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Discovery of 7-Tetrahydropyran-2-yl Chromans: β-Site Amyloid Precursor Protein Cleaving Enzyme 1 (BACE1) Inhibitors That Reduce Amyloid β-Protein (Aβ) in the Central Nervous System

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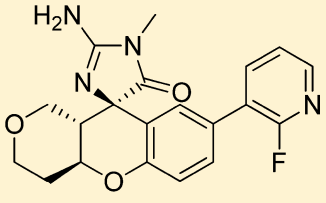
Supporting Information

ABSTRACT: In an attempt to increase selectivity vs Cathepsin D (CatD) in our BACE1 program, a series of 1,3,4,4a,10,10a-hexahydropyrano[4,3-b]chromene analogues was developed. Three different Asp-binding moieties were examined: spirocyclic acyl guanidines, aminooxazolines, and aminothiazolines in order to modulate potency, selectivity, efflux, and permeability. Using structure-based design, substitutions to improve binding to both the S3 and S2′ sites of BACE1 were explored. An acyl guanidine moiety provided the most potent analogues. These compounds demonstrated 10−420 fold selectivity for BACE1 vs CatD, and were highly potent in a cell assay measuring $A\beta_{1-40}$ production (5−99 nM). They also suffered from high efflux. Despite this undesirable property, two of the acyl guanidines achieved free brain concentrations ($C_{free,brain}$) in a guinea pig PD model sufficient to cover their cell IC_{50}s. Moreover, a significant reduction of $A\beta_{1-40}$ in guinea pig, rat, and cyno CSF (58%, 53%, and 63%, respectively) was observed for compound **62**.



62

CSF $A\beta_{1-40}$ Reduction

Guinea Pig:	58%
Rat:	53%
Cyno:	63%

INTRODUCTION

During the last few decades, breakthroughs in medicine have resulted in a decline of deaths associated with many major diseases. In contrast, deaths due to Alzheimer's disease have steadily increased, in part due to a growing elderly population.[1] The worldwide prevalence of dementia-related diseases, of which Alzheimer's is the most common, was estimated to be 36 million people in 2010, and this number is expected to almost double every 20 years.[2] Currently there is a great need for disease-modifying treatments, as the current standards of care (acetylcholinesterase inhibitors and NMDA antagonists) do not delay disease progression.[3]

Targeting the formation of the amyloid peptide (Aβ) has received considerable attention as a strategy for treating Alzheimer's disease.[4] Much research has revolved around inhibiting the two major proteases responsible for processing the amyloid-β precursor protein (APP), γ and β-site APP cleaving enzyme 1 (BACE1).[5] We chose to pursue BACE1 inhibitors because of the preclinical[6] and genetic[7] evidence supporting its role in production of Aβ in the brain. More recently, it was demonstrated that a rare mutation in the APP, which reduces the efficiency of BACE1 cleavage, correlated with a dramatic improvement in cognition among elderly Scandinavians.[8] Compelling evidence for targeting BACE1 comes from clinical trial results from Eli Lilly[9] and Merck[10] in which significant decreases in CSF Aβ were observed after dosing with BACE1 inhibitors. In addition to APP, BACE1 processes other substrates that may limit its usefulness as a therapeutic target.[11] Nonetheless, Merck's BACE1 inhibitor MK-8931 has been well tolerated[12] and is currently undergoing evaluation in phase II/III trials.[13]

One of the greatest challenges in developing BACE1 inhibitors for Alzheimer's disease is the need to cross the blood−brain barrier. In our discovery program, efforts were focused on compounds with physicochemical properties[14] that might give adequate brain levels to elicit a pharmacodynamic response. In conflict with this goal, our most potent BACE1 inhibitors[15] contained either an acyl guanidine (**1**) or aminooxazoline/aminothiazoline (**2** and **3**, respectively) warhead (Figure 1), which was necessary for potent interactions with the catalytic Asps in the active site (Figure 2). These warheads added two hydrogen bond donors and increased the total polar surface area (tPSA) of our compounds, both of which increased efflux liability and made obtaining brain penetrant molecules more challenging.[14] In addition to potency

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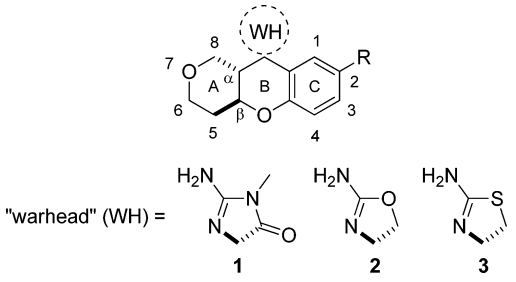

Figure 1. Numbering system and ring designation to be used for 1,3,4,4a,10,10a-hexahydropyrano[4,3-b]chromene analogues ("7-THP chromans") discussed in this article. Spirocyclic warheads (WH): acyl guanidine (**1**), aminooxazoline (**2**), and aminothiazoline (**3**).

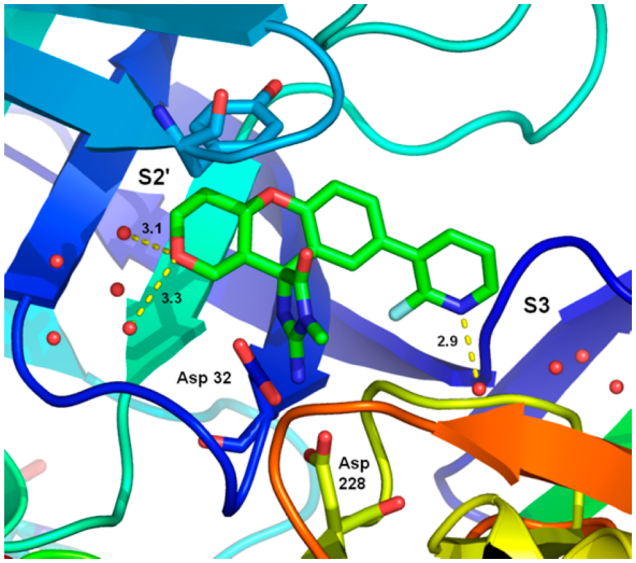

Figure 2. X-ray cocrystal structure of BACE1 and **62**. The two key active site aspartic acids, Asp32 and Asp228, are labeled. The distances between water oxygens (depicted as red spheres) and **62** are given in angstroms.

and brain penetration, we also desired compounds with >100-fold selectivity against another aspartyl protease, Cathepsin D (CatD), which has been implicated in multiple physiologically important roles.[16] In an effort to overcome these challenges, we hypothesized that modification of our previously described BACE1 inhibitors[15] by incorporation of a 7-THP A-ring would generate 1,3,4,4a,10,10a-hexahydropyrano[4,3-b]chromenes (**1**–**3**; hereafter referred to as "7-THP chromans"), which might be highly potent vs BACE1 and >100-fold selective against CatD, due to differences in their active sites (see Supporting Information). The SAR and PK for these 7-THP chromans, which culminated with advancement of **62** to PD studies and its effect on lowering Aβ in the CSF will be the main focus of this article.

CHEMISTRY

Scheme 1 describes the synthesis of spirocyclic acyl guanidines of Table 1. Enamine **5** was prepared via the condensation of morpholine and pyranone **4**. Subsequent addition of **5** to aldehyde **6** followed by intramolecular aminal formation provided compound **7**. Swern oxidation of **7**, followed by in situ elimination afforded chromene-one **8**. L-Selectride mediated reduction then gave chromanone **9**, which was isolated as the pure trans diastereomer after trituration with hexanes. Compound **9** was then subjected to the Bucherer–Bergs reaction, which gave diastereomer **10** as the major isomer, which was enriched to >80% diastereomeric purity by trituration with hot *i*-PrOH. Methylation of hydantoin **10** provided compound **11**, which was subsequently converted to the thiohydantoin **12** with Lawesson's reagent. Oxidation/aminolysis of thiohydantion **12** afforded intermediate **13**. Analogues in Table 1 (**56**–**68**) were prepared via Suzuki couplings with **13**. Racemic compounds **13** and **61** were resolved by chiral SFC to provide compounds **55**, **62**, and **63** (Table 1). Compound **59** was prepared from enantiopure intermediate **55**. A cocrystal structure of **62** with BACE1 (Figure 2) was obtained, and by analogy, the stereochemistries for compounds of Table 1 (**55**–**68**) were assigned.

Analogues in Table 2, containing aminooxazoline or aminothiazoline warheads, were prepared according to Scheme 2. Chromanone **9** (from Scheme 1) was reacted with Tebbe's reagent to afford alkene **14**. Using published conditions,[17] alkene **14** was converted to aminooxazoline **15** (X = O) or aminothiazoline **16** (X = S), obtained as a mixture of stereoisomers. Diastereomers of **15** (X = O) were separated by silica gel chromatography to provide **15a** and **15b** in nearly equivalent amounts. Diastereomers **16** were carried forward as a mixture and separated by silica chromatography at the final product stage. Analogous to compounds of Table 1, Suzuki coupling of intermediates **15** or **16** with various boronic acids provided compounds **69**–**76**. The stereochemical assignments for **15a** and **15b**, and by analogy, compounds **69**–**74** (Table 2) were made based on the cocrystal structure of compound **72** with BACE1.[18] The alternate diastereomers of aminothiazolines **75** and **76** were not isolated. The stereochemistries for compounds **75** and **76** (Table 2) were determined by cocrystal structure of compound **76** with BACE1.[18]

Aminooxazolines **77**–**82** (Table 3), substituted with a methyl group at the β-position, were synthesized according to Scheme 3. Salicylaldehyde **18** was cyclized to hemiacetal **19** in the presence of Et_3N and 3-methylbut-2-enal **17**. Diol **20** was formed upon treatment with $NaBH_4$ and could then be converted to ketone **21** in the presence of MnO_2. Treatment with MOMCl provided MOM-protected ether **22**. Cyclic ether **24** was synthesized by deprotonation of **22** with LHMDS and trapping with TMSCl to provide intermediate **23** (not isolated), which was then treated with $TiCl_4$ to give **24** as the cis ring junction diastereomer. This cis ketone was then epimerized by deprotonation with LHMDS followed by kinetic reprotonation at −78 °C with ethyl salicylate to provide ketone **25** as a 5:1 mixture of trans/cis diastereomers. Olefin **26** was formed upon treatment with Tebbe's reagent. The spirocyclic aminooxazolines **27** were then formed by treatment with I_2 and AgOCN followed by the addition of aq. NH_4OH, in an analogous fashion to compounds **15** (Scheme 2). A mixture of diastereomers was obtained and carried forward to final product. Suzuki coupling then provided compounds **77**–**82**, which were isolated as pure diastereomers by silica gel chromatography or as single enantiomers after chiral SFC purification.

3-Fluoro chromans **92** and **93** (Table 4) were synthesized from **28** in an analogous fashion to compounds **77**–**82** according to Scheme 3. Relative stereochemistries for compounds **92** and **93** were determined by NMR comparison with compounds **77**–**82**, for which absolute stereochemistry had been unambiguously determined by cocrystal structure of **79** with BACE1.

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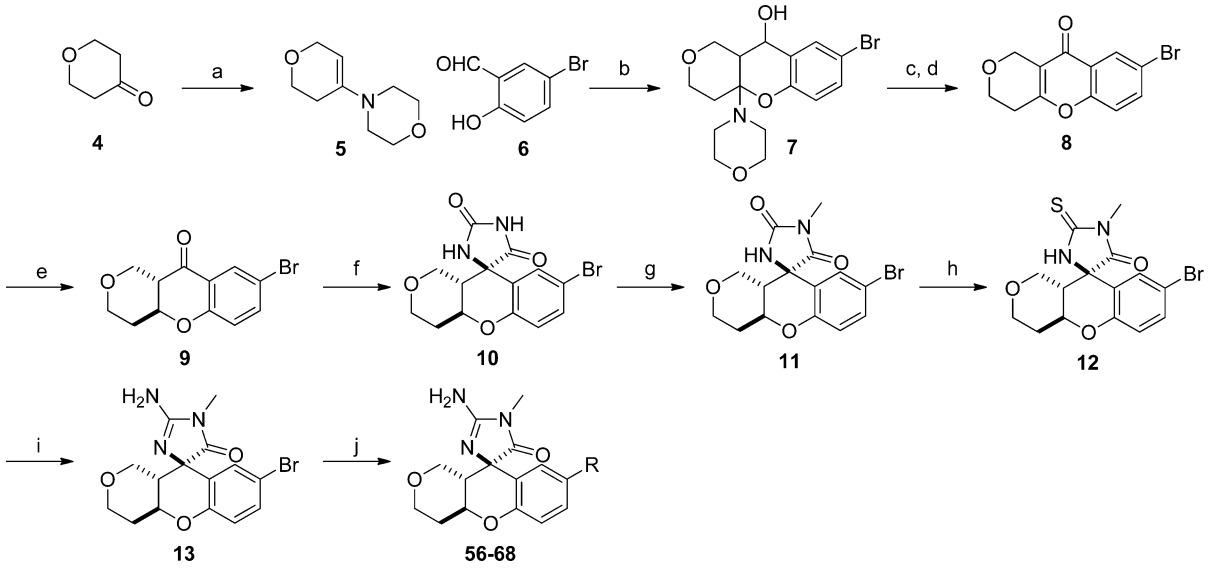
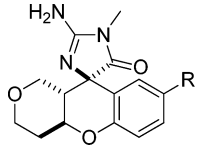
Scheme 1[a]



[a]Reagents and conditions: (a) morpholine, toluene, reflux, Dean−Stark trap; (b) toluene, rt; (c) oxalyl chloride, DMSO, Et_3N, DCM, −78 °C; (d) HOAc, rt; (e) L-selectride, THF, −78 °C; (f) KCN, $(NH_4)_2CO_3$, $NaHSO_3$, EtOH, 130 °C; (g) MeI, K_2CO_3, DMF, rt; (h) Lawesson's reagent, toluene, 90 °C; (i) *t*-BuOOH, aq. NH_4OH, MeOH, 50 °C; (j) $RB(OH)_2$, $Pd[P(Ph)_3]_4$, aq. Na_2CO_3, dioxane, 90 °C. All chiral compounds depicted above were racemic, except for compounds **59**, **62**, and **63** as discussed below.

