m/z* = 523.2 (M + H), 262.2 (M/2 + H), t = 3.052 min; 10-95% MeOH in H_2O, 10 min, *m/z* = 523.2 (M + H), 262.2 (M/2 + H), t = 4.873 min.

(S)-N-(4-(((pyridin-2-ylmethyl)amino)methyl)benzyl)-N-(((R)-1,2,3,4-tetrahydroisoquinolin-3-yl)methyl)-5,6,7,8-tetrahydroquinolin-8-amine (**44a**): Reductive amination was carried out according to General Reductive Amination Protocol D using amine **41** and 2-pyridinecarboxaldehyde. Purified via column chromatography (CombiFlash, 24 g column, 30 mL/min) eluting with the following gradient to yield a yellow foam (181 mg, 0.300 mmol, 62% yield): 0-3 min, 0% 100:10:1 DCM/MeOH/NH_4OH in DCM; 3-18 min, 0-100% 100:10:1 DCM/MeOH/NH_4OH in DCM; 18-30 min, 100% 100:10:1 DCM/MeOH/NH_4OH. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.58 (d, *J* = 5.0 Hz, 1H), 8.43 (d, *J* = 10.0 Hz, 1H), 7.65 (dt, *J* = 1.8 Hz, *J* = 7.9 Hz, 1H), 7.34 (d, *J* = 8.0 Hz, 1H), 7.29-7.31 (m, 2H), 7.25 (m, 3H), 7.17 (dd, *J* = 4.5 Hz, *J* = 7.5 Hz, 1H), 7.04-7.06 (m, 2H), 6.79-7.00 (m, 3H), 4.65-4.79 (m, 1H), 4.44-4.50 (m, 1H), 3.99-4.11 (m, 1H), 3.95 (s, 2H), 3.89-3.92 (m, 1H), 3.84 (app d, *J* = 4.0 Hz, 2H), 3.56-3.71 (m, 2H), 3.02-3.09 (m, 1H), 2.91-2.99 (m, 1H), 2.72-2.87 (m, 1H), 2.52-2.69 (m, 3H), 2.11-2.26 (m, 2H), 1.94-1.95 (m, 1H), 1.77-1.80 (m, 1H), 1.58-1.69 (m, 1H), 1.53 (s, 4.5H), 1.46 (s, 4.5H). HRMS (NSI)

m/z = 604.36452 (M + H); Theo. for $C_{38}H_{45}N_5O_2$ + H = 604.36460. LC-MS (ESI-API) 75-95% MeOH in H_2O, 5 min, *m/z* = 604.2 (M + H), 302.6 (M/2 + H), t = 0.535 min; 25-95% MeOH in H_2O, 8 min, *m/z* = 604.2 (M + H), 302.7 (M/2 + H), t = 4.604 min. BOC group removal was carried out according to the General Deprotection Protocol. Purified via column chromatography (CombiFlash, 12 g column, 25 mL/min) eluting with 100:10:1 DCM/MeOH/NH_4OH to yield a white foam (106 mg, 0.210 mmol, 70% yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.56-8.57 (m, 1H), 8.51 (d, *J* = 4.5 Hz, 1H), 7.63 (dt, *J* = 1.8 Hz, *J* = 7.8 Hz, 1H), 7.43 (d, *J* = 8.0 Hz, 2H), 7.29-7.34 (m, 4H), 7.15-7.17 (m, 1H), 7.02-7.07 (m, 3H), 6.94-7.01 (m, 2H), 4.42 (d, *J* = 14.0 Hz, 1H), 4.10 (dd, *J* = 6.5 Hz, *J* = 10.0 Hz, 1H), 3.94 (dd, *J* = 15.0 Hz, *J* = 17.5 Hz, 2H), 3.92 (s, 2H), 3.84 (s, 2H), 3.68 (d, *J* = 14.5 Hz, 1H), 2.73-2.91 (m, 3H), 2.63-2.71 (m, 2H), 2.55 (dd, *J* = 3.5 Hz, *J* = 16.0 Hz, 1H), 2.35-2.44 (m, 2H), 2.11-2.15 (m, 2H), 1.91-1.98 (m, 2H), 1.62-1.71 (m, 1H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 159.9, 159.0, 149.4, 146.9, 140.8, 138.6, 136.5, 135.8, 134.8, 134.2, 129.1, 128.5 (2C), 128.3 (2C), 126.5, 125.9, 125.5, 122.4, 122.0, 121.4, 62.2, 59.4, 58.0, 54.6, 53.4, 52.1, 48.7, 33.9, 29.7, 29.5, 22.2. HRMS (NSI) *m/z* = 504.31269 (M + H); Theo. for $C_{33}H_{37}N_5$ + H = 504.31217. LC-MS (ESI-API) 25-95% MeOH in H_2O, 8 min, *m/z* = 504.2 (M + H), 252.6 (M/2 + H), t = 0.852 min; 10-95% MeOH in H_2O, 10 min, *m/z* = 504.2 (M + H), 252.6 (M/2 + H), t = 3.286 min.

(S)-N-(4-(((pyridin-3-ylmethyl)amino)methyl)benzyl)-N-(((R)-1,2,3,4-tetrahydroisoquinolin-3-yl)methyl)-5,6,7,8-tetrahydroquinolin-8-amine (**44b**): Reductive amination was carried out according to General Reductive Amination Protocol D using amine **41** and 3-pyridinecarboxaldehyde. Purified via column chromatography (CombiFlash, 24 g column, 30 mL/min) eluting with the following gradient to yield a white foam (179 mg, 0.296 mmol, 61% yield): 0-3 min, 0% 100:10:1 DCM/MeOH/NH_4OH in DCM; 3-18 min, 0-100% 100:10:1

DCM/MeOH/NH_4OH in DCM; 18-30 min, 100% 100:10:1 DCM/MeOH/NH_4OH. ^1H NMR (500 MHz, $CDCl_3$) δ 8.59 (br s, 1H), 8.52 (dd, *J* = 1.5 Hz, *J* = 4.5 Hz, 1H), 8.43 (d, *J* = 9.5 Hz, 1H), 7.72 (dt, *J* = 1.8 Hz, *J* = 7.8 Hz, 1H), 7.30-7.32 (m, 2H), 7.27 (dd, *J* = 5.0 Hz, *J* = 7.5 Hz, 1H), 7.22-7.25 (m, 3H), 7.04-7.05 (m, 2H), 6.78-7.00 (m, 3H), 4.65-4.78 (m, 1H), 4.45-4.52 (m, 1H), 3.86-4.09 (m, 2H), 3.82 (s, 2H), 3.58-3.80 (m, 4H), 2.71-3.04 (m, 3H), 2.53-2.70 (m, 3H), 2.11-2.24 (m, 1H), 1.95 (m, 1H), 1.65-1.80 (m, 3H), 1.53 (s, 4.5H), 1.46 (s, 4.5H). HRMS (NSI) *m/z* = 604.36444 (M + H); Theo. for $C_{38}H_{45}N_5O_2$ + H = 604.36460. LC-MS (ESI-API) 75-95% MeOH in H_2O, 5 min, *m/z* = 604.2 (M + H), 302.6 (M/2 + H), t = 0.592 min; 25-95% MeOH in H_2O, 8 min, *m/z* = 604.2(M + H), 302.6 (M/2 + H), t = 4.309 min. BOC group removal was carried out according to the General Deprotection Protocol. Purified via column chromatography (CombiFlash, 12 g column, 25 mL/min) eluting with 100:10:1 DCM/MeOH/NH_4OH to yield a white foam (137 mg, 0.272 mmol, 92% yield). ^1H NMR (500 MHz, $CDCl_3$) δ 8.57 (m, 1H), 8.50-8.52 (m, 2H), 7.67-7.70 (m, 1H), 7.44 (d, *J* = 7.5 Hz, 2H), 7.33 (d, *J* = 7.5 Hz, 1H), 7.24-7.29 (m, 3H), 7.02-7.08 (m, 3H), 6.92-7.01 (m, 2H), 4.39 (d, *J* = 14.0 Hz, 1H), 4.10 (dd, *J* = 6.8 Hz, *J* = 10.3 Hz, 1H), 3.94 (dd, *J* = 14.8 Hz, *J* = 18.8 Hz, 2H), 3.80 (s, 2H), 3.79 (s, 2H), 3.67 (d, *J* = 15.0 Hz, 1H), 2.90 (dd, *J* = 2.5 Hz, *J* = 13.0 Hz, 1H), 2.74-2.80 (m, 2H), 2.63-2.67 (m, 2H), 2.55 (dd, *J* = 3.0 Hz, *J* = 16.0 Hz, 1H), 2.43 (dd, *J* = 10.5 Hz, *J* = 13.0 Hz, 1H), 2.37 (dd, *J* = 11.0 Hz, *J* = 16.0 Hz, 1H), 2.12-2.16 (m, 1H), 1.91-1.99 (m, 3H), 1.62-1.71 (m, 1H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 158.9, 149.8, 148.5, 146.9, 141.0, 138.4, 136.5, 135.9, 135.8, 134.7, 134.2, 129.1, 128.6 (2C), 128.1 (2C), 126.5, 125.9, 125.5, 123.5, 121.5, 62.3, 59.4, 58.1, 53.1, 52.1, 50.4, 48.7, 33.9, 29.6, 29.5, 22.2. HRMS (NSI) *m/z* = 504.31292 (M + H); Theo. for $C_{33}H_{37}N_5$ + H = 504.31217. LC-MS (ESI-API) 25-95% MeOH in H_2O, 8 min, *m/z* = 504.2 (M + H), 252.6

(M/2 + H), t = 0.663 min; 10-95% MeOH in H_2O, 10 min, *m/z* = 504.2 (M + H), 252.6 (M/2 + H), t = 2.495 min.

(S)-N-(4-(((pyridin-4-ylmethyl)amino)methyl)benzyl)-N-(((R)-1,2,3,4-tetrahydroisoquinolin-3-yl)methyl)-5,6,7,8-tetrahydroquinolin-8-amine (**44c**): Reductive amination was carried out according to General Reductive Amination Protocol D using amine **41** and 4-pyridinecarboxaldehyde. Purified via column chromatography (CombiFlash, 24 g column, 30 mL/min) eluting with the following gradient to yield a white foam (192 mg, 0.318 mmol, 65% yield): 0-3 min, 0% 100:10:1 DCM/MeOH/NH_4OH in DCM; 3-18 min, 0-100% 100:10:1 DCM/MeOH/NH_4OH in DCM; 18-30 min, 100% 100:10:1 DCM/MeOH/NH_4OH. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.56-8.57 (m, 2H), 8.44 (d, *J* = 10.0 Hz, 1H), 7.31-7.32 (m, 4H), 7.22-7.26 (m, 3H), 7.04-7.05 (m, 2H), 6.78-7.00 (m, 3H), 4.65-4.78 (m, 1H), 4.46-4.52 (m, 1H), 3.87-4.09 (m, 2H), 3.83 (s, 2H), 3.56-3.79 (4H), 2.72-3.03 (m, 3H), 2.53-2.70 (m, 3H), 2.12-2.25 (m, 1H), 1.94 (m, 1H), 1.65-1.81 (m, 3H), 1.53 (s, 4.5H), 1.46 (s, 4.5H). HRMS (NSI) *m/z* = 604.36456 (M + H); Theo. for $C_{38}H_{45}N_5O_2$ + H = 604.36460. LC-MS (ESI-API) 75-95% MeOH in H_2O, 5 min, *m/z* = 604.2 (M + H), 302.8 (M/2 + H), t = 0.553 min; 25-95% MeOH in H_2O, 8 min, *m/z* = 604.2 (M + H), 302.6 (M/2 + H), t = 4.253 min. Deprotection was carried out according to the General Deprotection Protocol. Purified via column chromatography (CombiFlash, 12 g column, 25 mL/min) eluting with 100:10:1 DCM/MeOH/NH_4OH to yield a white foam (132 mg, 0.262 mmol, 82% yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.54-8.55 (m, 2H), 8.52 (d, *J* = 4.5 Hz, 1H), 8.45 (d, *J* = 8.0 Hz, 2H), 7.34 (d, *J* = 7.5 Hz, 1H), 7.27-7.29 (m, 4H), 6.99-7.08 (m, 3H), 6.93-7.00 (m, 2H), 4.38 (d, *J* = 14.0 Hz, 1H), 4.11 (dd, *J* = 6.8 Hz, *J* = 9.8 Hz, 1H), 3.94 (app t, *J* = 15.8 Hz, 2H), 3.80 (s, 2H), 3.79 (s, 2H), 3.66 (d, *J* = 15.0 Hz, 1H), 2.90 (dd, *J* = 2.3 Hz, *J* = 13.3 Hz, 1H), 2.74-2.80 (m, 1H), 2.62-2.68 (m, 2H), 2.55 (dd, *J* = 3.0 Hz, *J* = 16.0 Hz, 1H), 2.43 (dd,

