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Design and Synthesis of Potent, Orally Efficacious Hydroxyethylamine Derived β-Site Amyloid Precursor Protein Cleaving Enzyme (BACE1) Inhibitors

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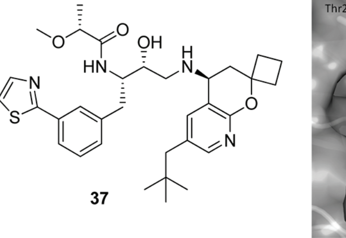
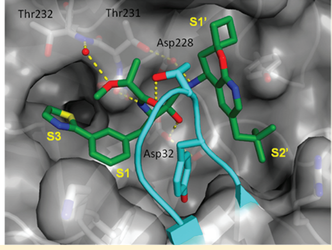
Supporting Information

ABSTRACT: We have previously shown that hydroxyethylamines can be potent inhibitors of the BACE1 enzyme and that the generation of BACE1 inhibitors with CYP 3A4 inhibitory activities in this scaffold affords compounds (e.g., **1**) with sufficient bioavailability and pharmacokinetic profiles to reduce central amyloid-β peptide (Aβ) levels in wild-type rats following oral dosing. In this article, we describe further modifications of the P1-phenyl ring of the hydroxyethylamine series to afford potent, dual BACE1/CYP 3A4 inhibitors which demonstrate improved penetration into the CNS. Several of these compounds caused robust reduction of Aβ levels in rat CSF and brain following oral dosing, and compound **37** exhibited an improved cardiovascular safety profile relative to **1**.





INTRODUCTION

Alzheimer's disease (AD) is a terminal neurodegenerative disorder of the brain that begins with mild cognitive and short-term memory impairment that gradually progresses to complete loss of cognitive function and ultimately death.[1] While AD is the most common form of dementia in the elderly, affecting more that 5 million people in the U.S. and over 20 million people worldwide, current treatment options are limited and do little to alleviate the symptoms of the disease. As a result, a disease-modifying therapy for AD represents a large and growing unmet medical need.[2] A large body of evidence suggests that AD results from the accumulation of β-amyloid peptides, particularly $A\beta_{40}$ and $A\beta_{42}$, in the brain. These peptides can aggregate to form toxic, soluble oligomers or further aggregate to form insoluble senile plaques, the histological hallmark of AD.[3] While it remains unclear to what extent each of these species contributes to the overall pathology of the disease, the available evidence suggests that inhibition of Aβ formation is an important and direct strategy for therapeutic intervention in AD.[4]

Aβ is generated via initial proteolytic cleavage of membrane-bound amyloid precursor protein (APP) by the aspartyl protease BACE1 (β-site APP cleaving enzyme) to give a soluble *N*-terminal domain (β-sAPP) and a membrane-bound C-terminal domain (C99). Further processing of C99 by another aspartyl protease, γ-secretase, affords soluble Aβ peptides of varying length. Since its identification as β-secretase in 1999,[5] BACE1 has received considerable attention as a therapeutic target in AD research.[6] BACE1 knockout mice do not produce Aβ from APP in the brain but are viable, suggesting that targeted inhibition of the BACE1 enzyme would prevent formation of Aβ without significant on-target

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toxicities.[7] Given the central role of the enzyme in Aβ formation and the viability of knockout mice, the use of a CNS-penetrant small-molecule inhibitor of BACE1 to reduce Aβ levels in the brain is an attractive approach toward a disease-modifying therapy for AD.[8]

In previous articles, we have described the synthesis and evaluation of a series of BACE1 inhibitors featuring a hydroxyethylamine (HEA) scaffold as the transition state isostere to bind the critical catalytic aspartic acid residues of the BACE1 enzyme. While select compounds from these series did demonstrate the ability to reduce central Aβ concentrations following peripheral dosing in Sprague–Dawley (SD) rats, the pharmacological effects of these compounds were limited by their low intrinsic stability (as measured in liver microsome preparations) and poor penetration into the CNS as a result of P-glycoprotein (Pgp) mediated efflux.[9] Consequently, significant CNS access and efficacy required either high doses or codosing with a CYP 3A4 inhibitor. In an effort to obviate these shortcomings, a strategy was adopted wherein CYP inhibitory activities were incorporated into our HEA-derived BACE1 inhibitors, leading to the identification of **1**, a potent BACE1 inhibitor which demonstrated moderate in vivo clearance and reduced Pgp-mediated efflux (Figure 1).[10] In the course of

BACE Enzyme IC_{50} = 18 nM
BACE HEK Cell IC_{50} = 14 nM
Human/Rat Pgp Efflux Ratio = 4/6
Rat CL = 0.79 L/h/Kg
F = 130%
Cyp 3A4 IC_{50} = 50 nM
hERG IC_{50} = 23.7 μM
$EC_{50, \text{unbound in plasma}}$ for Aβ reduction in CSF = 176 nM

Figure 1. Properties of BACE1 inhibitor **1**.

evaluating inhibitor **1** for off-target cardiovascular (CV) liabilities in dog, it was found that this compound exhibited a narrow therapeutic index due to drug-induced tachycardia of an unknown mechanism.

In this article, we report on the design and synthesis of a series of compounds aimed at improving the CV-safety margin of hydroxyethylamine BACE1 inhibitors exemplified by **1**. Given the uncertainties of the underlying cause of the observed off-target CV effects, our strategy to improve upon this series focused on decreasing the unbound plasma drug concentration required to achieve significant reduction of CNS Aβ. This strategy thus relied on our ability to improve the functional cellular potency of our inhibitors and/or reduce their susceptibility to Pgp-mediated efflux and thereby increase their distribution into the CNS. Our previous work had demonstrated that the highly optimized azachroman ring system was required to maintain potent cellular activities in HEA BACE1 inhibitors which demonstrated suitable properties for CNS penetration.[9] This work also revealed that the BACE1 enzyme was much more tolerant of modification to the P1-phenyl group, hence our efforts to improve upon **1** focused on modifying this region of the inhibitor.[10] In keeping with our previously described strategy to design hydroxyethylamines which demonstrated suitable metabolic stability, we designed compounds incorporating *N*-containing heterocycles and alkynes, functional groups commonly known to inhibit CYP 3A4.[11] This work led to the identification of potent, highly orally efficacious BACE1 inhibitors with CYP 3A4 inhibitory activities, some of which demonstrated significantly improved cardiovascular safety margins relative to **1**.

■ CHEMISTRY

The compounds described in Tables 1 and 3 were prepared as shown in Scheme 1. Sulfinyl imine **2** was treated with a number of different benzyl Grignard reagents to afford sulfinamides **4**.[12] Simultaneous deprotection of the *tert*-butyl sulfinyl and primary *tert*-butyldimethylsilyl (TBS) ether groups was accomplished by exposure of compounds **4** to hydrogen chloride in cold ethanol, and the resulting intermediates were reprotected to give the Boc-carbamates **5**. Dess–Martin oxidation[13] of the primary alcohols to the corresponding aldehydes was followed by sodium triacetoxyborohydride-mediated reductive amination with amine **6**[9] to afford protected hydroxyethylamines **7**. Simultaneous removal of the *N*-Boc and TBS-groups in methanolic hydrochloric acid was followed by direct exposure of the crude amine hydrochlorides to 1-(1*H*-imidazol-1-yl)-2-methoxyethanone (**8**) in the presence of Hünig's base to afford the hydroxyethylamines **9**. At this point, palladium-catalyzed cross-coupling reactions were used to form the final biaryl products **11–30**. In the case of compounds **22**, **23**, and **27**, the cross-coupling reactions were performed directly on intermediates **7** to generate compounds **10g–i**. Sequential deprotection and acylation, as described above, afforded the corresponding final products.

The compounds described in Table 4 were used to evaluate the SAR around the acyl group of the inhibitors, and the synthesis of these compounds is illustrated in Scheme 2. Negishi coupling[14] of aryl bromide **5a** and 2-thiazolyl zinc bromide in 1,4-dioxane at room temperature afforded biaryl **31** in 92% yield. Following oxidation of the primary alcohol unit of **31** with the Dess–Martin periodinane, reductive amination with amine **6** in the presence of sodium triacetoxyborohydride afforded protected hydroxyethylamine **32** in 83% yield. Deprotection of the *N*-Boc and TBS-groups was again accomplished by exposure of **32** to warm methanolic hydrochloric acid. The crude intermediate amine was directly acylated by treatment with a preformed solution of the appropriate acyl imidazole in dichloromethane at 0 °C to afford inhibitors **34–43**.

■ RESULTS

Our efforts to identify HEA-derived BACE1 inhibitors which exhibited improved safety profiles relied extensively upon our previously established SAR.[9,10] As outlined above, our strategy involved the generation of dual potent BACE1/CYP 3A4 inhibitors. Although not ideal, this strategy was deemed necessary so as to maintain sufficiently high drug plasma levels. In addition, to ensure adequate exposure within the CNS, analogues with high permeability and low efflux ratios were targeted. Evaluation of compounds such as **1**, containing the optimized right-hand side azachroman ring system, had demonstrated that potent inhibitors of both BACE1 and CYP 3A4 could be generated by appropriate substitution of the P1-phenyl ring at the 3- and 4-positions.[10] Furthermore, incorporation of an ethereal oxygen at the α-position of the acyl group had been shown to attenuate Pgp-mediated efflux.[9] Upon the basis of these observations, we elected to initiate our studies by keeping the acyl group constant as the α-methoxy acetamide and varying the substituent attached to the P1-

Scheme 1. Preparation of P1-Biaryl BACE1 Inhibitors[a]

a $R_1=R_3=R_4=R_5=R_6=H$; $R_2=Br$
b $R_1=R_3=R_5=H$; $R_2=Cl$; $R_4=F$
c $R_1=R_4=R_5=H$; $R_2=Cl$; $R_3=F$
d $R_1=R_3=R_4=H$; $R_1=F$; $R_4=Cl$
e $R_1=R_2=R_4=R_5=H$; $R_3=Cl$
f $R_3=R_4=R_5=H$; $R_1=F$; $R_2=Cl$

10g $R_1=F$; R_2=2-thiazolyl; $R_3=H$
10h $R_1=F$; R_2=TMS-ethynyl; $R_3=H$
10i $R_1=R_2=H$; R_3=TMS-ethynyl

[a]Reagents: (a) R-MgBr, THF, −70 °C; (b) HCl, 1,4-dioxane-EtOH, −20 °C, Et_3N, Boc_2O, CH_2Cl_2, −20 to 23 °C; (c) (i) Dess−Martin periodinane, $NaHCO_3$, CH_2Cl_2, 0 to 23 °C (ii) **6**, $(EtO)_3CH$, $NaBH(OAc)_3$, CH_2Cl_2, 23 °C; (d) (i) HCl, 1,4-dioxane-CH_3OH, 40 °C, (ii) **8**, *i*-Pr_2NEt, CH_2Cl_2, 23 °C; (e) Ar-$SnBu_3$ or R-CC-$SnBu_3$, $Pd_2(dba)_3$, X-Phos, CsF, 1,4-dioxane, 160 °C, *μ*-wave; (f) Ar-$SnBu_3$, $Pd(PPh_3)_4$, 1,4-dioxane, 140 °C, *μ*-wave; (g) R-CC-ZnCl, $Pd_2(dba)_3$, X-Phos, THF, 120 °C, sealed vessel.

phenyl ring to identify inhibitors with enhanced potency and reduced efflux relative to compound **1**.

After extensive screening, we identified a series of biaryl inhibitors which functioned as potent dual BACE1/CYP 3A4 inhibitors. More specifically, it was found that the incorporation of a variety of *N*-containing heterocycles provided compounds which compared favorably with our previously identified HEA-containing BACE1 inhibitors (i.e., **1**). As illustrated in Table 1, a range of hetereocycles were tolerated at this position, and incorporation of pyridyl (**11**−**12**), oxazolyl (**13**), or thiazolyl (**15**−**18**) groups generated potent BACE1 inhibitors as measured in both the enzymatic and cellular assays. A notable exception is *N*-methylimidazole **14** with reduced potency in the enzymatic assay but inexplicably good potency in the cellular assay. Alkynyl-substitution was also well tolerated, with the terminal aryl acetylene **19** being roughly 3−5-fold more potent than internal alkyne **20**. Substitution at the 4-position of the P1-phenyl ring with either the 2-thiazolyl group (**21**) or terminal alkyne (**22**) generated less potent inhibitors. Generally, the relative intrinsic metabolic stability within the series could be correlated to the estimated potency of the inhibitors on the CYP 3A4 enzyme. For example, 4-pyridyl compound **11** was significantly more potent on CYP 3A4 (IC_{50} = 160 nM) than the 2-pyridyl regioisomer **12** (IC_{50} = 400 nM), and this difference is reflected in greater turnover of **12** in human liver microsomes. Further, compounds with estimated IC_{50}s on CYP 3A4 below 50 nM demonstrated consistently low microsomal turnover. Overall, inhibitors substituted with a pyridyl group were less metabolically stable than compounds containing other heterocycles examined. While compounds bearing 2-thiazolyl (**15** and **21**) or 5-thiazolyl (**16**) groups demonstrated good intrinsic metabolic stability, 4-thiazolyl substitution (**17**) generated a compound with high microsomal turnover. Introduction of a methyl group on the thiazole ring, as in 4-methyl-2-thiazolyl analogue **18**, significantly decreased metabolic stability. Similarly, an internal alkyne (**20**) was much less stable relative to terminal aryl acetylenes (**19** and **22**).

Given that a primary focus of this work was to identify a series of BACE1 inhibitors which demonstrated improved penetration into the CNS, potent inhibitors were also assessed for their passive permeability and potential for Pgp-mediated efflux. Interestingly, as a whole, this series of compounds displayed excellent passive permeability and did not show a tendency for significant Pgp-mediated efflux. Most of the

Scheme 2. Preparation of Acetamide Analogues[a]

[a]Reagents: (a) $Pd(Pt\text{-}Bu_3)_2$ (5 mol %), $ZnCl_2$ (2 equiv), 2-thiazolyl zinc bromide (1.2 equiv), dioxane, 23 °C; (b) (i) Dess–Martin periodinane, $NaHCO_3$, CH_2Cl_2, 0 to 23 °C, (ii) **6**, $(EtO)_3CH$, $NaBH(OAc)_3$, CH_2Cl_2, 23 °C; (c) HCl, dioxane-CH_3OH, 40 °C; (d) carboxylic acid, carbonyl diimidazole, *i*-Pr_2NEt, CH_2Cl_2, 0 °C.

compounds showed higher efflux by rat Pgp relative to the human transporter; however, in most cases, these values were within 3-fold of one another. The three compounds which were recognized and transported by Pgp were the 4-pyridyl, 2-oxazolyl, and *N*-methylimidazole derivatives (**11**, **13**, and **14**). Compound **17** showed a moderate potential for rat Pgp-mediated efflux but was less prone to transport by the human transporter.

Compounds **13**, **15**, **16**, and **19** offered the best balance of functional potency and intrinsic metabolic stability, so these compounds were profiled in an acute rat pharmacodynamic model measuring levels of $A\beta_{40}$ in rat CSF and brain. Each compound was dosed by oral gavage at 30 mg/kg to male Sprague–Dawley rats (Table 2). After 4 h, 2-thiazolyl substituted compound **15** and terminal alkyne **19** demonstrated robust reduction of $A\beta_{40}$ levels in the CSF (75% and 76%, respectively) while oxazole **13** and 5-thiazolyl isomer **16** led to a much less pronounced effect of CSF $A\beta_{40}$ levels (44% and 27%, respectively). At the 4 h time point, reductions of $A\beta_{40}$ levels in the brain were similar to that observed in the CSF for compounds **13** and **19** (34% and 68%, respectively), but surprisingly compound **15** only afforded a 49% reduction in brain $A\beta_{40}$ levels. The magnitude of these effects could be correlated with the concentration of inhibitor in the CSF, a surrogate for brain free fraction.[15] Although all compounds exhibited good total plasma levels (2.5–7 μM) at the 4 h time point, compounds **15** and **19** achieved CSF concentrations of greater than 8-fold at their respective in vitro cellular potencies. On the other hand, CSF levels of **13** were 14 nM, a considerably reduced exposure relative to its in vitro potency, which likely explains the moderate efficacy observed with this inhibitor. Similarly, the modest $A\beta$ reduction observed following oral administration of **16** can be explained by the low drug levels in the CSF (2 nM). While it is unclear why the concentration of 5-thiazolyl isomer **16** was low in CSF at the 4 h time point, the lower passive permeability (9.9×10^{-6} cm/s) and higher Pgp-mediated efflux (efflux ratio_{rat} = 8.5) of oxazole **13** explain the low levels of drug observed in the CSF for this inhibitor.

With the knowledge that compounds **15** and **19**, featuring 3-(2-thiazolyl)phenyl and 3-(ethynyl)phenyl P1-groups, respectively, produced robust effects in our PD model, we further explored the SAR of dual BACE1/CYP 3A4 inhibitors bearing these groups. We anticipated that by identifying additional compounds with more potent in vivo $A\beta$ reduction in our PD model, we would increase our chances of identifying BACE1 inhibitors with significantly improved cardiovascular safety margins relative to inhibitors like **1**.

Previous work had demonstrated that the incorporation of a fluorine atom on the P1-phenyl ring can result in significant improvements on the potencies and pharmacokinetic profiles of hydroxyethylamine BACE1 inhibitors,[9] so we next examined fluorinated analogues of compounds **15** and **19** (Table 3). Within the series of compounds containing the 2-thiazolyl moiety, incorporation of a fluorine at the 2 position of the phenyl ring led to a compound (**23**) with diminished BACE1 potency. Alternatively, compounds containing a fluorine at the 4, 5, or 6 position (**24**–**26**) demonstrated similar levels of

Table 1. Substituted P1-Phenyl BACE1 Inhibitors

Cpd	R	IC_{50} (nM)			metabolic stability ((μL/min)/mg)[d]			Pgp-Efflux[f]	
		BACE1[a]	HEK[a,b]	CYP 3A4[c]	RLM	HLM	P_{app}[e]	Rat	Human
11		33	9.8	160	103	182	22	11	3.9
12		9.3	18	400	292	706	32	1.3	2.0
13		9.6	12	100	37	43	9.9	8.5	5.9
14		64	5.5	50	14	16	12	50	50
15		4.1	6.5	100	66	54	23	2.7	1.1
16		5.2	8.2	30	75	74	24	4.1	1.2
17		6.9	9.8	100	115	221	21	8.3	2.3
18		5.7	14	100	311	347	23	0.6	0.6
19		3.4	7.1	30	45	43	24	2.3	0.9
20		15	22	30	303	81	20	1.1	0.3
21		29	26	30	63	68	20	0.5	0.6
22		47	99	100	67	28	18	4	5.9

[a] IC_{50} values were averaged values determined by at least two independent experiments. [b] Human embryonic kidney cells. [c] IC_{50} values were estimated based on a single-concentration experiment. Compound concentration = 3 μM [d] Rat liver microsomal (RLM) and human liver microsomal (HLM) clearance. Compound concentration = 1 μM. Microsomal protein concentration = 250 μg/mL. [e] Apparent permeability measured in parental LLC-PK1 cells. Values are an average of apical to basolateral (A to B) and basolateral to apical (B to A) velocities and are reported as 10^{-6} cm/s. [f] Efflux measured in LLC-PK1 cells transfected with either rat MDR1A/1B or human MDR1 and are reported as a ratio of (B to A)/(A to B).

potency in the enzymatic assay as that shown by the parent compound (**15**). Of note was compound **25**, which demonstrated a roughly 3-fold improvement in our enzymatic assay. Similar trends were observed within the acetylene series

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Table 2. Reduction of CSF Aβ_{40} in Wild-Type Rats

compd[a]	CSF Aβ_{40} reduction[b] (%)	brain Aβ_{40} reduction[b] (%)	CSF compd conc (μM)[c]	plasma compd conc (μM)[d]	cell IC$_{50}$ (nM)
13	44	34	0.014 ± 0.016	7.08 ± 4.11	12
15	75	49	0.052 ± 0.032	3.27 ± 0.43	6.5
16	27	ND	0.002 ± 0.003	2.51 ± 1.30	8.2
19	76	68	0.078 ± 0.032	3.54 ± 0.48	7.1

[a]Compounds were dosed orally at 30 mg/kg in male Sprague−Dawley rats. n = 5 animals per group. [b]CSF and brain Aβ_{40} measured at t = 4 h. ND = not determined. [c]CSF drug concentration measured at t = 4 h. [d]Plasma drug concentration measured at t = 4 h.