Table 1. SAR of 7-THP Chromans Containing a Spirocyclic Acyl Guanidine[a]



		IC_{50} (nM)					
compd	R	BACE1[b]	CatD[c]	CatD/BACE1	cell IC_{50} (nM)[d]	efflux ratio[e]	P_{app} AB[f]
55[g]	Br	670	24000	36	1200	3.4	22
56	3-methoxyphenyl	28	1800	64	27	47	25
57	3-(difluoromethoxy)phenyl	23	780	34	21	49	16
58	3-cyanophenyl	31	2700	87	14	37	18
59[g]	3-cyanophenyl	19	960	51	7.3	69	14
60	3-chloro-5-fluorophenyl	31	2300[h]	74	44	28	11
61	2-fluoropyridin-3-yl	27	1100	41	9.9	91	9.8
62[g]	2-fluoropyridin-3-yl	9.5	840	88	5.4	67	9.0
63[i]	2-fluoropyridin-3-yl	490	14000	29	550	140	8.2
64	5-fluoropyridin-3-yl	33	6200	190	16	56	6.6
65	5-chloropyridin-3-yl	27	3200	120	11	170	16
66	5-methoxypyridin-3-yl	28	5400	190	8.3	61	3.7
67	5-cyanopyridin-3-yl	24	6300	260	21	61	2.4
68	pyrimidin-5-yl	39	11000	280	39	12	1.2

[a]Compounds were racemic, with relative stereochemistry as depicted, unless specified otherwise. All assay data was obtained at least in triplicate, and the values listed are the average from multiple experiments. [b]BACE1 enzyme assay performed at 20 nM substrate concentration. [c]Cathepsin D enzyme assay was performed using enzyme derived from liver cells, unless specified otherwise. [d]Cell assay measuring $A\beta_{1-40}$ production. [e]Efflux ratio is the (B to A)/(A to B) value derived from LLC-PK1 cells transfected with human MDR1 (P-gp). Compounds were tested at 1 μM concentration. Control P-gp substrate, digoxin, had an average efflux ratio of 51 ± 30. [f]P_{app} AB was determined using the LLC-PK1 parental cell line, in units of 10^{-6} cm/s. [g]Single enantiomer with *R* configuration at the spirocyclic center and *S*,*S* configuration at the ring fusion. [h]Cathepsin D enzyme assay was performed using enzyme derived from spleen cells. [i]Single enantiomer with *S* configuration at the spirocyclic center and *R*,*R* configuration at the ring fusion.

Scheme 4 depicts the synthesis of compounds **83**−**85** (Table 3), substituted with a methyl group at the 8-position. Acetyl-substituted chromenone **40** was prepared using published procedures for related hydroxy aryl ethanones.[19] For the synthesis of these analogues, as well as for compounds **90** and **91** (Scheme 6), the THP A-ring was built via a hetero Diels−Alder reaction (e.g., **40** to **41**). The stereochemistry of adduct **41** was not unambiguously determined, thus the depicted stereochemistry is analogous to structurally related hetero Diels−Alder products described previously.[20] Compound **42**

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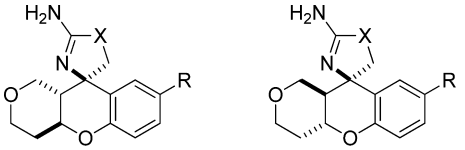
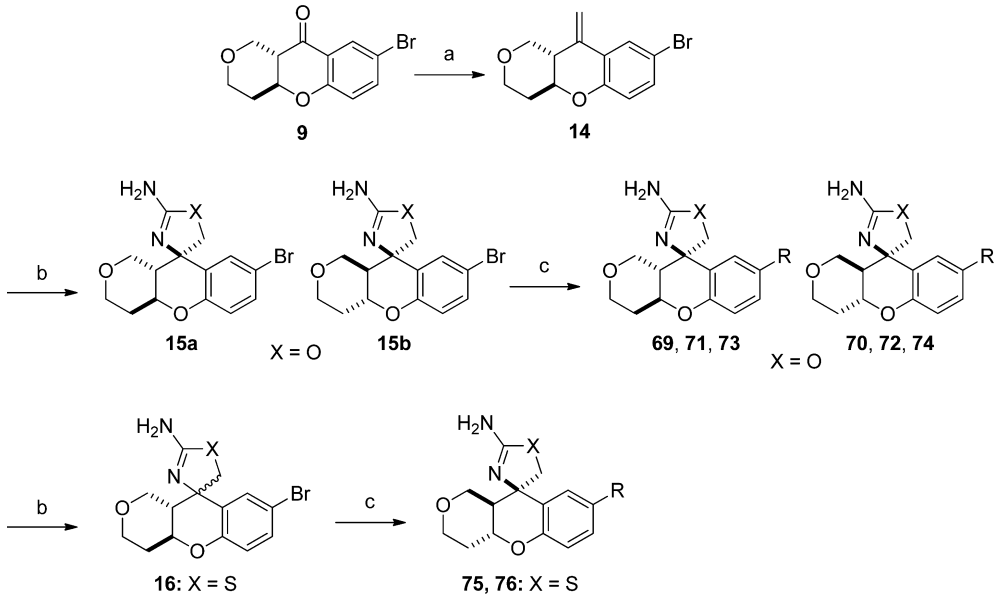
Table 2. SAR of 7-THP Chromans Containing Spirocyclic Aminooxazolines or Aminothiazolines[a]



				IC$_{50}$ (nM)					
compd	X	diastereomer	R	BACE1[b]	CatD[c]	CatD/BACE1	cell IC$_{50}$ (nM)[d]	efflux ratio[e]	P_{app} AB[f]
69	O	A	5-chloropyridin-3-yl	760	12000	16	230	4.3	29
70	O	B	5-chloropyridin-3-yl	440	120000	270	120	2.6	31
71	O	A	2-fluoropyridin-3-yl	1300	15000[g]	12	670	2.8	31
72	O	B	2-fluoropyridin-3-yl	830	170000[g]	205	270	2.1	22
73	O	A	pyrimidin-5-yl	2500	76000[g]	30	650	4.9	13
74	O	B	pyrimidin-5-yl	2000	>200000[g]	>100	940	5.5	9.7
75	S	B	5-chloropyridin-3-yl	220	57000	260	79	1.7	22
76	S	B	2-fluoropyridin-3-yl	330	96000	290	180	1.6	31

[a]Compounds were racemic, with relative stereochemistry as depicted, unless specified otherwise. All assay data was obtained at least in triplicate, and the values listed are the average from multiple experiments. [b]BACE1 enzyme assay performed at 20 nM substrate concentration. [c]Cathepsin D enzyme assay was performed using enzyme derived from liver cells, unless specified otherwise. [d]Cell assay measuring Aβ_{1-40} production. [e]Efflux ratio is the (B to A)/(A to B) value derived from LLC-PK1 cells transfected with human MDR1 (P-gp). Compounds were tested at 1 μM concentration. Control P-gp substrate, digoxin, had an average efflux ratio of 51 ± 30. [f]P_{app} AB was determined using the LLC-PK1 parental cell line, in units of 10^{-6} cm/s. [g]Cathepsin D assay was performed using enzyme derived from spleen cells.

Scheme 2[a]



[a]Reagents and conditions: (a) Tebbe's reagent, THF, rt; (b) I_2, AgXCN (X = O or S), aq. NH_4OH, CH_3CN, THF, rt; for X = O, diastereomers were separated; (c) $RB(OH)_2$, $Pd[P(Ph)_3]_4$, aq. Na_2CO_3, dioxane, 90 °C, for X = S, single diastereomers were isolated. All chiral compounds depicted above were racemic.

was generated as a single diastereomer by DIBALH reduction of **41**. Triethyl silane with boron trifluoride etherate effected reduction of the acetal functionality of **42** to afford THP **43**. The relative stereochemistry for **43** as well as for the other compounds in Scheme 4 were assigned from cocrystal structures of final products **83**–**85** with BACE1.[18] Methyl hydantoin **44** was prepared from THP **43** in an analogous manner to compound **11** (Scheme 1), and obtained as a 60:40 mixture of trans diastereomers at the ring fusion. The two trans diastereomers were separated by silica gel chromatography to provide a single diastereomer **44**, which was converted to final product **83** in an analogous fashion to compounds **56**–**68** (Scheme 1). Compounds **84** and **85** were similarly prepared from THP **43** as described for **69**–**76** (Scheme 2), and the diastereomers were separated at final product by silica gel chromatography.

7-THP chromans **86**–**89** (Table 3) substituted with a methyl group at the α-position were formed according to Scheme 5. Chromanone **9**, prepared as described in Scheme 1, was methylated using LDA with HMPA to yield **45** in >90% diastereomeric purity after silica gel chromatography. Conversion of **45** to products **86**–**88** was executed analogous to compounds **56**–**68** (Scheme 1). The Bucherer–Bergs reaction of ketone **45** (Scheme 5, step b) gave >85% diastereomerically

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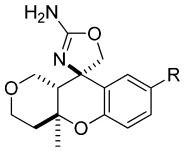
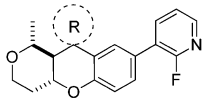
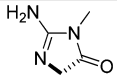
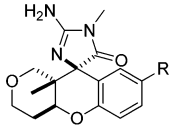
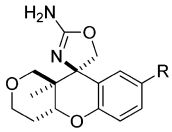
Table 3. SAR of 7-THP Chromans with Methyl Substitution at the α, β, and 8-Positions[a]

Compd	Substructure	R	IC_{50} (nM)		CatD/ BACE1	Cell IC_{50} (nM)[d]	Efflux Ratio[e]	P_{app} AB[f]
			BACE1[b]	CatD[c]				
77[g]		3-cyanophenyl	300	69000	230	35	4.0	27
78[g]		3-chloro-5-fluorophenyl	54	7500	140	41	1.5	8.8
79		5-chloropyridin-3-yl	190	140000	740	23	4.3	23
80		5-fluoropyridin-3-yl	530	160000	300	99	4.4	25
81		2-fluoropyridin-3-yl	220	42000	190	92	6.0	33
82		pyrimidin-5-yl	420	>200000	>480	180	7.1	11
83			11	4600	420	17	49	8.8
84			230	57000	250	170	2.4	34
85			120	47000	390	170	1.1	31
86		3-cyanophenyl	29	1700	59	6.8	64	16
87		2-fluoropyridin-3-yl	24	490	25	5.1	79	9.3
88		pyrimidin-5-yl	33	2600	79	13	38	1.4
89		2-fluoropyridin-3-yl	1100	>200000	>180	400	1.8	30

[a]Compounds were racemic, with relative stereochemistry as depicted, unless specified otherwise. All assay data was obtained at least in triplicate, and the values listed are the average from multiple experiments. [b]BACE1 enzyme assay performed at 20 nM substrate concentration. [c]Cathepsin D enzyme assay was performed using enzyme derived from liver cells. [d]Cell assay measuring $A\beta_{1-40}$ production. [e]Efflux ratio is the (B to A)/(A to B) value derived from LLC-PK1 cells transfected with human MDR1 (P-gp). Compounds were tested at 1 μM concentration. Control P-gp substrate, digoxin, had an average efflux ratio of 51 ± 30. [f]P_{app} AB was determined using the LLC-PK1 parental cell line, in units of 10^{-6} cm/s. [g]Single enantiomer with *R* configuration at the spirocyclic center and *S*,*S* configuration at the ring fusion.

pure spirocyclic hydantoin (not depicted), which was subsequently transformed (Scheme 5, steps c–f) to products **86**–**88**. The stereochemistry of ketone **45** and products **86**–**88** was determined by cocrystal structure of analogue **87** with BACE1.[18] Product **89** was prepared analogous to compounds from Scheme 2. Formation of the aminooxazoline moiety (Scheme 5, step h) was >90% diastereoselective giving the relative stereochemistry depicted for **89**, which was confirmed by cocrystal structure with BACE1.[18]

Spirocyclic acyl guanidines **90** and **91** (Table 4) with fluoro substitution at the C-ring's 3-position were prepared according to Scheme 6. In order to avoid competing nucleophilic aromatic substitution (S_NAr) with a potentially reactive 3-fluoro 2-bromo chromanone during the Bucherer–Bergs reaction (Scheme 6, step h), we chose to utilize a methoxy group in place of the bromo substituent previously used as a handle for Suzuki couplings in Schemes 1–5. Baeyer–Villiger oxidation of starting material **46** yielded **47**, which was subjected to a Fries rearrangement to give phenyl ethanone **48**. Reaction of **48** with triphosgene afforded aldehyde **49**, which was converted to **50** via a hetero Diels–Alder reaction analogous to compound **41** in Scheme 4. Hydrogenation of olefin **50** lead to partial reduction of the ketone functionality, so the crude was stirred with MnO_2 in DCM to provide **51**. Removal of the acetal functionality was accomplished with Et_3SiH/BF_3-Et_2O to give ketone **52**, which was transformed to **53** (mixture of

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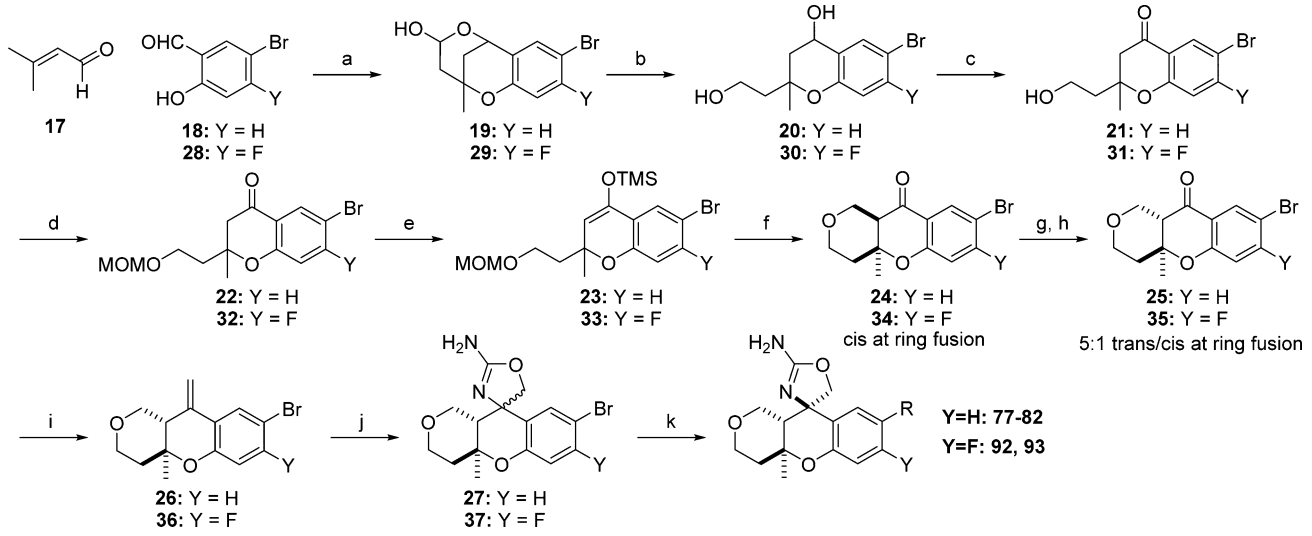
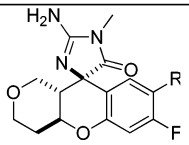
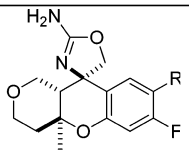
Scheme 3[a]



[a]Reagents and conditions: (a) Et_3N, dioxane, 55 °C; (b) $NaBH_4$, MeOH, THF, 0 °C; (c) MnO_2, DCM, rt; (d) MOMCl, i-Pr_2EtN, DCM, rt; (e) LHMDS, TMSCl, THF, −70 °C; (f) $TiCl_4$, DCM, −40 °C; (g) LHMDS, THF, −78 °C; (h) ethyl 2-hydroxybenzoate, THF, −78 °C; (i) Tebbe's reagent, THF, −78 °C to rt; (j) I_2, AgOCN, aq. NH_4OH, CH_3CN, EtOAc, 40 °C; (k) $RB(OH)_2$, $Pd[P(Ph)_3]_4$, aq. Na_2CO_3, dioxane, 90 °C; diastereomers were separated. All chiral compounds depicted above were racemic, except for compounds **77**, **78**, **92**, and **93** as discussed below.