J = 11.0 Hz, *J* = 12.5 Hz, 1H), 2.37 (dd, *J* = 11.3 Hz, *J* = 15.8 Hz, 1H), 2.13-2.17 (m, 1H), 1.92-1.99 (m, 3H), 1.64-1.71 (m, 2H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 158.9, 149.9 (2C), 149.5, 146.9, 141.1, 138.3, 136.5, 135.7, 134.7, 134.2, 129.1, 128.6 (2C), 128.1 (2C), 126.5, 125.9, 125.5, 123.1 (2C), 121.5, 62.4, 59.4, 58.1, 53.1, 52.1, 51.8, 48.7, 33.9, 29.5, 29.5, 22.2. HRMS (NSI) *m/z* = 504.31252 (M + H); Theo. for $C_{33}H_{37}N_5$ + H = 504.31217. LC-MS (ESI-API) 25-95% MeOH in H_2O, 8 min, *m/z* = 504.2 (M + H), 252.6 (M/2 + H), t = 0.636 min; 10-95% MeOH in H_2O, 10 min, *m/z* = 504.2 (M + H), 252.7 (M/2 + H), t = 2.506 min.

(E)-N1-((tetrahydro-2H-pyran-4-yl)methyl)-N4-(((R)-1,2,3,4-tetrahydroisoquinolin-3-yl)methyl)-N4-((S)-5,6,7,8-tetrahydroquinolin-8-yl)but-2-ene-1,4-diamine (**45**): Reductive amination was carried out according to General Reductive Amination Protocol D using amine **23** and aldehyde **10c**. Purified via column chromatography (CombiFlash, 24 g column, 30 mL/min) eluting with the following gradient to yield a clear oil (120 mg, 0.214 mmol, 40% yield): 0-3 min, 0% 100:10:1 DCM/MeOH/NH_4OH in DCM; 3-18 min, 0-100% 100:10:1 DCM/MeOH/NH_4OH in DCM; 18-30 min, 100% 100:10:1 DCM/MeOH/NH_4OH. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.39 (d, *J* = 14.0 Hz, 1H), 7.24 (d, *J* = 7.0 Hz, 1H), 7.05-7.10 (m, 2H), 6.97 (m, 3H), 5.58-5.70 (m, 2H), 4.45-4.69 (m, 2H), 4.04-4.09 (m, 1H), 3.95-3.97 (m, 3H), 3.35-3.41 (m, 3H), 3.09-3.19 (m, 3H), 2.91-3.00 (m, 2H), 2.65-2.71 (m, 1H), 2.49-2.61 (m, 3H) 2.46 (d, *J* = 6.5 Hz, 2H), 1.91-1.98 (m, 2H), 1.53-1.77 (m, 6H), 1.49 (s, 9H), 1.28 (ddd, *J* = 4.5 Hz, *J* = 12.3 Hz, *J* = 24.0 Hz, 2H). HRMS (NSI) *m/z* = 561.37977 (M + H); Theo. for $C_{34}H_{48}N_4O_3$ + H = 561.37992. LC-MS (ESI-API) 50-95% MeOH in H_2O, 8 min, *m/z* = 561.2 (M + H), 281.2 (M/2 + H), t = 0.793 min; 25-95% MeOH in H_2O, 8 min, *m/z* = 561.2 (M + H), 281.2 (M/2 + H), t = 3.987 min. BOC group removal was carried out according to the General Deprotection Protocol. Purified via column chromatography (CombiFlash, 12 g column, 25 mL/min) eluting with the

following gradient to yield a clear oil (74.0 mg, 0.161 mmol, 75% yield): 0-3 min, 0% 100:10:1 DCM/MeOH/NH_4OH in DCM; 3-18 min, 0-100% 100:10:1 DCM/MeOH/NH_4OH in DCM; 18-30 min, 100% 100:10:1 DCM/MeOH/NH_4OH. ^1H NMR (500 MHz, $CDCl_3$) δ 8.46 (dd, *J* = 1.3 Hz, *J* = 4.8 Hz, 1H), 7.32 (m, 1H), 7.07-7.09 (m, 2H), 7.02-7.06 (m, 2H), 6.97-7.01 (m, 1H), 5.69-5.78 (m, 2H), 4.10 (dd, *J* = 6.3 Hz, *J* = 9.8 Hz, 1H), 4.01 (d, *J* = 10.5 Hz, 1H), 3.94 (dt, *J* = 2.0 Hz, *J* = 11.5 Hz, 2H), 3.82 (d, *J* = 15.0 Hz, 1H), 3.63 (dd, *J* = 5.3 Hz, *J* = 14.3 Hz, 1H), 3.32-3.38 (m, 3H), 3.22 (d, *J* = 4.5 Hz, 2H), 2.87 (dd, *J* = 3.5 Hz, *J* = 13.0 Hz, 1H), 2.71-2.80 (m, 2H), 2.65-2.68 (m, 1H), 2.60 (dd, *J* = 3.5 Hz, *J* = 16.0 Hz, 1H), 2.47 (d, *J* = 7.0 Hz, 2H), 2.37-2.44 (m, 3H), 2.05-2.09 (m, 1H), 1.94-2.01 (m, 1H), 1.87-1.93 (m, 1H), 1.63-1.74 (m, 2H), 1.58-1.61 (m, 2H), 1.22-1.30 (m, 3H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 158.7, 146.9, 136.6, 135.7, 134.8, 134.0, 131.9, 130.3, 129.2, 126.5, 126.0, 125.6, 121.5, 68.0 (2C), 61.3, 57.2, 56.6, 55.7, 52.1, 51.7, 48.7, 35.5, 33.9, 31.4 (2C), 29.5, 28.1, 22.0. HRMS (NSI) *m/z* = 461.32697 (M + H); Theo. for $C_{29}H_{40}N_4O$ + H = 461.32749. LC-MS (ESI-API) 25-95% MeOH in H_2O, 8 min, *m/z* = 461.2 (M + H), 231.2 (M/2 + H), t = 0.535 min; 10-95% MeOH in H_2O, 10 min, *m/z* = 461.2 (M + H), 231.2 (M/2 + H), t = 1.198 min.

(E)-N1-(4,4-difluorocyclohexyl)-N4-(((R)-1,2,3,4-tetrahydroisoquinolin-3-yl)methyl)-N4-((S)-5,6,7,8-tetrahydroquinolin-8-yl)but-2-ene-1,4-diamine (**46b**): Reductive amination was carried out according to General Reductive Amination Protocol E using amine **23** and ketone **12b**. Purified via column chromatography (CombiFlash, 24 g column, 30 mL/min) eluting with the following gradient to yield a clear oil (143 mg, 0.246 mmol, 51% yield): 0-3 min, 0% 100:10:1 DCM/MeOH/NH_4OH in DCM; 3-18 min, 0-100% 100:10:1 DCM/MeOH/NH_4OH in DCM; 18-30 min, 100% 100:10:1 DCM/MeOH/NH_4OH. ^1H NMR (500 MHz, $CDCl_3$) δ 8.39 (d, *J* = 13.5 Hz, 1H), 7.25 (d, *J* = 7.0 Hz, 1H), 7.06-7.10 (m, 2H), 6.98 (m, 3H), 5.58-5.70 (m, 2H), 4.46-4.70

(m, 2H), 4.07 (app t, *J* = 15.5 Hz, 1H), 3.96 (br s, 1H), 3.36-3.37 (m, 1H), 3.17 (d, *J* = 5.5 Hz, 2H), 3.12 (dd, *J* = 5.8 Hz, *J* = 14.3 Hz, 1H), 2.91-3.00 (m, 2H), 2.51-2.71 (m, 5H), 2.06-2.13 (m, 2H), 1.86-1.99 (m, 4H), 1.67-1.80 (m, 4H), 1.57-1.64 (m, 1H), 1.42-1.53 (m, 11H). HRMS (NSI) *m/z* = 581.36530 (M + H); Theo. for $C_{34}H_{46}F_2N_4O_2$ + H = 581.36616. LC-MS (ESI-API) 50-95% MeOH in H_2O, 8 min, *m/z* = 581.2 (M + H), 291.2 (M/2 + H), t = 1.106 min; 25-95% MeOH in H_2O, 8 min, *m/z* = 581.2 (M + H), 291.1 (M/2 + H), t = 4.471 min. BOC group removal was carried out according to the General Deprotection Protocol. Purified via column chromatography (CombiFlash, 12 g column, 25 mL/min) eluting with the following gradient to yield a clear oil (116 mg, 0.241 mmol, 98% yield): 0-3 min, 0% 100:10:1 DCM/MeOH/NH_4OH in DCM; 3-18 min, 0-100% 100:10:1 DCM/MeOH/NH_4OH in DCM; 18-30 min, 100% 100:10:1 DCM/MeOH/NH_4OH. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.46 (dd, *J* = 1.0 Hz, *J* = 4.5 Hz, 1H), 7.32 (dd, *J* = 0.5 Hz, *J* = 7.5 Hz, 1H), 7.06-7.10 (m, 2H), 2.99 (m, 3H), 5.68-5.78 (m, 2H), 4.10 (dd, *J* = 6.3 Hz, *J* = 9.8 Hz, 1H), 4.01 (d, *J* = 15.0 Hz, 1H), 3.82 (d, *J* = 15.0 Hz, 1H), 3.62 (dd, *J* = 6.0 Hz, *J* = 14.0 Hz, 1H), 3.33 (dd, *J* = 5.3 Hz, *J* = 14.3 Hz, 1H), 3.24 (d, *J* = 5.5 Hz, 2H), 2.87 (dd, *J* = 3.5 Hz, *J* = 13.0 Hz, 1H), 2.71-2.80 (m, 2H), 2.70 (m, 1H), 2.58-2.65 (m, 2H), 2.38-2.44 (m, 2H), 1.92-2.13 (m, 5H), 1.86-1.91 (m, 3H), 1.65-1.79 (m, 3H), 1.42-1.49 (m, 2H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 158.6, 146.9, 136.6, 135.6, 134.7, 134.0, 131.9, 130.3, 129.2, 126.5, 126.0, 125.6, 123.4 (t, *J* = 239.4 Hz), 121.5, 61.2, 57.2, 56.5, 53.5, 52.1, 48.9, 48.6, 33.9, 31.8 (t, *J* = 24.4 Hz, 2C), 29.5, 28.9 (t, *J* = 2.5 Hz), 28.9 (t, *J* = 3.8 Hz), 27.9, 22.0. ^{19}F NMR (376 MHz, $CDCl_3$, TFA standard) δ -96.8 (app d, *J* = 233.1 Hz), -101.1 (app d, *J* = 206.8 Hz). HRMS (NSI) *m/z* = 481.31303 (M + H); Theo. for $C_{29}H_{38}F_2N_4$ + H = 481.31373. LC-MS (ESI-API) 25-95% MeOH in H_2O, 8 min, *m/z* = 481.2 (M + H), 241.2 (M/2 + H), t = 0.591 min; 10-95% MeOH in H_2O, 10 min, *m/z* = 481.2 (M + H), 241.2 (M/2 + H), t = 2.382 min.