Table 3. P1-Fluorophenyl BACE1 Inhibitors

Cpd	P1	IC$_{50}$ (nM)		metabolic stability ((µL/min)/mg)[c]			Pgp-Efflux[e]	
		BACE1[a]	HEK[a,b]	RLM	HLM	P$_{app}$[d]	Rat	Human
15		4.1	6.5	66	54	23	2.7	1.1
23		71	100	91	69	19	5.7	3
24		6.1	15	62	14	28	3.2	0.6
25		1.9	7.3	68	53	23	1.1	0.6
26		9.5	18	55	63	11	1.5	1.4
19		3.4	7.1	45	43	24	2.3	0.9
27		11	144	71	62	18	5.7	4.1
28		3.7	12	97	57	23	2	1
29		1.9	7.3	95	42	34	1.9	1.3
30		2.9	11	71	38	14	4.1	2.3

[a]IC$_{50}$ values were averaged values determined by at least two independent experiments. [b]Human embryonic kidney cells. [c]Rat liver microsomal (RLM) and human liver microsomal (HLM) clearance. Compound concentration = 1 μM. Microsomal protein concentration = 250 μg/mL. [d]Apparent permeability measured in parental LLC-PK1 cells. Values are an average of apical to basolateral (A to B) and basolateral to apical (B to A) velocities and are reported as 10^{-6} cm/s. [e]Efflux measured in LLC-PK1 cells transfected with either rat MDR1A/1B or human MDR1 and are reported as a ratio of (B to A)/(A to B).

Table 4. SAR of the *N*-Acyl Unit of P1-3-(2-Thiazolyl)phenyl BACE1 Inhibitors

Cpd	R	IC_{50} (nM)		metabolic stability ((μL/min)/mg)[c]			Pgp-Efflux[e]	
		BACE1[a]	HEK[a,b]	RLM	HLM	P_{app}[d]	Rat	Human
34	CH_3	3.7	5.6	70	71	22	4.9	9.4
35	CH_3	11	414	45	39	18	5.6	3.0
15	O	4.1	22	66	54	23	2.7	1.1
36	O	5.7	8.1	41	29	33	0.7	0.7
37	O	5.5	9.9	51	35	17	1.7	1.1
38	O	7.2	17	15	42	22	2.2	1.4
39	O	16	18	88	38	23	0.7	0.8
40	O O	9.8	9.1	41	33	24	3.0	1.8
41	O	6.3	11	35	46	17	2.1	1.8
42	O	9.2	5.9	44	64	21	0.8	0.5
43	HO	3.2	408	32	26	9.3	9.6	14

[a] IC_{50} values were averaged values determined by at least two independent experiments. [b] Human embryonic kidney cells. [c] Rat liver microsomal (RLM) and human liver microsomal (HLM) clearance. Compound concentration = 1 μM. Microsomal protein concentration = 250 μg/mL. [d] Apparent permeability measured in parental LLC-PK1 cells. Values are an average of apical to basolateral (A to B) and basolateral to apical (B to A) velocities and are reported as 10^{-6} cm/s. [e] Efflux measured in LLC-PK1 cells transfected with either rat MDR1A/1B or human MDR1 and are reported as a ratio of (B to A)/(A to B).

of compounds. Notably, compound **27**, which contains a fluorine at the 2-position, showed a modest loss in potency, while the 3-fluoro derivative **29** showed a small improvement in BACE1 potency. In general, the enzyme-to-cell potency shifts were marginally lower in the fluorinated derivatives (celluar IC_{50}/enzyme IC_{50} = 1.4−3.8) compared to parent compound **15** (cellular IC_{50}/enzyme IC_{50} = 5.5). As was observed with the aryl thiazoles, 4-fluoro substitution in the acetylene series (**28**) did not lead to appreciable changes in potency. It should be noted that within both series of compounds, introduction of fluorine had no discernible effect on either metabolic stability or efflux potential. It is worth pointing out, however, that compounds substituted with a fluorine at the 6-position (**26** and **30**) showed a tendency toward reduced passive permeability relative to their *des*-fluoro analogues.

Having established that incorporation of a 2-thiazolyl group onto the 3-position of the P1-phenyl ring of hydroxyethylamine compounds produced potent BACE1 inhibitors with moderate intrinsic metabolic stability, high permeability, and low Pgp-mediated efflux, we next examined systematic variation of the *N*-acyl group for further optimization. As shown in Table 4, the *N*-acetyl analogue (**34**) displayed increased BACE1 potency but suffered from significantly increased efflux ratios. This supports our previous observations that the Pgp-mediated efflux could be attenuated by incorporating an ethereal oxygen in the amide tether.[9,10] Increasing the chain length of the acyl group (**35**) resulted in a diminished human efflux ratio, but the

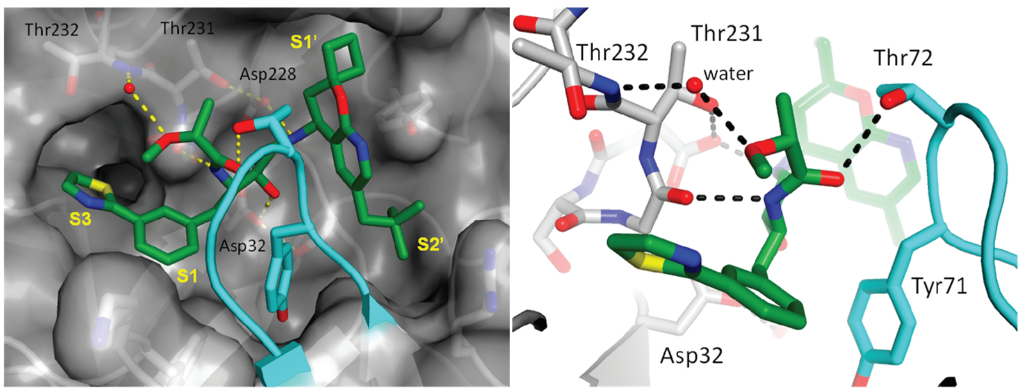

Figure 2. Cocrystal structure of BACE1 complexed with **37**. (a) Interactions of **37** with the BACE1 enzyme and extension of the *meta*-thiazole unit into S3. (b) Interactions of the methoxyacetamide group with BACE1.

Table 5. Reduction of CSF Aβ in Sprague−Dawley Rats[a]

compd	CSF $A\beta_{40}$ reduction[b] (%)	brain $A\beta_{40}$ reduction[b] (%)	CSF compd conc (μM)[c]	plasma compd conc (μM)[d]	rat Pgp-efflux ratio
15	75	49	0.052 ± 0.032	3.27 ± 0.43	2.7
19	76	68	0.078 ± 0.032	3.54 ± 0.48	2.3
25	64	45	0.035 ± 0.018	11.7 ± 1.53	1.1
34	0	ND	0.002 ± 0.003	5.06 ± 1.06	4.9
37	69	59	0.085 ± 0.022	3.11 ± 0.18	1.7
41	61	20	0.046 ± 0.046	4.18 ± 1.99	2.1

[a]Compounds were dosed orally at 30 mg/kg in male Sprague−Dawley rats. n = 5 animals per group. [b]CSF and brain $A\beta_{40}$ measured at t = 4 h. ND = not determined. [c]CSF drug concentration measured at t = 4 h. [d]Plasma drug concentration measured at t = 4 h.

modestly increased lipophilicity dramatically increased the observed enzyme to cell shift. Alkyl-substitution at the α-position, as in α-methoxypropanamide **37** and tetrahydrofuran-2-carboxamide epimers **41** and **42**, generated compounds with similar potency, stability, and permeability properties as methoxyacetamide **15**, although α-methoxypropanamide epimer **38** and α-ethoxypropanamide epimer **39** were slightly less potent and **39** was slightly less stable in rat liver microsomes as well. Incorporation of additional oxygenation, as in 2,2-dimethoxyacetamide **40**, was also tolerated. Addition of a free alcohol to the acetamide (**43**) resulted in a compound with good potency on the BACE1 enzyme, but this compound suffered from an increased enzyme to cell shift, reduced passive permeability, and elevated efflux ratios.

An X-ray cocrystal structure of BACE1 with compound **37** is represented in Figure 2.[16] As expected,[9,10] the azachroman and neopentyl groups of the molecule occupy the S1′ and S2′ regions of BACE1, respectively, and the hydroxyethylamine unit engages in hydrogen bonding interactions with the catalytic aspartic acid residues, Asp32 and Asp228. While the phenyl group of the inhibitor occupies the S1 region of BACE1 as observed previously, the crystal structure indicates that the preferred *meta*-orientation of the 2-thiazolyl group allows it to fill the S3 pocket. This observation is consistent with the higher BACE1 potency of P1 *meta*-substituted inhibitors (e.g., **15** and **19**) relative to their *para*-substituted counterparts (**21** and **22**, respectively). Additionally, the methoxyacetamide unit of **37** appears to bind differently than the corresponding group of inhibitor **1**. In the latter compound, a bidentate interaction occurs between the methoxy and carbonyl groups of the acetamide with Thr72.[10] In the case of **37**, only the carbonyl of the acetamide engages Thr72, while the methoxy group hydrogen bonds to Thr232 via a water molecule. The different orientation of the methoxy group observed in the crystal structure of **37** and BACE1 may occur to accommodate the binding of the 2-thiazolyl group in the S3 pocket.

In Vivo Model for Reduction of CNS Aβ Levels. Studies with potent BACE1 inhibitors in rats had demonstrated that the extent of Aβ-reduction in the brain and CSF could be correlated with the concentrations of inhibitor in the CSF, a surrogate measurement for unbound drug in the brain. Within an acute setting, the dosing of peripherally restricted BACE1 inhibitors resulted in reduced plasma Aβ concentrations; however, the levels of Aβ in the brain and CSF remained unperturbed.[9] Although distribution of a compound into the CNS is governed by a number of complex factors, compounds with moderate-to-high passive permeability ($\geq 10 \times 10^{-6}$ cm/s) and low transporter-mediated (e.g., Pgp) efflux (efflux ratios <3) generally have a better likelihood of achieving good CNS exposures.[17] Most of the compounds featuring either a 2-thiazolyl-phenyl or ethynylphenyl P1 group that we examined exhibited low (<3) rat and human efflux ratios. Exceptions to this trend are straight-chain alkyl amides **34** and **35** and hydroxypropanamide **43**. These analogues displayed higher human and rat efflux ratios (generally >3) that are likely to decrease CNS penetration. Of the methoxyacetamides examined, only the oxazole **13** and the 6-fluorophenyl derivatives **26** and **30** exhibited low passive permeabilities ($<15 \times 10^{-6}$ cm/s), and all but one of the acyl analogues examined in Table 4 (hydroxypropanamide **43**) showed good passive permeability. On the basis of these considerations, a select group of compounds from Tables 1−4 were evaluated further in the acute rat pharmacodynamic model (Table 5). In general it was found that compounds that achieved CSF concentrations at or over their functional IC_{50}s resulted in robust reduction (60−76%) of CSF $A\beta_{40}$ levels. As mentioned above, Pgp-mediated efflux can limit the exposure of compounds in the CNS, and this trend is exemplified by

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Table 6. Plasma Protein Binding and Pharmacokinetic Parameters in Male Sprague−Dawley Rats

	iv[a]			po[b]				
compd	CL (L/h/kg)	V_{ss} (L/kg)	$t_{1/2}$ (h)	$AUC_{0\to\infty}$ (ng·h/mL)	C_{max} (ng/mL)	t_{max} (h)	F (%)	plasma protein binding (%)[c]
15	1.46	2.19	8.96	9030	1887	3.33	120	96.3
19	0.71	1.54	5.30	24250	4863	5.33	100	ND
25	1.03	1.92	8.81	5335	4440	4.00	210	98.4
37	0.87	1.22	5.33	34770	5290	5.33	297	98.6

[a]Dosed iv at 2 mg/kg as a solution in DMSO. [b]Dosed po at 10 mg/kg as an aqueous suspension in 1% Tween 80 in 2% HMPC. [c]ND = not determined.

acetamide **34**, a compound with good passive permability but which is susceptible to active transport by Pgp. Dosed orally at 30 mg/kg, this compound achieved only low levels (0.002 μM) in the CSF at the 4 h time point despite plasma concentrations of ca. 5.06 μM. As a result, **34** produced no measurable change in CSF $A\beta_{40}$ levels. In contrast, (*R*)-2-methoxypropanamide **37** displayed similar passive permeability but did not appear to be a substrate for Pgp-mediated efflux. Despite lower plasma concentrations (3.11 μM), **37** achieved 42-fold higher CSF levels (0.085 μM) than **34**, producing a robust 69% reduction in CSF $A\beta$ 4 h after oral dosing.

Pharmacokinetic Profiles in Rat. The pharmacokinetic properties of a group of these compounds were assessed in rat following both intravenous (iv) and oral (po) dosing. As summarized in Table 6, thiazole-containing compounds **15**, **25**, and **37** and aryl acetylene **19** displayed moderate clearance (0.71−1.46 L/h/kg) and half-lives (ca. 5−9 h) in male Sprague−Dawley rats when dosed iv. As a consequence of their CYP 3A4 inhibitory activities, these compounds each demonstrated high plasma exposure following a 10 mg/kg oral dose in addition to excellent bioavailabilities (>100%). Compound **37** had a particularly high $AUC_{0\to\infty}$ of 34470 ng·h/mL, and the thiazole-containing compounds showed similar plasma protein binding in the range of 96.3−98.6%.

Determination of In Vivo Plasma EC_{50} for Central $A\beta_{40}$ Reduction. On the basis of their favorable pharmacokinetic and pharmacodynamic properties, compounds **15** and **37** were further evaluated in dose−response studies at a 4 h time point. As shown in Table 7, the total plasma concentrations required

Table 7. Effective Concentrations in Plasma for CSF $A\beta_{40}$ Reduction in Male Sprague−Dawley Rats 4 h after Oral Dosing[a]

compd	$EC_{50,\ total}$ for CSF $A\beta_{40}$ reduction (μM)	$EC_{50,\ unbound}$ for CSF $A\beta_{40}$ reduction (μM)
15	1.85	0.0066
37	1.62	0.0135

[a]Each compound was dosed at 10, 30, and 100 mg/kg, po as an aqueous suspension in 1% Tween 80 in 2% HMPC. Plasma drug concentrations and CSF $A\beta_{40}$ concentrations measured at t = 4 h. For each group, n = 5.

to produce a 50% reduction in CSF $A\beta_{40}$ for both compounds were determined to be very similar and in the low single-digit micromolar range ($EC_{50,\ total}$ = 1.85 μM for **15**; $EC_{50,\ total}$ = 1.62 μM for **37**). When corrected for plasma protein binding at these concentrations, the effective unbound plasma concentrations were found to be in the low nanomolar range ($EC_{50,\ unbound}$ = 6.6 nM and 13.5 nM for **15** and **37**, respectively). Compounds **15** and **37** produced robust reductions in CSF $A\beta_{40}$ at significantly lower unbound plasma concentrations than those required for compounds such as **1** ($EC_{50,\ unbound}$ = 176 nM), likely as a result of reduced Pgp-mediated efflux and consequently higher CNS exposures. Therefore, this series represented a substantial advantage over earlier HEA BACE1 inhibitors in terms of efficacy in our rat pharmacodynamic model.

Pharmacokinetic Profile and Cardiovascular Safety Evaluation of Inhibitor 37 in Male Beagle Dogs. To study the cardiovascular profile of **37** in an anesthetized dog model, we first determined the pharmacokinetic parameters of this compound in dogs. Upon intravenous administration to male beagles, **37** displayed moderate clearance (0.23 L/h/kg) and V_{ss} (0.586 L/kg), resulting in a short half-life of 2.8 h (Table 8).

Table 8. Plasma Protein Binding and Pharmacokinetic Parameters of 37 in Male Beagle Dogs[a]

compd	$AUC_{0\to\infty}$ (ng·h/mL)	CL (L/h/kg)	V_{ss} (L/kg)	$t_{1/2}$ (h)	plasma protein binding (%)
37	11970	0.23	0.58	2.82	98.6

[a]Dosed iv at 2 mg/kg as a solution in 5% propylene glycol, 95% D5W at pH = 4.0.

On the basis of this information, compound **37** was administered intravenously as a series of three 60 min infusions at doses of 1.5, 5, and 20 mg/kg to two chloralose-anesthetized dogs; blood samples were taken throughout the infusion period to quantify exposure. At the end of each infusion period, the unbound plasma concentrations increased in a dose-dependent manner and were determined (average) to be 37.5, 219, and 793 nM, respectively. No significant effects on mean arterial pressure, heart rate, cardiac output, or systemic vascular resistance were observed by the end of the second infusion, at which point unbound plasma concentrations of **37** were ca. 16-fold over the effective unbound plasma concentration for a 50% reduction in CSF $A\beta$ in the acute rat pharmacodynamic model. Upon infusion of the high dose, concentrations of **37** were ca. 60-fold over the $EC_{50,unbound}$, and reductions in mean arterial pressure (15%) and systemic vascular resistance (43%) were observed along with reflex tachycardia (50%). The wider safety margin represented a significant improvement in CV safety for **37** compared to lead compound **1**.

CONCLUSIONS

Dual BACE1/CYP 3A4 inhibitors were prepared via modification of the hydroxyethylamine scaffold to incorporate *N*-heterocyclic or alkynyl groups at the 3-position of a P1-phenyl ring. α-Alkoxy acetamides bearing 2-thiazolyl or ethynyl groups on the P1-phenyl ring provided the best balance of functional potency, intrinsic metabolic stability, high passive permeability, and low efflux ratios. By virtue of their CYP 3A4-

inhibiting properties, these compounds exhibited good pharmacokinetic profiles in both rat and dog. When dosed orally in an acute rat pharmacodynamic model, several compounds were shown to produce robust decreases in CSF and brain $A\beta_{40}$ levels following a single 30 mg/kg dose. More rigorous dose−response efficacy studies demostrated that compounds **15** and **37** reduced CSF $A\beta_{40}$ levels by 50% at effective unbound plasma concentrations in the range of 6−13 nM. Compound **37** was further profiled in an anesthetized dog model for cardiovascular hazard identification and was found to produce no significant changes in cardiovascular function at concentrations exceeding 16-fold the effective unbound plasma concentration required for 50% reduction of CSF $A\beta_{40}$ levels in rats, a significant improvement over BACE1 inhibitors exemplified by **1**. Further studies describing pharmacodynamic and toxicology studies of these compounds will be reported in due course.

■ EXPERIMENTAL SECTION

BACE1 Enzymatic Assay. BACE1 enzymatic activity was determined by the enhancement of fluorescence intensity upon enzymatic cleavage of the fluorescence resonance energy transfer substrate. The BACE1 recognition and cleavage sequence of the substrate is derived from the reported literature,[18] and the fluorophore and quencher dyes are attached to side chain of Lys residues at the termini of the substrate peptide. The human recombinant BACE1[19] assay was performed in 50 mM acetate, pH 4.5/8% DMSO/100 μM Genepol/0.002% Brij-35. In dose−response IC_{50} assays, 10 point 1:3 serial dilutions of compound in DMSO were preincubated with the enzyme for 60 min at room temperature. Subsequently, the substrate was added to initiate the reaction. After 60 min at room temperature, the reaction was stopped by addition of 0.1 M Tris base to raise the pH above the enzyme active range, and the increase of fluorescence intensity was measured on Safire II microplate reader (Tecan, Männedorf, Switzerland).