Table 4. SAR of 7-THP 3-Fluoro Chromans[a]

Compd	Substructure	R	IC_{50} (nM) BACE1[b]	IC_{50} (nM) CatD[c]	CatD/BACE1	Cell IC_{50} (nM)[d]	Efflux Ratio[e]	P_{app} AB[f]	MLM[g] Predicted CL_{hep} (mL/min/kg)
90		3-(difluoro-methoxy)phenyl	61	600	9.8	99	16	7.7	21
91		2-fluoropyridin-3-yl	66	940	14	19	48	7.1	40
92[h]		5-chloropyridin-3-yl	60	11000	180	61	11	22	65
93[h]		2-fluoropyridin-3-yl	71	3700	52	90	2.2	27	84

[a]Compounds were racemic, with relative stereochemistry as depicted, unless specified otherwise. All assay data was obtained at least in triplicate, and the values listed are the average from multiple experiments. [b]BACE1 enzyme assay performed at 20 nM substrate concentration. [c]Cathepsin D enzyme assay was performed using enzyme derived from liver cells. [d]Cell assay measuring $A\beta_{1-40}$ production. [e]Efflux ratio is the (B to A)/(A to B) value derived from LLC-PK1 cells transfected with human MDR1 (P-gp). Compounds were tested at 1 μM concentration. Control P-gp substrate, digoxin, had an average efflux ratio of 51 ± 30. [f]P_{app} AB was determined using the LLC-PK1 parental cell line, in units of 10^{-6} cm/s. [g]MLM: mouse liver microsomes. [h]Single enantiomer with *R* configuration at the spirocyclic center and *S*,*S* configuration at the ring fusion.

diastereomers) analogous to compound **13** in Scheme 1. Acyl guanidine **53** was carried forward as a mixture to give triflate **54** after HBr-mediated deprotection of the methoxy group, protection as an amidine, and conversion of the phenol to a triflate. The amidine protecting group made the diastereomers of **54** more amenable to separation by silica gel chromatography. Final products **90** and **91** were generated by Suzuki couplings with triflate **54**, and the amidine protecting group was simultaneously removed under the Suzuki reaction conditions. The relative stereochemistry for racemates **90** and **91** was assigned based on NMR comparison with compounds **56**−**68** (Scheme 1), for which the stereochemistry had been unambiguously determined by cocrystal structure of compound **62** with BACE1 (Figure 2).

RESULTS AND DISCUSSION

Structure−Activity Relationships (SAR). 7-THP chroman analogues were evaluated for activity in BACE1 and CatD enzyme assays, a cell assay for production of $A\beta_{1-40}$, and for efflux ratio and permeability in an MDR1-transfected LLC-PK1 cell line. Identification of compounds with <100 nM cell potency, >100-fold selectivity for CatD (due to its role in multiple physiological functions[16]), an efflux ratio <2 (to minimize P-gp liability), and apparent cell permeability in the apical to basolateral direction (P_{app} AB) > 10×10^{-6} cm/s was the goal. Coverage ratio[21] ($C_{free,brain}$/cell IC_{50}) was a key assessment that incorporated PK, potency, and brain penetration. Although we were unable to identify a compound

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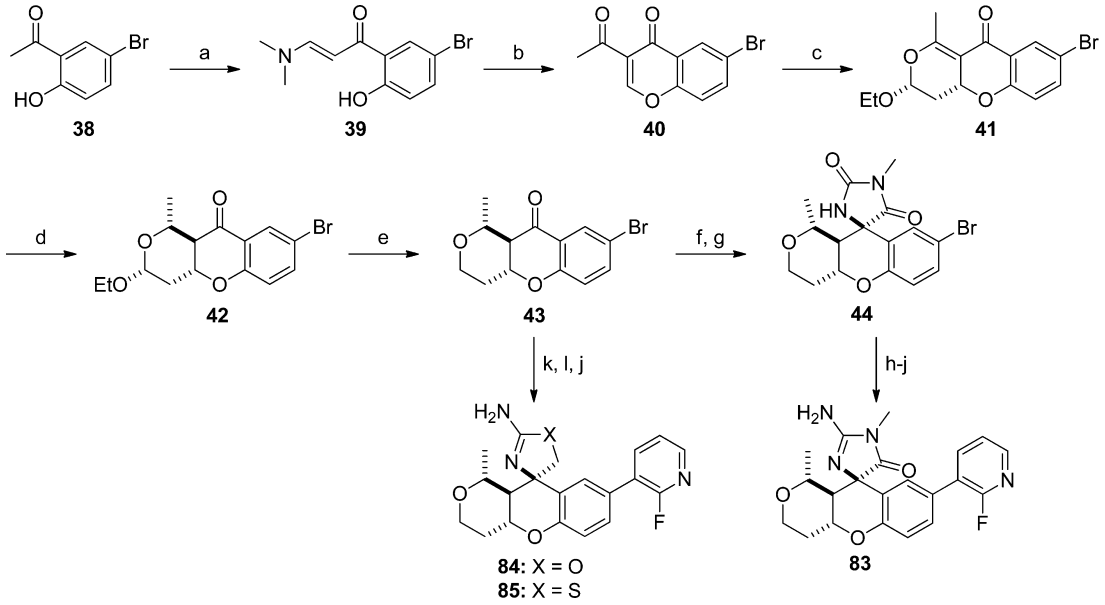
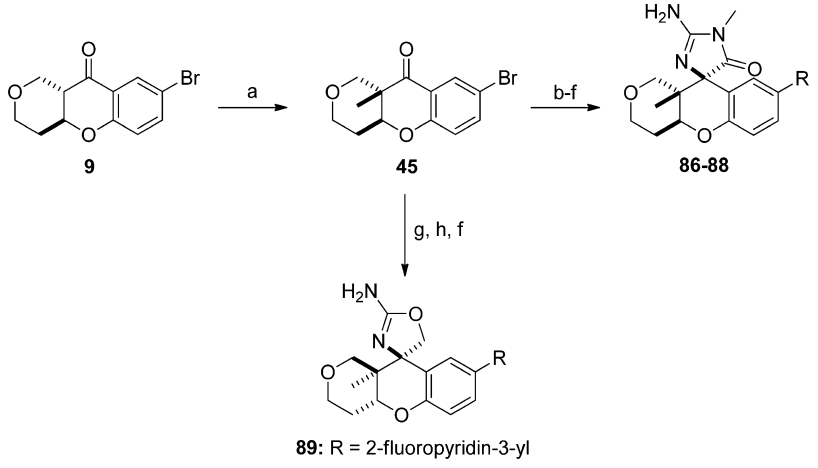
Scheme 4[a]



[a]Reagents and conditions: (a) DMF-dimethyl acetal, toluene, reflux; (b) Ac_2O, py, rt; (c) ethyl vinyl ether, 100 °C; (d) DIBALH, THF, −78 °C; (e) Et_3SiH, BF_3-Et_2O, DCM, rt; (f) KCN, $(NH_4)_2CO_3$, $NaHSO_3$, EtOH, 130 °C; (g) MeI, K_2CO_3, DMF, rt; diastereomers were separated; (h) Lawesson's reagent, toluene, 90 °C; (i) *t*-BuOOH, aq. NH_4OH, MeOH, 50 °C; (j) (2-fluoropyridin-3-yl)boronic acid, $Pd[P(Ph)_3]_4$, aq. Na_2CO_3, dioxane, 90 °C; (k) Tebbe's reagent, THF, rt; (l) I_2, AgXCN (X = O or S), aq. NH_4OH, CH_3CN, THF, rt. All chiral compounds depicted above were racemic.

Scheme 5[a]



[a]Reagents and conditions: (a) LDA, HMPA, MeI, THF, −78 °C; (b) KCN, $(NH_4)_2CO_3$, $NaHSO_3$, EtOH, 130 °C; (c) MeI, K_2CO_3, DMF, rt; (d) Lawesson's reagent, toluene, 90 °C; (e) *t*-BuOOH, aq. NH_4OH, MeOH, 50 °C; (f) $RB(OH)_2$, $Pd[P(Ph)_3]_4$, aq. Na_2CO_3, dioxane, 90 °C; (g) Tebbe's reagent, THF, rt; (h) I_2, AgOCN, aq. NH_4OH, CH_3CN, THF, rt. All chiral compounds depicted above were racemic.

meeting all of these criteria, we discovered that some of our compounds were still able to attain sufficient coverage ratios to decrease Aβ in the CSF (Table 7 and Figure 5, vide infra). We utilized the SAR from Tables 1−4 to select compounds with different profiles of cell potency, efflux, and permeability to evaluate in mouse PK. The results from mouse PK (Table 5), in particular the coverage ratio, then guided the optimization and selection of compounds to progress to PD studies.

From our previous work[15] and others,[22] we expected the three different Asp-binding warheads to anchor inhibitors in the BACE1 active site such that substitution off the aromatic C-ring with various R groups would gain potency by projection into the S3 pocket (Figure 2). This was found to be the case, as compound **55** (R = Br) was >17-fold less potent than compounds in which R was a larger aromatic moiety. As a result of this preferred binding orientation, enantiomers were expected to have dramatically different potencies. Indeed, pure enantiomer **63** was 52-fold less potent for BACE1 than its antipode **62**. The various aromatic R groups of Tables 1−4 were chosen based on our earlier SAR[15] and supported by crystallography, so as to maximize binding affinity to BACE1. Thus, compounds **56**−**62** and **64**−**68** were all <40 nM in the enzyme assay. These compounds were also highly potent in the cell assay, wherein the two most potent racemic analogues were **61** and **66** (R = 2-fluoropyridin-3-yl and 5-methoxypyridin-3-yl; IC_{50}s = 9.9 and 8.3 nM, respectively).

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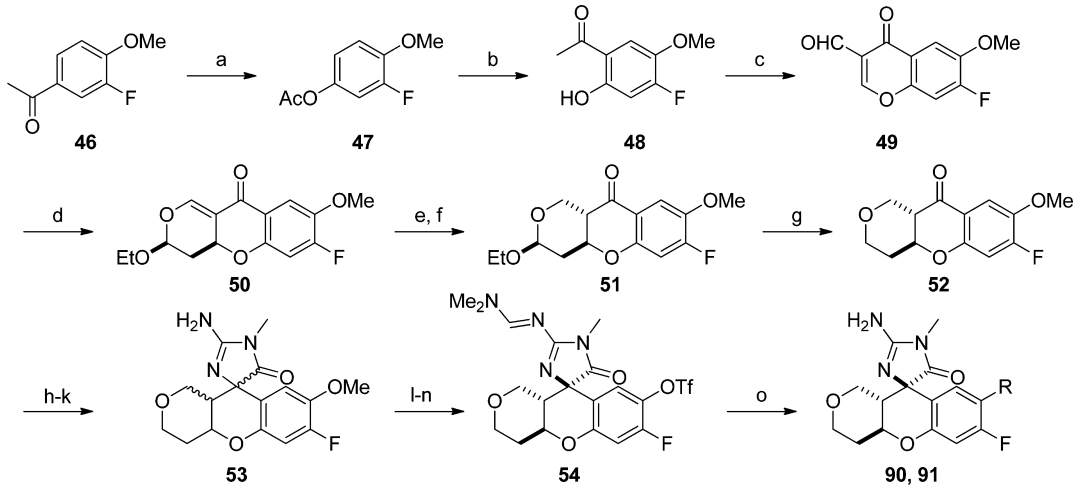
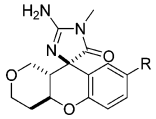
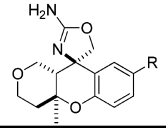
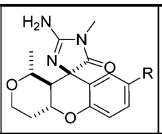
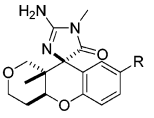
Scheme 6[a]



[a]Reagents and conditions: (a) *m*-CPBA, DCM, 40 °C; (b) TfOH, 60 °C; (c) triphosgene, DMF, DCE, rt; (d) ethyl vinyl ether, 100 °C; (e) Pd/C, EtOH, H_2 (50 psi), rt; (f) MnO_2, DCM, rt; (g) Et_3SiH, BF_3—Et_2O, DCM, rt; (h) KCN, $(NH_4)_2CO_3$, $NaHSO_3$, EtOH, 130 °C; (i) MeI, K_2CO_3, DMF, rt; (j) Lawesson's reagent, toluene, 90 °C; (k) *t*-BuOOH, aq. NH_4OH, MeOH, 50 °C; (l) 48% aq. HBr, 100 °C; (m) DMF—dimethyl acetal, DMF, rt; (n) Tf_2NPh, Et_3N, DCM, rt; diastereomers were separated; (o) $RB(OH)_2$, $Pd[P(Ph)_3]_4$, aq. Na_2CO_3, dioxane, 90 °C. All chiral compounds depicted above were racemic.