(E)-N1-(tetrahydro-2H-pyran-4-yl)-N4-(((R)-1,2,3,4-tetrahydroisoquinolin-3-yl)methyl)-N4-((S)-5,6,7,8-tetrahydroquinolin-8-yl)but-2-ene-1,4-diamine (**46c**): Reductive amination was carried out according to General Reductive Amination Protocol E using amine **23** and ketone **12c**. Purified via column chromatography (CombiFlash, 24 g column, 30 mL/min) eluting with the following gradient to yield a clear oil (160 mg, 0.160 mmol, 61% yield): 0-3 min, 0% 100:10:1 DCM/MeOH/NH_4OH in DCM; 3-18 min, 0-100% 100:10:1 DCM/MeOH/NH_4OH in DCM; 18-30 min, 100% 100:10:1 DCM/MeOH/NH_4OH. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.38 (d, *J* = 13.5 Hz, 1H), 7.24 (d, *J* = 5.5 Hz, 1H), 7.05-7.09 (m, 2H), 6.97 (m, 3H), 5.59-5.70 (m, 2H), 4.45-4.69 (m, 2H), 4.07 (app t, *J* = 14.5 Hz, 1H), 3.95-3.97 (m, 3H), 3.34-3.39 (m, 3H), 3.20 (d, *J* = 4.5 Hz, 2H), 3.11 (dd, *J* = 5.3 Hz, *J* = 14.3 Hz, 1H), 2.91-3.00 (m, 2H), 2.50-2.68 (m, 5H), 1.91-1.98 (m, 2H), 1.70-1.82 (m, 4H), 1.57-1.64 (m, 1H), 1.45-1.54 (m, 9H), 1.36-1.41 (m, 2H). HRMS (NSI) *m/z* = 547.36397 (M + H); Theo. for $C_{33}H_{46}N_4O_3$ + H = 547.36427. LC-MS (ESI-API) 50-95% MeOH in H_2O, 8 min, *m/z* = 547.2 (M + H), 274.2 (M/2 + H), t = 0.859 min; 25-95% MeOH in H_2O, 8 min, *m/z* = 547.2 (M + H), 274.2 (M/2 + H), t = 4.085 min. BOC group removal was carried out according to the General Deprotection Protocol. Purified via column chromatography (CombiFlash, 12 g column, 25 mL/min) eluting with the following gradient to yield a clear oil (126 mg, 0.282 mmol, 96% yield): 0-3 min, 0% 100:10:1 DCM/MeOH/NH_4OH in DCM; 3-18 min, 0-100% 100:10:1 DCM/MeOH/NH_4OH in DCM; 18-30 min, 100% 100:10:1 DCM/MeOH/NH_4OH. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.46 (dd, *J* = 1.3 Hz, *J* = 4.8 Hz, 1H), 7.32 (dd, *J* = 0.8 Hz, *J* = 7.8 Hz, 1H), 7.06-7.10 (m, 2H), 6.98-7.05 (m, 3H), 5.69-5.79 (m, 2H), 4.10 (dd, *J* = 6.3 Hz, *J* = 9.8 Hz, 1H), 4.01 (d, *J* = 15.0 Hz, 1H), 3.92-3.97 (m, 2H), 3.81 (d, *J* = 15.0 Hz, 1H), 3.63 (dd, *J* = 5.3 Hz, *J* = 14.3 Hz, 1H), 3.31-3.38 (m, 3H), 3.27 (d, *J* = 5.0 Hz, 2H), 2.88 (dd, *J* = 3.5 Hz, *J* = 13.0 Hz, 1H), 2.58-2.80 (m, 6H), 2.38-2.44 (m, 2H), 2.04-2.09 (m, 1H),

1.94-2.01 (m, 1H), 1.87-1.93 (m, 1H), 1.79-1.82 (m, 2H), 1.65-1.74 (m, 1H), 1.34-1.42 (m, 2H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 158.6, 146.8, 136.5, 135.6, 134.6, 133.9, 131.7, 130.3, 129.1, 126.4, 125.9, 125.5, 121.4, 66.9, 66.8, 61.0, 57.1, 56.4, 53.3, 52.0, 48.6, 48.0, 33.8, 33.7 (2C), 29.4, 27.9, 21.9. HRMS (NSI) *m/z* = 447.31120 (M + H); Theo. for $C_{28}H_{38}N_4O$ + H = 447.31184. LC-MS (ESI-API) 25-95% MeOH in H_2O, 8 min, *m/z* = 447.2 (M + H), 224.2 (M/2 + H), t = 0.523 min; 10-95% MeOH in H_2O, 10 min, *m/z* = 447.2 (M + H), 224.2 (M/2 + H), t = 1.126 min.

(E)-N1-(4,4-dimethylcyclohexyl)-N4-(((R)-1,2,3,4-tetrahydroisoquinolin-3-yl)methyl)-N4-((S)-5,6,7,8-tetrahydroquinolin-8-yl)but-2-ene-1,4-diamine (**46g**): Reductive amination was carried out according to General Reductive Amination Protocol E using amine **23** and ketone **12g**. Purified via column chromatography (CombiFlash, 24 g column, 30 mL/min) eluting with the following gradient to yield a clear oil (173 mg, 0.302 mmol, 63% yield): 0-3 min, 0% 100:10:1 DCM/MeOH/NH_4OH in DCM; 3-18 min, 0-100% 100:10:1 DCM/MeOH/NH_4OH in DCM; 18-30 min, 100% 100:10:1 DCM/MeOH/NH_4OH. ^{1}H NMR (500 MHz, $CDCl_3$) δ 4.39 (d, *J* = 14.5 Hz, 1H), 7.24 (d, *J* = 7.5 Hz, 1H), 7.05-7.10 (m, 2H), 6.97 (m, 3H), 5.58-5.67 (m, 2H), 4.44-4.69 (m, 2H), 4.07 (app t, *J* = 16.5 Hz, 1H), 3.97 (app t, *J* = 7.0 Hz, 1H), 3.34-3.41 (m, 1H), 3.18 (d, *J* = 5.0 Hz, 2H), 3.11 (dd, *J* = 5.5 Hz, *J* = 14.0 Hz, 1H), 2.91-3.00 (m, 2H), 2.65-2.72 (m, 1H), 2.48-2.62 (m, 3H), 2.35-2.39 (m, 1H), 1.92-1.98 (m, 2H), 1.58-1.77 (m, 5H), 1.50 (s, 9H), 1.36-1.39 (m, 2H), 1.15-1.29 (m, 4H), 0.89 (s, 3H), 0.89 (s, 3H). HRMS (NSI) *m/z* = 573.41600 (M + H); Theo. for $C_{36}H_{52}N_4O_2$ + H = 573.41630. LC-MS (ESI-API) 25-95% MeOH in H_2O, 8 min, *m/z* = 573.2 (M + H), 287.2 (M/2 + H), t = 2.102 min; 25-95% MeOH in H_2O, 8 min, *m/z* = 574.2 (M + H), 287.2 (M/2 + H), t = 5.200 min. BOC group removal was carried out according to the General Deprotection Protocol. Purified via column chromatography (CombiFlash, 12 g

column, 25 mL/min) eluting with the following gradient to yield a clear oil (27.0 mg, 0.057 mmol, 19% yield): 0-3 min, 0% 100:10:1 DCM/MeOH/NH_4OH in DCM; 3-18 min, 0-100% 100:10:1 DCM/MeOH/NH_4OH in DCM; 18-30 min, 100% 100:10:1 DCM/MeOH/NH_4OH. ^1H NMR (500 MHz, $CDCl_3$) δ 8.45 (dd, *J* = 1.0 Hz, *J* = 4.5 Hz, 1H), 7.31 (d, *J* = 7.0 Hz, 1H), 7.06-7.09 (m, 2H), 6.98-7.05 (m, 3H), 5.69-5.77 (m, 2H), 4.10 (dd, *J* = 6.3 Hz, *J* = 9.8 Hz, 1H), 4.00 (d, *J* = 15.0 Hz, 1H), 3.81 (d, *J* = 15.0 Hz, 1H), 3.59-3.63 (m, 1H), 3.28-3.32 (m, 1H), 3.21-3.25 (m, 2H), 2.88 (dd, *J* = 3.0 Hz, *J* = 13.0 Hz, 1H), 2.70-2.79 (m, 2H), 2.64-2.67 (m, 1H), 2.59 (dd, *J* = 3.5 Hz, *J* = 16.0 Hz, 1H), 2.36-2.44 (m, 5H), 2.04-2.09 (m, 1H), 1.94-2.01 (m, 1H), 1.87-1.92 (m, 1H), 1.66-1.74 (m, 3H), 1.33-1.38 (m, 2H), 1.21-1.29 (m, 3H), 1.12-1.19 (m, 1H), 0.88 (s, 3H), 0.87 (s, 3H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 158.8, 147.0, 136.7, 135.8, 134.8, 134.1, 131.6, 130.8, 129.2, 126.6, 126.0, 125.6, 121.6, 61.2, 57.2, 56.6, 53.6, 52.2, 48.8, 48.7, 38.0, 38.0, 33.9, 32.4, 30.3, 29.6, 29.4 (2C), 28.1, 24.8, 22.1. HRMS (NSI) *m/z* = 473.36329 (M + H); Theo. for $C_{31}H_{44}N_4$ + H = 473.36387. LC-MS (ESI-API) 25-95% MeOH in H_2O, 8 min, *m/z* = 473.2 (M + H), 237.2 (M/2 + H), t = 2.547 min; 10-95% MeOH in H_2O, 10 min, *m/z* = 473.2 (M + H), 237.2 (M/2 + H), t = 4.437 min.