Cell-Based Assay. Human embryonic kidney cells (HEK293) stably expressing APP_{SW} were plated at a density of 100K cells/well in 96-well plates (Costar). The cells were cultivated for 6 h at 37 °C and 5% CO_2 in DMEM supplemented with 10% FBS. Cells were incubated overnight with test compounds at concentrations ranging from 0.0005 to 10 μM. Following incubation with the test compounds, the conditioned media was collected and the Aβ40 levels were determined using a sandwich ELISA. The IC_{50} was calculated from the percent of control Aβ40 as a function of the concentration of the test compound. The sandwich ELISA to detect Aβ40 was performed in 96-well microtiter plates, which were precoated with goat antirabbit IgG (Pierce). The capture and detection antibody pair that was used to detect Aβ40 from cell supernatants consists of affinity purified pAβ40 (Invitrogen) and biotinylated 6E10 (Covance), respectively. Conditioned media was incubated with capture antibody overnight at 4 °C, followed by washing. The detecting antibody incubation was for 3 h at 4 °C, again followed by the wash steps as described previously. The plate was developed using Delfia reagents (Streptavidin−Europium and Enhancement solution (Perkin-Elmer)), and time-resolved fluorescence was measured on an EnVision multilabel plate reader (Perkin-Elmer).

Permeability Assay. The wild-type cell line LLC-PK1 (porcine renal epithelial cells, WT-LLC-PK1) was purchased from American Type Culture Collection (ATCC, Manassass, VA). Transfections of WT-LLC-PK1 cells with human *MDR*1 gene (hMDR1-LLC-PK1) and rat *mdr1a* gene (rMdr1a-LLC-PK1) were generated. Cells were grown in Medium 199 supplemented with 10% fetal bovine serum.[20] Cells were seeded onto matrigel-coated transwell filter membranes at a density of 90000 cells/well. Media change was performed on day 3. Compound incubations were performed 5−6 days post seeding. All cultures were incubated at 37 °C in a humidified (95% relative humidity) atmosphere of 5% CO_2/95% air.

Prior to the transport experiment,[21] culture medium was aspirated from both apical and basolateral wells and cells were rinsed with warmed (37 °C) Hank's balanced salt solution supplemented with 10 mM Hepes at pH 7.4 (HHBSS, Invitrogen, Grand Island, NY). HHBSS was removed from wells prior to dosing with test drugs at 5 μM in transport buffer (HHBSS containing 0.1% bovine serum albumin). Then 150 μL of transport buffer were added to receiver chambers prior to dosing in triplicate to apical or basolateral chambers. The dosed transwell plates containing the cell monolayers were incubated for 2 h at 37 °C on a shaking platform. At the end of the incubation period, 100 μL samples were collected from receiver reservoirs and analyzed by LC-MS/MS on an API4000 (Applied Biosystem, Foster City, CA) triple quadruple mass spectrometer interfaced with turbo IonSpray operated in positive mode using Analyst 1.4.2 software.

The apparent permeability coefficient (P_{app}) of all tested agents was estimated from the slope of a plot of cumulative amount of the agent versus time based on the following equation:

$$P_{app} = (dQ/dt)/(AC_0)$$

where dQ/dt is the penetration rate of the agent (ng/s), A is the surface area of the cell layer on the Transwell (0.11 cm^2), and C_0 is the initial concentration of the test compound (ng/mL). Efflux ratio (ER) was calculated from the basolateral-to-apical permeability divided by the apical-to-basolateral permeability: ER = P_{app} B > A/P_{app} A > B.

X-Ray Crystal Structure. The catalytic domain of human BACE1 (residues 14−453) was overexpressed in *Escherichia coli* and refolded from inclusion bodies following a procedure described previously.[22] Purified protein was concentrated to ∼8 mg/mL and 0.5 mM compound **37** was added to the sample, which was allowed to incubate on ice for 2 h. BACE1 was crystallized by hanging drop vapor diffusion at room temperature by mixing 1.0 uL of protein solution with 1.0 uL of reservoir solution containing 16% PEG 8000 and 0.1 M sodium citrate (pH 5.0). Diffraction data were collected at the Advanced Light Source, beamline 5.0.2, using λ = 1.0000 Å and an ADSC Q315R CCD detector. Data were reduced using HKL2000.[23] The crystals belong to the space group $P2_1$ with approximate unit cell dimensions of a = 82.7 Å, b = 104.8 Å, and c = 103.9 Å. The structure was solved by molecular replacement using PHASER[24] and employing a human BACE1 structure with the same space group (PDB 3DUY) as a search model. The structure was refined with REFMAC5, and model building was performed with COOT.[25,26] Data collection and refinement statistics appear in the Supporting Information.

Pharmacodynamic Assay. Male Sprague−Dawley rats (175−200 g) were purchased from Harlan and were maintained on a 12 h light/dark cycle with unrestricted access to food and water until use. Rats were administered compound by oral gavage at the appropriate dose. Rats were euthanized with CO_2 inhalation for 2 min, and cisterna magna was quickly exposed by removing the skin and muscle above it. CSF (50−100 μL) was collected with a 30 gauge needle through the dura membrane covering the cisterna magna. Blood was withdrawn by cardiac puncture and plasma obtained by centrifugation for drug exposures. Brains were removed and, along with the CSF, immediately frozen on dry ice and stored at −80 °C until use. The frozen brains were subsequently homogenized in 10 volumes of (w/v) of 0.5% Triton X-100 in TBS with protease inhibitors. The homogenates were centrifuged at 100000 rpm for 30 min at 4 °C. The supernatants were analyzed for Aβ40 levels by immunoassay as follows: Meso Scale 96-well avidin plates were coated with Biotin-4G8 (Covance) and detected with ruthenium-labeled Fab specific for Aβ40. Plates were read in MSD Sector6000 imager according to manufacturer's recommended protocol (Meso Scale Discovery, Inc.). Aβ40 concentrations were plotted using Graphpad Prism and analyzed by one-way ANOVA followed by Dunnett's multiple comparison analysis to compare drug-treated animals to vehicle-treated controls.

CYP3A4 Inhibition Assay. Pooled human liver microsomes (0.1 mg/mL) were incubated at 37 °C in a phosphate buffer (pH 7.4) with the selective CYP3A substrate midazolam at a concentration of 2.5 μM in the presence and absence of test compound (at about a 3 μM

concentration). The reaction was started with the addition of NADPH (1 mM final concentration). Incubations were stopped after 10 min by the addition of organic solvent and 1-hydroxymidazolam. Metabolite formation was measured by HPLC-MS. The ability of the test compound to inhibit the activity of CYP3A4 was determined. Estimated IC_{50}s were calculated based on the ratio of the concentration of metabolite in the presence of test compound to the concentration of metabolite in the absence of test compound.

Chemistry. Unless otherwise noted, all materials were obtained from commercial suppliers and used without further purification. Sulfinyl imine **2** and azachroman **6** were prepared as described in ref 9. Benzyl Grignard reagents were purchased from Rieke Metals, Inc. as solutions in ether. Anhydrous solvents were obtained from Aldrich or EM Science and used directly. All reactions involving air- or moisture-sensitive reagents were performed under a nitrogen or argon atmosphere. All microwave assisted reactions were conducted with a Smith synthesizer from Personal Chemistry, Uppsala, Sweden. Silica gel chromatography was performed using either glass columns packed with silica gel (230−400 mesh, EMD Chemicals, Gibbstown, NJ) or prepacked silica gel cartridges (Biotage or ISCO). ^{1}H NMR spectra were recorded on a Bruker AV-400 (400 MHz) spectrometer at ambient temperature or on a Varian 400 MHz spectrometer. Chemical shifts are reported in parts per million (ppm, δ units) downfield from tetramethylsilane. Data are reported as follows: chemical shift, multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, br = broad, m = multiplet), coupling constants, and number of protons. Purity for final compounds was greater than 95% unless otherwise noted and was measured using Agilent 1100 series high performance liquid chromatography (HPLC) systems with UV detection at 254 nm (system A, Agilent Zorbax Eclipse XDB-C8 4.6 mm ×150 mm, 5 μm, 5−100% CH_3CN in H_2O with 0.1% TFA for 15 min at 1.5 mL/min; system B, Waters Xterra 4.6 mm ×150 mm, 3.5 μm, 5−95% CH_3CN in H_2O with 0.1% TFA for 15 min at 1.0 mL/min). Exact mass confirmation was performed on an Agilent 1100 series high performance liquid chromatography (HPLC) system (Santa Clara, CA, USA) by flow injection analysis, eluting with a binary solvent system A and B (A, water with 0.1% FA; B, ACN with 0.1% FA) under isocratic conditions (50% A/50% B) at 0.2 mL/min with MS detection by an Agilent G1969A time-of-flight (TOF) mass spectrometer (Santa Clara, CA, USA).

(*S*)-*N*-((2*S*,3*S*)-1-(3-Bromophenyl)-3,4-bis(*tert*-butyldimethylsilyloxy)butan-2-yl)-2-methylpropane-2-sulfinamide (4a). A 2 L flask was charged with (*S*,*E*)-*N*-((*S*)-2,3-bis(*tert*-butyldimethylsilyloxy)propylidene)-2-methylpropane-2-sulfinamide (**2**) (50.00 g, 119 mmol) and THF (300 mL), and the resulting solution was cooled to −65 °C. (3-Bromobenzyl)magnesium chloride (948 mL of a 0.25 M solution in diethyl ether, 237 mmol) was added dropwise via cannula over 2.5 h such that the internal temperature did not rise above −60 °C. The resulting yellow slurry was stirred for 2 h at −65 °C, at which time TLC analysis indicated consumption of the starting material. The reaction mixture was warmed −25 °C, at which point a saturated aq ammonium chloride solution (200 mL) was added. The resulting white solids were dissolved upon the addition of water (150 mL). The mixture was warmed to room temperature, and the layers were separated. The aqueous layer was extracted with ethyl acetate (250 mL). The organic layers were combined, and the combined solution was dried over sodium sulfate, filtered, and concentrated to afford a yellow oil. The crude product was purified by chromatography on silica gel (0−10% EtOAc/hexane) to afford (*S*)-*N*-((2*S*,3*S*)-1-(3-bromophenyl)-3,4-bis(*tert*-butyldimethylsilyloxy)butan-2-yl)-2-methylpropane-2-sulfinamide (**4a**) as a pale-yellow oil that formed a waxy solid upon standing at room temperature. ^{1}H NMR (400 MHz, chloroform-*d*) δ ppm 7.29−7.36 (m, 2 H), 7.12 (ddd, *J* = 15.43, 7.65, 7.53 Hz, 2 H), 4.18 (ddd, *J* = 8.16, 5.90, 2.51 Hz, 1 H), 3.71−3.80 (m, 1 H), 3.67 (dd, *J* = 10.29, 5.77 Hz, 1 H), 3.49−3.60 (m, 2 H), 2.90 (dd, *J* = 14.05, 4.02 Hz, 1 H), 2.58 (dd, *J* = 13.80, 10.79 Hz, 1 H), 0.99 (s, 9 H), 0.94 (s, 9 H), 0.93 (s, 9 H), 0.18 (s, 3 H), 0.07−0.14 (m, 6 H), 0.01 (s, 3 H). MS *m*/*z* = 592.2 $[M + H]^+$. Calcd for $C_{26}H_{51}BrNO_3SSi_2$: 592.2.

(*S*)-*N*-((2*S*,3*S*)-3,4-Bis(*tert*-butyldimethylsilyloxy)-1-(3-chloro-5-fluorophenyl)butan-2-yl)-2-methylpropane-2-sulfinamide (4b). The title compound was prepared via a method analogous to the preparation of compound **4a** using (3-chloro-5-fluorobenzyl)-magnesium chloride, giving a pale-yellow oil (24.0 g, 71% yield). ^{1}H NMR (300 MHz, chloroform-*d*) δ ppm 6.96 (s, 1 H), 6.91 (dt, *J* = 8.40, 2.08 Hz, 1 H), 6.78 (dt, *J* = 9.21, 1.75 Hz, 1 H), 4.19 (ddd, *J* = 8.51, 5.88, 2.41 Hz, 1 H), 3.62−3.82 (m, 2 H), 3.43−3.62 (m, 2 H), 2.90 (dd, *J* = 14.10, 3.73 Hz, 1 H), 2.58 (dd, *J* = 14.10, 10.60 Hz, 1 H), 1.01 (s, 9 H), 0.94 (s, 9 H), 0.93 (s, 9 H), 0.19 (s, 3 H), 0.07−0.15 (m, 9 H).

(*S*)-*N*-((2*S*,3*S*)-3,4-Bis(*tert*-butyldimethylsilyloxy)-1-(3-chloro-4-fluorophenyl)butan-2-yl)-2-methylpropane-2-sulfinamide (4c). The title compound was prepared via a method analogous to the preparation of compound **4a** using (3-chloro-4-fluorobenzyl)-magnesium bromide, giving a pale yellow oil (1.4 g, 80% yield). ^{1}H NMR (300 MHz, chloroform-*d*) δ ppm 7.14−7.22 (m, 1 H), 6.96−7.08 (m, 2 H), 4.12−4.22 (m, 1 H), 3.51−3.79 (m, 4 H), 2.88 (dd, *J* = 14.25, 3.58 Hz, 1 H), 2.56 (dd, *J* = 14.10, 10.45 Hz, 1 H), 1.00 (s, 9 H), 0.94 (s, 9 H), 0.93 (s, 9 H), 0.18 (s, 3 H), 0.06−0.14 (m, 9 H).

(*S*)-*N*-((2*S*,3*S*)-3,4-Bis(*tert*-butyldimethylsilyloxy)-1-(5-chloro-2-fluorophenyl)butan-2-yl)-2-methylpropane-2-sulfinamide (4d). The title compound was prepared via a method analogous to the preparation of compound **4a** using (5-chloro-2-fluorobenzyl)-magnesium bromide, giving a pale-yellow oil (4.0 g, 63% yield). ^{1}H NMR (300 MHz, chloroform-*d*) δ ppm 7.05−7.19 (m, 2 H), 6.82−6.98 (m, 1 H), 4.22 (ddd, *J* = 8.37, 5.88, 2.27 Hz, 1 H), 3.75 (ddd, *J* = 10.19, 4.71, 2.34 Hz, 1 H), 3.47−3.72 (m, 3 H), 2.65−2.88 (m, 2 H), 1.27 (s, 9 H), 0.97 (s, 9 H), 0.94 (s, 9 H), 0.20 (s, 3 H), 0.08−0.14 (m, 9 H).

(*S*)-*N*-((2*S*,3*S*)-3,4-Bis(*tert*-butyldimethylsilyloxy)-1-(4-chlorophenyl)butan-2-yl)-2-methylpropane-2-sulfinamide (4e). The title compound was prepared via a method analogous to the preparation of compound **4a** using 4-chlorobenzylmagnesium chloride, giving a pale-yellow oil (86 g, 77% yield). ^{1}H NMR (400 MHz, chloroform-*d*) δ ppm 7.23 (d, *J* = 8.53 Hz, 2 H), 7.03−7.13 (m, 2 H), 4.12−4.21 (m, 1 H), 3.70−3.82 (m, 1 H), 3.67 (dd, *J* = 10.04, 5.52 Hz, 1 H), 3.48−3.61 (m, 2 H), 2.89 (dd, *J* = 14.56, 4.02 Hz, 1 H), 2.60 (dd, *J* = 14.05, 10.54 Hz, 1 H), 0.98 (s, 9 H), 0.94 (s, 9 H), 0.93 (s, 9 H), 0.18 (s, 3 H), 0.07−0.13 (m, 9 H).

(*S*)-*N*-((2*S*,3*S*)-3,4-Bis(*tert*-butyldimethylsilyloxy)-1-(3-chloro-2-fluorophenyl)butan-2-yl)-2-methylpropane-2-sulfinamide (4f). The title compound was prepared via a method analogous to the preparation of compound **4a** using (3-chloro-2-fluorobenzyl)-magnesium bromide, giving a pale-yellow oil (24.5 g, 77% yield). ^{1}H NMR (400 MHz, acetonitrile-d_3) δ ppm 7.29 (ddd, *J* = 7.97, 7.09, 1.76 Hz, 1 H), 7.14−7.22 (m, 1 H), 7.03−7.10 (m, 1 H), 4.13 (ddd, *J* = 7.87, 5.72, 2.15 Hz, 1 H), 3.59−3.77 (m, 4 H), 2.84−2.91 (m, 2 H), 0.94 (s, 9 H), 0.94 (s, 9 H), 0.90 (s, 9 H), 0.17 (s, 3 H), 0.13 (s, 3 H), 0.12 (s, 6 H).

***tert*-Butyl (2*S*,3*S*)-1-(3-Bromophenyl)-3-(*tert*-butyldimethylsilyloxy)-4 Hydroxybutan-2-ylcarbamate (5a).** A solution of (*S*)-*N*-((2*S*,3*S*)-1-(3-bromophenyl)-3,4-bis(*tert*-butyldimethylsilyloxy)-butan-2-yl)-2-methylpropane-2-sulfinamide (**4a**) (5.00 g, 8.43 mmol) in ethanol (60 mL) at −40 °C was added hydrogen chloride (10.5 mL of a 4N solution in 1,4-dioxane, 42.2 mmol) dropwise over 3 min such that the maintained the temperature did not rise above −35 °C. The reaction was briefly warmed to −5 °C to dissolve the white precipitate that had formed, and then the resulting homogeneous solution was maintained at −20 °C for 16 h. After this time, *N*,*N*-diisopropylethyl-amine (10.3 mL, 59.0 mmol) and di-*tert*-butyl dicarbonate (3.68 g, 16.9 mmol) were added in sequence, and the reaction was warmed to 23 °C. After stirring for 30 min, the reaction mixture was diluted with dichloromethane (100 mL) and poured into a 50% aq ammonium chloride solution (500 mL). The layers were separated, and the aqueous layer was extracted with dichloromethane (3 × 100 mL). The organic layers were combined, and the combined solution was washed with water (100 mL), washed with brine (100 mL), dried over sodium sulfate, filtered, and concentrated. Purification of the crude product by chromatography on silica gel (0−25% EtOAc/Hexane) afforded *tert*-butyl (2*S*,3*S*)-1-(3-bromophenyl)-3-(*tert*-butyldimethylsilyloxy)-4 hy-

droxybutan-2-ylcarbamate (**5a**) (2.75 g, 68.7% yield) as a colorless oil. ^{1}H NMR (400 MHz, chloroform-*d*) δ ppm 7.32−7.39 (m, 2 H), 7.09−7.20 (m, 2 H), 4.67 (d, *J* = 8.51 Hz, 1 H), 4.02−4.24 (m, 1 H), 3.93 (br s, 1 H), 3.69−3.77 (m, 2 H), 3.59−3.68 (m, 1 H), 2.99 (dd, *J* = 14.28, 4.40 Hz, 1 H), 2.69 (dd, *J* = 13.55, 9.54 Hz, 1 H), 1.37 (s, 9 H), 0.94 (s, 9 H), 0.11 (d, *J* = 1.86 Hz, 6 H).

***tert*-Butyl (2*S*,3*S*)-3-(*tert*-Butyldimethylsilyloxy)-1-(3-chloro-5-fluorophenyl)-4 Hydroxybutan-2-ylcarbamate (5b).** The title compound was prepared from **4b** via a method analogous to the preparation of compound **5a**, giving a colorless oil (36.0 g, 88% yield). ^{1}H NMR (300 MHz, chloroform-*d*) δ ppm 7.00 (s, 1 H), 6.94 (dt, *J* = 8.48, 2.05 Hz, 1 H), 6.84 (d, *J* = 9.35 Hz, 1 H), 4.68 (d, *J* = 8.92 Hz, 1 H), 3.99−4.24 (m, 1 H), 3.91 (tt, *J* = 9.59, 5.02 Hz, 1 H), 3.57−3.80 (m, 2 H), 2.99 (dd, *J* = 14.25, 4.17 Hz, 1 H), 2.60−2.75 (m, 1 H), 2.37 (br s, 1 H), 1.36 (s, 9 H), 1.19 (s, 9 H), 0.11 (s, 6 H).