Table 5. Mouse PK: iv, po Dose[a]

Substructure										
R										
Compd		**58**[b]	**60**[b]	**61**[e]	**64**[d]	**65**[c]	**78**[b]	**79**[b]	**83**[b]	**87**[c]
Cell IC_{50} (nM)		14	44	9.9	16	11	41	23	17	5.1
P_{app} AB (10^{-6}cm/s)		18	11	9.8	6.6	16	8.8	23	8.8	9.3
Efflux ratio[k]		37	28	91	56	170	1.5	4.3	49	79
$F_{u,plasma}$ mouse		0.11	0.019	0.21	0.32	0.14	0.012	0.11	0.20	0.22
$F_{u,brain}$ mouse		0.024	0.0018	0.090	0.095	0.031	0.0031	0.033	0.098	0.046
CL (mL/min/kg)	$CL_{hep,in\ vitro}$[g]	17	32	46	45	54	57	60	51	55
	$CL_{hep,syst}$	35	22	70	140	110	48	34	72	51
	$CL_{hep,syst,u}$[h]	320	1200	330	440	790	4000	310	360	230
F%		103[l]	67	86	38	19	43	76	75	15
$C_{free,plasma}$ (nM)	1h	210	8.7	320	160	26	9.5	220	260	53
	4h	170	17	75	17	7.5	3.7	120	63	25
$C_{free,brain}$ (nM)	1h	3.0	0.28	11	4.4	1.0	7.2	18	7.1	1.9
	4h	4.3	0.49	8.4	<2.3[i]	<0.80[i]	2.5	5.8	<0.75[i]	3.7
Free b/p	1h	0.014	0.032	0.034	0.028	0.038	0.76	0.082	0.027	0.036
	4h	0.025	0.029	0.11	<0.14[i]	<0.11[i]	0.68	0.048	<0.012[i]	0.15
Coverage Ratio[j]	1h	0.21	0.0064	1.1	0.28	0.091	0.18	0.78	0.42	0.37
	4h	0.31	0.011	0.85	<0.14[i]	<0.073[i]	0.061	0.25	<0.044[i]	0.73

[a]Racemic compounds (unless specified otherwise) were dosed at 3 mg/kg iv and 10 mg/kg po in male CD-1 mice: 3 animals/cohort. [b]iv and po formulation: 40% PEG 400/10% EtOH/50% H_2O. [c]iv and po formulation: 30% captisol in H_2O. [d]iv formulation: 50 mM citrate in saline (pH 4). po formulation: 1% carboxymethyl cellulose (CMC)/0.5% Tween/5 mM citrate in H_2O. [e]iv formulation: 40% PEG 400/10% EtOH/50% H_2O. po formulation: 1% CMC/0.5% Tween/5 mM citrate in H_2O. [f]Single enantiomer with *R* configuration at the spirocyclic center and *S,S* configuration at the ring fusion. [g]Compounds (1 μM) incubated with mouse liver microsomes for 20 min at 37 °C in the presence of NADPH. [h]$CL_{hep,syst,u}$ = $CL_{hep,syst}/F_{u,plasma}$. [i]Compound levels were BLQ. [j]Coverage ratio = $C_{free,brain}$/cell IC_{50}. [k]Control P-gp substrate, digoxin, had an average efflux ratio of 51 ± 30. [l]See ref 28.

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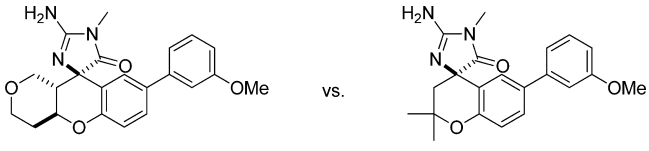
Consistent with our earlier SAR,[15] selectivity against CatD for compounds of Table 1 generally improved with more polar R groups. The most selective were **64** and **66**–**68** (190–280-fold), and these compounds had low cLogPs, which ranged from −1.0 to 0.3. In addition to selectivity from the more polar aromatic R groups, the THP A-ring also contributed to selectivity for BACE1. For example, 7-THP chroman **56** was both more potent against BACE1 and less potent against CatD than the corresponding dimethyl chroman (Figure 3). This result is consistent with data on other chromans from our previous work.[15b]



56

BACE1: 28 nM
CatD: 1800 nM
CatD/BACE1: 64

BACE1: 68 nM
CatD: 300 nM
CatD/BACE1: 4.4

Figure 3. Comparison of 7-THP chroman **56** with a less selective dimethyl chroman (both compounds were racemic).[15b]

From a cocrystal crystal structure of **62** with BACE1 (Figure 2), the THP A-ring projects into the S2′ pocket and the A-ring oxygen interacts with a well-defined water network. Comparisons of this structure with the published structure of CatD (pdb code: 1LYA) show that there are many differences between the two active sites, both in the flexible loop and in the S1 and S2′ pockets (see Figure S1, Supporting Information). It is likely that several of these structural differences, including the interaction of the A-ring with the water network, contribute to the selectivity of these compounds for BACE1 over CatD.

None of the spirocyclic acyl guanidines of Tables 1, 3, and 4 met our goal of an efflux ratio <2. We partially attributed the large efflux ratios to the compounds' tPSAs (77–110) and the presence of two hydrogen bond donors, both of which are likely to increase P-gp mediated efflux.[14] As one might expect, there was a general trend for compounds containing more polar R groups (e.g., compounds **66**–**68**) to be less permeable. However, we appreciate that recognition by transporters such as P-gp is a whole molecule property since **60** (R = 3-chloro-5-fluorophenyl) had an 8-fold greater efflux ratio than **55** (R = Br), despite the fact that their tPSAs are both 77 and that they both contain acyl guanidines.

We hypothesized that reducing tPSA by changing from the acyl guanidine (e.g., **61**, tPSA = 90) to the aminooxazoline (e.g., **71**, tPSA = 78) or aminothiazoline moieties (e.g., **76**, tPSA = 69) would have a positive impact on both efflux and permeability. To our satisfaction, compounds of Table 2 all exhibited lower efflux ratios and greater permeability. It was recently reported that efflux ratio was directly correlated with pK_a for a series of BACE1 inhibitors;[23] however, we observed the opposite trend in this work. For example, acylguanidine **62** (efflux ratio = 67) had a measured pK_a of 5.8, whereas aminooxazolines **78**, **84**, and **89** (efflux ratios < 2.5) had pK_a values of 6.7, 6.4, and 6.4, respectively. Apparently the lower tPSA values for the aminooxazolines had a greater influence on efflux ratio than pK_a.

Unfortunately, changing to the alternate Asp binders resulted in an 8–64-fold decrease in BACE1 potency with a commensurate drop in cell potency. For aminooxazolines **69**–**74**, the two diastereomers (type A and B) with opposite trans stereochemistry at the ring fusion were synthetically accessible (via Scheme 2), whereas only diastereomer B for aminothiazolines **75** and **76** was generated in sufficient quantity for isolation. Significant differences in selectivity were observed between pairs of trans diastereomers **69**–**74**. Diastereomers of type B were found to be significantly more selective for BACE1 than type A, supporting our previous hypothesis that the THP A-ring contributes to CatD selectivity. Changing from the acyl guanidine in type A diastereomers **65**, **61**, and **68** to the aminooxazoline in **69**, **71**, and **73**, respectively, resulted in decreased selectivity. Because these aminooxazolines and aminothiazolines provided lower efflux ratios and improved permeability relative to the acyl guanidines, we were prompted to further explore the SAR around the THP A-ring with a goal of increasing potency. On the basis of the cocrystal structure for **62** (Figure 2), we identified the α-, β-, and 8-positions on the THP A-ring as sites for substitution.

Since the flexible loops of BACE1 and CatD are very different (see Figure S1, Supporting Information), it was suggested that addition of a methyl group to the β-position of the A-ring might increase CatD selectivity. Aminooxazolines with methyl substitution at the β-position (Table 3)[24] demonstrated improved enzyme and cell potencies relative to the corresponding diastereomers from Table 2. The β-methyl group also provided a dramatic increase in selectivity for BACE1 (e.g., **81**, 190-fold, vs **71**, 12-fold). Moreover, compound **82** was inactive against CatD (40% inhibition at 200 μM).

Low efflux ratios and high permeability were maintained for **77**–**82**. Methyl substitution at the 8-position[25] also resulted in improved enzyme and cell potencies for analogues **84** and **85** relative to the corresponding diastereomers **72** and **76**. However, incorporation of the 8-methyl group was not sufficient to provide potency comparable to the corresponding acyl guanidine **83**. Selectivities for 8-methyl analogues **84** and **85** were similar to des-methyl analogues **72** and **76** and easily met our criteria for selectivity (>100-fold). Methyl substitution at the α-position for compound **89**[26] resulted in a modest decrease in potency relative to comparator **72**. The α-methyl substituted acyl guanidines **86**–**88** had similar enzyme potencies to the corresponding des-methyl analogues in Table 1 (**58**, **61**, and **68**), but gained a ∼2–3-fold improvement in cell potency. The α-methyl group offered no significant benefit toward reducing efflux for acyl guanidines **86**–**88**. Although methyl substitution at the β- and 8-positions of the THP A-ring generally improved potency relative to their des-methyl counterparts, analogues prepared with aminooxazolines or aminothiazolines still had inferior potency relative to the corresponding acyl guanidines.

Compounds from Tables 1–3 were generally predicted to have high hepatic clearance based on a mouse liver microsome (MLM) assay, and several analogues selected for mouse PK also suffered from high systemic clearance (Table 5). It is known that replacement of hydrogen with fluorine can improve metabolic stability.[27] On the basis of observations made from our previous work on chromans,[15b,c] we hypothesized that substitution of the C-ring's 3-position with a fluorine would improve metabolic stability. To test this hypothesis, 3-fluoro chromans of Table 4 were prepared as described in Schemes 3 and 6. Contrary to what we hoped, stability in MLMs was not substantially different for the two acyl guanidines **90** and **91** (CL_{hep} = 21 and 40 mL/min/kg, respectively) compared to

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des-fluoro analogues **57** and **61** (CL_{hep} = 10 and 46 mL/min/kg, respectively). In like manner, the two aminooxazolines **92** and **93** also demonstrated no improvement in microsome stability by comparison with **79** and **81** (CL_{hep} = 60 and 61 mL/min/kg, respectively). The enzyme and cell potencies for the two acyl guanidines **90** and **91** were ∼2−5-fold less than those for des-fluoro analogues **57** and **61**. Aminooxazolines **92** and **93** were tested as eutomers, so their enzyme and cell potencies were likewise not substantially different (∼1−3-fold) from the analogous racemates **79** and **81**, after correcting for the difference in enantiomeric purity. A more marked effect of fluorine substitution was on selectivity, as compounds **90**−**93** demonstrated a ∼3−4-fold decrease relative to their des-fluoro versions. In light of these disappointing results, fluorine substitution at the 3-position was not pursued on the other 7-THP chroman cores from Tables 2 and 3.

As the acyl guanidines (Tables 1, 3, and 4) suffered from high efflux, we wanted to further improve their cell potencies; and by doing so, potentially increase coverage ratios. From our previously published work,[15b] we had determined that replacing the chroman-based scaffold (Figure 4, X = O) with a

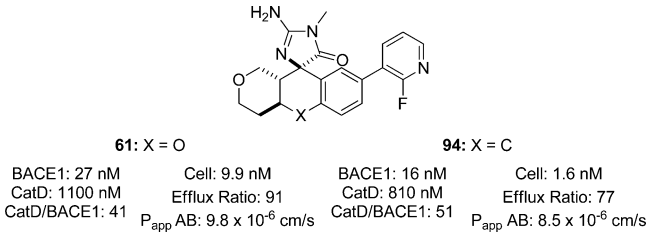

Figure 4. Comparison of racemic compounds **61** and **94**.

tetrahydronaphthalene (THN; Figure 4, X = C) generally resulted in greater cell potency. Indeed, compound **94** was ∼6-fold more potent in our cell assay than **61**, even though their enzyme potencies were within 2-fold of each other. Besides a difference in cell potency, **94** and **61** had similar selectivity, were both highly effluxed, and both had acceptable permeability. Unfortunately, the THN-based 7-THP analogues were synthetically less accessible than the corresponding chromans, and they were not pursued further.

Pharmacokinetic Studies in Mice. From the 7-THP chromans described above, we selected seven acyl guanidines **58**, **60**, **61**, **64**, **65**, **83**, and **87** and two aminooxazolines **78** and **79** to evaluate in mouse PK (Table 5). In spite of the efflux liability for the acyl guanidines, we wanted to assess their coverage ratios ($C_{free,brain}$/cell IC_{50}) to prioritize analogues for progression to pharmacodynamic studies. Aminooxazolines **78** and **79** were selected to determine if their lower efflux ratios (1.5 and 4.3, respectively) would translate to greater brain levels.

Compounds were dosed iv and po (3 and 10 mg/kg, respectively) in male CD-1 mice, with plasma samples withdrawn and brains harvested (oral groups only) at 1 and 4 h time points (Table 5). Generally, MLM's underpredicted hepatic clearance for the acyl guanidines, with most having moderate to high systemic clearance. Compounds **64** and **65** were cleared faster than hepatic blood flow, resulting in brain levels that were below level of quantitation (BLQ) at the 4 h time point. Despite having the lowest in vivo clearance, **60** had free brain levels that were 91-fold lower than its cell IC_{50} at the 4 h time point, due in part to a very low free fraction ($F_{u,brain}$ = 0.0018). In contrast, acyl guanidines **58**, **61**, and **87** were moderate to high clearance compounds, but they had ∼10−50-fold greater free fraction in the brain than **60**. A large free fraction contributed to their achieving coverage ratios of 0.31−0.85 at the 4 h time point, in spite of moderate to high clearance and high efflux. However, free brain to plasma (b/p) ratios for **58**, **61**, and **87** were poor (0.025−0.15, 4 h time point), likely due to their high efflux. Oral bioavailability (*F*%) for **58** and **61** was very good (*F*%: 103[28] and 86, respectively), but **87** had *F*% of 15, even though its permeability was comparable to **61**. It might be that addition of the α-methyl group in **87** lead to increased first pass metabolism, but this was unverified. Though **83** had similar PK and brain tissue binding to structurally related **61**, its brain levels were below the level of quantitation (BLQ) at the 4 h time point for reasons not readily apparent.

Aminooxazolines **78** and **79** demonstrated moderate systemic clearance that was better than predicted in vitro, and both had low to moderate *F*%. Compound **79** achieved free brain concentrations comparable to **61**, but because of its being ∼2-fold less potent, the coverage ratio was inferior. Aminooxazoline **78**, one of the least effluxed (efflux ratio = 1.5) of the 7-THP chromans in this work, also demonstrated a superior free b/p ratio (0.68, 4 h time point). However, its moderate cell potency (IC_{50} = 41 nM) and high brain tissue binding ($F_{u,brain}$ = 0.0031) contributed to a poor coverage ratio of 0.061 at the 4 h time point.