(E)-N1-(pyridin-2-ylmethyl)-N4-(((R)-1,2,3,4-tetrahydroisoquinolin-3-yl)methyl)-N4-((S)-5,6,7,8-tetrahydroquinolin-8-yl)but-2-ene-1,4-diamine (**47a**): Reductive amination was carried out according to General Reductive Amination Protocol D using amine **23** and 2-pyridinecarboxaldehyde. Purified via column chromatography (CombiFlash, 24 g column, 30 mL/min) eluting with the following gradient to yield a yellow foam (161 mg, 0.291 mmol, 54% yield): 0-3 min, 0% 100:10:1 DCM/MeOH/NH_4OH in DCM; 3-18 min, 0-100% 100:10:1 DCM/MeOH/NH_4OH in DCM; 18-30 min, 100% 100:10:1 DCM/MeOH/NH_4OH. ^1H NMR (500 MHz, $CDCl_3$) δ 8.56 (dd, *J* = 0.8 Hz, *J* = 4.8 Hz, 1H), 8.38 (d, *J* = 12.5 Hz, 1H), 7.64 (dt, *J* = 1.8

Hz, *J* = 7.6 Hz, 1H), 7.24-7.29 (m, 2H), 7.16 (dd, *J* = 5.3 Hz, *J* = 6.8 Hz, 1H), 7.04-7.07 (m, 2H), 6.97 (m, 3H), 5.61-5.71 (m, 2H), 4.45-4.69 (m, 2H), 4.07 (app t, *J* = 16.5 Hz, 1H), 3.98 (m, 1H), 3.86 (s, 2H), 3.32-3.40 (m, 1H), 3.18-3.25 (m, 2H), 3.11 (dd, *J* = 5.5 Hz, *J* = 14.5 Hz, 1H), 2.91-3.02 (m, 2H), 2.51-2.72 (m, 4H), 1.92-2.00 (m, 3H), 1.74 (dd, *J* = 10.0 Hz, *J* = 21.0 Hz, 1H), 1.57-1.65 (m 1H), 1.49 (s, 9H). HRMS (NSI) *m/z* = 554.34870 (M + H); Theo. for $C_{34}H_{43}N_5O_2$ + H = 554.34895. LC-MS (ESI-API) 50-95% MeOH in H_2O, 8 min, *m/z* = 554.2 (M + H), 277.6 (M/2 + H), t = 1.029 min; 25-95% MeOH in H_2O, 8 min, *m/z* = 554.2 (M + H), 277.6 (M/2 + H), t = 4.339 min. BOC group removal was carried out according to the General Deprotection Protocol. Purified via column chromatography (CombiFlash, 12 g column, 25 mL/min) eluting with 100:10:1 DCM/MeOH/NH_4OH to yield a yellow oil (130 mg, 0.287 mmol, 99% yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.53-8.54 (m, 1H), 8.45-8.46 (m, 1H), 7.58-7.62 (m, 1H), 7.31 (d, *J* = 8.0 Hz, 1H), 7.25-7.27 (m, 1H), 7.13-7.15 (m, 1H), 7.05-7.09 (m, 2H), 7.01-7.05 (m, 2H), 6.98-6.99 (m, 1H), 5.73-5.81 (m, 2H), 4.10 (dd, *J* = 6.5 Hz, *J* = 9.5 Hz, 1H), 4.00 (d, *J* = 15.5 Hz, 1H), 3.88 (s, 2H), 3.83 (d, *J* = 15.0 Hz, 1H), 3.68 (dd, *J* = 2.8 Hz, *J* = 15.8 Hz, 1H), 3.33-3.36 (m, 1H), 3.28-3.29 (m, 2H), 2.86 (dd, *J* = 3.0 Hz, *J* = 13.0 Hz, 1H), 2.58-2.79 (m, 5H), 2.37-2.43 (m, 2H), 2.05-2.09 (m, 1H), 1.87-1.99 (m, 2H), 1.65-1.73 (m, 1H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 159.7, 158.6, 149.3, 146.8, 136.5, 136.4, 135.7, 134.7, 133.9, 132.0, 130.1, 129.1, 126.4, 125.9, 125.4, 122.4, 121.9, 121.4, 61.1, 57.2, 56.4, 54.6, 52.0, 51.0, 48.6, 33.8, 29.4, 28.1, 21.9. HRMS (NSI) *m/z* = 454.29750 (M + H); Theo. for $C_{29}H_{35}N_5$ + H = 454.29652. LC-MS (ESI-API) 25-95% MeOH in H_2O, 8 min, *m/z* = 454.2 (M + H), 227.6 (M/2 + H), t = 0.551 min; 10-95% MeOH in H_2O, 10 min, *m/z* = 454.2 (M + H), 227.8 (M/2 + H), t = 2.148 min.

(E)-N1-(pyridin-3-ylmethyl)-N4-(((R)-1,2,3,4-tetrahydroisoquinolin-3-yl)methyl)-N4-((S)-5,6,7,8-tetrahydroquinolin-8-yl)but-2-ene-1,4-diamine (**47b**): Reductive amination was carried

out according to General Reductive Amination Protocol D using amine **23** and 3-pyridinecarboxaldehyde. Purified via column chromatography (CombiFlash, 24 g column, 30 mL/min) eluting with the following gradient to yield a yellow foam (188 mg, 0.340 mmol, 63% yield): 0-3 min, 0% 100:10:1 DCM/MeOH/NH_4OH in DCM; 3-18 min, 0-100% 100:10:1 DCM/MeOH/NH_4OH in DCM; 18-30 min, 100% 100:10:1 DCM/MeOH/NH_4OH. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.54 (d, *J* = 1.5 Hz, 1H), 8.51 (dd, *J* = 1.8 Hz, *J* = 4.8 Hz, 1H), 8.39 (d, *J* = 15.5 Hz, 1H), 7.67 (m, 1H), 7.24-7.26 (m, 2H), 7.03-7.06 (m, 2H), 6.97 (m, 3H), 5.60-5.70 (m, 2H), 4.46-4.70 (m, 2H), 4.09 (app t, *J* = 16.0 Hz, 1H), 3.97 (dd, *J* = 6.0 Hz, *J* = 8.0 Hz, 1H), 3.75 (s, 2H), 3.31-3.38 (m, 1H), 3.18 (d, *J* = 5.5 Hz, 2H), 3.11 (dd, *J* = 5.8 Hz, *J* = 14.3 Hz, 1H), 2.92-3.01 (m, 2H), 2.52-2.72 (m, 4H), 1.92-2.00 (m, 2H), 1.74 (dd, *J* = 10.0 Hz, *J* = 21.0 Hz, 1H), 1.58-1.66 (m, 2H), 1.48 (s, 9H). HRMS (NSI) *m/z* = 554.34895 (M + H); Theo. for $C_{34}H_{43}N_5O_2$ + H = 554.34895. LC-MS (ESI-API) 50-95% MeOH in H_2O, 8 min, *m/z* = 554.2 (M + H), 277.6 (M/2 + H), t = 0.932 min; 25-95% MeOH in H_2O, 8 min, *m/z* = 554.2 (M + H), 277.6 (M/2 + H), t = 4.144 min. BOC group removal was carried out according to the General Deprotection Protocol. Purified via column chromatography (CombiFlash, 12 g column, 25 mL/min) eluting with 100:10:1 DCM/MeOH/NH_4OH to yield a yellow oil (138 mg, 0.304 mmol, 90% yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.53 (d, *J* = 2.0 Hz, 1H), 8.49 (dd, *J* = 1.8 Hz, *J* = 4.8 Hz, 1H), 8.46 (d, *J* = 5.0 Hz, 1H), 7.64-7.66 (m, 1H), 7.31 (d, *J* = 8.0 Hz, 1H), 7.22 (dd, *J* = 4.8 Hz, *J* = 7.8 Hz, 1H), 7.06-7.09 (m, 2H), 7.01-7.05 (m, 2H), 6.98-6.99 (m, 1H), 5.70-5.80 (m, 2H), 4.10 (dd, *J* = 6.3 Hz, *J* = 9.8 Hz, 1H), 4.00 (d, *J* = 15.0 Hz, 1H), 3.82 (d, *J* = 15.0 Hz, 1H), 3.78 (s, 2H), 3.63-3.68 (m, 1H), 3.34 (dd, *J* = 4.8 Hz, *J* = 13.8 Hz, 1H), 3.25 (d, *J* = 5.0 Hz, 2H), 2.87 (dd, *J* = 3.5 Hz, *J* = 13.5 Hz, 1H), 2.58-2.79 (m, 5H), 2.37-2.43 (m, 2H), 2.05-2.09 (m, 1H), 1.87-2.00 (m, 2H), 1.65-1.73 (m, 1H) 1.34 (br s, 1H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 158.6, 149.7, 148.5,

146.8, 136.5, 135.9, 135.7, 135.6, 134.7, 134.0, 132.3, 129.8, 129.1, 126.4, 125.9, 125.5, 123.4, 121.5, 61.1, 57.2, 56.4, 52.0, 50.8, 50.6, 48.6, 33.8, 29.4, 28.1, 21.9. HRMS (NSI) *m/z* = 454.29690 (M + H); Theo. for $C_{29}H_{35}N_5$ + H = 454.29652. LC-MS (ESI-API) 25-95% MeOH in H_2O, 8 min, *m/z* = 454.2 (M + H), 227.6 (M/2 + H), t = 0.534 min; 10-95% MeOH in H_2O, 10 min, *m/z* = 454.2 (M + H), 227.6 (M/2 + H), t = 1.015 min.

(E)-N1-(pyridin-4-ylmethyl)-N4-(((R)-1,2,3,4-tetrahydroisoquinolin-3-yl)methyl)-N4-((S)-5,6,7,8-tetrahydroquinolin-8-yl)but-2-ene-1,4-diamine (**47c**): Reductive amination was carried out according to General Reductive Amination Protocol D using amine **23** and 4-pyridinecarboxaldehyde. Purified via column chromatography (CombiFlash, 24 g column, 30 mL/min) eluting with the following gradient to yield a yellow foam (175 mg, 0.316 mmol, 59% yield): 0-3 min, 0% 100:10:1 DCM/MeOH/NH_4OH in DCM; 3-18 min, 0-100% 100:10:1 DCM/MeOH/NH_4OH in DCM; 18-30 min, 100% 100:10:1 DCM/MeOH/NH_4OH. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.54 (dd, *J* = 1.5 Hz, *J* = 4.5 Hz, 2H), 8.38 (d, *J* = 14.5 Hz, 1H), 7.25 (m, 3H), 7.04-7.06 (m, 2H), 6.97 (m, 3H), 5.61-5.71 (m, 2H), 4.47-4.70 (m, 2H), 4.09 (app t, *J* = 15.8 Hz, 1H), 3.96-3.97 (m, 1H), 3.76 (s, 2H), 3.31-3.37 (m, 1H), 3.10-3.17 (m, 3H), 2.91-3.01 (m, 2H), 2.53-2.68 (m, 4H), 1.91-2.00 (m, 2H), 1.71-1.77 (m, 2H), 1.53-1.65 (m, 1H), 1.48 (s, 9H). HRMS (NSI) *m/z* = 554.34869 (M + H); Theo. for $C_{34}H_{43}N_5O_2$ + H = 554.34895. LC-MS (ESI-API) 50-95% MeOH in H_2O, 8 min, *m/z* = 554.2 (M + H), 277.6 (M/2 + H), t = 0.861 min; 25-95% MeOH in H_2O, 8 min, *m/z* = 554.2 (M + H), 277.6 (M/2 + H), t = 3.979 min. BOC group removal was carried out according to the General Deprotection Protocol. Purified via column chromatography (CombiFlash, 12 g column, 25 mL/min) eluting with 100:10:1 DCM/MeOH/NH_4OH to yield a yellow oil (137 mg, 0.302 mmol, 96% yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.50-8.51 (m, 2H), 8.45 (dd, *J* = 1.5 Hz, *J* = 4.5 Hz, 1H), 7.31 (d, *J* = 7.5 Hz,

1H), 7.22-7.23 (m, 2H), 7.07-7.10 (m, 2H), 7.01-7.06 (m, 2H), 6.98-6.99 (m, 1H), 5.70-5.80 (m, 2H), 4.10 (dd, *J* = 6.5 Hz, *J* = 9.5 Hz, 1H), 4.00 (d, *J* = 15.5 Hz, 1H), 3.82 (d, *J* = 15.0 Hz, 1H), 3.78 (s, 2H), 3.64 (dd, *J* = 5.8 Hz, *J* = 14.3 Hz, 1H), 3.34 (dd, *J* = 5.0 Hz, *J* = 14.0 Hz, 1H), 3.25 (d, *J* = 5.5 Hz, 2H), 2.87 (dd, *J* = 3.5 Hz, *J* = 13.0 Hz, 1H), 2.58-2.80 (m, 5H), 2.37-2.43 (m, 2H), 2.04-2.09 (m, 1H), 1.87-2.00 (m, 2H), 1.65-1.73 (m, 1H), 1.40 (br s, 1H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 158.6, 149.8 (2C), 149.4, 146.9, 136.6, 135.7, 134.7, 134.0, 132.4, 129.7, 129.1, 126.4, 125.9, 125.5, 123.0 (2C), 121.5, 61.2, 57.2, 56.5, 52.1, 51.9, 50.9, 48.7, 33.9, 29.5, 28.1, 21.9. HRMS (NSI) *m/z* = 454.29778 (M + H); Theo. for $C_{29}H_{35}N_5$ + H = 454.29652. LC-MS (ESI-API) 25-95% MeOH in H_2O, 8 min, *m/z* = 454.2 (M + H), 227.6 (M/2 + H), t = 0.528 min; 10-95% MeOH in H_2O, 10 min, *m/z* = 454.2 (M + H), 227.8 (M/2 + H), t = 0.834 min.