***tert*-Butyl (2*S*,3*S*)-3-(*tert*-Butyldimethylsilyloxy)-1-(3-chloro-4-fluorophenyl)-4 Hydroxybutan-2-ylcarbamate (5c).** The title compound was prepared from **4c** via a method analogous to the preparation of compound **5a**, giving a colorless oil (0.49 g, 84% yield). ^{1}H NMR (300 MHz, chloroform-*d*) δ ppm 7.24 (d, *J* = 7.45 Hz, 1 H), 6.99−7.12 (m, 2 H), 4.67 (d, *J* = 8.77 Hz, 1 H), 3.83−3.97 (m, *J* = 9.37, 9.37, 4.71, 4.46 Hz, 1 H), 3.58−3.79 (m, 3 H), 2.98 (dd, *J* = 14.18, 4.24 Hz, 1 H), 2.65 (dd, *J* = 14.18, 10.08 Hz, 1 H), 2.40 (t, *J* = 6.07 Hz, 1 H), 1.36 (s, 9 H), 0.90−0.95 (m, 9 H), 0.07−0.15 (m, 6 H). MS *m*/*z* = 470.1 [M + Na]$^+$. Calcd for $C_{21}H_{35}ClFNNaO_4Si$: 470.1.

***tert*-Butyl (2*S*,3*S*)-3-(*tert*-Butyldimethylsilyloxy)-1-(5-chloro-2-fluorophenyl)-4 hydroxybutan-2-ylcarbamate (5d).** The title compound was prepared from **4d** via a method analogous to the preparation of compound **5a**, giving a colorless oil (2.9 g, 92% yield). ^{1}H NMR (300 MHz, chloroform-*d*) δ ppm 7.06−7.25 (m, 2 H), 6.97 (t, *J* = 8.99 Hz, 1 H), 4.77 (d, *J* = 9.06 Hz, 1 H), 4.00−4.31 (m, 1 H), 3.84−4.00 (m, 1 H), 3.56−3.85 (m, 2 H), 3.01 (dd, *J* = 14.47, 3.65 Hz, 1 H), 2.52−2.82 (m, 1 H), 2.32 (t, *J* = 6.14 Hz, 1 H), 1.36 (s, 9 H), 0.94 (s, 9 H), 0.12 (s, 6 H).

***tert*-Butyl (2*S*,3*S*)-3-(*tert*-Butyldimethylsilyloxy)-1-(4-chlorophenyl)-4 hydroxybutan-2-ylcarbamate (5e).** The title compound was prepared from **4e** via a method analogous to the preparation of compound **5a**, giving a colorless oil (17.8 g, 84% yield). ^{1}H NMR (400 MHz, chloroform-*d*) δ ppm 7.22−7.33 (m, 2 H), 7.14 (d, *J* = 8.41 Hz, 2 H), 4.66 (d, *J* = 8.41 Hz, 1 H), 3.87−4.01 (m, 1 H), 3.51−3.78 (m, 2 H), 2.98 (dd, *J* = 14.18, 4.40 Hz, 1 H), 2.68 (dd, *J* = 13.40, 10.07 Hz, 1 H), 2.39 (br s, 1 H), 1.31−1.42 (m, 9 H), 0.93 (s, 9 H), 0.11 (s, 6 H).

***tert*-Butyl (2*S*,3*S*)-3-(*tert*-Butyldimethylsilyloxy)-1-(3-chloro-2-fluorophenyl)-4 hydroxybutan-2-ylcarbamate (5f).** The title compound was prepared from **4f** via a method analogous to the preparation of compound **5a**, giving a colorless oil (17.1 g, 88% yield). ^{1}H NMR (400 MHz, acetonitrile-d_3) δ ppm 7.31 (t, *J* = 7.29 Hz, 1 H), 7.15−7.25 (m, 1 H), 7.01−7.12 (m, 1 H), 5.16 (d, *J* = 9.29 Hz, 1 H), 3.98−4.16 (m, 1 H), 3.84−3.97 (m, 1 H), 3.75 (q, *J* = 4.86 Hz, 1 H), 3.52−3.63 (m, 1 H), 3.03 (dd, *J* = 13.30, 2.93 Hz, 1 H), 2.87 (t, *J* = 5.82 Hz, 1 H), 2.66 (t, *J* = 12.52 Hz, 1 H), 1.27 (br s, 9 H), 0.93 (s, 9 H), 0.11 (s, 6 H).

***tert*-Butyl (2*S*,3*R*)-1-(3-Bromophenyl)-3-(*tert*-butyldimethylsilyloxy)-4-((*S*)-6′-neopentyl-3′,4′-dihydrospiro[cyclobutane-1,2′-pyrano[2,3-*b*]pyridine]-4′-ylamino)butan-2-ylcarbamate (7a).** A solution of *tert*-butyl (2*S*,3*S*)-1-(3-bromophenyl)-3-(*tert*-butyldimethylsilyloxy)-4-oxobutan-2-ylcarbamate (**5a**) (0.68 g, 1.4 mmol) and (*S*)-6′-neopentyl-3′,4′-dihydrospiro[cyclobutane-1,2′-pyrano[2,3-*b*]pyridin]-4′-amine (**6**) (0.37 g, 1.4 mmol) in dichloromethane (10 mL) was treated with trimethyl orthoformate (1.3 mL, 12 mmol). The solution was stirred for 1 h, then sodium triacetoxyborohydride (1.2 g, 5.8 mmol) was added, and the resulting mixture was stirred for an additional hour. The reaction mixture was diluted with dichloromethane (25 mL) and saturated aq Rochelle's salt solution (50 mL). The biphasic mixture was stirred vigorously for 1 h, and then the layers were separated. The aq layer was extracted with dichloromethane (2 × 25 mL). The organic extracts were combined, and the combined solution was washed with water, washed with brine, dried over sodium sulfate, filtered, and concentrated. The crude product was purified by chromatography on silica gel (0−30% EtOAc/Hexane) to afford *tert*-butyl (2*S*,3*R*)-1-(3-bromophenyl)-3-(*tert*-butyldimethylsilyloxy)-4-((*S*)-6′-neopentyl-3′,4′-dihydrospiro-[cyclobutane-1,2′-pyrano[2,3-*b*]pyridine]-4′-ylamino)butan-2-ylcarbamate (**7a**) (0.845 g, 82% yield) as a white solid. ^{1}H NMR (400 MHz, chloroform-*d*) δ ppm 7.89 (br s, 1 H), 7.57 (br s, 1 H), 7.37 (s, 2 H), 7.10−7.21 (m, 2 H), 3.75−4.10 (m, 3 H), 2.87−3.00 (m, 1 H), 2.81 (d, *J* = 7.82 Hz, 2 H), 2.50−2.66 (m, 1 H), 2.29−2.47 (m, 2 H), 2.17−2.29 (m, 1 H), 2.04−2.15 (m, 1 H), 1.92−2.03 (m, 1 H), 1.65−1.82 (m, 1 H), 1.39 (br s, 9 H), 0.90 (s, 18 H), 0.83−0.89 (m, 6 H), 0.07 (br s, 3 H), 0.05 (br s, 3 H). MS *m*/*z* = 716.2 [M + H]$^+$. Calcd for $C_{37}H_{59}BrN_3O_4Si$: 716.4.

***tert*-Butyl (2*S*,3*R*)-3-(*tert*-Butyldimethylsilyloxy)-1-(3-chloro-5-fluorophenyl)-4-((*S*)-6′-neopentyl-3′,4′-dihydrospiro[cyclobutane-1,2′-pyrano[2,3-*b*]pyridine]-4′-ylamino)butan-2-ylcarbamate (7b).** The title compound was prepared from **5b** and **6** via a method analogous to the preparation of **7a**, giving a white solid (2.3 g, 99% yield). MS *m*/*z* = 690.2 [M + H]$^+$. Calcd for $C_{37}H_{58}ClFN_3O_4Si$: 690.4.

***tert*-Butyl (2*S*,3*R*)-3-(*tert*-Butyldimethylsilyloxy)-1-(3-chloro-4-fluorophenyl)-4-((*S*)-6′-neopentyl-3′,4′-dihydrospiro[cyclobutane-1,2′-pyrano[2,3-*b*]pyridine]-4′-ylamino)butan-2-ylcarbamate (7c).** The title compound was prepared from **5c** and **6** via a method analogous to the preparation of **7a**, giving a white solid (3.8 g, 83% yield). MS *m*/*z* = 690.3 [M + H]$^+$. Calcd for $C_{37}H_{58}ClFN_3O_4Si$: 690.4.

***tert*-Butyl (2*S*,3*R*)-3-(*tert*-Butyldimethylsilyloxy)-1-(5-chloro-2-fluorophenyl)-4-((*S*)-6′-neopentyl-3′,4′-dihydrospiro[cyclobutane-1,2′-pyrano[2,3-*b*]pyridine]-4′-ylamino)butan-2-ylcarbamate (7d).** The title compound was prepared from **5d** and **6** via a method analogous to the preparation of **7a**, giving a white solid (0.13 g, 78% yield). MS *m*/*z* = 690.3 [M + H]$^+$. Calcd for $C_{37}H_{58}ClFN_3O_4Si$: 690.4.

***tert*-Butyl (2*S*,3*R*)-3-(*tert*-Butyldimethylsilyloxy)-1-(4-chlorophenyl)-4-((*S*)-6′-neopentyl-3′,4′-dihydrospiro[cyclobutane-1,2′-pyrano[2,3-*b*]pyridine]-4′-ylamino)butan-2-ylcarbamate (7e).** The title compound was prepared from **5e** and **6** via a method analogous to the preparation of **7a**, giving a white solid (0.50 g, 54% yield). MS *m*/*z* = 671.8 [M + H]$^+$. Calcd for $C_{37}H_{59}ClN_3O_4Si$: 672.4.

***tert*-Butyl (2*S*,3*R*)-3-(*tert*-Butyldimethylsilyloxy)-1-(3-chloro-2-fluorophenyl)-4-((*S*)-6′-neopentyl-3′,4′-dihydrospiro[cyclobutane-1,2′-pyrano[2,3-*b*]pyridine]-4′-ylamino)butan-2-ylcarbamate (7f).** The title compound was prepared from **5f** and **6** via a method analogous to the preparation of **7a**, giving a white solid (7.9 g, 72% yield). MS *m*/*z* = 690.1 [M + H]$^+$. Calcd for $C_{37}H_{58}ClFN_3O_4Si$: 690.4.

***N*-((2*S*,3*R*)-1-(3-Bromophenyl)-3-hydroxy-4-((*S*)-6′-neopentyl-3′,4′-dihydrospiro[cyclobutane-1,2′-pyrano[2,3-*b*]pyridine]-4′-ylamino)butan-2-yl)-2-methoxyacetamide (9a).** A solution of (2*S*,3*R*)-1-(3-bromophenyl)-3-(*tert*-butyldimethylsilyloxy)-4-((*S*)-6′-neopentyl-3′,4′-dihydrospiro[cyclobutane-1,2′-pyrano[2,3-*b*]pyridine]-4′-ylamino)butan-2-ylcarbamate (**7a**) (1.45 g, 2.00 mmol) in methanol (5 mL) was treated with hydrogen chloride (10 mL of a 4N solution in 1,4-dioxane, 40 mmol), and the resulting solution was stirred for 16 h. The reaction mixture was concentrated in vacuo, and the residue was taken up in dichloromethane (25 mL) and *N*,*N*-diisopropylethylamine (7.8 mL, 44 mmol) to give a clear solution. A solution of 1-(1*H*-imidazol-1-yl)-2-methoxyethanone (1.0 mL, 2.0 mmol) in dichloromethane (10 mL) was added, and the reaction mixture was to stirred at room temperature for 15 h. The reaction mixture was washed with a saturated aq sodium bicarbonate solution (50 mL), the layers were separated, and the aqueous layer was extracted with dichloromethane (2 × 25 mL). The organic layers were combined, and the combined solution was washed with water (50 mL), washed with brine (50 mL), dried over sodium sulfate, filtered, and concentrated. The crude product was purified by chromatography on silica gel (0−5% MeOH/DCM) to afford *N*-((2*S*,3*R*)-1-(3-bromophenyl)-3-hydroxy-4-((*S*)-6′-neopentyl-3′,4′-dihydrospiro-[cyclobutane-1,2′-pyrano[2,3-*b*]pyridine]-4′-ylamino)butan-2-yl)-2-methoxyacetamide (**9a**) (1.090 g, 94% yield) as a white solid. ^{1}H NMR (400 MHz, acetonitrile-d_3) δ ppm 7.79 (d, *J* = 1.76 Hz, 1 H), 7.70 (dd, *J* = 2.40, 1.03 Hz, 1 H), 7.40−7.45 (m, 1 H), 7.32−7.39 (m,

1 H), 7.17–7.24 (m, 2 H), 6.89 (d, *J* = 10.17 Hz, 1 H), 4.02–4.13 (m, 1 H), 3.90 (dd, *J* = 10.47, 5.48 Hz, 1 H), 3.53–3.79 (m, 4 H), 3.36–3.46 (m, 1 H), 3.25 (s, 2 H), 3.09 (dd, *J* = 14.04, 3.86 Hz, 1 H), 2.65–2.82 (m, 3 H), 2.43 (s, 4 H), 2.32–2.42 (m, 1 H), 2.17–2.24 (m, 1 H), 2.08–2.13 (m, 1 H), 1.87 (br s, 1 H), 1.71–1.79 (m, 1 H), 1.65 (ddd, *J* = 13.25, 10.51, 1.17 Hz, 1 H), 0.88 (s, 9 H). MS *m/z* = 574.2 [M + H]$^+$. Calcd for $C_{29}H_{41}BrN_3O_4$: 574.2.

***N*-((2*S*,3*R*)-1-(3-Chloro-5-fluorophenyl)-3-hydroxy-4-((*S*)-6′-neopentyl-3′,4′-dihydrospiro[cyclobutane-1,2′-pyrano[2,3-*b*]pyridine]-4′-ylamino)butan-2-yl)-2-methoxyacetamide (9b).** The title compound was prepared from **7b** via a method analogous to the preparation of **9a**, giving a white solid (0.64 g, 85% yield).

***N*-((2*S*,3*R*)-1-(3-Chloro-4-fluorophenyl)-3-hydroxy-4-((*S*)-6′-neopentyl-3′,4′-dihydrospiro[cyclobutane-1,2′-pyrano[2,3-*b*]pyridine]-4′-ylamino)butan-2-yl)-2-methoxyacetamide (9c).** The title compound was prepared from **7c** via a method analogous to the preparation of **9a**, giving a white solid (0.87 g, 64% yield). MS *m/z* = 548.2 [M + H]$^+$. Calcd for $C_{29}H_{40}ClFN_3O_4$: 548.3.

***N*-((2*S*,3*R*)-1-(5-Chloro-2-fluorophenyl)-3-hydroxy-4-((*S*)-6′-neopentyl-3′,4′-dihydrospiro[cyclobutane-1,2′-pyrano[2,3-*b*]pyridine]-4′-ylamino)butan-2-yl)-2-methoxyacetamide (9d).** The title compound was prepared from **7d** via a method analogous to the preparation of **9a**, giving a yellow glass (1.2 g, 61% yield). MS *m/z* = 548.1 [M + H]$^+$. Calcd for $C_{29}H_{40}ClFN_3O_4$: 548.3.

***N*-((2*S*,3*R*)-1-(4-Chlorophenyl)-3-hydroxy-4-((*S*)-6′-neopentyl-3′,4′-dihydrospiro[cyclobutane-1,2′-pyrano[2,3-*b*]pyridine]-4′-ylamino)butan-2-yl)-2-methoxyacetamide (9e).** The title compound was prepared from **7e** via a method analogous to the preparation of **9a**, giving a light-yellow solid. (0.81 g, 70% yield). MS *m/z* = 530.2 [M + H]$^+$. Calcd for $C_{29}H_{41}ClN_3O_4$: 530.3.

***tert*-Butyl (2*S*,3*R*)-3-(*tert*-Butyldimethylsilyloxy)-1-(2-fluoro-3-(thiazol-2-yl)phenyl)-4-((*S*)-6′-neopentyl-3′,4′-dihydrospiro[cyclobutane-1,2′-pyrano[2,3-*b*]pyridine]-4′-ylamino)butan-2-ylcarbamate (10g).** A solution of *tert*-butyl (2*S*,3*R*)-3-(*tert*-butyldimethylsilyloxy)-1-(3-chloro-2-fluorophenyl)-4-((*S*)-6′-neopentyl-3′,4′-dihydrospiro[cyclobutane-1,2′-pyrano[2,3-*b*]pyridine]-4′-ylamino)butan-2-ylcarbamate (**7f**) (3.97 g, 5.75 mmol), dicyclohexyl-(2′,4′,6′-triisopropylbiphenyl-2-yl)phosphine (1.64 g, 3.45 mmol), and $Pd_2(dba)_3$ (0.79 g, 0.86 mmol) in THF (15 mL) was degassed by sparging with N_2 (g) for 15 min. 2-(Tributylstannyl)thiazole (3.23 g, 8.63 mmol) was added, and the resulting mixture was heated to 135 °C for 40 min in a microwave reactor. The reaction mixture was cooled and filtered through a plug of silica gel with the aid of ethyl acetate. The filtrate was concentrated, and the residue was purified by chromatography on silica gel (10–60% EtOAc/hexane) to give *tert*-butyl (2*S*,3*R*)-3-(*tert*-butyldimethylsilyloxy)-1-(2-fluoro-3-(thiazol-2-yl)phenyl)-4-((*S*)-6′-neopentyl-3′,4′-dihydrospiro[cyclobutane-1,2′-pyrano[2,3-*b*]pyridine]-4′-ylamino)butan-2-ylcarbamate (**10g**) as a white solid (3.90 g, 92% yield). ^{1}H NMR (400 MHz, acetonitrile-d_3) δ ppm 8.04–8.17 (m, 1 H), 7.87–7.98 (m, 1 H), 7.80 (s, 1 H), 7.66 (s, 1 H), 7.63 (d, *J* = 3.33 Hz, 1 H), 7.36 (t, *J* = 8.17 Hz, 1 H), 7.22 (t, *J* = 7.68 Hz, 1 H), 5.51 (d, *J* = 8.61 Hz, 1 H), 4.01–4.25 (m, 1 H), 3.84–3.97 (m, 2 H), 3.07 (dd, *J* = 14.33, 4.65 Hz, 1 H), 2.94 (dd, *J* = 12.62, 3.91 Hz, 1 H), 2.69–2.82 (m, 2 H), 2.33–2.41 (m, 2 H), 2.21–2.33 (m, 1 H), 2.07–2.21 (m, 2 H), 1.84–1.93 (m, 1 H), 1.69–1.82 (m, 1 H), 1.60 (br s, 1 H), 1.22 (s, 9 H), 0.92 (s, 9 H), 0.85 (s, 9 H), 0.10 (s, 3 H), 0.09 (s, 3 H). MS *m/z* = 739.2 [M + H]$^+$. Calcd for $C_{40}H_{60}FN_4O_4SSi$: 739.4.