Pharmacokinetics for 62 in Multiple Species. Compound **61** demonstrated the best coverage ratio in the mouse (Table 5: 1.1, 1 h; 0.85, 4 h), and its eutomer **62** passed multiple in vitro toxicological hurdles (e.g., hERG patch clamp >100 μM, IC_{50} >10 μM against CYP's, clean in Ames and micronucleus assays, and highly selective in receptor, kinase, and protease panels). Thus, the PK for **62** in multiple species (Table 6) was evaluated to enable in vivo toxicology as well as PD studies in guinea pig, rat, and cyno. There was a good in vitro−in vivo correlation (IVIVC) of microsome and hepatocyte stability with in vivo clearances observed for mouse and rat. For cyno, there was a good IVIVC for microsomes; however, hepatocytes underpredicted clearance. In contrast, guinea pig demonstrated very high in vivo clearance with no apparent IVIVC. Oral bioavailability was good for mouse and rat (*F*% = 61 and >100, respectively), but poor for guinea pig and cyno (*F*% = 17 and 10, respectively). Unbound levels of **62** in plasma were ∼10−50-fold greater than in brain for mouse and rat, and ∼10−30-fold greater than CSF levels in rat and cyno, likely a result of the compound's high efflux. Because of the exceptionally high clearance in guinea pig, it was not surprising that plasma levels of **62** were >20-fold lower than for rat and cyno. However, the difference between plasma and brain/CSF levels (∼1−7-fold) was significantly smaller in guinea pig than the other species. In spite of this disconnect, CSF concentrations for **62** in rat, guinea pig, and cyno, the three species chosen for PD studies (Table 7 and Figure 5) were greater than cell IC_{50} at both time points.

Pharmacodynamic Studies. Enantiopure compounds **59** and **62** were selected for PD studies in guinea pig (Table 7), and **62** was also evaluated in rat and cyno PD models (Table 7 and Figure 5, respectively). The maximum reduction observed in guinea pig CSF $A\beta_{1-40}$ was 58% at the 3 h time point, for both the 60 and 100 mg/kg doses of **62**. For guinea pig, there was a crude correlation between free brain concentration of **59** and **62** and PD response, with the maximum response (52%

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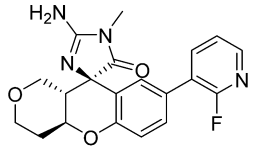
Table 6. PK for Eutomer 62 in Multiple Species: iv, po Dose



62

MW: 382
cLogP: 0.10
PSA: 90

Cell IC_{50}: 5.4 nM
CatD/BACE1: 88
P_{app} AB (10^{-6} cm/s): 9.0
Efflux Ratio: 67
Solubility (pH 7.2): 220 μg/mL

species		mouse[a]	rat[b]	guinea pig[c]	cyno[d]
dose (mg/kg)		IV: 3	IV: 3	IV: 3	IV: 0.5
		PO: 10	PO: 60	PO: 60	PO: 100
$F_{u,plasma}$		0.20	0.22	0.37	0.39
$F_{u,brain}$		0.14	0.12	0.077	0.13
CL (mL/min/kg)	$CL_{hep,int}$[g]	36	35	9.7	5.8
	$CL_{hep,in\ vitro}$[h]	38	23	17	25
	$CL_{hep,syst}$	41	30	110	22
F%		61	>100	17	10
$C_{free,plasma}$ (nM)	**1h**	300	3100	140	3200[f]
	4h	78	2200[e]	23[e]	1700
$C_{free,brain}$ (nM)	**1h**	24	68	19	
	4h	15	66[e]	34[e]	
free b/p	**1h**	0.080	0.022	0.14	
	4h	0.19	0.030[e]	1.5[e]	
C_{CSF} (nM)	**1h**		290	69	110[f]
	4h		150[e]	23[e]	84
CSF/free plasma	**1h**		0.094	0.49	0.034[f]
	4h		0.068[e]	1.0[e]	0.049

[a]iv formulation: 5 mM citrate in saline. po formulation: 1% CMC/0.5% Tween/5 mM citrate in H_2O. Male CD-1 mice: 3 animials/cohort. [b]iv formulation: 30% captisol/5 mM citrate in saline. po formulation: 1% CMC/0.5% Tween/5 mM citrate in H_2O. Male Sprague–Dawley rats: 3 animals/cohort. [c]iv formulation: 30% captisol/5 mM citrate in saline. po formulation: 1% CMC/0.5% Tween/5 mM citrate in H_2O. Male Hartley guinea pigs: 4 animals/iv route and 6 animals/po route. [d]iv formulation: PEG400/EtOH/water (40/10/50) solution. po formulation: 1% carboxymethylcellulose sodium (CMC), 0.5% Tween 80, 5 mM citrate buffer, suspension. Female cynos: 3 animals/dosing route. [e]Measured at the 3 h time point. [f]Measured at the 2 h time point. [g]Compounds (2 μM) incubated with cryopreserved hepatocytes for 60 and 120 min at 37 °C. [h]Compounds (1 μM) incubated with mouse liver microsomes for 20 min at 37 °C in the presence of NADPH.

and 58%, respectively) for both compounds corresponding with their peak brain concentrations at the 3 h time point. In rat, **62** had a relatively constant free brain concentration between 46–84 nM, which lead to a sustained PD effect out to the 12 h time point. Even though **59** had ~10-fold greater unbound plasma levels than **62** in guinea pig, it had a lower free brain concentration due in part to increased brain tissue binding ($F_{u,brain}$ = 0.021 for **59** vs 0.077 for **62**). Despite a lower coverage ratio, **59** demonstrated a comparable PD effect to **62** at both time points. For compound **62**, coverage ratios corresponding with maximum CSF Aβ_{1-40} reduction for guinea pig and rat were significantly different (6.3 vs 15, respectively). This discrepancy may be due to interspecies differences in the APP sequence.[29]

For cyno, the maximum observed reduction in CSF Aβ_{1-40} for **62** was 63% at 8 h, which lagged behind its maximum CSF concentration of 120 nM at 2 h (Figure 5). As with rat, there was a sustained PD response in cyno, with a 49% reduction in CSF Aβ_{1-40} at the 24 h time point. However, the PD response in cyno persisted even though the CSF concentration for **62** had decreased by 24-fold from its peak concentration after 24 h. By the 48 h time point, cyno CSF Aβ_{1-40} levels had returned to baseline. Full details of this cyno PD study are published elsewhere.[30]

SUMMARY

Using structure-based design, we substituted a 1,3,4,4a,10,10a-hexahydropyrano[4,3-b]chromene ("7-THP chroman") core to improve affinity for BACE1 and selectivity against CatD. Three different warheads (spirocyclic acyl guanidine, aminooxazoline, and aminothiazoline), which anchored the molecules in the active site by binding to the catalytic Asp residues, were examined for their effect on potency, selectivity, efflux, and permeability. The acyl guanidine moiety provided the most potent analogues, but at the expense of high efflux ratios and lower permeability than the two alternative warheads. On the basis of crystallography and modeling of the S2′ site, methyl substitution was explored at three different positions (α-, β-, and 8-positions) around the 7-THP A-ring in order to boost potency and selectivity. Although we did identify BACE1 inhibitors with superior potency and selectivity relative to their des-methyl counterparts, the improvements in potency were unable to provide compounds (e.g., **78** and **79**) with free brain levels sufficient to cover their cell IC_{50} values in vivo. Substitution with various aromatic R groups, which provided potency and selectivity through binding to the S3 site, resulted in the identification of lead molecule **62** (R = 2-fluoropyridin-3-yl). In spite of high efflux (efflux ratio = 67), **62** demonstrated the best coverage ratio of the various 7-THP chromans evaluated in mouse PK. Furthermore, **62** significantly reduced CSF Aβ_{1-40} in three different species (i.e., guinea pig, rat, and cyno). Our initial criteria for selectivity against CatD had been >100-fold, but to minimize risks associated with inhibition of CatD in vivo,[16] we chose to pursue compounds with even greater selectivity. Guided by modeling and the SAR presented in this work, we discovered a series of THP A-ring chromans with much improved selectivity (>1000-fold against CatD), which will be the subject of a future publication.

EXPERIMENTAL SECTION

Chemistry. Column chromatography was done on a Biotage system (Manufacturer: Dyax Corporation) having a silica gel column, on a silica SepPak cartridge (Waters), or using silica gel 60 (EMD brand, 230–400 mesh) (unless otherwise stated). Preparative TLC purification was performed using PLC-type silica gel 60, F_{254}, 20 × 20 cm plates (EMD brand; 0.5, 1.0, or 2.0 mm thickness). ^{1}H NMR were recorded on a Varian or Bruker instrument operating at 400 MHz. ^{1}H NMR spectra were obtained as $CDCl_3$, CD_3OD, D_2O, $(CD_3)_2SO$, $(CD_3)_2CO$, C_6D_6, and CD_3CN solutions (reported in ppm), using tetramethylsilane (0.00 ppm) or residual solvent ($CDCl_3$, 7.26 ppm; CD_3OD, 3.31 ppm; D_2O, 4.79; $(CD_3)_2SO$, 2.50 ppm; $(CD_3)_2CO$, 2.05 ppm; C_6D_6, 7.16 ppm; CD_3CN, 1.94 ppm) as the reference standard. When peak multiplicities are reported, the following abbreviations are used: s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet), br (broadened), dd (doublet of doublets), and dt (doublet of triplets). Coupling constants, when given, are reported in Hertz (Hz). All temperatures are set forth in degrees Celsius. Low-resolution mass spectral (MS) data were obtained on an Agilent Technologies 6120

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Table 7. PD in Guinea Pig and Rat

compd	formulation	species	PO dose (mg/kg)	time point (h)	$C_{free,plasma}$ (nM)	$C_{free,brain}$ (nM)	b/p free	C_{CSF} (nM)	coverage ratio[a]	CSF $A\beta_{1-40}$ Reduction (%)
62	1% CMC/0.5% Tween/5 mM citrate in H_2O	guinea pig[b]	30[c]	3	5.8	9.5	1.6	9.9	1.8	20
			60[d]	1	140	19	0.14	69	3.5	35
				3	23	34	1.5	23	6.3	58
				5	7.0	12	1.7	6.9	2.2	7.0
				8	<3.0[f]	5.2	>1.7	1.6	0.96	23
				12	<3.0[f]	11	>3.7	2.6	2.0	15
			100[c]	3	38	50	1.3	56	9.3	58
		rat[e]	60[c]	1	3100	68	0.022	290	13	26
				3	2200	66	0.030	150	12	40
				5	2700	79	0.029	200	15	53
				8	1700	84	0.049	130	16	38
				12	960	46	0.048	85	8.5	30
59	40/10/50 PEG/EtOH/H_2O	guinea pig[b]	60	3[d]	260	12[g]	0.046	90	1.6	52
				5[c]	79	6.7[g]	0.085	40	0.92	15

[a]Coverage ratio = $C_{free,brain}$/cell IC_{50}. [b]Male Hartley guinea pigs. [c]Eight animals/cohort. [d]Six animals/cohort. [e]Male Sprague–Dawley rats. [f]Compound levels were BLQ. [g]Three animals were used to determine brain concentration.

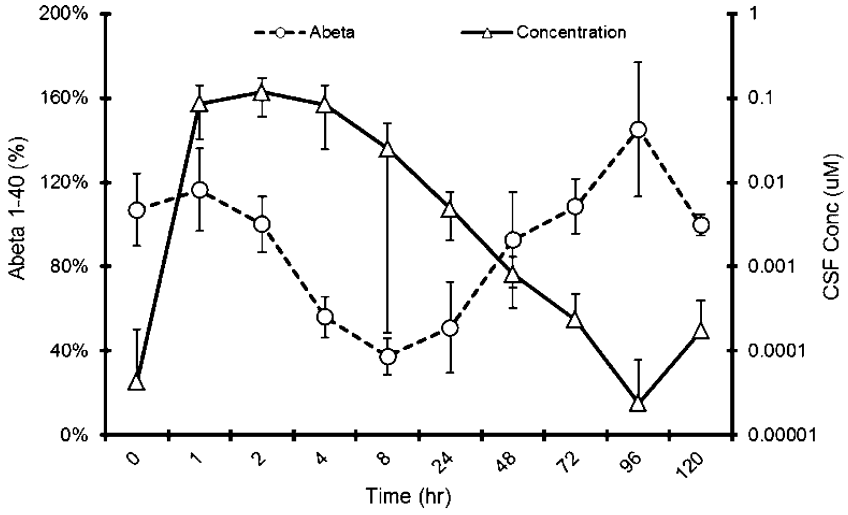

Figure 5. Cyno CSF concentration of $A\beta_{1-40}$ (dotted line) and compound **62** (solid line). Po formulation: 1% carboxymethylcellulose sodium (CMC), 0.5% Tween 80, 5 mM citrate buffer, suspension; 10 male cynomolgus monkeys, crossover design (n = 10 vehicle, n = 10 at 100 mg/kg).

series quadrupole LC–MS with UV detection at 220 and 254 nm and multimode ionization using atmospheric-pressure chemical ionization (APCI) or ESI. The reactions set forth below were done generally under a positive pressure of nitrogen or argon or with a drying tube (unless specified otherwise) in anhydrous solvents, and the reaction flasks were typically fitted with rubber septa for the introduction of substrates and reagents via syringe. Glassware was oven-dried and/or heat dried using a heat gun while under vacuum. Reagents were purchased from commercial suppliers such as Sigma-Aldrich, Alfa Aesar, TCI, or Acros and were used without further purification unless otherwise indicated. Anhydrous solvents (e.g., THF, DMF, DMA, DMSO, MeOH, DCM, and toluene) were purchased from EMD chemicals (DriSolv line) or from Sigma-Aldrich and used directly. Unless indicated otherwise, final compounds were purified to ≥95% purity as determined by high-performance liquid chromatography (HPLC) or liquid chromatography mass spectrometry (LC–MS). HPLC purity was measured using a Varian ProStar with UV detection at 220 and 254 nm, a Waters YMC ODS-AQ C18 3.0 mm × 50 mm, 3.0 μm, 5–95% CH_3CN in H_2O with 0.1% NH_4OAc for 5 min at 1.0 mL/min. LC–MS purity was measured using an Agilent Technologies 6120 series LC–MS with UV detection at 220 and 254 nm, a Halo C18 2.1 mm × 30 mm, 2.7 μm, 5–95% CH_3CN in H_2O with 0.1% NH_4OAc for 3 min at 1.0 mL/min. pK_a values were determined at Analiza using a 24 point parallel capillary electrophoresis method with quantitation by UV absorbance at 229 nm.

4-(3,6-Dihydro-2*H*-pyran-4-yl)morpholine (5). A mixture of dihydro-2*H*-pyran-4(3H)-one **4** (100 g, 999 mmol) and morpholine (131 mL, 1498 mmol) in toluene (333 mL) was refluxed under Dean–Stark trap overnight. More than 1 equiv of water was collected. This reaction mixture was then concentrated down to give crude **5** (169 g) as an oil, which was carried forward to the next step without purification.