2,2-dimethyl-N1-((tetrahydro-2H-pyran-4-yl)methyl)-N4-(((R)-1,2,3,4-tetrahydroisoquinolin-3-yl)methyl)-N4-((S)-5,6,7,8-tetrahydroquinolin-8-yl)butane-1,4-diamine (**48**): Reductive amination was carried out according to General Reductive Amination Protocol D using amine **33** and aldehyde **10c**. Purified via column chromatography (CombiFlash, 24 g column, 30 mL/min) eluting with the following gradient to yield a clear oil (216 mg, 0.366 mmol, 73% yield): 0-3 min, 0% 100:10:1 DCM/MeOH/NH_4OH in DCM; 3-18 min, 0-100% 100:10:1 DCM/MeOH/NH_4OH in DCM; 18-30 min, 100% 100:10:1 DCM/MeOH/NH_4OH. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.35 (br s, 1H), 7.25 (d, *J* = 7.5 Hz, 1H), 7.08-7.11 (m, 2H), 7.04-7.05 (m, 1H), 6.95-6.97 (m, 2H), 4.38-4.68 (m, 2H), 3.88-4.08 (m, 4H), 3.37 (dt, *J* = 1.8 Hz, *J* = 11.8 Hz, 2H), 2.91-3.07 (m, 2H), 2.70-2.72 (m, 1H), 2.44-2.64 (m, 7H), 2.22-2.26 (m, 2H), 1.94-1.96 (m, 2H), 1.74-1.79 (m, 1H), 1.60-1.66 (m, 4H), 1.50 (s, 9H), 1.21-1.43 (m, 4H) 0.81 (s, 6H). HRMS (NSI) *m/z* = 591.42700 (M + H); Theo. for $C_{36}H_{54}N_4O_3$ + H = 591.42687. LC-MS (ESI-API) 50-95% MeOH in H_2O, 8 min, *m/z* = 591.3 (M + H), 296.2 (M/2 + H), t = 0.895 min; 25-95% MeOH in

H_2O, 8 min, *m/z* = 591.2 (M + H), 296.2 (M/2 + H), t = 4.240 min. BOC group removal was carried out according to the General Deprotection Protocol. Purified via column chromatography (CombiFlash, 12 g column, 25 mL/min) eluting with the following gradient to yield a clear oil (133 mg, 0.271 mmol, 74% yield): 0-3 min, 0% 100:10:1 DCM/MeOH/NH_4OH in DCM; 3-18 min, 0-100% 100:10:1 DCM/MeOH/NH_4OH in DCM; 18-30 min, 100% 100:10:1 DCM/MeOH/NH_4OH. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.45 (dd, *J* = 1.5 Hz, *J* = 4.5 Hz, 1H), 7.31-7.33 (m, 1H), 7.06-7.10 (m, 2H), 7.02-7.05 (m, 2H), 6.99-7.02 (m, 1H), 4.02-4.09 (m, 2H), 3.88-3.93 (m, 3H), 3.33 (ddt, *J* = 2.2 Hz, *J* = 3.7 Hz, *J* = 11.8 Hz, 2H), 3.10 (dt, *J* = 4.5 Hz, *J* = 12.3 Hz, 1H), 2.94 (dd, *J* = 3.3 Hz, *J* = 13.3 Hz, 1H), 2.58-2.81 (m, 5H), 2.45 (d, *J* = 6.5 Hz, 2H), 2.34-2.42 (m, 2H), 2.31 (d, *J* = 5.0 Hz, 2H), 2.04-2.08 (m, 1H), 1.96-2.02 (m, 1H), 1.87-1.94 (m, 1H), 1.69-1.76 (m, 1H), 1.59-1.68 (m, 4H), 1.42 (dt, *J* = 5.0 Hz, *J* = 12.5 Hz, 1H), 1.24 (ddd, *J* = 4.5 Hz, *J* = 11.8 Hz, *J* = 24.8 Hz, 2H), 0.87 (s, 3H), 0.87 (s, 3H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 158.8, 146.8, 136.5, 135.7, 134.8, 133.9, 129.1, 126.5, 125.9, 125.5, 121.4, 68.0 (2C), 61.5, 60.6, 57.8, 57.2, 52.3, 50.0, 54.8, 39.8, 35.2, 33.9, 33.6, 31.4, 31.4, 29.5, 29.0, 26.1, 26.0, 21.9. HRMS (NSI) *m/z* = 491.37384 (M + H); Theo. for $C_{31}H_{46}N_4O$ + H = 491.37444. LC-MS (ESI-API) 25-95% MeOH in H_2O, 8 min, *m/z* = 491.2 (M + H), 246.2 (M/2 + H), t = 0.597 min; 10-95% MeOH in H_2O, 10 min, *m/z* = 491.2 (M + H), 246.2 (M/2 + H), t = 2.390 min.

N1-(4,4-difluorocyclohexyl)-2,2-dimethyl-N4-(((R)-1,2,3,4-tetrahydroisoquinolin-3-yl)methyl)-N4-((S)-5,6,7,8-tetrahydroquinolin-8-yl)butane-1,4-diamine (**49b**): Reductive amination was carried out according to General Reductive Amination Protocol E using amine **33** and ketone **12b**. Purified via column chromatography (CombiFlash, 24 g column, 35 mL/min) eluting with the following gradient to yield a clear oil (146 mg, 0.239 mmol, 60% yield): 0-3 min, 0% 100:10:1 DCM/MeOH/NH_4OH in DCM; 3-18 min, 0-100% DCM/MeOH/NH_4OH in DCM; 18-

30 min, 100% DCM/MeOH/NH_4OH in DCM. ^1H NMR (500 MHz, $CDCl_3$) δ 8.36 (br s, 1H), 7.25-7.27 (m, 1H), 7.06-7.12 (m, 3H), 6.97-6.98 (m, 2H), 4.39-4.69 (m, 2H), 3.92-4.10 (m, 2H), 2.91-3.08 (m, 2H), 2.49-2.70 (m, 7H), 2.26 (m, 2H), 2.07-2.10 (m, 2H), 1.96 (br s, 2H), 1.58-1.79 (m, 6H), 1.42-1.54 (m, 12H), 1.25-1.30 (m, 2H), 0.81 (s, 6H). HRMS (NSI) *m/z* = 611.41292 (M + H); Theo. for $C_{36}H_{52}F_2N_4O_2$ + H = 611.41311. LC-MS (ESI-API) 50-95% MeOH in H_2O, 8 min, *m/z* = 612.2 (M + H), 306.2 (M/2 + H), t = 1.405 min; 25-95% MeOH in H_2O, 8 min, *m/z* = 611.2 (M + H), 306.2 (M/2 + H), t = 4.764 min. BOC group removal was carried out according to the General Deprotection Protocol. Purified via column chromatography (CombiFlash, 12 g column, 25 mL/min) eluting with the following gradient to yield a slightly yellow foam (117 mg, 0.229 mmol, 96% yield): 0-3 min, 0% 100:10:1 DCM/MeOH/NH_4OH in DCM; 3-18 min, 0-100% 100:10:1 DCM/MeOH/NH_4OH in DCM; 18-30 min, 100% 100:10:1 DCM/MeOH/NH_4OH. ^1H NMR (500 MHz, $CDCl_3$) δ 8.45 (dd, *J* = 1.0 Hz, *J* = 4.5 Hz, 1H), 7.33 (d, *J* = 7.5 Hz, 1H), 7.07-7.11 (m, 2H), 6.99-7.06 (m, 3H), 4.03-4.09 (m, 2H), 3.90 (d, *J* = 15.0 Hz, 1H), 2.94-3.02 (m, 2H), 2.72-2.81 (m, 2H), 2.69 (app t, *J* = 3.5 Hz, 1H), 2.60-2.66 (m, 3H), 2.50-2.54 (m, 1H), 2.39-2.45 (m, 1H), 2.27-2.37 (m, 3H), 1.96-2.10 (m, 5H), 1.87-1.94 (m, 1H), 1.78-1.83 (m, 2H), 1.68-1.77 (m, 3H), 1.61 (dt, *J* = 4.5 Hz, *J* = 12.5 Hz, 1H), 1.38-1.49 (m, 3H), 0.87 (s, 3H), 0.86 (s, 3H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 158.9, 146.9, 136.6, 135.7, 134.8, 134.1, 129.2, 126.6, 126.0, 125.6, 123.7 (t, *J* = 246.3 Hz), 121.5, 61.6, 57.9, 57.9, 54.5, 52.4, 49.9, 48.8, 39.7, 33.9, 33.5, 31.6 (t, *J* = 23.8 Hz), 31.6 (t, *J* = 23.1 Hz), 29.5, 28.9-29.0 (m, 3C), 26.1, 26.0, 22.0. ^{19}F NMR (376 MHz, $CDCl_3$, TFA standard) δ -96.0 (app d, *J* = 236.9 Hz), -97.9 (app d, *J* = 218.1 Hz). HRMS (NSI) *m/z* = 511.36172 (M + H); Theo. for $C_{31}H_{44}F_2N_4$ + H = 511.36068. LC-MS (ESI-API) 25-95% MeOH in H_2O, 8 min, *m/z* = 511.2 (M + H), 256.2 (M/2

+ H), t = 0.709 min; 10-95% MeOH in H_2O, 10 min, *m/z* = 511.2 (M + H), 256.2 (M/2 + H), t = 2.970 min.

2,2-dimethyl-N1-(tetrahydro-2H-pyran-4-yl)-N4-(((R)-1,2,3,4-tetrahydroisoquinolin-3-yl)methyl)-N4-((S)-5,6,7,8-tetrahydroquinolin-8-yl)butane-1,4-diamine (**49c**): Reductive amination was carried out according to General Reductive Amination Protocol E using amine **23** and ketone **12c**. Purified via column chromatography (CombiFlash, 24 g column, 35 mL/min) eluting with the following gradient to yield a clear oil (125 mg, 0.217 mmol, 54% yield): 0-3 min, 0% 100:10:1 DCM/MeOH/NH_4OH in DCM; 3-18 min, 0-100% DCM/MeOH/NH_4OH in DCM; 18-30 min, 100% DCM/MeOH/NH_4OH in DCM. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.36 (br s, 1H), 7.25-7.27 (m, 1H), 7.06-7.12 (m, 3H), 6.96-7.00 (m, 2H), 4.39-4.68 (m, 2H), 3.94-4.09 (4H), 3.38 (app t, *J* = 11.5 Hz, 2H), 2.91-3.08 (m, 2H), 2.70-2.72 (m, 1H), 2.48-2.64 (m, 6H), 2.29 (m, 2H), 1.96 (m, 2H), 1.77 (m, 3H), 1.63-1.67 (m, 1H), 1.50 (s, 9H), 1.26-1.46 (m, 5H), 0.82 (s, 6H). HRMS (NSI) *m/z* = 577.41135 (M + H); Theo. for $C_{35}H_{52}N_4O_3$ + H = 577.41122. LC-MS (ESI-API) 50-95% MeOH in H_2O, 8 min, *m/z* = 577.3 (M + H), 289.2 (M/2 + H), t = 1.049 min; 25-95% MeOH in H_2O, 8 min, *m/z* = 577.3 (M + H), 289.2 (M/2 + H), t = 4.382 min. Deprotection was carried out according to the General Deprotection Protocol. Purified via column chromatography (CombiFlash, 12 g column, 25 mL/min) eluting with the following gradient to yield a slightly yellow foam (100 mg, 0.210 mmol, 97% yield): 0-3 min, 0% 100:10:1 DCM/MeOH/NH_4OH in DCM; 3-18 min, 0-100% 100:10:1 DCM/MeOH/NH_4OH in DCM; 18-30 min, 100% 100:10:1 DCM/MeOH/NH_4OH. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.45 (dd, *J* = 1.0 Hz, *J* = 4.5 Hz, 1H), 7.33 (dd, *J* = 0.5 Hz, *J* = 7.5 Hz, 1H), 7.07-7.09 (2H), 6.99-7.06 (m, 3H), 4.08 (dd, *J* = 6.3 Hz, *J* = 9.8 Hz, 1H), 4.04 (d, *J* = 15.0 Hz, 1H), 3.93 (dt, *J* = 3.5 Hz, *J* = 11.5 Hz, 2H), 3.89 (d, *J* = 15.0 Hz, 1H), 3.36 (tt, *J* = 2.0 Hz, *J* = 11.5 Hz, 2H), 2.95-3.06 (m, 2H),