***tert*-Butyl (2*S*,3*R*)-3-(*tert*-Butyldimethylsilyloxy)-1-(2-fluoro-3-((trimethylsilyl)ethynyl)phenyl)-4-((*S*)-6′-neopentyl-3′,4′-dihydrospiro[cyclobutane-1,2′-pyrano[2,3-*b*]pyridine]-4′-ylamino)butan-2-ylcarbamate (10h).** A solution of *tert*-butyl (2*S*,3*R*)-3-(*tert*-butyldimethylsilyloxy)-1-(3-chloro-2-fluorophenyl)-4-((*S*)-6′-neopentyl-3′,4′-dihydrospiro[cyclobutane-1,2′-pyrano[2,3-*b*]-pyridine]-4′-ylamino)butan-2-ylcarbamate (**7f**) (3.97 g, 5.75 mmol), dicyclohexyl(2′,4′,6′-triisopropylbiphenyl-2-yl)phosphine (1.64 g, 3.45 mmol), and $Pd_2(dba)_3$ (0.79 g, 0.86 mmol) in THF (15 mL) was degassed by sparging with N_2 (g) for 15 min. Trimethyl(2-(tributylstannyl)ethynyl)silane (3.34 g, 8.63 mmol) was added, and the resulting mixture was heated to 135 °C for 40 min in a microwave reactor. The reaction mixture was cooled and filtered through a plug of silica gel with the aid of ethyl acetate. The filtrate was concentrated, and the residue was purified by chromatography on silica gel (10–60% EtOAc/hexane) to give *tert*-butyl (2*S*,3*R*)-3-(*tert*-butyldimethylsilyloxy)-1-(2-fluoro-3-((trimethylsilyl)ethynyl)phenyl)-4-((*S*)-6′-neopentyl-3′,4′-dihydrospiro[cyclobutane-1,2′-pyrano[2,3-*b*]pyridine]-4′-ylamino)butan-2-ylcarbamate as a white solid (3.67 g, 85% yield). ^{1}H NMR (400 MHz, acetonitrile-d_3) δ ppm 7.81 (d, *J* = 2.15 Hz, 1 H), 7.65 (s, 1 H), 7.19–7.33 (m, 2 H), 7.06 (t, *J* = 7.63 Hz, 1 H), 5.42 (d, *J* = 9.39 Hz, 1 H), 3.96–4.09 (m, 1 H), 3.78–3.96 (m, 2 H), 2.86–3.06 (m, 2 H), 2.68–2.83 (m, 1 H), 2.63 (t, *J* = 13.01 Hz, 1 H), 2.32–2.42 (m, 2 H), 2.20–2.32 (m, 1 H), 2.04–2.20 (m, 2 H), 1.84–1.93 (m, 1 H), 1.69–1.82 (m, 2 H), 1.23–1.32 (m, 9 H), 0.90–0.98 (m, 9 H), 0.83–0.90 (m, 9 H), 0.20–0.30 (m, 9 H), 0.10 (s, 3 H), 0.09 (s, 3 H).

***tert*-Butyl (2*S*,3*R*)-3-(*tert*-Butyldimethylsilyloxy)-4-((*S*)-6′-neopentyl-3′,4′-dihydrospiro[cyclobutane-1,2′-pyrano[2,3-*b*]pyridine]-4′-ylamino)-1-(4-((trimethylsilyl)ethynyl)phenyl)-butan-2-ylcarbamate (10i).** The title compound was prepared from **7e** via a method analogous to the preparation of **10h**, giving a white solid (0.30 g, 93% yield). ^{1}H NMR (400 MHz, acetonitrile-d_3) δ ppm 7.81 (br s, 1 H), 7.54–7.68 (m, 1 H), 7.33 (d, *J* = 8.02 Hz, 2 H), 7.21 (d, *J* = 8.12 Hz, 2 H), 5.57 (d, *J* = 10.27 Hz, 1 H), 3.92–4.03 (m, 1 H), 3.79–3.92 (m, 2 H), 2.84–2.98 (m, 2 H), 2.67–2.75 (m, 1 H), 2.57–2.67 (m, 1 H), 2.31–2.40 (m, 3 H), 2.22–2.31 (m, 1 H), 2.14–2.23 (m, 3 H), 1.83–1.92 (m, 1 H), 1.77 (d, *J* = 2.54 Hz, 2 H), 1.30 (s, 9 H), 0.90 (s, 9 H), 0.88 (s, 9 H), 0.22 (s, 9 H), 0.04–0.09 (m, 6 H). *m/z* = 734.4 [M + H]$^+$. Calcd for $C_{42}H_{68}N_3O_4Si_2$: 734.5.

***N*-((2*S*,3*R*)-3-Hydroxy-4-((*S*)-6′-neopentyl-3′,4′-dihydrospiro[cyclobutane-1,2′-pyrano[2,3-*b*]pyridine]-4′-ylamino)-1-(3-(pyridin-4-yl)phenyl)butan-2-yl)-2-methoxyacetamide (11).** A solution of *N*-((2*S*,3*R*)-1-(3-bromophenyl)-3-hydroxy-4-((*S*)-6′-neopentyl-3′,4′-dihydrospiro[cyclobutane-1,2′-pyrano[2,3-*b*]pyridine]-4′-ylamino)butan-2-yl)-2-methoxyacetamide (**9a**) (0.30 g, 0.52 mmol), 4-(tributylstannyl)pyridine (0.21 g, 0.57 mmol), and tetrakis-(tripheynlphosphine)palladium (0.12 g, 0.10 mmol) in 1,4-dioxane (5 mL) was heated to 140 °C for 30 min in a microwave reactor. The reaction mixture was cooled, stirred with 10% aq potassium fluoride solution (10 mL), and extracted with EtOAc (3 × 10 mL). The organic layers were combined, and the combined solution was washed with brine, dried over magnesium sulfate, filtered, and concentrated. The residue was purified by reverse-phase chromatography (10–90% CH_3CN/H_2O with 0.1% TFA). Pure fractions containing product were combined in saturated aq sodium bicarbonate solution and extracted with DCM. The organic extract was dried over sodium sulfate, filtered, and evaporated to give *N*-((2*S*,3*R*)-3-hydroxy-4-((*S*)-6′-neopentyl-3′,4′-dihydrospiro[cyclobutane-1,2′-pyrano[2,3-*b*]pyridine]-4′-ylamino)-1-(3-(pyridin-4-yl)phenyl)butan-2-yl)-2-methoxyacetamide (**11**) (0.105 g, 35% yield) as a white solid. ^{1}H NMR (400 MHz, acetonitrile-d_3) δ ppm 8.61 (d, *J* = 6.16 Hz, 2 H), 7.79 (d, *J* = 2.25 Hz, 1 H), 7.70 (dd, *J* = 2.40, 1.03 Hz, 1 H), 7.54–7.61 (m, 4 H), 7.41 (dt, *J* = 1.00, 7.62 Hz, 1 H), 7.33 (dt, *J* = 7.53, 1.00 Hz, 1 H), 6.93 (d, *J* = 9.39 Hz, 1 H), 4.11–4.25 (m, 1 H), 3.91 (dd, *J* = 10.51, 5.53 Hz, 1 H), 3.74 (dd, *J* = 15.16 Hz, 1 H), 3.60–3.65 (m, 1 H), 3.58 (dd, *J* = 15.20 Hz, 1 H), 3.14–3.23 (m, 3 H), 2.76–2.89 (m, 2 H), 2.67–2.75 (m, 1 H), 2.32–2.47 (m, 4 H), 2.06–2.26 (m, 6 H), 1.81–1.91 (m, 1 H), 1.61–1.78 (m, 2 H), 0.86 (s, 9 H). HRMS (ESI+) *m/z* = 573.3427 [M + H]$^+$. Calcd for $C_{34}H_{44}N_4O_4$: 573.3425.

***N*-((2*S*,3*R*)-3-Hydroxy-4-((*S*)-6′-neopentyl-3′,4′-dihydrospiro[cyclobutane-1,2′-pyrano[2,3-*b*]pyridine]-4′-ylamino)-1-(3-(pyridin-2-yl)phenyl)butan-2-yl)-2-methoxyacetamide (12).** The title compound was prepared from **9a** and 2-(tributylstannyl)-pyridine via a method analogous to the preparation of **12**, giving a white solid (0.088 g, 32% yield). ^{1}H NMR (300 MHz, chloroform-*d*) δ ppm 8.69 (dt, *J* = 4.75, 1.35 Hz, 1 H), 7.82–7.99 (m, 3 H), 7.64–7.80 (m, 2 H), 7.56 (d, *J* = 1.61 Hz, 1 H), 7.42 (t, *J* = 7.60 Hz, 1 H), 7.18–7.35 (m, 2 H), 6.62 (d, *J* = 9.06 Hz, 1 H), 4.09–4.28 (m, *J* = 8.51, 8.20, 8.20, 4.46 Hz, 1 H), 3.89–3.99 (m, 1 H), 3.66–3.89 (m, 2 H), 3.47–3.62 (m, 1 H), 3.16–3.29 (m, 4 H), 2.94–3.07 (m, 1 H), 2.83 (dd, *J* = 12.35, 4.75 Hz, 1 H), 2.65 (dd, *J* = 12.42, 3.95 Hz, 1 H), 2.54 (t, *J* = 10.23 Hz, 1 H), 2.41 (s, 2 H), 2.33 (dd, *J* = 13.15, 5.55 Hz, 1 H), 2.12–2.28 (m, 1 H), 2.06 (ddd, *J* = 7.71, 4.24, 3.98 Hz, 1 H), 1.87–

1.99 (m, 1 H), 1.60−1.86 (m, 3 H), 0.89 (s, 9 H). HRMS (ESI+) *m/z* = 573.3439 [M + H]$^+$. Calcd for $C_{34}H_{45}N_4O_4$: 573.3425.

***N*-((2*S*,3*R*)-3-Hydroxy-4-((*S*)-6′-neopentyl-3′,4′-dihydrospiro[cyclobutane-1,2′-pyrano[2,3-*b*]pyridine]-4′-ylamino)-1-(3-(oxazol-2-yl)phenyl)butan-2-yl)-2-methoxyacetamide (13).** A solution of *N*-((2*S*,3*R*)-1-(3-bromophenyl)-3-hydroxy-4-((*S*)-6′-neopentyl-3′,4′-dihydrospiro[cyclobutane-1,2′-pyrano[2,3-*b*]pyridine]-4′-ylamino)butan-2-yl)-2-methoxyacetamide (**9a**) (0.28 g, 0.50 mmol), 2-(tributylstannyl)oxazole (0.20 g, 0.55 mmol), and tetrakis-(tripheynlphosphine)palladium (0.12 g, 0.10 mmol) in 1,4-dioxane (5 mL) was heated to 140 °C for 30 min in a microwave reactor. The reaction mixture was cooled, stirred with 10% aq potassium fluoride solution (10 mL), and extracted with EtOAc (3 × 10 mL). The organic layers were combined, and the combined solution was washed with brine, dried over magnesium sulfate, filtered, and concentrated. The residue was purified by reverse-phase chromatography (10−90% CH_3CN/H_2O with 0.1% TFA). Pure fractions containing product were combined in saturated aq sodium bicarbonate solution and extracted with DCM. The organic extract was dried over sodium sulfate, filtered, and evaporated to give *N*-((2*S*,3*R*)-3-hydroxy-4-((*S*)-6′-neopentyl-3′,4′-dihydrospiro[cyclobutane-1,2′-pyrano[2,3-*b*]pyridine]-4′-ylamino)-1-(3-(oxazol-2-yl)phenyl)butan-2-yl)-2-methoxyacetamide (**13**) (0.065 g, 23% yield) as a white solid. MS (ESI+) *m/z* = 563.3226 [M + H]$^+$. Calcd for $C_{32}H_{43}N_4O_5$: 563.3218.

***N*-((2*S*,3*R*)-3-Hydroxy-1-(3-(1-methyl-1*H*-imidazol-2-yl)-phenyl)-4-((*S*)-6′-neopentyl-3′,4′-dihydrospiro[cyclobutane-1,2′-pyrano[2,3-*b*]pyridine]-4′-ylamino)butan-2-yl)-2-methoxyacetamide (14).** A solution of *N*-((2*S*,3*R*)-1-(3-bromophenyl)-3-hydroxy-4-((*S*)-6′-neopentyl-3′,4′-dihydrospiro[cyclobutane-1,2′-pyrano[2,3-*b*]pyridine]-4′-ylamino)butan-2-yl)-2-methoxyacetamide (**9a**) (0.30 g, 0.52 mmol), dicyclohexyl(2′,4′,6′-triisopropylbiphenyl-2-yl)phosphine (0.075 g, 0.157 mmol), $Pd_2(dba)_3$ (0.79 g, 0.86 mmol), and cesium fluoride (0.397 g, 2.61 mmol) in 1,4-dioxane (4 mL) was degassed by sparging with N_2 (g) for 15 min. 1-Methyl-2-(tributylstannyl)-1*H*-imidazole (0.484 g, 1.31 mmol) was added, and the mixture was heated to 160 °C for 1 h in a microwave reactor. The reaction mixture was cooled to room temperature and filtered through a silica gel plug with 10% MeOH/EtOAc. The filtrate was concentrated, and the residue was purified by reverse-phase chromatography (10−90% CH_3CN/H_2O with 0.1% TFA). Pure fractions containing product were combined in saturated aq sodium bicarbonate solution and extracted with DCM. The organic extract was dried over sodium sulfate, filtered, and evaporated to give *N*-((2*S*,3*R*)-3-hydroxy-1-(3-(1-methyl-1*H*-imidazol-2-yl)phenyl)-4-((*S*)-6′-neopentyl-3′,4′-dihydrospiro[cyclobutane-1,2′-pyrano[2,3-*b*]pyridine]-4′-ylamino)butan-2-yl)-2-methoxyacetamide (**14**) (0.12 g, 40% yield) as a white solid. ^{1}H NMR (400 MHz, acetonitrile-d_3) δ ppm 7.81 (s, 1 H), 7.74 (s, 1 H), 7.36 (t, *J* = 7.34 Hz, 2 H), 7.25−7.32 (m, 2 H), 7.23 (d, *J* = 7.73 Hz, 2 H), 6.91 (d, *J* = 9.19 Hz, 1 H), 4.12−4.22 (m, 1 H), 3.96 (dd, *J* = 9.24, 5.04 Hz, 1 H), 3.74 (d, *J* = 15.26 Hz, 1 H), 3.64 (s, 1 H), 3.62 (d, *J* = 1.56 Hz, 1 H), 3.56−3.61 (m, 1 H), 3.20 (s, 3 H), 3.14−3.19 (m, 1 H), 2.70−2.86 (m, 5 H), 2.35−2.46 (m, 7 H), 2.20−2.25 (m, 2 H), 1.83−1.90 (m, 1 H), 1.63−1.76 (m, 2 H), 0.87 (s, 9 H). HRMS (ESI+) *m/z* = 576.3555 [M + H]$^+$. Calcd for $C_{33}H_{45}N_5O_4$: 576.3534.

***N*-((2*S*,3*R*)-3-Hydroxy-4-((*S*)-6′-neopentyl-3′,4′-dihydrospiro[cyclobutane-1,2′-pyrano[2,3-*b*]pyridine]-4′-ylamino)-1-(3-(thiazol-2-yl)phenyl)butan-2-yl)-2-methoxyacetamide (15).** The title compound was prepared from **9a** and 2-(tributylstannyl)-thiazole via a method analogous to the preparation of **14**, giving a white solid (0.066 g, 22% yield). ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 7.76 (d, *J* = 2.15 Hz, 1 H), 7.67 (d, *J* = 1.66 Hz, 1 H), 7.63 (d, *J* = 9.49 Hz, 1 H), 7.14−7.31 (m, 6 H), 4.92 (d, *J* = 5.77 Hz, 1 H), 4.10 (s, 1 H), 4.09 (dd, *J* = 13.89, 3.62 Hz, 1 H), 3.90−3.98 (m, 1 H), 3.72−3.80 (m, 1 H), 3.68 (dt, *J* = 7.90, 6.52 Hz, 1 H), 3.51−3.60 (m, 1 H), 3.30 (s, 1 H), 3.17 (d, *J* = 5.28 Hz, 1 H), 3.01 (dd, *J* = 13.89, 3.62 Hz, 1 H), 2.64−2.73 (m, 1 H), 2.53−2.64 (m, 1 H), 2.38 (s, 3 H), 2.28−2.36 (m, 1 H), 2.13−2.24 (m, 1 H) 2.04−2.13 (m, 1 H), 1.90−2.03 (m, 1 H), 1.83 (dd, *J* = 10.12, 7.09 Hz, 1 H), 1.58−1.77 (m, 4 H), 0.85 (s, 9 H). HRMS (ESI+) *m/z* = 579.2992 [M + H]$^+$. Calcd for $C_{32}H_{43}N_4O_4S$: 579.2989.

***N*-((2*S*,3*R*)-3-Hydroxy-4-((*S*)-6′-neopentyl-3′,4′-dihydrospiro[cyclobutane-1,2′-pyrano[2,3-*b*]pyridine]-4′-ylamino)-1-(3-(thiazol-5-yl)phenyl)butan-2-yl)-2-methoxyacetamide (16).** The title compound was prepared from **9a** and 5-thiazolyltributylstannane via a method analogous to the preparation of **14**, giving a white solid (0.16 g, 55% yield). ^{1}H NMR (400 MHz, acetonitrile-d_3) δ ppm 7.86 (t, *J* = 1.57 Hz, 1 H), 7.85 (d, *J* = 3.23 Hz, 1 H), 7.79−7.83 (m, 2 H), 7.73−7.75 (m, 1 H), 7.50 (d, *J* = 3.33 Hz, 1 H), 7.38 (t, *J* = 7.58 Hz, 1 H), 7.30−7.34 (m, 1 H), 6.96 (d, *J* = 9.68 Hz, 1 H), 4.10−4.20 (m, 1 H), 4.00−4.09 (m, 1 H), 3.74 (d, *J* = 15.26 Hz, 1 H), 3.64−3.71 (m, 1 H), 3.59 (d, *J* = 15.26 Hz, 1 H), 3.16−3.23 (m, 4 H), 2.78−2.88 (m, 2 H), 2.36−2.49 (m, 3 H), 2.07−2.26 (m, 5 H), 1.84 (s, 1 H), 1.67−1.79 (m, 2 H), 1.18 (s, 2 H), 0.87 (s, 9 H). HRMS (ESI+) *m/z* = 579.3001 [M + H]$^+$. Calcd for $C_{32}H_{43}N_4O_4S$: 579.2989.

***N*-((2*S*,3*R*)-3-Hydroxy-4-((*S*)-6′-neopentyl-3′,4′-dihydrospiro[cyclobutane-1,2′-pyrano[2,3-*b*]pyridine]-4′-ylamino)-1-(3-(thiazol-4-yl)phenyl)butan-2-yl)-2-methoxyacetamide (17).** The title compound was prepared from **9a** and 4-(tributylstannyl)-thiazole via a method analogous to the preparation of **14**, giving a white solid (0.078 g, 44% yield). ^{1}H NMR (400 MHz, acetonitrile-d_3) δ ppm 7.87 (t, *J* = 1.52 Hz, 1 H), 7.77−7.82 (m, 3 H), 7.75 (d, *J* = 1.96 Hz, 1 H), 7.71 (dd, *J* = 2.35, 0.98 Hz, 1 H), 7.35 (t, *J* = 7.68 Hz, 1 H), 7.22 (ddd, *J* = 7.80, 1.34, 1.12 Hz, 1 H), 6.93 (d, *J* = 9.49 Hz, 1 H), 4.11−4.20 (m, 1 H), 3.93 (dd, *J* = 10.32, 5.43 Hz, 1 H), 3.72 (d, *J* = 15.16 Hz, 1 H), 3.61−3.65 (m, 1 H), 3.52 (d, *J* = 15.20 Hz, 1 H), 3.14−3.21 (m, 4 H), 2.78−2.85 (m, 2 H), 2.70−2.76 (m, 1 H), 2.35−2.46 (m, 4 H), 2.07−2.25 (m, 5 H), 1.80−1.91 (m, 1 H), 1.62−1.78 (m, 2 H), 0.87 (s, 9 H). HRMS (ESI+) *m/z* = 579.2998 [M + H]$^+$. Calcd for $C_{32}H_{43}N_4O_4S$: 579.2989.