8-Bromo-4a-morpholino-1,3,4,4a,10,10a-hexahydropyrano-[4,3-b]chromen-10-ol (7). A mixture of **5** (178.1 g, 1052 mmol) and 5-bromo-2-hydroxybenzaldehyde **6** (211.6 g, 1052 mmol) in toluene (351 mL) was stirred overnight at rt. A white solid crashed out and was filtered off. This was washed with toluene (50 mL). The solid product was collected and dried to give **7** (306.8 g, 79% yield) as a 2:1 mixture of diastereomers, which were not separated. ^1H NMR (400 MHz, $CDCl_3$) isomer 1 (major diastereomer) δ 7.55 (m, 1H), 7.29 (dd, J = 3, 9 Hz, 1H), 6.71 (d, J = 9 Hz, 1H), 5.10 (d, J = 6 Hz, 1H), 4.08 (m, 1H), 3.96 (m, 1H), 3.61–3.85 (m, 2H), 3.58 (m, 4H), 3.31 (t, J = 11 Hz, 1H), 2.59–2.84 (m, 5H), 1.83 (m, 2H); isomer 2 (minor diastereomer) δ 7.47 (m, 1H), 7.35 (dd, J = 3, 9 Hz, 1H), 6.79 (d, J =

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9 Hz, 1H), 4.08 (m, 1H), 3.96 (m, 1H), 3.61−3.85 (m, 2H), 3.58 (m, 4H), 3.02 (*t*, J = 11 Hz, 1H), 2.59−2.84 (m, 5H), 2.00 (m, 2H), 1.89 (m, 1H).

8-Bromo-3,4-dihydropyrano[4,3-b]chromen-10(1*H*)-one (8). DMSO (204 mL, 2878 mmol) was added dropwise to oxalyl chloride (1726.9 mmol) in DCM (8 L) at −78 °C. This was added such that the temperature did not rise above −65 °C. This was then stirred for 40 min at −78 °C. Compd **7** (533 g, 1439 mmol) was added as a solid (temperature did not rise), and this was stirred for 2 h at −78 °C. The solid did not fully go into solution. Triethylamine (602 mL, 4317 mmol) was added dropwise (some exotherm was seen; however, the reaction temperature did not get above −65 °C). This was stirred for 30 min at −78 °C. During the entire course of the reaction, the mixture was continually purged with N_2, which exited the flask via a line fed into a bleach trap. The mixture was then concentrated down. Glacial acetic acid (1000 mL) was added to the mixture. The material went into solution initially; however, after 5 min of stirring, product began to crash out. The material was stirred overnight at rt. White solid had crashed out and this was filtered. The white solid was washed with glacial acetic acid (200 mL). This gave **8** (340.8 g, 84% yield) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.31 (d, J = 3 Hz, 1H), 7.74 (dd, J = 2, 9 Hz, 1H), 7.32 (d, J = 9 Hz, 1H), 4.65 (m, 2H), 4.02 (m, 2H), 2.79 (m, 2H).

(4a*S,10a*S**)-8-Bromo-1,4,4a,10a-tetrahydropyrano[4,3-b]chromen-10(3*H*)-one (9).** L-Selectride (587 mL, 587 mmol, 1 M in THF) was added to a mixture of **8** (150 g, 534 mmol) in DCM (2809 mL) at −78 °C. This was stirred for 45 min. TLC showed that the reaction was complete. The mixture was placed in an ice bath. Aqueous Rochelle's salt (0.5 M) was added to the mixture as it was warming to 0 °C. This was then worked up with EtOAc/water. The organics were extracted twice, washed with brine, dried (Na_2SO_4), and concentrated. The crude was then triturated with hexanes to give **9** (100 g, 66% yield) in >95% diastereomeric purity by ^{1}H NMR. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.96 (d, J = 3 Hz, 1H), 7.57 (dd, J = 3, 9 Hz, 1H), 6.89 (d, J = 9 Hz, 1H), 4.51 (m, 1H), 4.32 (m, 1H), 4.12 (m, 1H), 3.45 (td, J = 3, 12 Hz, 1H), 3.38 (m, 1H), 2.83 (m, 1H), 2.05−2.20 (m, 2H).

(4*S,4a′*S**,10a′*S**)-8′-Bromo-3′,4′,4a′,10a′-tetrahydro-1′*H*-spiro[imidazolidine-4,10′-pyrano[4,3-b]chromene]-2,5-dione (10).** A mixture of **9** (75 g, 265 mmol), KCN (34.5 g, 530 mmol), ammonium carbonate (204 g, 2119 mmol), and $NaHSO_3$ (11.0 g, 106 mmol) in EtOH (265 mL) was heated to 130 °C overnight in a steel bomb with stirring. The mixture was poured into an Erlenmeyer flask with side arm in an ice bath. The flask was purged with N_2, and the outlet line was fed into a 2 N NaOH soln to quench HCN. To the flask was carefully added concentrated HCl until the pH was about 1. This was then stirred in an ice bath for 1 h while purging with N_2. The resulting white solid was filtered off and collected. This solid was dried and then taken up in i-PrOH (500 mL) and heated at reflux for 30 min. This was then cooled to rt and then to 5 °C in an ice bath. The white solid was filtered and washed with i-PrOH (50 mL) to give **10** (44.8 g, 43% yield) in 84% diastereomeric purity by ^{1}H NMR. ^{1}H NMR (400 MHz, $(CD_3)_2SO$) δ 11.14 (s, 1H), 8.45 (s, 1H), 7.44 (dd, J = 2, 9 Hz, 1H), 7.22 (d, J = 2 Hz, 1H), 6.89 (d, J = 9 Hz, 1H), 4.74 (td, J = 5, 11 Hz, 1H), 3.97 (m, 2H), 3.40 (m, 1H), 3.06 (t, J = 11 Hz, 1H), 2.10 (m, 2H), 1.69 (m, 1H).

(4*R,4a′*S**,10a′*S**)-8′-Bromo-1-methyl-3′,4′,4a′,10a′-tetrahydro-1′*H*-spiro[imidazolidine-4,10′-pyrano[4,3-b]chromene]-2,5-dione (11).** A round bottomed flask plus stir bar was charged with DMF (100 mL) and **10** (16.3 g, 46.2 mmol). The mixture was cooled in an ice bath under N_2, and K_2CO_3 (9.6 g, 69 mmol) and iodomethane (2.9 mL, 46 mmol) were added sequentially. It was stirred in the ice bath for 10 min, and then removed from the bath and allowed to stir at rt for 2 h. The reaction mixture was diluted with EtOAc (300 mL) and water (200 mL). The phases were separated and re-extracted with aqueous EtOAc (150 mL). Combined organic phases were washed with water (200 mL), brine (200 mL), dried ($MgSO_4$), filtered, and concentrated. Yield of **11**: 15.9 g (94% yield). Product was approximately 85% pure based on HPLC, and was carried forward to the next step without purification. ^{1}H NMR (400 MHz, $(CD_3)_2SO$) δ 8.72 (s, 1H), 7.42 (dd, J = 2, 9 Hz, 1H), 7.28 (d, J = 2 Hz, 1H), 6.89 (d, J = 9 Hz, 1H), 4.76 (td, J = 5, 11 Hz, 1H), 3.97 (m, 1H), 3.88 (m, 1H), 3.37 (m, 1H), 2.97 (t, J = 11 Hz, 1H), 2.88 (s, 3H), 2.13 (m, 2H), 1.68 (m, 1H).

(4*R,4a′*S**,10a′*S**)-8′-Bromo-1-methyl-2-thioxo-3′,4′,4a′,10a′-tetrahydro-1′*H*-spiro[imidazolidine-4,10′-pyrano[4,3-b]chromen]-5-one (12).** A thick walled, glass pressure vessel with **11** (15.9 g, 43.3 mmol), Lawesson's reagent (10.5 g, 26.0 mmol), and toluene (150 mL) was charged. The mixture was degassed with N_2 for several minutes. The mixture was then heated to 90 °C for 15 h with stirring. Reaction had gone to approx 50% conversion by HPLC and ^{1}H NMR. Then more Lawesson's reagent (3.5 g, 0.2 equiv) was added and heated to 100 °C for an additional 22 h. HPLC indicated >95% consumption of starting material. After cooling to rt, much solid had formed. The suspension was cooled in a freezer at 5 °C for 2 h, then solids were filtered (14.9 g) and washed with toluene. The mother liquor was saved. This solid (14.9 g) was mostly desired product by ^{1}H NMR, and it was partially purified by silica gel plug, eluting with 10% Et_2O in DCM. The mother liquor that had been saved was partitioned between EtOAc (200 mL) and satd. aq. $NaHCO_3$ (200 mL). Phases were separated, and the aqueous phase was re-extracted with EtOAc (150 mL). Combined organic phases were washed with brine (200 mL), dried ($MgSO_4$), filtered, and concentrated to obtain 15.3 g of crude material. By ^{1}H NMR, this crude contained approx 20% desired product. To improve upon the product yield, it was chromatographed on a Biotage Flash 75L system, eluting with 5% Et_2O/DCM isocratic. From this column recovered 2.1 g additional product, which was combined with the 13 g of product purified by silica gel plug, to obtain 15.1 g (59% yield) of **12**. ^{1}H NMR (400 MHz, $(CD_3)_2SO$) δ 10.76 (s, 1H), 7.47 (dd, J = 2, 9 Hz, 1H), 7.07 (d, J = 2 Hz, 1H), 6.93 (d, J = 9 Hz, 1H), 4.75 (td, J = 5, 11 Hz, 1H), 3.97 (m, 1H), 3.73 (m, 1H), 3.38 (m, 1H), 3.14 (s, 3H), 2.98 (t, J = 11 Hz, 1H), 2.19 (m, 2H), 1.70 (m, 1H).

(4*R,4a′*S**,10a′*S**)-2-Amino-8′-bromo-1-methyl-3′,4′,4a′,10a′-tetrahydro-1′*H*-spiro[imidazole-4,10′-pyrano-[4,3-b]chromen]-5(1*H*)-one (13).** A round bottomed flask plus stir bar with **12** (15.1 g, 39.4 mmol), MeOH (200 mL), 70% aqueous *t*-butyl hydroperoxide (38 mL, 276 mmol), and 30% aq. NH_4OH (77 mL, 591 mmol) was charged. The mixture was heated to 50 °C for 2 h with stirring. After cooling to rt, the mixture was diluted with water (20 mL) and concentrated (but not to dryness) in vacuo. The residue was diluted with EtOAc (150 mL), and phases were separated and re-extracted with aqueous EtOAc (2 × 75 mL). Combined organic phases were washed with brine (150 mL), dried ($MgSO_4$), filtered, and concentrated. Purified crude (15.6 g) by silica gel chromatography on a Biotage Flash 75L system, eluting with 7−10% MeOH/DCM. A total of 7.4 g (47%) of **13** as a white powde was obtained, in >95% diastereomeric purity by ^{1}H NMR. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.27 (dd, J = 2, 9 Hz, 1H), 7.01 (d, J = 2 Hz, 1H), 6.76 (d, J = 9 Hz, 1H), 4.85 (td, J = 5, 11 Hz, 1H), 4.68 (br s, 2H), 4.05 (m, 1H), 3.94 (m, 1H), 3.45 (m, 1H), 3.05 (s, 3H), 3.00 (t, J = 11 Hz, 1H), 2.11 (m, 2H), 1.82 (m, 1H).

(4a*S,10a*S**)-8-Bromo-10-methylene-1,3,4,4a,10,10a-hexahydropyrano[4,3-b]chromene (14).** A round bottomed flask plus stir bar with **9** (544 mg, 1.92 mmol) and anhyd. THF (5 mL) was charged and cooled to 0 °C under N_2, and added Tebbe's reagent (5.7 mL, 2.9 mmol, 0.5 M in toluene). This mixture was stirred for 2 h at rt. Then, it was cooled in an ice bath and added MeOH (5 mL) very carefully (vigorous exotherm and bubbling), then added aq. 2 N NaOH (5 mL) dropwise. To enable stirring, DCM (10 mL) was added. The biphasic suspension was stirred for 15 min at rt. The biphase was filtered to remove solids through celite, rinsing with DCM. Phases were separated and re-extracted with aqueous DCM (5 mL). Combined organics were washed with brine (20 mL), dried ($MgSO_4$), filtered, and concentrated. Purified crude by Biotage Flash 40 silica gel chromatography, eluting with 10−15% EtOAc/hexanes. Yield of **14**: 168 mg (30%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.63 (d, J = 2 Hz, 1H), 7.27 (dd, J = 2, 9 Hz, 1H), 6.74 (d, J = 9 Hz, 1H), 5.48 (d, J = 2 Hz, 1H), 4.71 (d, J = 2 Hz, 1H), 4.44 (m, 1H), 4.12 (m, 1H),

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3.79−3.89 (m, 1H), 3.48 (m, 1H), 3.35 (m, 1H), 2.51 (m, 1H), 2.09 (m, 1H), 1.96 (m, 1H).

(4*R,4a′*S**,10a′*S**)-8′-Bromo-3′,4′,4a′,10a′-tetrahydro-1′*H*,5*H*-spiro[oxazole-4,10′-pyrano[4,3-b]chromen]-2-amine (15a) and (4*R**,4a′*R**,10a′*R**)-8′-Bromo-3′,4′,4a′,10a′-tetrahydro-1′*H*,5*H*-spiro[oxazole-4,10′-pyrano[4,3-b]chromen]-2-amine (15b).** This analogue was prepared from **14**, as described for 8-bromo-3,4,4a,10a-tetrahydro-1H,5′H-spiro[pyrano[4,3-b]chromene-10,4′-thiazol]-2′-amine **16**, replacing silver thiocyanate with silver cyanate. Diastereomers were partially separated by preparative TLC (2 mm thickness), eluting with 10% MeOH/DCM. Mixed fractions were repurified by Biotage Flash 40 column, eluting with a gradient of 1:1 EtOAc/hexanes, neat EtOAc, then 2.5%−5% MeOH/EtOAc. Yield of **15a**: 62 mg (16%); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.42 (d, J = 2 Hz, 1H), 7.25 (dd, J = 2, 9 Hz, 1H), 6.67 (d, J = 9 Hz, 1H), 4.57 (br s, 2H), 4.39 (d, J = 9 Hz, 1H), 4.10 (m, 2H), 3.97 (d, J = 9 Hz, 1H), 3.46 (m, 1H), 3.23 (t, J = 11 Hz, 1H), 2.15 (m, 2H), 1.91 (m, 2H). Yield of **15b**: 49 mg (15%); ^{1}H NMR (400 MHz, $CDCl_3$ + CD_3OD) δ 7.31 (d, J = 2 Hz, 1H), 7.26 (dd, J = 2, 9 Hz, 1H), 6.72 (d, J = 9 Hz, 1H), 4.54 (d, J = 9 Hz, 1H), 4.36 (d, J = 9 Hz, 1H), 4.19 (td, J = 5, 11 Hz, 1H), 4.05 (m, 2H), 3.52 (m, 1H), 3.37 (m, 1H), 2.16 (m, 1H), 1.88 (m, 2H).