2.71-2.82 (m, 2H), 2.69 (app t, *J* = 3.8 Hz, 1H), 2.49-2.66 (m, 4H), 2.39-2.47 (m, 1H), 2.32-2.38 (m, 3H), 2.04-2.09 (m, 1H), 1.96-2.02 (m, 1H), 1.87-1.95 (m, 1H), 1.67-1.79 (m, 3H), 1.62 (dt, *J* = 4.5 Hz, *J* = 12.5 Hz, 1H), 1.42 (dt, *J* = 5.0 Hz, *J* = 12.0 Hz, 1H), 1.30-1.38 (m, 2H), 0.87 (s, 3H), 0.86 (s, 3H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 158.8, 146.9, 136.6, 135.7, 134.8, 134.0, 129.2, 126.6, 126.0, 125.6, 121.5, 67.0 (2C), 61.6, 57.9, 57.0, 54.8, 52.4, 49.9, 48.8, 39.6, 34.0, 34.0, 33.9, 33.4, 29.6, 28.8, 26.1, 26.0, 22.0. HRMS (NSI) *m/z* = 477.35898 (M + H); Theo. for $C_{30}H_{44}N_4O$ + H = 477.35879. LC-MS (ESI-API) 25-95% MeOH in H_2O, 8 min, *m/z* = 477.2 (M + H), 239.2 (M/2 + H), t = 0.558 min; 10-95% MeOH in H_2O, 10 min, *m/z* = 477.2 (M + H), 239.2 (M/2 + H), t = 2.310 min.

N1-(4,4-dimethylcyclohexyl)-2,2-dimethyl-N4-(((R)-1,2,3,4-tetrahydroisoquinolin-3-yl)methyl)-N4-((S)-5,6,7,8-tetrahydroquinolin-8-yl)butane-1,4-diamine (**49g**): Reductive amination was carried out according to General Reductive Amination Protocol E using amine **23** and ketone **12g**. Purified via column chromatography (CombiFlash, 24 g column, 35 mL/min) eluting with the following gradient to yield a clear oil (133 mg, 0.221 mmol, 55% yield): 0-3 min, 0% 100:10:1 DCM/MeOH/NH_4OH in DCM; 3-18 min, 0-100% DCM/MeOH/NH_4OH in DCM; 18-30 min, 100% DCM/MeOH/NH_4OH in DCM. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.35 (br s, 1H), 7.25-7.27 (m, 1H), 7.06-7.11 (m, 3H), 6.95-6.98 (m, 2H), 4.38-4.68 (m, 2H), 3.90-4.09 (m, 2H), 2.92-3.09 (m, 2H), 2.71-2.73 (m, 1H), 2.57-2.64 (m, 3H), 2.46-2.50 (m, 2H), 2.22-2.28 (m, 3H), 1.95-1.96 (m, 2H), 1.75-1.79 (m, 1H), 1.63-1.65 (m, 3H), 1.50 (s, 9H), 1.36-1.46 (m, 3H), 1.15-1.33 (m, 6H), 0.90 (s, 3H), 0.90 (s, 3H), 0.78 (s, 6H). HRMS (NSI) *m/z* = 603.46280 (M + H); Theo. for $C_{38}H_{58}N_4O_2$ + H = 603.46325. LC-MS (ESI-API) 50-95% MeOH in H_2O, 8 min, *m/z* = 604.4 (M + H), 302.2 (M/2 + H), t = 2.457 min; 25-95% MeOH in H_2O, 8 min, *m/z* = 604.4 (M + H), 302.2 (M/2 + H), t = 5.593 min. BOC group removal was carried out according

to the General Deprotection Protocol. Purified via column chromatography (CombiFlash, 12 g column, 25 mL/min) eluting with the following gradient to yield a slightly yellow foam (109 mg, 0.217 mmol, 98% yield, 90-95% purity): 0-3 min, 0% 100:10:1 DCM/MeOH/NH_4OH in DCM; 3-18 min, 0-100% 100:10:1 DCM/MeOH/NH_4OH in DCM; 18-30 min, 100% 100:10:1 DCM/MeOH/NH_4OH. ^1H NMR (500 MHz, $CDCl_3$) δ 8.45 (dd, *J* = 1.5 Hz, *J* = 4.5 Hz, 1H), 7.30-7.33 (m, 1H), 7.07-7.10 (m, 2H), 7.00-7.07 (m, 3H), 4.09 (dd, *J* = 6.0 Hz, *J* = 9.5 Hz, 1H), 4.04 (d, *J* = 15.0 Hz, 1H), 3.90 (d, *J* = 15.0 Hz, 1H), 2.94-3.02 (m, 3H), 2.73-2.81 (m, 2H), 2.69 (app t, *J* = 3.5 Hz, 1H), 2.59-2.66 (m, 3H), 2.32-2.47 (m, 4H), 2.21-2.31 (m, 1H), 2.04-2.09 (m, 1H), 1.96-2.02 (m, 1H), 1.87-1.95 (m, 1H), 1.68-1.76 (m, 1H), 1.58-1.66 (m, 3H), 1.39-1.45 (m, 1H), 1.35 (d, *J* = 12.5 Hz, 2H), 1.12-1.27 (m, 4H), 0.88 (s, 3H), 0.87 (s, 3H), 0.87 (s, 3H), 0.86 (s, 3H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 158.9, 146.8, 136.5, 135.8, 134.9, 134.0, 129.2, 126.5, 125.9, 125.5, 121.4, 61.5, 58.1, 57.9, 57.8, 52.4, 49.8, 48.8, 39.7, 38.0, 33.9, 33.4 (2C), 32.3, 30.2, 29.5 (2C), 28.9, 26.1, 26.1, 25.0, 22.0. HRMS (NSI) *m/z* = 503.41093 (M + H); Theo. for $C_{33}H_{50}N_4$ + H = 503.41082. LC-MS (ESI-API) 25-95% MeOH in H_2O, 8 min, *m/z* = 503.3 (M + H), 252.2 (M/2 + H), t = 2.503 min; 10-95% MeOH in H_2O, 10 min, *m/z* = 503.3 (M + H), 252.2 (M/2 + H), t = 4.340 min.

Biological Methods

CXCR4 and mAChR Calcium Flux Assays: Exemplary compounds were tested for their ability to induce or inhibit Ca^{+2} flux in CCRF-CEM cells. The experimental procedures, which follow, have been carried out with all compounds. Human T lymphoblast cells (CCRF-CEM) expressing endogenous CXCR4 and mAChR were grown in suspension culture and plated in clear bottom 384-well microplates (Greiner bio-one Cat# 789146) in assay buffer [Hank's Buffered Saline Solution (Gibco Cat# 14025-092) supplemented with 20 mM HEPES (Gibco

Cat# 15630-080) and 0.1% fatty-acid free BSA (Sigma Cat# A9205)] at 40,000 cells per well. The cells were loaded with equal volume of Ca^{+2} indicator dye (AAT Bioquest Inc, Cat# 34601) for 30 min at 37˚C. The cells were then equilibrated to room temperature for 15 min before assay. Test compounds solubilized and serially diluted in DMSO were transferred to 384-well plates (Matrix Cat# 4307). The serially diluted compounds were diluted to working concentrations with the same assay buffer to 0.5% DMSO. They were added to the cells by FDSS6000 (Hamamatsu) at final concentrations ranging from 25,000 nM to 0.423 nM. Activity of the compounds to induce Ca^{+2} flux was monitored by FDSS in the "agonist mode" for 90 s. For "antagonist mode" assessment, the cells were subsequently incubated for 25 min at rt. SDF-1α (R&D System Cat# 350-NS/CF) or acetylcholine was then added at a final concentration of 5 nM and 2,000 nM, respectively, to stimulate the cells. Inhibition of SDF-1α and acetylcholine-induced Ca^{+2} flux was monitored by FDSS6000 for 90 s. Activation data for test compounds over a range of concentrations were plotted as percentage activation of the standard agonist (100% = maximum response triggered by a saturating concentration of SDF-1α, i.e., 160 nM). After correcting for background, EC_{50} values were determined. The EC_{50} is defined as the concentration of test compound that produces 50% of the maximal response, and EC_{50} values were quantified using the 4-parameter logistic equation to fit the data. Inhibition data for test compounds over a range of concentrations were plotted as percentage inhibition of the standard agonist activity. The IC_{50} is defined as the concentration of test compound that inhibits 50% of the maximal response, and IC_{50} values were quantified using the 4-parameter logistic equation to fit the data. None of the compounds tested demonstrated agonist activity in the Ca^{+2} flux assay (EC_{50} > 30 μM). In contrast, compounds demonstrated a range of potencies for inhibition of SDF-1α-induced Ca^{+2} flux.

PAMPA Assays: Test compounds and controls were utilized as 10 mM stocks in 100% DMSO. Compounds were diluted 1:100 in pH 7.4 or pH 5.5 donor well buffer (pION CAT # 110151) providing a 100 µM assay solution in 1% DMSO. Compound diluted in donor well buffer was transferred to a Whatman Unifilter plate and filtered prior to dispensing 200 µl into the donor well of the assay plate (pION CAT #110163). The PAMPA membrane was formed by pipetting 4 µl of the lipid solution (pION CAT #110169) onto the filter plate (VWR CAT #13503). The membrane was then covered with 200 µl of acceptor well buffer at pH 7.4 (pION CAT #110139). The PAMPA assay plate (donor side and acceptor side) was combined and allowed to incubate at rt for 4 h. The plate was then disassembled and spectrophotometer plates (VWR CAT #655801) were filled (150 µl/well). The donor, acceptor, reference, and blank plates were read by the SpectraMax UV plate reader. Data was captured by the pION software which analyzed the spectra and generated Pc values.