***N*-((2*S*,3*R*)-3-Hydroxy-1-(3-(4-methylthiazol-2-yl)phenyl)-4-((*S*)-6′-neopentyl-3′,4′-dihydrospiro[cyclobutane-1,2′-pyrano-[2,3-*b*]pyridine]-4′-ylamino)butan-2-yl)-2-methoxyacetamide (18).** The title compound was prepared from **9a** and 4-methyl-2-(tributylstannyl)thiazole via a method analogous to the preparation of **14**, giving a white solid (0.097 g, 38% yield). ^{1}H NMR (400 MHz, acetonitrile-d_3) δ ppm 7.80 (ddd, *J* = 3.45, 1.91, 1.79 Hz, 2 H), 7.75 (dt, *J* = 7.63, 1.52 Hz, 1 H), 7.71 (dd, *J* = 2.25, 0.78 Hz, 1 H), 7.36 (t, *J* = 7.63 Hz, 1 H), 7.28−7.32 (m, 1 H), 7.04 (d, *J* = 1.08 Hz, 1 H), 6.96 (d, *J* = 9.59 Hz, 1 H), 4.09−4.19 (m, 1 H), 3.95 (dd, *J* = 10.71, 5.72 Hz, 1 H), 3.74 (d, *J* = 15.25 Hz, 1 H), 3.61−3.66 (m, 1 H), 3.58 (d, *J* = 15.26 Hz, 1 H), 3.21 (s, 3 H), 3.20−3.23 (m, 1 H), 3.18 (dd, *J* = 14.04, 3.86 Hz, 1 H), 2.77−2.86 (m, 2 H), 2.70−2.77 (m, 1 H), 2.35−2.45 (m, 7 H), 2.09−2.26 (m, 4 H), 1.81−1.90 (m, 1 H), 1.63−1.79 (m, 2 H), 0.86 (s, 9 H). HRMS (ESI+) *m/z* = 593.3163 [M + H]$^+$. Calcd for $C_{33}H_{45}N_4O_4S$: 593.3146.

***N*-((2*S*,3*R*)-1-(3-Ethynylphenyl)-3-hydroxy-4-((*S*)-6′-neopentyl-3′,4′-dihydrospiro[cyclobutane-1,2′-pyrano[2,3-*b*]-pyridine]-4′-ylamino)butan-2-yl)-2-methoxyacetamide (19).** *n*-Butyllithium (1.96 mL of a 2.5 M solution in hexane, 4.9 mmol) was added dropwise to a solution of ethynltrimethylsilane (0.68 mL, 4.9 mmol) in THF (1 mL) at −78 °C, and the resulting mixture was stirred for 15 min. A solution of zinc chloride (4.9 mL of a 1.0 M solution in THF, 4.9 mmol) wass added dropwise. The mixture was warmed to room temperature and stirred for 30 min. This mixture was added to a vessel containing $Pd_2(dba)_3$ (0.032 mg, 0.035 mmol), dicyclohexyl(2′,4′,6′-triisopropylbiphenyl-2-yl)phosphine (0.10 g, 0.21 mmol), and *N*-((2*S*,3*R*)-1-(3-bromophenyl)-3-hydroxy-4-((*S*)-6′-neopentyl-3′,4′-dihydrospiro[cyclobutane-1,2′-pyrano[2,3-*b*]pyridine]-4′-ylamino)butan-2-yl)-2-methoxyacetamide (**9a**). The vessel was sealed and heated to 120 °C for 2 h, cooled to room temperature, and diluted with a saturated aq Rochelle's salt solution (20 mL). After stirring for 1 h, biphasic mixture was diluted with EtOAc (50 mL), washed with 2N aq NaOH (25 mL), washed with brine, dried over magnesium sulfate, filtered, and concentrated. The crude reside was dissolved in methanol (10 mL) and treated with cesium fluoride (1.1 g, 7.0 mmol). The mixture was heated to 50 °C for 1 h and then concentrated. The crude product was purified by chromatography on a silica gel (10−50% EtOAc/hexane) to give *N*-((2*S*,3*R*)-1-(3-ethynylphenyl)-3-hydroxy-4-((*S*)-6′-neopentyl-3′,4′-dihydrospiro[cyclobutane-1,2′-pyrano[2,3-*b*]-pyridine]-4′-ylamino)butan-2-yl)-2-methoxyacetamide (**19**) as a white solid (0.12 g, 33% yield). ^{1}H NMR (400 MHz, acetonitrile-d_3) δ ppm

7.82 (d, J = 1.96 Hz, 1 H), 7.74 (d, J = 1.47 Hz, 1 H), 7.25−7.37 (m, 4 H), 6.90 (d, J = 9.29 Hz, 1 H), 3.74 (d, J = 16.0 Hz, 1 H), 3.60−3.66 (m, 1 H), 3.59 (d, J = 15.9 Hz, 1 H), 3.27 (s, 1 H), 3.24 (s, 2 H), 3.11 (dd, J = 14.04, 3.86 Hz, 1 H), 2.78−2.83 (m, 3 H), 2.36−2.49 (m, 4 H), 2.08−2.27 (m, 8 H), 1.83−1.92 (m, 1 H), 1.66−1.79 (m, 2 H), 0.88 (s, 9 H). HRMS (ESI+) m/z = 520.3167 [M + H]$^+$. Calcd for $C_{31}H_{42}N_3O_4$: 520.3160.

***N*-((2*S*,3*R*)-3-Hydroxy-4-((*S*)-6′-neopentyl-3′,4′-dihydrospiro-[cyclobutane-1,2′-pyrano[2,3-*b*]pyridine]-4′-ylamino)-1-(3-(prop-1-ynyl)phenyl)butan-2-yl)-2-methoxyacetamide (20).** The title compound was prepared from **9a** and tributyl(prop-1-ynyl)stannane via a method analogous to the preparation of **14**, giving a white solid (0.058 g, 45% yield). MS m/z = 534.2 [M + H]$^+$. Calcd for $C_{32}H_{44}N_3O_4$: 534.3.

***N*-((2*S*,3*R*)-3-Hydroxy-4-((*S*)-6′-neopentyl-3′,4′-dihydrospiro-[cyclobutane-1,2′-pyrano[2,3-*b*]pyridine]-4′-ylamino)-1-(4-(thiazol-2-yl)phenyl)butan-2-yl)-2-methoxyacetamide (21).** A solution of *N*-((2*S*,3*R*)-1-(4-chlorophenyl)-3-hydroxy-4-((*S*)-6′-neopentyl-3′,4′-dihydrospiro[cyclobutane-1,2′-pyrano[2,3-*b*]pyridine]-4′-ylamino)butan-2-yl)-2-methoxyacetamide (**9e**) (0.20 g, 0.38 mmol), dicyclohexyl(2′,4′,6′-triisopropylbiphenyl-2-yl)phosphine (0.36 g, 0.76 mmol), and $Pd_2(dba)_3$ (0.345 g, 0.377 mmol) in 1,4-dioxane (4.0 mL) was degassed by sparging with N_2 (g) for 15 min. 2-(Tributylstannyl)-thiazole (0.494 g, 1.32 mmol) was added, and the mixture was heated to 160 °C for 1 h in a microwave reactor. The reaction mixture was cooled to room temperature and filtered through a silica gel plug with 10% MeOH/DCM. The filtrate was concentrated, and the residue was purified by reverse-phase chromatography (10−90% CH_3CN/H_2O with 0.1% TFA). Fractions containing product were combined and evaporated to give the triflate salt of *N*-((2*S*,3*R*)-3-hydroxy-4-((*S*)-6′-neopentyl-3′,4′-dihydrospiro[cyclobutane-1,2′-pyrano[2,3-*b*]pyridine]-4′-ylamino)-1-(4-(thiazol-2-yl)phenyl)butan-2-yl)-2-methoxyacetamide (**21**) as a light-yellow solid (0.10 g, 33% yield). ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 9.00−9.30 (s, 1 H), 7.70−8.02 (m, 6 H), 7.34 (d, J = 8.02 Hz, 2 H), 5.90 (s, 1 H), 4.77 (s, 1 H), 3.90−4.13 (m, 2 H), 3.74 (d, J = 15.10 Hz, 1 H), 3.63 (d, J = 15.15 Hz, 1 H), 2.99−3.26 (m, 5 H), 2.73−2.92 (m, 3 H), 2.60 (dd, J = 13.45, 6.90 Hz, 1 H), 2.36−2.47 (m, 3 H), 2.10−2.29 (m, 2 H), 2.02 (dd, J = 17.80, 6.94 Hz, 2 H), 1.79−1.91 (m, 1 H), 1.66−1.79 (m, 1 H), 0.87 (s, 9 H). HRMS (ESI+) m/z = 579.3001 [M + H]$^+$. Calcd for $C_{32}H_{43}N_4O_4S$: 579.2990.

***N*-((2*S*,3*R*)-1-(4-Ethynylphenyl)-3-hydroxy-4-((*S*)-6′-neopentyl-3′,4′-dihydrospiro[cyclobutane-1,2′-pyrano[2,3-*b*]-pyridine]-4′-ylamino)butan-2-yl)-2-methoxyacetamide (22).** A solution of *tert*-butyl (2*S*,3*R*)-3-(*tert*-butyldimethylsilyloxy)-4-((*S*)-6′-neopentyl-3′,4′-dihydrospiro[cyclobutane-1,2′-pyrano[2,3-*b*]pyridine]-4′-ylamino)-1-(4-((trimethylsilyl)ethynyl)phenyl)butan-2-ylcarbamate (**10i**) (0.42 g, 0.63 mmol) in THF (6 mL) was treated with TBAF (0.63 mL of a 1 M solution in THF, 0.63 mmoL), and the resulting solution was stirred for 30 min. The volatiles were evaporated in vacuo, and the residue was dissolved in methanol (3 mL) and a solution of 4N hydrogen chloride in dioxane (0.63 mL, 2.5 mmol). The solution was heated to 50 °C for 1 h and then cooled to room temperature. The cooled mixture was diluted with ether, and the resulting solid was allowed to settle. The ether was decanted, and the remaining solid was taken up in 2N aq sodium hydroxide solution and extracted with EtOAc. The organic extract was washed with brine, dried over magnesium sulfate, filtered, and evaporated to afford intermediate (2*R*,3*S*)-3-amino-4-(4-ethynylphenyl)-1-((*S*)-6′-neopentyl-3′,4′-dihydrospiro[cyclobutane-1,2′-pyrano[2,3-*b*]pyridine]-4′-ylamino)butan-2-ol. A portion of the intermediate amine (0.135 g, 0.300 mmol) was combined with *N*,*N*-diisopropylethylamine (0.08 mL, 0.45 mmol) in DCM (3 mL). Separately, a solution of carbonyl diimidazole (0.05 g, 0.30 mmol) and *N*,*N*-diisopropylethylamine (0.05 mL, 0.40 mmol) in DCM (1 mL) was treated with 2-methoxyacetic acid. The resulting mixture was stirred for 1 h and then was added to the solution of amine. The combined mixture was stirred for 1 h at room temperature and then was concentrated. The residue was purified by chromatography on silica gel (0−5% MeOH/DCM) to give *N*-((2*S*,3*R*)-1-(4-ethynylphenyl)-3-hydroxy-4-((*S*)-6′-neopentyl-3′,4′-dihydrospiro[cyclobutane-1,2′-pyrano[2,3-*b*]pyridine]-4′-ylamino)butan-2-yl)-2-methoxyacetamide (**22**) as a white solid (0.067 g, 43% yield). ^{1}H NMR (400 MHz, acetonitrile-d_3) δ ppm 7.80 (d, J = 2.05 Hz, 1 H), 7.69 (dd, J = 2.40, 1.03 Hz, 1 H), 7.36−7.43 (m, 2 H), 7.18−7.26 (m, 2 H), 6.82−6.91 (m, 1 H), 4.09−4.16 (m, 1 H), 3.90 (dd, J = 10.27, 5.38 Hz, 1 H), 3.73 (d, J = 15.26 Hz, 1 H), 3.58−3.64 (m, 2 H), 3.61 (d, J = 15.25 Hz, 1 H), 3.33 (s, 1 H), 3.11 (dd, J = 14.08, 4.01 Hz, 1 H), 2.65−2.85 (m, 4 H), 2.33−2.45 (m, 5 H), 2.18−2.28 (m, 1 H), 2.07−2.15 (m, 4 H), 1.81−1.91 (m, 1 H), 1.61−1.80 (m, 2 H), 0.88 (s, 9 H). HRMS (ESI+) m/z = 520.3169 [M + H]$^+$. Calcd for $C_{31}H_{42}N_3O_4$: 520.3160.

***N*-((2*S*,3*R*)-1-(2-Fluoro-3-(thiazol-2-yl)phenyl)-3-hydroxy-4-((*S*)-6′-neopentyl-3′,4′-dihydrospiro[cyclobutane-1,2′-pyrano-[2,3-*b*]pyridine]-4′-ylamino)butan-2-yl)-2-methoxyacetamide (23).** A solution of *tert*-butyl (2*S*,3*R*)-3-(*tert*-butyldimethylsilyloxy)-1-(2-fluoro-3-(thiazol-2-yl)phenyl)-4-((*S*)-6′-neopentyl-3′,4′-dihydrospiro[cyclobutane-1,2′-pyrano[2,3-*b*]pyridine]-4′-ylamino)-butan-2-ylcarbamate (**10i**) (3.90 g, 5.30 mmol) in methanol (10 mL) was treated with a solution of 4N hydrogen chloride in dioxane (26 mL, 106 mmol), and the resulting mixture was heated to 50 °C for 3 h. The reaction mixture was cooled, diluted with ether (300 mL), and aged for 12 h. The ether was decanted, and the remaining solid was dried under vacuum to give the 4.24 g of the hydrochloride salt of (2*R*,3*S*)-3-amino-4-(2-fluoro-3-(thiazol-2-yl)phenyl)-1-((*S*)-6′-neopentyl-3′,4′-dihydrospiro[cyclobutane-1,2′-pyrano[2,3-*b*]pyridine]-4′-ylamino)butan-2-ol. A portion of this intermediate amine hydrchloride (1.41 g, 2.1 mmol) was combined with *N*,*N*-diisopropylethylamine (2.2 mL, 13 mmol) in DCM (8 mL). Separately, a solution of carbonyl diimidazole (0.34 g, 2.1 mmol) in DCM (8 mL) was treated with 2-methoxyacetic acid. The resulting mixture was stirred for 1 h and then was added to the solution of amine. The combined mixture was stirred for 1 h at room temperature and then was concentrated. The residue was purified by chromatography on silica gel (0−5% MeOH/DCM) to give *N*-((2*S*,3*R*)-1-(2-fluoro-3-(thiazol-2-yl)phenyl)-3-hydroxy-4-((*S*)-6′-neopentyl-3′,4′-dihydrospiro[cyclobutane-1,2′-pyrano[2,3-*b*]-pyridine]-4′-ylamino)butan-2-yl)-2-methoxyacetamide (**23**) (0.76 g, 60%). ^{1}H NMR (400 MHz, acetonitrile-d_3) δ ppm 8.12 (td, J = 7.48, 1.76 Hz, 1 H), 7.93 (dd, J = 3.23, 2.54 Hz, 1 H), 7.79 (d, J = 1.76 Hz, 1 H), 7.71 (dd, J = 2.40, 1.03 Hz, 1 H), 7.64 (d, J = 3.33 Hz, 1 H), 7.35 (td, J = 7.38, 1.86 Hz, 1 H), 7.22 (t, J = 7.73 Hz, 1 H), 6.98 (d, J = 8.80 Hz, 1 H), 4.14−4.24 (m, 1 H), 3.92 (dd, J = 10.47, 5.58 Hz, 1 H), 3.71 (d, J = 15.18 Hz, 1 H), 3.63−3.68 (m, 1 H), 3.58 (d, J = 15.20 Hz, 1 H), 3.27 (d, J = 5.18 Hz, 2 H), 3.21−3.25 (m, 3 H), 2.89 (ddd, J = 1.37, 10.66, 13.98 Hz, 1 H), 2.83 (dd, J = 12.42, 5.86 Hz, 1 H), 2.73 (dd, J = 12.33, 4.31 Hz, 1 H), 2.35−2.48 (m, 4 H), 2.08−2.26 (m, 3 H), 1.82−1.91 (m, 1 H), 1.61−1.82 (m, 3 H), 0.87 (s, 9 H). HRMS (ESI+) m/z = 597.2901 [M + H]$^+$. Calcd for $C_{32}H_{42}FN_4O_4$: 597.2896.

***N*-((2*S*,3*R*)-1-(4-Fluoro-3-(thiazol-2-yl)phenyl)-3-hydroxy-4-((*S*)-6′-neopentyl-3′,4′-dihydrospiro[cyclobutane-1,2′-pyrano-[2,3-*b*]pyridine]-4′-ylamino)butan-2-yl)-2-methoxyacetamide (24).** The triflate salt of the title compound was prepared from **9c** and 2-(tributylstannyl)thiazole via a method analogous to the preparation of **21**, giving a light-yellow oil (0.109 g, 38% yield). ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 8.09 (dd, J = 7.38, 2.10 Hz, 1 H), 7.99 (dd, J = 3.28, 2.40 Hz, 1 H), 7.91 (d, J = 3.23 Hz, 1 H), 7.58−7.81 (m, 3 H), 7.22−7.38 (m, 2 H), 4.97 (d, J = 5.87 Hz, 1 H), 3.99−4.16 (m, 1 H), 3.86 (br s, 1 H), 3.52−3.75 (m, 3 H), 3.14 (s, 3 H), 3.10 (dd, J = 13.60, 3.62 Hz, 1 H), 2.74 (dd, J = 13.64, 10.42 Hz, 1 H), 2.56−2.69 (m, 2 H), 2.27−2.40 (m, 4 H), 2.14−2.23 (m, 1 H), 2.08 (t, J = 7.97 Hz, 3 H), 1.77−1.88 (m, 1 H), 1.60−1.77 (m, 2 H), 0.83 (s, 9 H). HRMS (ESI+) m/z = 597.2899 [M + H]$^+$. Calcd for $C_{32}H_{41}FN_4O_4S$: 597.2896.

***N*-((2*S*,3*R*)-1-(4-Fluoro-3-(thiazol-2-yl)phenyl)-3-hydroxy-4-((*S*)-6′-neopentyl-3′,4′-dihydrospiro[cyclobutane-1,2′-pyrano-[2,3-*b*]pyridine]-4′-ylamino)butan-2-yl)-2-methoxyacetamide (25).** The triflate salt of the title compound was prepared from **9b** and 2-(tributylstannyl)thiazole via a method analogous to the preparation of **21**, giving a light-yellow solid (0.31 g, 58% yield). ^{1}H NMR (400 MHz, acetonitrile-d_3) δ ppm 7.87 (d, J = 3.23 Hz, 1 H), 7.79 (d, J = 2.05 Hz, 1 H), 7.70 (dd, J = 2.35, 0.98 Hz, 1 H), 7.67 (t, J = 1.37 Hz, 1 H), 7.52−7.60 (m, 2 H), 7.09 (ddd, J = 9.73, 2.30, 1.47 Hz, 1 H), 6.96 (d, J = 9.68 Hz, 1 H), 4.11−4.23 (m, J = 9.92, 9.92, 6.33, 3.86 Hz, 1

H), 3.91 (dd, J = 10.32, 5.33 Hz, 1 H), 3.53−3.79 (m, 4 H), 3.22 (s, 3 H), 3.13−3.21 (m, 1 H), 2.65−2.91 (m, 3 H), 2.34−2.47 (m, 4 H), 2.16−2.28 (m, 2 H), 2.05−2.17 (m, 2 H), 1.79−1.91 (m, 1 H), 1.57−1.79 (m, 2 H), 0.87 (s, 9 H). HRMS (ESI+) m/z = 597.2904 [M + H]$^+$. Calcd for $C_{32}H_{41}FN_4O_4S$: 597.2896.