8-Bromo-3,4,4a,10a-tetrahydro-1*H*,5′*H*-spiro[pyrano[4,3-b]-chromene-10,4′-thiazol]-2′-amine (16). A stirred solution of **14** (168 mg, 0.598 mmol) in diethyl ether (2 mL) was cooled to 0 °C under N_2. In a separate flask, silver thiocyanate (397 mg, 2.39 mmol) was suspended in CH_3CN (1 mL), and to this suspension, iodine (303 mg, 1.20 mmol) in THF (1 mL) was added, and the resulting mixture was shaken for 30 s. The suspension was then poured into the alkene solution at 0 °C, and the vial was rinsed with CH_3CN and added to the reaction mixture. The reaction mixture was stirred at rt for 4 h. Aqueous NH_4OH (1 mL) was added directly to the reaction mixture, and it was stirred at rt for 2 h, then left sitting for 18 h at rt. Filtered salts through Celite, rinsing with EtOAc. The filtrate was washed with aq. satd. $Na_2S_2O_3$ (20 mL). After shaking and then separating the phases, the aqueous layer was re-extracted with EtOAc (20 mL). The combined organic layers were washed with brine (20 mL), dried ($MgSO_4$), filtered, and concentrated. Crude was partially purified by preparative TLC (2 mm thickness) eluting with 10% MeOH/DCM and then carried forward to the next step as a mixture of diastereomers.

8-Bromo-2-methyl-2,3,4,6-tetrahydro-2,6-methanobenzo-[b][1,5]dioxocin-4-ol (19). To a solution of 5-bromo-2-hydroxybenzaldehyde **18** (30 g, 150 mmol) and Et_3N (10.5 mL, 75 mmol) in dioxane (56 mL) and H_2O (19 mL) was added methylbut-2-enal **17** (12.6 g, 150 mmol). The mixture was stirred at 55 °C for 12 h. Saturated aq. $NaHCO_3$ was then added to the mixture and the aqueous phase was extracted with EtOAc. The organic layer was dried over Na_2SO_4, filtered, and concentrated to give the crude product, which was purified by column chromatography on silica gel (eluting with 1:1 EtOAc/hexanes) to give yellow solid **19** (26.5 g, 63%) as a single unknown diastereomer. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.30−7.26 (m, 2H), 6.69 (d, J = 10 Hz, 1H), 4.85−4.80 (m, 2H), 3.17 (d, J = 6 Hz, 1H), 2.20−2.10 (m, 2H), 1.81−1.77 (m, 1H), 1.60−1.55 (m, 1H), 1.45 (s, 3H).

6-Bromo-2-(2-hydroxyethyl)-2-methylchroman-4-ol (20). To a solution of **19** (20.5 g, 71.8 mmol) in MeOH (60 mL) and THF (240 mL) was added $NaBH_4$ (1.4 g, 35.9 mmol). The mixture was stirred at 0 °C for 2 h. The reaction was quenched with satd. aq. $NaHCO_3$ and extracted with DCM. The organic layer was dried over Na_2SO_4, filtered, and concentrated to give white solid **20** (16.6 g, 81%) as a single unknown diastereomer. ^{1}H NMR (400 MHz, CDCl3) δ 7.58 (dd, J = 0.8, 2 Hz, 1H), 7.28−7.25 (m, 1H), 6.68 (d, J = 9 Hz, 1H), 4.84−4.81 (m, 1H), 3.90−3.82 (m, 2H), 2.16−1.90 (m, 6H), 1.35 (s, 3H).

6-Bromo-2-(2-hydroxyethyl)-2-methylchroman-4-one (21). To a solution of **20** (16.6 g, 58 mmol) in DCM (300 mL) was added MnO_2 (25.2 g, 290 mmol). The reaction mixture was stirred at rt for 12 h. The reaction was filtered, and the filtrate was concentrated to give the crude product, which was purified by column chromatography on silica gel (2:1 hexanes/EtOAc) to give **21** (10.8 g, 65% yield) as a yellow solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.96 (d, J = 3 Hz, 1H), 7.56 (dd, J = 3, 9 Hz, 1H), 6.84 (d, J = 9 Hz, 1H), 3.89−3.86 (m, 2H), 2.93−2.64 (m, 2H), 2.11−1.95 (m, 2H), 1.45 (s, 3H).

6-Bromo-2-(2-(methoxymethoxy)ethyl)-2-methylchroman-4-one (22). To a solution of **21** (10.8 g, 38 mmol) and i-Pr_2EtN (13.5 mL, 76 mmol) in DCM (30 mL) was added MOMCl (4.4 mL, 57 mmol) at 0 °C. The reaction mixture was stirred at rt for 12 h. The reaction was then quenched with satd. aq. NH_4Cl and extracted with DCM. The organic layer was dried over Na_2SO_4, filtered, and concentrated to give the crude product, which was purified by column chromatography on silica gel (10:1 hexanes/EtOAc) to give **22** (10.1 g, 81%) as an oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.96 (d, J = 2 Hz, 1H), 7.55 (dd, J = 3, 9 Hz, 1H), 6.84 (d, J = 9 Hz, 1H), 4.59 (d, J = 0.8 Hz, 2H), 3.72−3.68 (m, 2H), 3.34 (s, 3H), 2.91−2.86 (m, 1H), 2.70−2.66 (m, 1H), 2.12−1.99 (m, 2H), 1.44 (s, 3H).

((6-Bromo-2-(2-(methoxymethoxy)ethyl)-2-methyl-2*H*-chromen-4-yl)oxy)trimethylsilane (23). To a solution of **22** (20.0 g, 60.9 mmol) in THF (300 mL) was added dropwise LHMDS (66.9 mL, 66.9 mmol, 1 M in THF) at −70 °C. The mixture was stirred for 10 min. Then TMSCl (7.26 g, 66.8 mmol) was added to the mixture. After 30 min, the reaction was quenched with 10% aq. $NaHCO_3$ at −70 °C, and extracted with DCM. The organic layer was dried over Na_2SO_4, filtered, and concentrated to afford crude **23**, which was carried to the next step without further purification.

(4a*S,10a*R**)-8-Bromo-4a-methyl-1,4,4a,10a-tetrahydropyrano[4,3-b]chromen-10(3*H*)-one (24).** To a solution of $TiCl_4$ (23.4 mL, 213 mmol) in DCM (200 mL) was added **23** in DCM (100 mL) at −40 °C. The mixture was stirred at −40 °C for 30 min. The mixture was then poured into satd. aq. $NaHCO_3$, extracted with DCM twice, washed with brine, and the organic layer was then dried over Na_2SO_4, filtered, and concentrated to give the crude product, which was purified by column chromatography on silica gel (10:1 hexanes/EtOAc) to give **24** (12.0 g, 67% yield, 2 steps), which was the pure cis stereoisomer at the ring fusion. ^{1}H NMR (400 MHz, CDCl3) δ 7.96 (d, J = 2 Hz, 1H), 7.59 (dd, J = 2, 8 Hz, 1H), 6.90 (d, J = 9 Hz, 1H), 3.89−3.82 (m, 3H), 3.60 (t, J = 11 Hz, 1H), 2.71 (dd, J = 11, 5 Hz, 1H), 2.00−1.96 (dt, J = 14, 3 Hz, 1H), 1.78 (ddd, J = 14, 11, 6 Hz, 1H), 1.37 (s, 3H).

(4a*S,10a*S**)-8-Bromo-4a-methyl-1,4,4a,10a-tetrahydropyrano[4,3-b]chromen-10(3*H*)-one (25).** Compound **24** (7.0 g, 24 mmol) was dissolved in THF (150 mL) and cooled to −78 °C. LHMDS (25 mL, 25 mmol, 1 M in THF) was then added slowly via syringe. After stirring for 10 min at −78 °C, the enolate-containing solution was added over 10 min via cannula to a solution of ethyl 2-hydroxybenzoate (14 mL, 94 mmol) in THF (100 mL) also cooled to −78 °C. After stirring for 10 min, the reaction mixture was quenched with satd. aq. NH_4Cl, partitioned between DCM and brine, and the phases were then separated. The organic layer was dried over Na_2SO_4, filtered, and concentrated. The crude material was then purified by silica gel eluting with a linear gradient of 0−20% EtOAc/heptane to provide a 5:1 mixture of trans/cis stereoisomers at the ring fusion. Yield of **25**: 6.3 g, 90%.

(4a*S,10a*S**)-8-Bromo-4a-methyl-10-methylene-1,3,4,4a,10,10a-hexahydropyrano[4,3-b]chromene (26).** Compound **25** (2.45 g, 8.25 mmol) was dissolved in THF (25 mL) and cooled to −78 °C. Tebbe's reagent (24.7 mL, 12.4 mmol, 0.5 M in toluene) was then added slowly via syringe. The reaction mixture was allowed to warm to rt overnight. The next morning, the reaction mixture was cooled to 0 °C and slowly quenched with MeOH (∼20 mL) followed by the addition of 1 N aq. NaOH (∼10 mL). After stirring 15 min at rt, the mixture was diluted with DCM (200 mL) and filtered through Celite. The filtrate was partitioned between additional DCM and brine, phases separated, and the organic layer was then dried over Na_2SO_4, filtered, and concentrated. The crude material was purified by silica gel eluting with a linear gradient of 0−30% EtOAc/heptane to provide **26** (1.47 g, 60% yield) as a 3:1 mixture of trans/cis stereoisomers at the ring fusion.

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8′-Bromo-4a′-methyl-3′,4′,4a′,10a′-tetrahydro-1′*H*,5*H*-spiro[oxazole-4,10′-pyrano[4,3-b]chromen]-2-amine (27). Iodine (1.38 g, 5.44 mmol) dissolved in EtOAc (39 mL) was added over 5 min to a suspension of **26** (1.46 g, 4.95 mmol) and silver cyanate (899 mg, 5.93 mmol) in EtOAc (6.0 mL) and CH_3CN (13.2 mL) at 0 °C. The reaction mixture was then warmed to rt and allowed to stir for 30 min and then heated at 40 °C for an additional 30 min. The heterogeneous reaction mixture was then filtered through celite and concentrated. The crude material was redissolved in THF (30 mL) and NH_4OH solution (20 mL). The mixture was then heated at 40 °C for 1 h. The reaction mixture was then partitioned between DCM and brine, phases separated, and the organic layer dried over Na_2SO_4, filtered, and concentrated. The crude material was then purified by silica gel eluting with a linear gradient of 0−6% MeOH/DCM + 1% NH_4OH solution to provide **27** (585 mg, 33% yield) as a mixture of diastereomers, which was carried on to the next reaction without additional purification.

5-Bromo-4-fluoro-2-hydroxybenzaldehyde (28). To a solution of 3-fluorophenol (100.0 g, 893 mmol) in anhydrous CH_3CN (1.0 L) was added $MgCl_2$ (254.7 g, 2.7 mol) portionwise at 0 °C. Et_3N (494 mL, 3.5 mol) was added dropwise to the mixture over 25 min, followed by portionwise addition of paraformaldehyde (160.7 g, 5.3 mol). After complete addition, the mixture was heated at reflux for 3 h. The mixture was cooled and quenched by the addition of cold conc. HCl (347 mL) and extracted with EtOAc. The combined EtOAc layers were washed with water and brine, dried over Na_2SO_4, filtered, and concentrated to give 4-fluoro-2-hydroxybenzaldehyde (100.0 g, 80% yield) as a yellow oil, which was carried forward without purification. To a solution of 4-fluoro-2-hydroxy-benzaldehyde (100.0 g, 714 mmol) in acetic acid (1.0 L) was added bromine (118.5 g, 750 mmol) dropwise at 10 °C. The mixture was stirred at rt overnight. To the reaction mixture was added dropwise satd. aq. Na_2SO_3 slowly at 0 °C until the brown color disappeared. The reaction was then poured into ice−water, the solid was collected by filtration and dried to give **28** (50.0 g, 26% yield, 2 steps) as a yellow solid. It was used in the next step without purification. ^{1}H NMR (400 MHz, $CDCl_3$) δ 11.34 (br, 1H), 9.77 (s, 1 H), 7.74 (dd, J = 3, 7 Hz, 1H), 6.79 (d, J = 10 Hz, 1H).

(*E*)-1-(5-Bromo-2-hydroxyphenyl)-3-(dimethylamino)prop-2-en-1-one (39). Similar to a published procedure for related compounds,[19] a mixture of 1-(5-bromo-2-hydroxyphenyl)ethanone **38** (50 g, 233 mmol) and DMF-dimethylacetal (42 g, 349 mmol) in dry toluene (250 mL) was refluxed for 3 h. After cooling to rt, the mixture was concentrated to half volume, and the resulting suspension was cooled in an ice bath, then the solids were filtered and washed with minimum amounts of toluene. Yield of **39**: 56 g (87%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 13.95 (s, 1H), 7.88 (d, J = 12 Hz, 1H), 7.76 (d, J = 2 Hz, 1H), 7.42 (dd, J = 2, 9 Hz, 1H), 6.83 (d, J = 9 Hz, 1H), 5.67 (d, J = 12 Hz, 1H), 3.20 (s, 3H), 2.99 (s, 3H).

3-Acetyl-6-bromo-4*H*-chromen-4-one (40). Similar to a published procedure for related compounds,[19] to a solution of **39** (56 g, 207 mmol) in dry pyridine (84 mL) was added acetic anhydride (196 mL), and the mixture was stirred at rt for 18 h. The mixture was concentrated on the rotovap to one-half volume at 80 °C. Cooled the resulting suspension to rt, and then filtered the solids. Washed solids with hexanes. Dried under high vacuum. Yield of **40**: 48 g (85%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.59 (s, 1H), 8.39 (d, J = 2 Hz, 1H), 7.81 (dd, J = 2, 9 Hz, 1H), 7.42 (d, J = 9 Hz, 1H), 2.73 (s, 3H).