CYP450 Assays: The CYP450 2D6 inhibition assay utilized microsomes from insect cells expressing human recombinant CYP450 2D6 enzyme, as well as the fluorogenic probe (AMMC, 3-[2-(*N*,*N*-diethyl-*N*-methylamino)ethyl]-7-methoxy-4-methylcoumarin) that produces fluorescent metabolite; both reagents were obtained from Thermo Fisher Scientific/Discovery Labware (Woburn, MA). Assays were performed in 1536-well microplates in a total volume of 5 µl. Automated liquid handling equipment (Thermo Multidrop Combi, LabCyte ECHO 550) was used in all steps of compound preparation and for assay reagent additions. Each compound was tested in duplicate at 7 concentrations ranging from 1 nM to 20 µM; the final concentration of DMSO in reactions was 0.2%. Positive controls were included in each experiment/run. Test compounds (10 nL/well) were first pre-incubated at 37˚C for 30 min with 2.5 µL of pre-warmed 2-fold-concentrated mixture of AMMC fluorogenic substrate (3 µM) and 12.5 nM rCYP450 2D6

enzyme in 100 mM potassium phosphate assay buffer pH 7.4. At the end of pre-incubation, the reactions were initiated by the addition of 2.5 μL of pre-warmed 2-fold-concentrated NADPH-regenerating system (16.2 nM NADP) in the same assay buffer. Assay plates were then incubated at 37˚C for 45 min. Following incubation, reactions were terminated by the addition of 3 μL of quench buffer (80% acetonitrile, 20% 0.5 M TRIS-base). Fluorescence intensity was measured using the Envision fluorescence plate reader (Perkin Elmer) at excitation and emission wavelengths of 405 and 460 nm, respectively, using a 430-nm cut-off filter. The end-point fluorescence readout was normalized to the fluorescence intensity of the reaction performed in the absence of the test substance (totals, 0% inhibition) and the mixture of reaction components in the presence of "Inhibitor Cocktail" (background, 100% inhibition). The IC_{50} value for each compound was derived from the fitted 20-point curve using a four-parameter logistic regression model.

Metabolic Stability Assays: The metabolic stability of each compound was assessed according to previously reported methods.[59]

ASSOCIATED CONTENT

Supporting Information

The supporting information contains synthetic methods used for the preparation of common intermediate **6**, including relevant compound characterization and X-ray crystallographic data. Molecular formula strings are included as well.

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Author Contributions

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ABBREVIATIONS

CXCR4, CXC chemokine receptor 4; CXCL12, CXC chemokine ligand 12; SDF-1, stromal cell-derived factor 1; AC, adenylyl cyclase; PLC, phospholipase C; HSC, hematopoietic stem

cell; FoxP3, forkhead box 3; CD8, cluster of differentiation 8; HCC, hepatocellular carcinoma; PD-1, programmed cell death protein 1; PD-L1, programmed death ligand 1; STAB, sodium triacetoxyborohydride; DIPEA, diisopropylethylamine; DIAD, diisopropyl azodicarboxylate; mAChR, muscarinic acetylcholine receptor; LC-MS/MS, liquid chromatography tandem-mass spectrometry.

REFERENCES

(1) Le, Y.; Zhou, Y.; Iribarren, P.; Wang, J. M. Chemokines and Chemokine Receptors: Their Manifold Roles in Homeostasis and Disease. *Cell. Mol. Immunol.* **2004**, *1(2)*, 95–104.

(2) Balkwill, F. Cancer and the Chemokine Network. *Nat. Rev. Cancer* **2004**, *4(7)*, 540–550.

(3) Rosenbaum, D. M.; Rasmussen, S. G. F.; Kobilka, B. K. The Structure and Function of G Protein-Coupled Receptors. *Nature* **2009**, *459*, 356–363.

(4) Giannone, F.; Malpeli, G.; Lisi, V.; Grasso, S.; Shukla, P.; Ramarli, D.; Sartoris, S.; Monsurró, V.; Krampera, M.; Amato, E.; Tridente, G.; Colombatti, M.; Parenti, M.; Innamorati, G. The Puzzling Uniqueness of the Heterotrimeric G_{15} Protein and Its Potential Beyond Hematopoiesis. *J. Mol. Endocrinol.* **2010**, *44*, 259–269.

(5) Teicher, B. A.; Fricker, S. P. CXCL12 (SDF-1)/CXCR4 Pathway in Cancer. *Clin. Cancer Res.* **2010**, *16(11)*, 2927–2931.

(6) Wright, D. E.; Bowman, E. P.; Wagers, A. J.; Butcher, E. C.; Weissman, I. L. Hematopoietic Stem Cells Are Uniquely Selective in Their Migratory Response to Chemokines. *J. Exp. Med.* **2002**, *195(9)*, 1145–1154.

(7) Ratajczak, M. Z.; Zuba-Surma, E.; Kucia, M.; Reca, R.; Wojakowski, W.; Ratajczak, J. The Pleiotropic Effects of the SDF-1–CXCR4 Axis in Organogenesis, Regeneration and Tumorigenesis. *Leukemia* **2006**, *20*, 1915–1924.

(8) Ma, Q.; Jones, D.; Borghesani, P. R.; Segal, R. A.; Nagasawa, T.; Kishimoto, T.; Bronson, R. T.; Springer, T. A. Impaired B-Lymphopoiesis, Myelopoiesis, and Derailed Cerebellar Neuron Migration in CXCR4- and SDF-1-Deficient Mice. *Proc. Natl. Acad. Sci. U. S. A.* **1998**, *95*, 9448–9453.

(9) Kucia, M.; Jankowski, K.; Reca, R.; Wysoczynski, M.; Bandura, L.; Allendorf, D. J.; Zhang, J.; Ratajczak, J.; Ratajczak, M. Z. CXCR4-SDF-1 Signalling, Locomotion, Chemotaxis and Adhesion. *J. Mol. Histol.* **2003**, *35*, 233–245.

(10) Moore, J. P.; Trkolat, A.; Dragic, T. Co-Receptors for HIV-1 Entry. *Curr. Opin. Immunol.* **1997**, *9(4)*, 551–562.

(11) Mishan, M. A.; Ahmadiankia, N.; Bahrami, A. R. CXCR4 and CCR7: Two Eligible Targets in Targeted Cancer Therapy. *Cell Biol. Int.* **2016**, *40*, 955–967.

(12) Domanska, U. M.; Kruizinga, R. C.; Nagengast, W. B.; Timmer-Bosscha, H.; Huls, G.; de Vries, E. G. E.; Walenkamp, A. M. E. A Review on CXCR4/CXCL12 Axis in Oncology: No Place to Hide. *Eur. J. Cancer* **2013**, *49*, 219–230.

(13) Scala, S.; Ottaiano, A.; Ascierto, P. A.; Cavalli, M.; Simeone, E.; Giuliano, P.; Napolitano, M.; Franco, R.; Botti, G.; Castello, G. Expression of CXCR4 Predicts Poor Prognosis in Patients with Malignant Melanoma. *Clin. Cancer Res.* **2005**, *11*, 1835–1841.

(14) Semaan, A.; Dietrich, D.; Bergheim, D.; Dietrich, J.; Kalff, J. C.; Branchi, V.; Matthaei, H.; Kristiansen, G.; Fischer, H.-P.; Goltz, D. CXCL12 Expression and PD-L1 Expression Serve as Prognostic Biomarkers in HCC and Are Induced by Hypoxia. *Virchows Arch.* **2017**, *470*, 185–196.

(15) Sultan, M.; Coyle, K. M.; Vidovic, D.; Thomas, M. L.; Gujar, S.; Marcato, P. Hide-and-Seek: The Interplay between Cancer Stem Cells and the Immune System. *Carcinogenesis* **2016**, *38(2)*, 107–118.

(16) Righi, E.; Kashiwagi, S.; Yuan, J.; Santosuosso, M.; Leblanc, P.; Ingraham, R.; Forbes, B.; Edelblute, B.; Collette, B.; Xing, D.; Kowalski, M.; Mingari, M. C.; Vianello, F.; Birrer, M.; Orsulic, S.; Dranoff, G.; Poznansky, M. C. CXCL12/CXCR4 Blockade

Induces Multimodal Antitumor Effects That Prolong Survival in an Immunocompetent Mouse Model of Ovarian Cancer. *Cancer Res.* **2011**, *71(16)*, 5522–5534.

(17) Petit, I.; Jin, D.; Rafii, S. The SDF-1-CXCR4 Signaling Pathway: A Molecular Hub Modulating Neo-Angiogenesis. *Trends Immunol.* **2007**, *28(7)*, 299–307.

(18) Zlotnik, A.; Burkhardt, A. M.; Homey, B. Homeostatic Chemokine Receptors and Organ-Specific Metastasis. *Nat. Rev. Immunol.* **2011**, *11(9)*, 597–606.

(19) Kucia, M.; Reca, R.; Miekus, K.; Wanzeck, J.; Wojakowski, W.; Janowska-Wieczorek, A.; Ratajczak, J.; Ratajczak, M. Z. Trafficking of Normal Stem Cells and Metastasis of Cancer Stem Cells Involve Similar Mechanisms: Pivotal Role of the SDF-1-CXCR4 Axis. *Stem Cells* **2005**, *23*, 879–894.

(20) Rubin, J. B.; Kung, A. L.; Klein, R. S.; Chan, J. A.; Sun, Y.; Schmidt, K.; Kieran, M. W.; Luster, A. D.; Segal, R. A. A Small-Molecule Antagonist of CXCR4 Inhibits Intracranial Growth of Primary Brain Tumors. *Proc. Natl. Acad. Sci. U. S. A.* **2003**, *100(23)*, 13513–13518.

(21) de Nigris, F.; Schiano, C.; Infante, T.; Napoli, C. CXCR4 Inhibitors: Tumor Vasculature and Therapeutic Challenges. *Recent Pat. Anticancer. Drug Discov.* **2012**, *7(3)*, 251–264.

(22) Uchida, D.; Onoue, T.; Kuribayashi, N.; Tomizuka, Y.; Tamatani, T.; Nagai, H.; Miyamoto, Y. Blockade of CXCR4 in Oral Squamous Cell Carcinoma Inhibits Lymph Node Metastases. *Eur. J. Cancer* **2011**, *47*, 452–459.

(23) Domanska, U. M.; Timmer-Bosscha, H.; Nagengast, W. B.; Oude Munnink, T. H.; Kruizinga, R. C.; Ananias, H. J. K.; Kliphuis, N. M.; Huls, G.; De Vries, E. G. E.; de Jong, I. J.; Walenkamp, A. M. E. CXCR4 Inhibition with AMD3100 Sensitizes Prostate Cancer to Docetaxel Chemotherapy. *Neoplasia* **2012**, *14(8)*, 709–718.

(24) De Clercq, E. The AMD3100 Story: The Path to the Discovery of a Stem Cell Mobilizer (Mozobil). *Biochem. Pharmacol.* **2009**, *77*, 1655–1664.

(25) Gupta, S. K.; Pillarisetti, K.; Thomas, R. A.; Aiyar, N. Pharmacological Evidence for

Complex and Multiple Site Interaction of CXCR4 with SDF-1α: Implications for Development of Selective CXCR4 Antagonists. *Immunol. Lett.* **2001**, *78*, 29–34.

(26) Fricker, S. P.; Anastassov, V.; Cox, J.; Darkes, M. C.; Grujic, O.; Idzan, S. R.; Labrecque, J.; Lau, G.; Mosi, R. M.; Nelson, K. L.; Qin, L.; Santucci, Z.; Wong, R. S. Y. Characterization of the Molecular Pharmacology of AMD3100: A Specific Antagonist of the G-Protein Coupled Chemokine Receptor, CXCR4. *Biochem. Pharmacol.* **2006**, *72*, 588–596.

(27) Chen, Y.; Ramjiawan, R. R.; Reiberger, T.; Ng, M. R.; Hato, T.; Huang, Y.; Ochiai, H.; Kitahara, S.; Unan, E. C.; Reddy, T. P.; Fan, C.; Huang, P.; Bardeesy, N.; Zhu, A. X.; Jain, R. K.; Duda, D. G. CXCR4 Inhibition in Tumor Microenvironment Facilitates Anti-Programmed Death Receptor-1 Immunotherapy in Sorafenib-Treated Hepatocellular Carcinoma in Mice. *Hepatology* **2015**, *61*, 1591–1602.