***N*-((2*S*,3*R*)-1-(2-Fluoro-5-(thiazol-2-yl)phenyl)-3-hydroxy-4-((*S*)-6′-neopentyl-3′,4′-dihydrospiro[cyclobutane-1,2′-pyrano-[2,3-*b*]pyridine]-4′-ylamino)butan-2-yl)-2-methoxyacetamide (26).** The triflate salt of the title compound was prepared from **9d** and 2-(tributylstannyl)thiazole via a method analogous to the preparation of **21**, giving a light-yellow solid (0.16 g, 40% yield). ^{1}H NMR (300 MHz, MeOH-d_4) δ ppm 8.04 (s, 2 H), 7.74−7.95 (m, 3 H), 7.61 (d, J = 3.36 Hz, 1 H), 7.20 (t, J = 9.06 Hz, 1 H), 4.06−4.25 (m, 2 H), 3.54−3.94 (m, 2 H), 3.33−3.46 (m, 2 H), 3.24 (s, 3 H), 3.09 (dd, J = 12.13, 8.62 Hz, 1 H), 2.84−3.01 (m, 1 H), 2.79 (dd, J = 13.15, 6.14 Hz, 1 H), 2.49−2.67 (m, 3 H), 2.10−2.42 (m, 4 H), 1.96−2.08 (m, 1 H), 1.74−1.96 (m, 1 H), 1.38 (dd, J = 6.50, 3.00 Hz, 1 H), 0.95 (s, 9 H). MS m/z = 597.0 [M + H]$^+$. Calcd for $C_{32}H_{41}FN_4O_4S$: 597.2

***N*-((2*S*,3*R*)-1-(3-Ethynyl-2-fluorophenyl)-3-hydroxy-4-((*S*)-6′-neopentyl-3′,4′-dihydrospiro[cyclobutane-1,2′-pyrano[2,3-*b*]pyridine]-4′-ylamino)butan-2-yl)-2-methoxyacetamide (27).** The title compound was prepared from **10h** via a method analogous to the preparation of **22**, giving a white solid (0.49 g, 51% yield). ^{1}H NMR (400 MHz, acetonitrile-d_3) δ ppm 7.79 (d, J = 1.76 Hz, 1 H), 7.71 (dd, J = 2.45, 1.08 Hz, 1 H), 7.36 (td, J = 7.19, 1.76 Hz, 1 H), 7.29 (td, J = 7.43, 1.66 Hz, 1 H), 7.07 (t, J = 7.73 Hz, 1 H), 6.92 (d, J = 9.29 Hz, 1 H), 4.07−4.21 (m, 1 H), 3.91 (dd, J = 10.47, 5.48 Hz, 1 H), 3.72 (d, J = 15.22 Hz, 1 H), 3.62−3.67 (m, 2 H), 3.61 (s, 1 H), 3.56 (d, J = 15.24 Hz, 1 H), 3.21−3.29 (m, 2 H), 3.16 (dd, J = 14.48, 4.21 Hz, 1 H), 2.67−2.85 (m, 3 H), 2.33−2.48 (m, 4 H), 2.06−2.26 (m, 5 H), 1.82−1.91 (m, 1 H), 1.70−1.80 (m, 1 H), 1.61−1.70 (m, 1 H), 0.88 (s, 9 H). HRMS (ESI+) m/z = 538.3078 [M + H]$^+$. Calcd for $C_{31}H_{41}FN_3O_4$: 538.3066.

***N*-((2*S*,3*R*)-1-(3-Ethynyl-4-fluorophenyl)-3-hydroxy-4-((*S*)-6′-neopentyl-3′,4′-dihydrospiro[cyclobutane-1,2′-pyrano[2,3-*b*]pyridine]-4′-ylamino)butan-2-yl)-2-methoxyacetamide (28).** A mixture of *N*-((2*S*,3*R*)-1-(3-chloro-4-fluorophenyl)-3-hydroxy-4-((*S*)-6′-neopentyl-3′,4′-dihydrospiro[cyclobutane-1,2′-pyrano[2,3-*b*]pyridine]-4′-ylamino)butan-2-yl)-2-methoxyacetamide (**9c**) (100 mg, 0.18 mmol), cesium carbonate (178 mg, 0.55 mmol), $PdCl_2(CH_3CN)_2$ (3.5 mg, 0.009 mmol), dicyclohexyl(2′,4′,6′-triisopropylbiphenyl-2-yl)phosphine (13 mg, 0.02 mmol), and ethynyltrimethylsilane (63 mg, 0.64 mmol) in acetonitrile (2 mL) was heated to 125 °C for 30 min in a microwave reactor. The mixture was stirred at room temperature for 16 h (until only *des*-TMS alkyne product could be detected by LCMS) before being filtered through Celite with the aid of methanol. The filtrate was concentrated, and the residue was purified by reverse-phase chromatography (10−90% CH_3CN/H_2O with 0.1% TFA). Fractions containing product were combined and evaporated to give the triflate salt of *N*-((2*S*,3*R*)-1-(3-ethynyl-4-fluorophenyl)-3-hydroxy-4-((*S*)-6′-neopentyl-3′,4′-dihydrospiro[cyclobutane-1,2′-pyrano[2,3-*b*]pyridine]-4′-ylamino)butan-2-yl)-2-methoxyacetamide (**28**) (35 mg, 25% yield). ^{1}H NMR (400 MHz, MeOH-d_4) δ ppm 7.98 (d, J = 1.57 Hz, 1 H), 7.87 (d, J = 1.60 Hz, 1 H), 7.18 (s, 1 H), 6.98−7.11 (m, 2 H), 4.78−4.85 (m, 1 H), 4.07 (ddd, J = 10.91, 7.63, 3.47 Hz, 1 H), 3.96−4.03 (m, 1 H), 3.85 (d, J = 15.26 Hz, 1 H), 3.67 (d, J = 15.36 Hz, 1 H), 3.58 (s, 1 H), 3.17−3.33 (m, 3 H), 3.04 (dd, J = 12.57, 9.24 Hz, 1 H), 2.68−2.82 (m, 2 H), 2.47−2.63 (m, 3 H), 2.25−2.36 (m, 1 H), 2.05−2.25 (m, 3 H), 1.79−2.04 (m, 3 H), 0.87 (s, 9 H). HRMS (ESI+) m/z = 538.3078 [M + H]$^+$. Calcd for $C_{31}H_{41}FN_3O_4$: 538.3066.

***N*-((2*S*,3*R*)-1-(3-Ethynyl-5-fluorophenyl)-3-hydroxy-4-((*S*)-6′-neopentyl-3′,4′-dihydrospiro[cyclobutane-1,2′-pyrano[2,3-*b*]pyridine]-4′-ylamino)butan-2-yl)-2-methoxyacetamide (29).** The title compound was prepared from **9b** and ethynyltrimethylsilane via a method analogous to the preparation of **19**, giving a cream-colored solid (0.73 g, 83% yield). ^{1}H NMR (400 MHz, chloroform-d) δ ppm 7.90 (d, J = 1.86 Hz, 1 H), 7.66 (br s, 1 H), 7.13 (s, 1 H), 7.05 (ddd, J = 8.93, 2.32, 1.17 Hz, 1 H), 6.95 (dt, J = 9.32, 1.80 Hz, 1 H), 6.63 (d, J = 9.10 Hz, 1 H), 4.19 (br s, 1 H), 3.99−4.13 (m, 1 H), 3.57−3.93 (m, 4 H), 3.32−3.34 (m, 3 H), 3.02−3.20 (m, 2 H), 2.84 (dd, J = 14.28, 9.10 Hz, 1 H), 2.78 (d, J = 4.60 Hz, 2 H), 2.51−2.65 (m, 1 H), 2.36−2.51 (m, 4 H), 2.16−2.35 (m, 2 H), 2.03−2.15 (m, 1 H), 1.91−2.03 (m, 1 H), 1.86 (t, J = 12.03 Hz, 1 H), 1.74 (dt, J = 11.25, 8.71 Hz, 1 H), 0.90 (s, 9 H). HRMS (ESI+) m/z = 538.3077 [M + H]$^+$. Calcd for $C_{31}H_{41}FN_3O_4$: 538.3066.

***N*-((2*S*,3*R*)-1-(5-Ethynyl-2-fluorophenyl)-3-hydroxy-4-((*S*)-6′-neopentyl-3′,4′-dihydrospiro[cyclobutane-1,2′-pyrano[2,3-*b*]pyridine]-4′-ylamino)butan-2-yl)-2-methoxyacetamide (30).** A solution of *N*-((2*S*,3*R*)-1-(5-chloro-2-fluorophenyl)-3-hydroxy-4-((*S*)-6′-neopentyl-3′,4′-dihydrospiro[cyclobutane-1,2′-pyrano[2,3-*b*]pyridine]-4′-ylamino)butan-2-yl)-2-methoxyacetamide (**9d**) (0.30 g, 0.55 mmol), dicyclohexyl(2′,4′,6′-triisopropylbiphenyl-2-yl)phosphine (0.14 g, 0.29 mmol), and $Pd_2(dba)_3$ (0.18 g, 0.19 mmol) in THF (4 mL) was degassed by sparging with N_2 (g) for 15 min. 1-Trimethyl(2-(tributylstannyl)ethynyl)silane (0.58 g, 1.59 mmol) was added, and the mixture was heated to 160 °C for 1 h in a microwave reactor. The reaction mixture was cooled to room temperature and filtered through a silica gel plug with 10% MeOH/EtOAc. The filtrate was evaporated, and the residue was purified by chromatography on silica gel (0−10% MeOH/DCM) to give 300 mg of the intermediate *N*-((2*S*,3*R*)-1-(2-fluoro-5-((trimethylsilyl)ethynyl)phenyl)-3-hydroxy-4-((*S*)-6′-neopentyl-3′,4′-dihydrospiro[cyclobutane-1,2′-pyrano[2,3-*b*]pyridine]-4′-ylamino)butan-2-yl)-2-methoxyacetamide. This material was combined with TBAF (1.0 mL of a 1 M solution in THF, 1.0 mmol) and THF (15 mL), and the resulting mixture was stirred for 15 h. The mixture was diluted with water (20 mL) and brine (20 mL) and extracted with EtOAc (50 mL). The organic extrac was dried over sodium sulfate, filtered, and evaporated. The residue was purified by reverse-phase chromatography (10−90% CH_3CN/H_2O with 0.1% TFA). Pure fractions containing product were combined in saturated aq sodium bicarbonate solution and extracted with DCM. The organic extract was dried over sodium sulfate, filtered, and evaporated to give *N*-((2*S*,3*R*)-1-(5-ethynyl-2-fluorophenyl)-3-hydroxy-4-((*S*)-6′-neopentyl-3′,4′-dihydrospiro[cyclobutane-1,2′-pyrano[2,3-*b*]pyridine]-4′-ylamino)butan-2-yl)-2-methoxyacetamide (**30**) as a white solid (51 mg, 19% yield). ^{1}H NMR (300 MHz, MeOH-d_4) δ ppm 7.80 (dd, J = 16.30, 1.68 Hz, 2 H), 7.42 (dd, J = 7.16, 2.19 Hz, 1 H), 7.34 (ddd, J = 8.44, 4.93, 2.12 Hz, 1 H), 7.03 (dd, J = 9.87, 8.55 Hz, 1 H), 4.26 (ddd, J = 10.63, 6.61, 3.80 Hz, 1 H), 4.03 (dd, J = 10.45, 5.33 Hz, 1 H), 3.60−3.86 (m, 3 H), 3.22 (dd, J = 13.96, 3.58 Hz, 1 H), 2.69−2.89 (m, 4 H), 2.39−2.57 (m, 5 H), 2.10−2.35 (m, 4 H), 1.87−2.04 (m, 1 H), 1.70−1.87 (m, 2 H), 0.92 (s, 9 H). HRMS (ESI+) m/z = 538.3072 [M + H]$^+$. Calcd for $C_{31}H_{41}FN_3O_4$: 538.3066.

***tert*-Butyl (2*S*,3*S*)-3-(*tert*-Butyldimethylsilyloxy)-4 hydroxy-1-(3-(thiazol-2-yl)phenyl)butan-2-ylcarbamate (31).** A dry 1 L round-bottom flask was charged with *tert*-butyl (2*S*,3*S*)-1-(3-bromophenyl)-3-(*tert*-butyldimethylsilyloxy)-4 hydroxybutan-2-ylcarbamate (**5a**) (51.2 g, 108 mmol) and 1,4-dioxane (110 mL). The mixture was stirred for 10 min, by which time most of the solid had dissolved. The solution was then covered with a blanket of Ar(g). Zinc chloride (29.4 g, 216 mmol) and bis(tri-*t*-butylphosphine)palladium-(0) (5.51 g, 10.8 mmol) were added in sequence. The mixture was sonicated for 1 min, and then 2-thiazolyl-zinc bromide (259 mL of a 0.5 M solution in THF, 129 mmol) was added in one portion. The resulting mixture was stirred for 16 h and then poured into a 1N aq citric acid solution (350 mL) with the aid of ethyl acetate (150 mL). The mixture was shaken vigorously, and then the layers were separated. The aqueous layer was extracted with ethyl acetate (150 mL). The organic layers were combined and washed with saturated aq sodium bicarbonate solution (200 mL) The layers were then separated, and the aqueous layer was extracted with EtOAc (150 mL). The combined organic extracts were then washed with water (150 mL) and brine (150 mL). The water and brine layers were combined and extracted with ethyl acetate (100 mL). The organic layers were again combined and dried over sodium sulfate, filtered, and concentrate. The crude product was purified by chromatography on silica gel to give *tert*-butyl (2*S*,3*S*)-3-(*tert*-butyldimethylsilyloxy)-4-hydroxy-1-(3-(thiazol-2-yl)phenyl)butan-2-ylcarbamate (**31**) (42.4 g, 92% yield). ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 7.82 (d, J = 3.33 Hz, 2 H), 7.74 (s, 1 H), 7.68 (d, J = 3.13 Hz, 2 H), 7.66 (s, 1 H), 7.30 (t, J = 7.63 Hz, 1 H), 7.19−7.26 (m, 1 H), 4.52 (t, J = 5.04 Hz, 1 H),

3.56–3.69 (m, 2 H), 3.38–3.46 (m, 1 H), 3.23–3.33 (m, 1 H), 2.87 (d, *J* = 13.50 Hz, 1 H), 2.48–2.57 (m, 1 H), 1.14 (s, 9 H), 0.81 (s, 9 H), 0.00 (s, 6 H). MS *m/z* = 479.2 [M + H]$^+$. Calcd for $C_{24\ h39}N_2O_4SSi$: 479.2.

***tert*-Butyl (2*S*,3*R*)-3-(*tert*-Butyldimethylsilyloxy)-4-((*S*)-6′-neopentyl-3′,4′-dihydrospiro[cyclobutane-1,2′-pyrano[2,3-*b*]pyridine]-4′-ylamino)-1-(3-(thiazol-2-yl)phenyl)butan-2-ylcarbamate (32).** A solution of *tert*-butyl (2*S*,3*S*)-3-(*tert*-butyldimethylsilyloxy)-4 hydroxy-1-(3-(thiazol-2-yl)phenyl)butan-2-ylcarbamate (7.925 g, 16.62 mmol) (**31**) and (*S*)-6′-neopentyl-3′,4′-dihydrospiro[cyclobutane-1,2′-pyrano[2,3-*b*]pyridin]-4′-amine (**6**) (4.502 g, 17.29 mmol) in DCM (100 mL) was treated with trimethyl orthoformate (14.7 mL, 133 mmol). The resulting solution was stirred for 1 h, and then sodium triacetoxyborohydride (8.456 g, 39.90 mmol) was added. The mixture was stirred for 4 h and then diluted with DCM (100 mL) and saturated aq Rochelle's salt solution (500 mL). The resulting biphasic mixture was stirred vigorously for 1 h, and then the layers were separated. The aq layer was extracted with DCM (250 mL), and the organic layers were combined. The combined solution was washed with brine (200 mL), dried over sodium sulfate, filtered, and concentrated. The crude product was purified by chromatography on silica gel (0–50% EtOAc/hexane) to afford *tert*-butyl (2*S*,3*R*)-3-(*tert*-butyldimethylsilyloxy)-4-((*S*)-6′-neopentyl-3′,4′-dihydrospiro-[cyclobutane-1,2′-pyrano[2,3-*b*]pyridine]-4′-ylamino)-1-(3-(thiazol-2-yl)phenyl)butan-2-ylcarbamate (**32**) (9.960 g, 83.09% yield) as a white solid. MS *m/z* = 721.4 [M + H]$^+$. Calcd for $C_{40}H_{61}N_4O_4SSi$: 721.4.

(2*R*,3*S*)-3-Amino-1-((*S*)-6′-neopentyl-3′,4′-dihydrospiro-[cyclobutane-1,2′-pyrano[2,3-*b*]pyridine]-4′-ylamino)-4-(3-(thiazol-2-yl)phenyl)butan-2-ol hydrochloride (33). A solution of *tert*-butyl (2*S*,3*R*)-3-(*tert*-butyldimethylsilyloxy)-4-((*S*)-6′-neopentyl-3′,4′-dihydrospiro[cyclobutane-1,2′-pyrano[2,3-*b*]pyridine]-4′-ylamino)-1-(3-(thiazol-2-yl)phenyl)butan-2-ylcarbamate (**32**) (500 mg, 0.824 mmol) in methanol (4 mL) was cooled to 0 °C in an ice-bath. Hydrogen chloride (10.3 mL of a 4N solution in 1,4-dioxane, 41.2 mmol) was added, the ice-bath was removed, and the mixture was strred for 2 h at room temperature. The volatiles were removed in vacuo to afford the hydrochloride salt of (2*R*,3*S*)-3-amino-1-((*S*)-6′-neopentyl-3′,4′-dihydrospiro[cyclobutane-1,2′-pyrano[2,3-*b*]pyridine]-4′-ylamino)-4-(3-(thiazol-2-yl)phenyl)butan-2-ol hydrochloride (**33**) (551 mg) as an off-white foam. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 10.45 (br s, 1 H), 9.69 (br s, 1 H), 8.18–8.54 (m, 4 H), 7.98 (s, 2 H), 7.94 (d, *J* = 3.23 Hz, 1 H), 7.86 (dt, *J* = 7.41, 1.38 Hz, 1 H), 7.81 (d, 1 H), 7.41–7.73 (m, 2 H), 4.78 (br s, 1 H), 4.47 (d, *J* = 10.17 Hz, 1 H), 3.28–3.44 (m, 1 H), 2.91–3.19 (m, 3 H), 2.68 (dd, *J* = 13.16, 5.62 Hz, 1 H), 2.43 (d, *J* = 3.91 Hz, 2 H), 2.13–2.25 (m, 2 H), 1.97–2.11 (m, 2 H), 1.84 (br s, 1 H), 1.60–1.79 (m, 1 H), 0.87 (s, 9 H). MS *m/z* = 507.2 [M + H]$^+$. Calcd for $C_{29}H_{39}N_4O_2Si$: 507.3.

***N*-((2*S*,3*R*)-3-Hydroxy-4-((*S*)-6′-neopentyl-3′,4′-dihydrospiro-[cyclobutane-1,2′-pyrano[2,3-*b*]pyridine]-4′-ylamino)-1-(3-(thiazol-2-yl)phenyl)butan-2-yl)acetamide (34).** The title compound was prepared from *N*-((2*S*,3*R*)-1-(3-bromophenyl)-3-hydroxy-4-((*S*)-6′-neopentyl-3′,4′-dihydrospiro[cyclobutane-1,2′-pyrano[2,3-*b*]-pyridine]-4′-ylamino)butan-2-yl)acetamide (prepared as **9a**) and 2-(tributylstannyl)thiazole via a method analogous to the preparation of **14**, giving a white solid (0.079 g, 48% yield). ^{1}H NMR (400 MHz, acetonitrile-d_3) δ ppm 7.86 (t, *J* = 1.52 Hz, 1 H), 7.85 (d, *J* = 3.23 Hz, 1 H), 7.82 (dt, *J* = 1.52, 7.40 Hz, 1 H), 7.78 (d, *J* = 1.76 Hz, 1 H), 7.70 (dd, *J* = 2.40, 1.03 Hz, 1 H), 7.50 (d, *J* = 3.33 Hz, 1 H), 7.35–7.40 (m, 1 H), 7.30–7.34 (m, 1 H), 6.48 (d, *J* = 8.90 Hz, 1 H), 4.01–4.10 (m, 1 H), 3.91 (dd, *J* = 10.56, 5.48 Hz, 1 H), 3.53 (td, *J* = 6.31, 4.50 Hz, 1 H), 3.16 (dd, *J* = 13.94, 3.77 Hz, 1 H), 2.65–2.82 (m, 2 H), 2.34–2.46 (m, 3 H), 2.08–2.24 (m, 8 H), 1.81–1.91 (m, 1 H), 1.69–1.77 (m, 3 H), 1.64 (ddd, *J* = 13.28, 10.64, 1.22 Hz, 1 H), 0.86 (s, 9 H). HRMS (ESI+) *m/z* = 549.2895 [M + H]$^+$. Calcd for $C_{31}H_{41}N_4O_3S$: 549.2885.

***N*-((2*S*,3*R*)-3-Hydroxy-4-((*S*)-6′-neopentyl-3′,4′-dihydrospiro-[cyclobutane-1,2′-pyrano[2,3-*b*]pyridine]-4′-ylamino)-1-(3-(thiazol-2-yl)phenyl)butan-2-yl)propionamide (35).** A mixture of (2*R*,3*S*)-3-amino-1-((*S*)-6′-neopentyl-3′,4′-dihydrospiro[cyclobutane-1,2′-pyrano[2,3-*b*]pyridine]-4′-ylamino)-4-(3-(thiazol-2-yl)phenyl)-butan-2-ol hydrochloride (**33**) (0.136 g, 0.221 mmol) and *N*,*N*-diisopropylethylamine (0.192 mL, 1.10 mmol) in DMF (3 mL) was treated with propionic acid (16.5 μL, 0.221 mmol) and 1-[bis(dimethylamino)methylene]-1*H*-1,2,3-triazolo[4,5-*b*]pyridinium 3-oxide hexafluorophosphate (84 mg, 0.22 mmol) in sequence. The resulting mixture was stirred for 2 h and then was purified directly by reverse-phase chromatography (10–90% CH_3CN/H_2O with 0.1% TFA). Pure fractions containing product were combined and evaporated to give the triflate salt of *N*-((2*S*,3*R*)-3-hydroxy-4-((*S*)-6′-neopentyl-3′,4′-dihydrospiro[cyclobutane-1,2′-pyrano[2,3-*b*]pyridine]-4′-ylamino)-1-(3-(thiazol-2-yl)phenyl)butan-2-yl)propionamide (**35**) as a white solid (93 mg, 53% yield). ^{1}H NMR (400 MHz, chloroform-*d*) δ ppm 8.03 (d, *J* = 4.30 Hz, 2 H), 7.94 (d, *J* = 3.52 Hz, 1 H), 7.88 (s, 1 H), 7.73 (d, *J* = 8.02 Hz, 1 H), 7.51 (d, *J* = 3.52 Hz, 1 H), 7.43 (t, *J* = 7.73 Hz, 1 H), 7.34 (d, *J* = 7.63 Hz, 1 H), 6.72 (d, *J* = 8.02 Hz, 1 H), 4.78 (dd, *J* = 11.15, 6.06 Hz, 1 H), 4.06–4.21 (m, 1 H), 3.93–4.04 (m, 1 H), 3.09–3.32 (m, 2 H), 2.78–3.05 (m, 2 H), 2.54–2.70 (m, 2 H), 2.45 (s, 2 H), 2.19–2.34 (m, 2 H), 2.06–2.19 (m, 4 H), 1.91–2.06 (m, 1 H), 1.68–1.83 (m, 1 H), 0.98 (t, *J* = 7.53 Hz, 3 H), 0.88 (s, 9 H). HRMS (ESI+) *m/z* = 563.3048 [M + H]$^+$. Calcd for $C_{32}H_{43}N_4O_3S$: 563.3041.

2-Ethoxy-*N*-((2*S*,3*R*)-3-hydroxy-4-((*S*)-6′-neopentyl-3′,4′-dihydrospiro[cyclobutane-1,2′-pyrano[2,3-*b*]pyridine]-4′-ylamino)-1-(3-(thiazol-2-yl)phenyl)butan-2-yl)acetamide (36). To a solution of 2-ethoxyacetic acid (0.439 g, 4.22 mmol) in DCM (10 mL) was added carbonyl diimidazole (0.584 g, 4.22 mmol), and the resulting mixture was stirred for 30 min. This solution was then added via cannula to a preformed solution of (2*R*,3*S*)-3-amino-1-((*S*)-6′-neopentyl-3′,4′-dihydrospiro[cyclobutane-1,2′-pyrano[2,3-*b*]pyridine]-4′-ylamino)-4-(3-(thiazol-2-yl)phenyl)butan-2-ol hydrochloride (**33**) (2.60 g, 4.22 mmol) and *N*,*N*-diisopropylethylamine (3.68 mL, 21.1 mmol) in DCM (50 mL). The mixture was stirred for 2 h at room temperature and then was diluted with DCM (30 mL), washed with saturated aq sodium bicarbonate solution (50 mL), washed with water (50 mL), washed with brine, dried over sodium sulfate, filtered, and evaporated. The crude product was purified by chromatography on silica gel (0–5% MeOH/DCM) to give 2-ethoxy-*N*-((2*S*,3*R*)-3-hydroxy-4-((*S*)-6′-neopentyl-3′,4′-dihydrospiro[cyclobutane-1,2′-pyrano[2,3-*b*]pyridine]-4′-ylamino)-1-(3-(thiazol-2-yl)phenyl)butan-2-yl)acetamide (**36**) as a white solid (1.76 g, 70%). ^{1}H NMR (400 MHz, MeOH-d_4) δ ppm 7.82–7.87 (m, 2 H), 7.72–7.81 (m, 3 H), 7.59 (d, *J* = 3.33 Hz, 1 H), 7.28–7.42 (m, 2 H), 4.28 (ddd, *J* = 10.37, 6.46, 4.30 Hz, 1 H), 4.00 (dd, *J* = 10.56, 5.48 Hz, 1 H), 3.61–3.87 (m, 3 H), 3.32–3.40 (m, 2 H), 3.22 (dd, *J* = 13.99, 4.21 Hz, 1 H), 2.72–2.92 (m, 3 H), 2.40–2.54 (m, 4 H), 2.10–2.32 (m, 3 H), 1.86–1.99 (m, 1 H), 1.70–1.84 (m, 2 H), 1.08 (t, *J* = 7.04 Hz, 3 H), 0.89 (s, 9 H). HRMS (ESI+) *m/z* = 593.3157 [M + H]$^+$. Calcd for $C_{33}H_{45}N_4O_4S$: 593.3146.

(*R*)-*N*-((2*S*,3*R*)-3-Hydroxy-4-((*S*)-6′-neopentyl-3′,4′-dihydrospiro[cyclobutane-1,2′-pyrano[2,3-*b*]pyridine]-4′-ylamino)-1-(3-(thiazol-2-yl)phenyl)butan-2-yl)-2-methoxypropanamide (37). The title compound was prepared from **33** and (*R*)-2-methoxypropanoic acid via a method analogous to the preparation of **36**, giving a white solid (0.74 g, 79% yield). ^{1}H NMR (400 MHz, MeOH-d_4) δ ppm 7.80–7.87 (m, 3 H), 7.72–7.80 (m, 2 H), 7.59 (d, *J* = 3.33 Hz, 1 H), 7.31–7.44 (m, 2 H), 4.25 (ddd, *J* = 10.91, 7.09, 3.91 Hz, 1 H), 4.02 (dd, *J* = 10.56, 5.28 Hz, 1 H), 3.74 (td, *J* = 7.04, 3.91 Hz, 1 H), 3.49 (q, *J* = 6.72 Hz, 1 H), 3.06 (s, 3 H), 2.66–2.90 (m, 3 H), 2.41–2.53 (m, 4 H), 2.09–2.31 (m, 3 H), 1.87–2.01 (m, 1 H), 1.66–1.84 (m, 2 H), 1.25–1.37 (m, 1 H), 1.14 (d, *J* = 6.85 Hz, 3 H), 0.83–0.95 (m, 9 H). HRMS (ESI+) *m/z* = 593.3151 [M + H]$^+$. Calcd for $C_{33}H_{45}N_4O_4S$: 593.3146.

(*S*)-*N*-((2*S*,3*R*)-3-Hydroxy-4-((*S*)-6′-neopentyl-3′,4′-dihydrospiro[cyclobutane-1,2′-pyrano[2,3-*b*]pyridine]-4′-ylamino)-1-(3-(thiazol-2-yl)phenyl)butan-2-yl)-2-methoxypropanamide (38). The title compound was prepared from **33** and (*S*)-2-methoxypropanoic acid via a method analogous to the preparation of **36**, giving a white solid (0.08 g, 73% yield). ^{1}H NMR (400 MHz, acetonitrile-d_3) δ ppm 7.86 (s, 1 H), 7.85 (d, *J* = 3.91 Hz, 1 H), 7.76–7.83 (m, 3 H), 7.50 (d, *J* = 3.23 Hz, 1 H), 7.38 (t, *J* = 7.53 Hz, 1 H), 7.31–7.35 (m, 1 H), 6.58 (d, *J* = 7.24 Hz, 1 H), 4.02–4.10 (m, 1 H), 3.89–3.98 (m, 1 H), 3.50–3.57 (m, 1 H), 3.32–3.46 (m, 2 H), 3.27 (d, *J* = 5.38 Hz, 2 H), 3.21 (dd, *J* = 14.13, 3.57 Hz, 1 H), 3.14 (s, 3 H), 2.80–2.88 (m, 1 H), 2.73 (dd, *J* = 14.18, 10.47 Hz, 2 H), 2.35–2.49

(m, 3 H), 2.18–2.26 (m, 3 H), 2.08–2.13 (m, 3 H), 1.81–1.91 (m, 1 H), 1.69–1.79 (m, 1 H), 1.59–1.67 (m, 1 H), 0.87 (s, 9 H). MS m/z = 593.2 [M + H]$^+$. Calcd for $C_{33}H_{45}N_4O_4S$: 593.3.

(*R*)-2-Ethoxy-*N*-((2*S*,3*R*)-3-hydroxy-4-((*S*)-6′-neopentyl-3′,4′-dihydrospiro[cyclobutane-1,2′-pyrano[2,3-*b*]pyridine]-4′-ylamino)-1-(3-(thiazol-2-yl)phenyl)butan-2-yl)propanamide (39). The title compound was prepared as a diastereomeric mixture from **33** and 2-ethoxypropanoic acid via a method analogous to the preparation of **35**. Following workup, chromatographic separation of the diastereomers afforded (*S*)-2-ethoxy-*N*-((2*S*,3*R*)-3-hydroxy-4-((*S*)-6′-neopentyl-3′,4′-dihydrospiro[cyclobutane-1,2′-pyrano[2,3-*b*]pyridine]-4′-ylamino)-1-(3-(thiazol-2-yl)phenyl)butan-2-yl)propanamide (107 mg, 14% yield) and (*R*)-2-ethoxy-*N*-((2*S*,3*R*)-3-hydroxy-4-((*S*)-6′-neopentyl-3′,4′-dihydrospiro[cyclobutane-1,2′-pyrano[2,3-*b*]pyridine]-4′-ylamino)-1-(3-(thiazol-2-yl)phenyl)butan-2-yl)propanamide (**39**) (119 mg, 15% yield). Data for the title compound: ^{1}H NMR (400 MHz, chloroform-*d*) δ ppm 7.84–7.92 (m, 3 H), 7.82 (d, J = 7.63 Hz, 1 H), 7.69 (br s, 1 H), 7.38 (t, J = 7.63 Hz, 1 H), 7.28–7.36 (m, 2 H), 6.60 (d, J = 8.80 Hz, 1 H), 4.12–4.26 (m, J = 8.85, 8.63, 8.63, 4.01 Hz, 1 H), 4.08 (br s, 1 H), 3.54–3.75 (m, 2 H), 3.22–3.37 (m, 2 H), 3.10 (dq, J = 9.17, 6.99 Hz, 1 H), 2.93 (dd, J = 14.48, 9.39 Hz, 1 H), 2.80–2.88 (m, 1 H), 2.68–2.80 (m, 1 H), 2.50–2.65 (m, 1 H), 2.43 (s, 3 H), 2.24–2.38 (m, 1 H), 2.20 (dddd, J = 12.25, 8.29, 4.11, 3.91 Hz, 1 H), 2.07 (dddd, J = 12.08, 8.27, 4.11, 3.72 Hz, 1 H), 1.89–2.02 (m, 1 H), 1.65–1.85 (m, 2 H), 1.30 (d, J = 6.85 Hz, 3 H), 0.98 (t, J = 6.94 Hz, 3 H), 0.90 (s, 9 H). HRMS (ESI+) m/z = 607.3312 [M + H]$^+$. Calcd for $C_{34\ h47}N_4O_4S$: 607.3302.

***N*-((2*S*,3*R*)-3-Hydroxy-4-((*S*)-6′-neopentyl-3′,4′-dihydrospiro[cyclobutane-1,2′-pyrano[2,3-*b*]pyridine]-4′-ylamino)-1-(3-(thiazol-2-yl)phenyl)butan-2-yl)-2,2-dimethoxyacetamide (40).** The title compound was prepared from **33** and 2,2-dimethoxyacetic acid via a method analogous to the preparation of **36**, giving a white solid (0.41 g, 49% yield). ^{1}H NMR (400 MHz, chloroform-*d*) δ ppm 7.83–7.92 (m, 3 H), 7.80 (d, J = 7.53 Hz, 1 H), 7.58 (s, 1 H), 7.28–7.43 (m, 3 H), 6.68 (d, J = 9.03 Hz, 1 H), 4.58 (s, 1 H), 4.08–4.23 (m, 1 H), 3.94 (d, J = 4.52 Hz, 1 H), 3.54–3.63 (m, 1 H), 3.31 (s, 3 H), 3.21–3.29 (m, 1 H), 3.03 (s, 3 H), 2.79–2.95 (m, 2 H), 2.71 (dd, J = 12.05, 3.51 Hz, 1 H), 2.47–2.62 (m, 1 H), 2.42 (s, 2 H), 2.24–2.39 (m, 2 H), 2.20 (ddd, J = 11.80, 4.52, 4.27 Hz, 1 H), 2.05 (ddd, J = 11.92, 8.41, 4.27 Hz, 1 H), 1.88–2.01 (m, 1 H), 1.62–1.85 (m, 2 H), 0.90 (s, 9 H). HRMS (ESI+) m/z = 609.3104 [M + H]$^+$. Calcd for $C_{33}H_{45}N_4O_5S$: 609.3095.

(*R*)-*N*-((2*S*,3*R*)-3-Hydroxy-4-((*S*)-6′-neopentyl-3′,4′-dihydrospiro[cyclobutane-1,2′-pyrano[2,3-*b*]pyridine]-4′-ylamino)-1-(3-(thiazol-2-yl)phenyl)butan-2-yl)tetrahydrofuran-2-carboxamide (41). The title compound was prepared from **33** and (*R*)-tetrahydrofuran-2-carboxylic acid via a method analogous to the preparation of **36**, giving a white solid (0.15 g, 82% yield). ^{1}H NMR (400 MHz, acetonitrile-d_3) δ ppm 7.73–7.87 (m, 5 H), 7.50 (d, J = 3.81 Hz, 1 H), 7.38 (t, J = 7.58 Hz, 1 H), 7.28–7.33 (m, 1 H), 7.07 (d, J = 8.51 Hz, 1 H), 3.98–4.10 (m, 2 H), 3.89–3.97 (m, 1 H), 3.75–3.84 (m, 1 H), 3.67–3.74 (m, 1 H), 3.57–3.65 (m, 1 H), 3.27 (d, J = 5.09 Hz, 1 H), 3.20 (dd, J = 14.23, 3.77 Hz, 1 H), 2.76–2.88 (m, 2 H), 2.67–2.74 (m, 1 H), 2.36–2.47 (m, 4 H), 2.24 (s, 7 H), 1.60–1.84 (m, 4 H), 0.87 (s, 9 H). HRMS (ESI+) m/z = 605.3160 [M + H]$^+$. Calcd for $C_{34\ h45}N_4O_4S$: 605.3146.

(*S*)-*N*-((2*S*,3*R*)-3-Hydroxy-4-((*S*)-6′-neopentyl-3′,4′-dihydrospiro[cyclobutane-1,2′-pyrano[2,3-*b*]pyridine]-4′-ylamino)-1-(3-(thiazol-2-yl)phenyl)butan-2-yl)tetrahydrofuran-2-carboxamide (42). The title compound was prepared from **33** and (*S*)-tetrahydrofuran-2-carboxylic acid via a method analogous to the preparation of **36**, giving a white solid (1.50 g, 47% yield). ^{1}H NMR (400 MHz, acetonitrile-d_3) δ ppm 7.84 (d, J = 3.23 Hz, 2 H), 7.71–7.82 (m, 3 H), 7.50 (d, J = 3.23 Hz, 1 H), 7.38 (t, J = 7.63 Hz, 1 H), 7.29–7.34 (m, 1 H), 6.96 (d, J = 11.15 Hz, 1 H), 4.10 (dd, J = 8.17, 5.53 Hz, 2 H), 3.94 (dd, J = 11.74, 5.18 Hz, 1 H), 3.68–3.75 (m, 1 H), 3.65 (t, J = 7.38 Hz, 1 H), 3.53–3.61 (m, 1 H), 3.27 (d, J = 5.28 Hz, 2 H), 3.21–3.26 (m, 1 H), 2.67–2.86 (m, 3 H), 2.35–2.49 (m, 4 H), 2.08–2.25 (m, 5 H), 1.82–1.92 (m, 1 H), 1.50–1.79 (m, 2 H), 1.16–1.42 (m, 2 H), 0.88 (s, 9 H). HRMS (ESI+) m/z = 605.3158 [M + H]$^+$. Calcd for $C_{34\ h45}N_4O_4S$: 605.3146.

(*R*)-2-Hydroxy-*N*-((2*S*,3*R*)-3-hydroxy-4-((*S*)-6′-neopentyl-3′,4′-dihydrospiro[cyclobutane-1,2′-pyrano[2,3-*b*]pyridine]-4′-ylamino)-1-(3-(thiazol-2-yl)phenyl)butan-2-yl)propanamide (43). The triflate salt of the title compound was prepared from **33** and (*R*)-2-hydroxypropanoic acid via method analogous to the preparation of **35**, giving a white solid (0.102 g, 55% yield). ^{1}H NMR (400 MHz, chloroform-*d*) δ ppm 8.17 (s, 1 H), 8.07 (s, 1 H), 7.97 (d, J = 3.52 Hz, 1 H), 7.87 (s, 1 H), 7.66 (d, J = 7.63 Hz, 1 H), 7.60 (d, J = 3.52 Hz, 1 H), 7.33–7.51 (m, 3 H), 4.78 (dd, J = 11.05, 5.77 Hz, 1 H), 4.03–4.20 (m, 2 H), 3.94 (t, J = 7.92 Hz, 1 H), 3.37 (d, J = 11.54 Hz, 1 H), 3.01–3.19 (m, 2 H), 2.94 (dd, J = 11.84, 7.92 Hz, 1 H), 2.56–2.71 (m, 2 H), 2.40–2.52 (m, 2 H), 2.20–2.37 (m, 2 H), 2.08–2.21 (m, 2 H), 2.01 (d, J = 11.35 Hz, 1 H), 1.78 (dt, J = 11.40, 8.78 Hz, 1 H), 1.31 (d, J = 6.85 Hz, 3 H), 0.88 (s, 9 H). HRMS (ESI+) m/z = 579.2993 [M + H]$^+$. Calcd for $C_{32}H_{43}N_4O_4S$: 579.2990.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

General information, experimentals and characterization, X-ray crystal structure information. This material is available free of charge via the Internet at http://pubs.acs.org.

Accession Codes

The PDB accession code for the X-ray cocrystal of BACE1 + compound **37** is 4DI2.

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Notes

The authors declare no competing financial interest.

■ ABBREVIATIONS USED

AD, Alzheimer's Disease; Aβ, β-amyloid peptide; APP, β-amyloid precursor protein; BACE1, β-site β-amyloid precursor protein cleaving enzyme; Boc, *tert*-butyloxycarbonyl; CSF, cerebrospinal fluid; CYP, cytochrome P450; CV, cardiovascular; FRET, fluorescence resonance energy transfer; HEA, hydroxyethylamine; Pgp, p-glycoprotein; TBS, *tert*-butyldimethylsilyl

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