(28) Mosi, R. M.; Anastassova, V.; Cox, J.; Darkes, M. C.; Idzan, S. R.; Labrecque, J.; Lau, G.; Nelson, K. L.; Patel, K.; Santucci, Z.; Wong, R. S. Y.; Skerlj, R. T.; Bridger, G. J.; Huskens, D.; Schols, D.; Fricker, S. P. The Molecular Pharmacology of AMD11070: An Orally Bioavailable CXCR4 HIV Entry Inhibitor. *Biochem. Pharmacol.* **2012**, *83*, 472–479.

(29) Nyunt, M. M.; Becker, S.; Macfarland, R. T.; Chee, P.; Scarborough, R.; Everts, S.; Calandra, G. B.; Hendrix, C. W. Pharmacokinetic Effect of AMD070, an Oral CXCR4 Antagonist, on CYP3A4 and CYP2D6 Substrates Midazolam and Dextromethorphan in Healthy Volunteers. *J. Acquir. Immune Defic. Syndr.* **2008**, *47(5)*, 559–565.

(30) Moyle, G.; DeJesus, E.; Boffito, M.; Wong, R. S.; Gibney, C.; Badel, K.; MacFarland, R.; Calandra, G.; Bridger, G.; Becker, S. Proof of Activity with AMD11070, an Orally Bioavailable Inhibitor of CXCR4-Tropic HIV Type 1. *Clin. Infect. Dis.* **2009**, *48*, 798–805.

(31) Skerlj, R.; Bridger, G.; McEachern, E.; Harwig, C.; Smith, C.; Wilson, T.; Veale, D.; Yee, H.; Crawford, J.; Skupinska, K.; Wauthy, R.; Yang, W.; Zhu, Y.; Bogucki, D.; Di Fluri, M.; Langille, J.; Huskens, D.; De Clercq, E.; Schols, D. Synthesis and SAR of Novel

CXCR4 Antagonists That Are Potent Inhibitors of T-Tropic (X4) HIV-1 Replication. *Bioorg. Med. Chem. Lett.* **2011**, *21*, 1414–1418.

(32) Skerlj, R. T.; Bridger, G. J.; Kaller, A.; McEachern, E. J.; Crawford, J. B.; Zhou, Y.; Atsma, B.; Langille, J.; Nan, S.; Veale, D.; Wilson, T.; Harwig, C.; Hatse, S.; Princen, K.; De Clercq, E.; Schols, D. Discovery of Novel Small Molecule Orally Bioavailable C-X-C Chemokine Receptor 4 Antagonists That Are Potent Inhibitors of T-Tropic (X4) HIV-1 Replication. *J. Med. Chem.* **2010**, *53(8)*, 3376–3388.

(33) Miller, J. F.; Gudmundsson, K. S.; D'Aurora Richardson, L.; Jenkinson, S.; Spaltenstein, A.; Thomson, M.; Wheelan, P. Synthesis and SAR of Novel Isoquinoline CXCR4 Antagonists with Potent Anti-HIV Activity. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 3026–3030.

(34) Jenkinson, S.; Thomson, M.; McCoy, D.; Edelstein, M.; Danehower, S.; Lawrence, W.; Wheelan, P.; Spaltenstein, A.; Gudmundsson, K. Blockade of X4-Tropic HIV-1 Cellular Entry by GSK812397, a Potent Noncompetitive CXCR4 Receptor Antagonist. *Antimicrob. Agents Chemother.* **2010**, *54(2)*, 817–824.

(35) Thoma, G.; Streiff, M. B.; Kovarik, J.; Glickman, F.; Wagner, T.; Beerli, C.; Zerwes, H.-G. Orally Bioavailable Isothioureas Block Function of the Chemokine Receptor CXCR4 In Vitro and In Vivo. *J. Med. Chem.* **2008**, *51*, 7915–7920.

(36) Tanaka, T.; Tsutsumi, H.; Nomura, W.; Tanabe, Y.; Ohashi, N.; Esaka, A.; Ochiai, C.; Sato, J.; Itotani, K.; Murakami, T.; Ohba, K.; Yamamoto, N.; Fujii, N.; Tamamura, H. Structure-Activity Relationship Study of CXCR4 Antagonists Bearing the Cyclic Pentapeptide Scaffold: Identification of the New Pharmacophore. *Org. Biomol. Chem.* **2008**, *6*, 4374–4377.

(37) Debnath, B.; Xu, S.; Grande, F.; Garofalo, A.; Neamati, N. Small Molecule Inhibitors of CXCR4. *Theranostics* **2013**, *3(1)*, 47–75.

(38) Zhao, H.; Prosser, A. R.; Liotta, D. C.; Wilson, L. J. Discovery of Novel *N*-Aryl Piperazine CXCR4 Antagonists. *Bioorganic Med. Chem. Lett.* **2015**, *25*, 4950–4955.

(39) Truax, V. M.; Zhao, H.; Katzman, B. M.; Prosser, A. R.; Alcaraz, A. A.; Saindane, M. T.; Howard, R. B.; Culver, D.; Arrendale, R. F.; Gruddanti, P. R.; Evers, T. J.; Natchus, M. G.; Snyder, J. P.; Liotta, D. C.; Wilson, L. J. Discovery of Tetrahydroisoquinoline-Based CXCR4 Antagonists. *ACS Med. Chem. Lett.* **2013**, *4*, 1025–1030.

(40) Mono-2-pyridylmethyl analog **8a** was alternatively prepared according to Truax, V. T.; Zhao, H.; Katzman, B. M.; Prosser, A. R.; Alcaraz, A. A.; Saindane, M. T.; Howard, R. B.; Culver, D.; Arrendale, R. F.; Gruddanti, P. R.; Evers, T. J.; Natchus, M. G.; Snyder, J. P.; Liotta, D. C.; Wilson, L. J. *ACS Med. Chem. Lett*. **2013**, *4*, 1025-1030.

(41) Kumpaty, H. J.; Bhattacharyya, S.; Rehr, E. W.; Gonzalez, A. M. Selective Access to Secondary Amines by a Highly Controlled Reductive Mono-*N*-Alkylation of Primary Amines. *Synthesis* **2003**, *14*, 2206–2210.

(42) Yang, Y.; Buchwald, S. L. Copper-Catalyzed Regioselective Ortho C-H Cyanation of Vinylarenes. *Angew. Chem. Int. Ed.* **2014**, *53*, 8677–8681.

(43) Adlington, R. M.; Baldwin, J. E.; Becker, G. W.; Chen, B.; Cheng, L.; Cooper, S. L.; Hermann, R. B.; Howe, T. J.; McCoull, W.; McNulty, A. M.; Neubauer, B. L.; Pritchard, G. J. Design, Synthesis, and Proposed Active Site Binding Analysis of Monocyclic 2-Azetidinone Inhibitors of Prostate Specific Antigen. *J. Med. Chem.* **2001**, *44(10)*, 1491–1508.

(44) Dess, D. B.; Martin, J. C. Readily Accessible 12-I-5' Oxidant for the Conversion of Primary and Secondary Alcohols to Aldehydes and Ketones. *J. Org. Chem.* **1983**, *48*, 4155–4156.

(45) Tu, Z.; Li, S.; Cui, J.; Xu, J.; Taylor, M.; Ho, D.; Luedtke, R. R.; Mach, R. H. Synthesis and Pharmacological Evaluation of Fluorine-Containing D_3 Dopamine Receptor Ligands. *J. Med. Chem.* **2011**, *54*, 1555–1564.

(46) Gierasch, T. M.; Shi, Z.; Verdine, G. L. Extensively Stereodiversified Scaffolds for Use in Diversity-Oriented Library Synthesis. *Org. Lett.* **2003**, *5(5)*, 621–624.

(47) Mitsunobu, O. The Use of Diethyl Azodicarboxylate and Triphenylphosphine in Synthesis

and Transformation of Natural Products. *Synthesis* **1981**, 1–28.

(48) Coleman, R. S.; Carpenterlb, A. J. Synthesis of the Aziridino[1,2-a]pyrrolidine Substructure of the Antitumor Agents Azinomycin A and B. *J. Org. Chem.* **1992**, *57*, 5813–5815.

(49) Cooper, S. P.; Booker-Milburn, K. I. A Palladium(II)-Catalyzed C-H Activation Cascade Sequence for Polyheterocycle Formation. *Angew. Chem. Int. Ed.* **2015**, *54*, 6496–6500.

(50) Parikh, J. R.; Doering, W. E. Sulfur Trioxide in the Oxidation of Alcohols by Dimethyl Sulfoxide. *J. Am. Chem. Soc.* **1967**, *89(21)*, 5505–5507.

(51) Moffett, R. B. 5,5-Dimethyl-2-Pyrrolidone. *Org. Synth.* **1952**, *32*, 59–60.

(52) Princen, K.; Hatse, S.; Vermeire, K.; De Clercq, E.; Schols, D. Evaluation of SDF-1/CXCR4-Induced Ca^{2+} Signaling by Fluorometric Imaging Plate Reader (FLIPR) and Flow Cytometry. *Cytom. Part A* **2003**, *51*, 35–45.

(53) Zhu, C.; Jiang, L.; Chen, T.-M.; Hwang, K.-K. A Comparative Study of Artificial Membrane Permeability Assay for High Throughput Profiling of Drug Absorption Potential. *Eur. J. Med. Chem.* **2002**, *37*, 399–407.

(54) Donato, M. T.; Jimenez, N.; Castell, J. V; Gomez-Lechon, M. Fluorescence-Based Assays for Screening Nine Cytochrome P450 (CYP450) Activities in Intact Cells Expressing Individual Human P450 Enzymes. *Drug Metab. Dispos.* **2004**, *32(7)*, 699–706.

(55) Yang, Z.; Zadjura, L. M.; Marino, A. M.; D'Arienzo, C. J.; Malinowski, J.; Gesenberg, C.; Lin, P.-F.; Colonno, R. J.; Wang, T.; Kadow, J. F.; Meanwell, N. A.; Hansel, S. B. Utilization of *in vitro* Caco-2 Permeability and Liver Microsomal Half-Life Screens in Discovering BMS-488043, a Novel HIV-1 Attachment Inhibitor with Improved Pharmacokinetic Properties. *J. Pharm. Sci.* **2010**, *99(4)*, 2135–2152.

(56) Storz, M. P.; Maurer, C. K.; Zimmer, C.; Wagner, N.; Brengel, C.; de Jong, J. C.; Lucas, S.; Müsken, M.; Häussler, S.; Steinbach, A.; Hartmann, R. W. Validation of PqsD as an Anti-Biofilm Target in Pseudomonas Aeruginosa by Development of Small-Molecule

Inhibitors. *J. Am. Chem. Soc.* **2012**, *134*, 16143–16146.

(57) O'Hagan, D.; Wang, Y.; Skibiński, M.; Slawin, A. M. Z. Influence of the Difluoromethylene Group (CF_2) on the Conformation and Properties of Selected Organic Compounds. *Pure Appl. Chem.* **2012**, *84(7)*, 1587–1595.

(58) Huchet, Q. A.; Kuhn, B.; Wagner, B.; Kratochwil, N. A.; Fischer, H.; Kansy, M.; Zimmerli, D.; Carreira, E. M.; Muller, K. Fluorination Patterning: A Study of Structural Motifs That Impact Physicochemical Properties of Relevance to Drug Discovery. *J. Med. Chem.* **2015**, *58*, 9041–9060.

(59) Kieltyka, K.; Zhang, J.; Li, S.; Vath, M.; Baglieri, C.; Ferraro, C.; Zvyaga, T. A.; Drexler, D. M.; Weller, H. N.; Shou, W. Z. A High-Throughput Bioanalytical Platform Using Automated Infusion for Tandem Mass Spectrometric Method Optimization and Its Application in a Metabolic Stability Screen. *Rapid Commun. Mass Spectrom.* **2009**, *23(11)*, 1579–1591.

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