(3*R,4a*R**)-8-Bromo-3-ethoxy-1-methyl-4,4a-dihydropyrano[4,3-b]chromen-10(3*H*)-one (41).** A stainless steel bomb plus stir bar with ethyl vinyl ether (169 mL, 1760 mmol) and **40** (47 g, 176 mmol) was charged and heated to 100 °C for 15 h. After cooling to rt, the reaction mixture was filtered, and the solids were washed with a minimum amount of EtOAc. Yield of **41**: 44 g (72%), isolated as a single diastereomer.[20] ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.05 (d, J = 2 Hz, 1H), 7.49 (dd, J = 2, 9 Hz, 1H), 6.83 (d, J = 9 Hz, 1H), 5.14 (dd, J = 2, 9 Hz, 1H), 5.07 (m, 1H), 3.99 (m, 1H), 3.67 (m, 1H), 2.53 (m, 1H), 2.38 (d, J = 2 Hz, 3H), 2.32 (m, 1H), 1.28 (t, J = 7 Hz, 3H).

(1*R,3*R**,4a*R**,10a*R**)-8-Bromo-3-ethoxy-1-methyl-1,4,4a,10a-tetrahydropyrano[4,3-b]chromen-10(3*H*)-one (42).** A round bottomed flask plus stir bar with **41** (43 g, 127 mmol) and THF (500 mL) was charged and cooled to −78 °C in a dry ice/acetone bath. To the mixture was added DIBALH (101 mL, 152 mmol, 1.5 M in toluene) dropwise, and it was stirred at −78 °C for 1 h. Reaction remained a suspension the entire time. The reaction mixture was quenched by inverse addition (via cannula) to Rochelle's salt (500 mL) and was stirred at rt. The mixture was worked up by extraction with EtOAc (2 × 500 mL). The combined organics were washed with brine (500 mL), dried ($MgSO_4$), filtered, and concentrated. The crude was purified by Biotage Flash 75 silica gel chromatography, eluting with 5−10% EtOAc/hexanes. Yield of **42**: 22.4 g (36%), which contained approximately 30% unreacted starting material, carried forward without further purification. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.91 (d, J = 2 Hz, 1H), 7.56 (dd, J = 2, 9 Hz, 1H), 6.87 (d, J = 9 Hz, 1H), 4.47 (dd, J = 2, 10 Hz, 1H), 4.36 (m, 1H), 3.98 (m, 1H), 3.69 (m, 1H), 3.57 (m, 1H), 2.49 (m, 1H), 2.43 (m, 1H), 2.00 (m, 1H), 1.62 (d, J = 6 Hz, 3H), 1.26 (t, J = 7 Hz, 3H).

(1*R,4a*R**,10a*R**)-8-Bromo-1-methyl-1,4,4a,10a-tetrahydropyrano[4,3-b]chromen-10(3*H*)-one (43).** A round bottomed flask plus stir bar with **42** (22.2 g, 65.1 mmol), DCM (200 mL), and Et_3SiH (51.8 mL, 325 mmol) was charged and cooled in an ice bath under N_2. Then BF_3-Et_2O (24.7 mL, 195 mmol) was added dropwise and stirred overnight at rt. The mixture was carefully quenched with satd. aq. $NaHCO_3$ (200 mL) and stirred for 1 h. Phases were separated and re-extracted with aqueous DCM (2 × 75 mL). Combined organic phases were washed with brine (200 mL), dried ($MgSO_4$), filtered, and concentrated. The crude was purified by Biotage Flash 65 silica gel chromatography, eluting with 10−20% EtOAc/hexanes. Yield of **43**: 13.6 g (60%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.91 (d, J = 2 Hz, 1H), 7.55 (dd, J = 2, 9 Hz, 1H), 6.86 (d, J = 9 Hz, 1H), 4.35 (m, 1H), 4.07 (m, 1H), 3.64 (m, 1H), 3.49 (m, 1H), 2.52 (dd, J = 9, 13 Hz, 1H), 2.13 (m, 2H), 1.57 (d, J = 6 Hz, 3H).

(1′*R,4*R**,4a′*R**,10a′*R**)-8′-Bromo-1,1′-dimethyl-3′,4′,4a′,10a′-tetrahydro-1′*H*-spiro[imidazolidine-4,10′-pyrano[4,3-b]chromene]-2,5-dione (44).** A stainless steel bomb plus stir bar with EtOH (10 mL) and **43** (3 g, 10 mmol) was charged. Next ammonium carbonate (4.9 g, 50 mmol), KCN (1.3 g, 20 mmol) and sodium hydrogensulfite (0.26 g, 2.5 mmol) were added. The reaction was heated to 130 °C for 16 h with stirring in an oil bath. After cooling to rt, reaction contents were transferred to an Erlenmeyer flask using EtOAc (20 mL) and water (10 mL) to aid in transfer. The mixture was chilled in an ice bath and carefully acidified with conc. HCl, then bubbled N_2 through mixture for 30 min to sparge HCN (near back of hood). Phases were separated, and the aqueous phase was re-extracted with EtOAc (2 × 10 mL). The combined organic phases were washed with brine (50 mL), dried ($MgSO_4$), filtered, and concentrated. The crude was subjected to a Biotage Flash 65 silica gel chromatography column, eluting with 5−10% MeOH/DCM. A 60:40 mixture of diastereomers was obtained and carried forward without separation as follows. A round bottomed flask plus stir bar with DMF (10 mL) and the two diastereomers (1.6 g, 4.36 mmol) was charged and cooled in an ice bath under N_2, then K_2CO_3 (0.903 g, 6.54 mmol) and MeI (0.217 mL, 3.49 mmol) were added sequentially. The mixture was stirred at rt overnight. The mixture was diluted with EtOAc (20 mL) and water (20 mL). Phases were separated and re-extracted with aqueous EtOAc (20 mL). Combined organic phases were washed with water (20 mL), brine (20 mL), dried ($MgSO_4$), filtered, and concentrated. Diastereomers were separated by Biotage Flash 40 silica gel chromatography, eluting with a gradient of 20% EtOAc/hexanes to 1:1 EtOAc/hexanes. Yield of **44**: 554 mg (15%, 2 steps). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.35 (m, 1H), 6.99 (m, 1H), 6.79 (d, J = 9 Hz, 1H), 6.24 (br s, 1H), 4.06 (m, 1H), 3.85 (m, 1H), 3.52 (m, 1H), 3.16 (s, 3H), 2.36 (m, 1H), 2.21 (m, 1H), 2.00 (m, 1H), 1.27 (m, 1H), 1.11 (d, J = 6 Hz, 3H).

(4a*S,10a*S**)-8-Bromo-10a-methyl-1,4,4a,10a-tetrahydropyrano[4,3-b]chromen-10(3*H*)-one (45).** A round bottomed flask plus stir bar with THF (25 mL) and i-Pr_2NH (2.40 mL, 17.1 mmol) was charged. The reaction vessel was cooled to −78 °C (dry ice/acetone) under N_2 and added *n*-BuLi (6.22 mL, 15.5 mmol, 2.5 M in hexanes). Next a solution of **9** (2.2 g, 7.77 mmol) in

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THF (10 mL) was added. The reaction vessel was transferred to a −10 °C ice/NaCl bath and stirred for 20 min in the bath. The vessel was recooled to −78 °C and added HMPA (9.46 mL, 54.4 mmol) followed by MeI (1.45 mL, 23.3 mmol) and stirred for 1 h at −78 °C. It was quenched by the addition of satd. aq. NH_4Cl (20 mL) at −78 °C. The mixture was removed from dry ice bath and stirred for 15 min as reaction mixture warmed. Phases were partitioned between EtOAc (50 mL) and water (50 mL). Phases were separated and re-extracted with aqueous EtOAc (30 mL). Combined organic phases were washed with water (50 mL), brine (50 mL), dried ($MgSO_4$), filtered, and concentrated. The crude was purified by Biotage Flash 40, eluting with 10% EtOAc/hexanes. Yield of **45**: 850 mg (31%). Isolated product was >90% diastereomerically pure by ^{1}H NMR. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.96 (d, J = 2 Hz, 1H), 7.57 (dd, J = 2, 9 Hz, 1H), 6.89 (d, J = 9 Hz, 1H), 4.30 (dd, J = 5, 12 Hz, 1H), 4.09 (m, 2H), 3.43 (m, 2H), 2.22 (m, 1H), 1.93 (m, 1H), 1.26 (s, 3H).

3-Fluoro-4-methoxyphenyl acetate (47). A stirred solution of 1-(3-fluoro-4-methoxyphenyl)ethanone **46** (50 g, 297 mmol) in DCM (1.2 L) was treated with *m*-CPBA (83.3 g, 372 mmol). The suspension was heated to 40 °C with stirring, and the suspension became a yellow solution. The reaction was stirred for 72 h at 40 °C, and TLC suggested only partial conversion. The reaction was cooled to rt, and an additional 80 g of *m*-CPBA was added in a single portion. The reaction was returned to 40 °C, and the reaction stirred for an additional 48 h. TLC confirmed conversion of starting material, and the reaction was cooled to rt and washed with aq. 1 N NaOH, repeating until organic phase was clear. The organic phase was then washed with brine, dried (Na_2SO_4), and concentrated to a yellow oil. Yield of **47**: 47.7 g (87%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.92 (d, J = 9 Hz, 1H), 6.83 (dd, J = 3, 11 Hz, 1H), 6.83 (m, 1H), 3.88 (s, 3H), 2.27 (s, 3H).

1-(4-Fluoro-2-hydroxy-5-methoxyphenyl)ethanone (48). Neat TfOH (194 g, 1295 mmol) was added dropwise by addition funnel into **47** (47.7 g, 259 mmol) with stirring at 0 °C. The reaction was heated to 60 °C for 1 h and cooled to rt. The reaction was poured carefully into an ice slurry (1 L). The resulting suspension was filtered, and the solid was partitioned between Et_2O and satd. aq. $NaHCO_3$. The organic phase was washed with brine, dried (Na_2SO_4), and concentrated under vacuum. Yield of **48**: 44.2 g, (93%) as a brown oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 12.31 (s, 1H), 7.23 (d, J = 9 Hz, 1H), 6.72 (d, J = 12 Hz, 1H), 3.89 (s, 3H), 2.60 (s, 3H).

7-Fluoro-6-methoxy-4-oxo-4*H*-chromene-3-carbaldehyde (49). A solution of triphosgene (71.2 g, 240 mmol) in DCE (160 mL) was added dropwise to a flask containing a mixture of DMF (254 mL, 2880 mmol) and DCE (300 mL) that was stirred in an ice bath. The reaction temperature was maintained below 25 °C. After addition, the reaction was cooled to 0 °C and treated with a solution of **48** (44.2 g, 240 mmol) in DCE (160 mL). The ice bath was removed and the reaction was allowed to warm to rt while monitoring by HPLC. After 5 h of stirring at rt, the reaction was poured into a 2 L ice slurry and stirred for an additional 2 h. The aqueous was extracted multiple times with DCE, and the combined organic extracts were washed with satd. aq. $NaHCO_3$ and brine, and then the organic phase was concentrated in vacuo. The resulting solid was placed in a vacuum oven and heated to 60 °C overnight. Yield of **49**: 28 g (53%) as a brown powder. ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.39 (s, 1H), 8.52 (s, 1H), 7.76 (d, J = 9 Hz, 1H), 7.29 (d, J = 10 Hz, 1H), 4.02 (s, 3H).

(3*S,4a*S**)-3-Ethoxy-7-fluoro-8-methoxy-4,4a-dihydropyrano[4,3-b]chromen-10(3*H*)-one (50).** A stirred suspension of **49** (31.4 g, 141 mmol) and ethyl vinyl ether (67.9 mL, 707 mmol) was heated at 100 °C in a Teflon-lined stainless steel bomb for 8 h with stirring. The heat was removed, and the reaction continued to stir an additional 7 h at rt. The resulting residue was crystallized from hot EtOH, and the solids were filtered. Yield of **50**: 16.6 g (40%) as a single diastereomer.[20] ^{1}H NMR (400 MHz, $(CD_3)_2SO$) δ 7.57 (d, J = 1 Hz, 1H), 7.41 (d, J = 10 Hz, 1H), 7.04 (d, J = 12 Hz, 1H), 5.39 (dd, J = 2, 10 Hz, 1H), 5.31 (m, 1H), 3.90 (m, 1H), 3.85 (s, 3H), 3.68 (m, 1H), 2.55 (m, 1H), 2.11 (m, 1H), 1.19 (t, J = 7 Hz, 3H).

(3*S,4a*S**,10a*S**)-3-Ethoxy-7-fluoro-8-methoxy-1,4,4a,10a-tetrahydropyrano[4,3-b]chromen-10(3*H*)-one (51).** A suspension of **50** (13.3 g, 45.3 mmol) in EtOH (100 mL) was treated with Pd/C (10 wt %, 0.8 g) and shaken in a Parr shaker under H_2 (50 psi) for 3 h. The reaction was filtered through GF/F paper, and the filtrate was concentrated. The solid was suspended in DCM (100 mL) and stirred with MnO_2 (7.9 g, 90.5 mmol) overnight. The crude was filtered and concentrated to provide **51** (12.1 g) as a white solid, which was carried forward crude without purification at this step.

(4a*S,10a*S**)-7-Fluoro-8-methoxy-1,4,4a,10a-tetrahydropyrano[4,3-b]chromen-10(3*H*)-one (52).** A solution of **51** (11.1 g, 37 mmol) in DCM (3 mL) was cooled to 0 °C and treated with Et_3SiH (18 mL, 112 mmol), then BF_3−Et_2O (9.2 mL, 75 mmol). The reaction was allowed to stir at rt overnight. The reaction was incomplete so an additional 3 equiv of Et_3SiH and 2 equiv of BF_3−Et_2O were added and the reaction continued to stir at rt. After 40 h, the reaction mixture was dissolved in EtOAc (30 mL) and MeOH (5 mL), then quenched with satd. aq. $NaHCO_3$ and stirred for 4 h. The organic phase was separated and washed with brine. The organic phase was dried (Na_2SO_4) and concentrated in vacuo to provide **52** (8.58 g), which was carried forward crude without purification at this step.

2-Amino-7′-fluoro-8′-methoxy-1-methyl-3′,4′,4a′,10a′-tetrahydro-1′*H*-spiro[imidazole-4,10′-pyrano[4,3-b]chromen]-5(1H)-one (53). Prepared from **52** using reaction conditions described for the synthesis of compounds **10**−**13**, crude **53** was purified by silica gel chromatography (gradient: 0−10% (80% DCM/19% MeOH/1% NH_4OH)/DCM) to obtain a 60:40 mixture of trans/cis stereoisomers at the ring fusion, which was carried forward without separation at this step. Yield: 1.70 g (12%, 4 steps). ^{1}H NMR (400 MHz, CDCl3) trans isomer, δ 6.65 (d, J = 12 Hz, 1H), 6.46 (m, 1H), 5.32 (br, 2H), 4.82 (td, J = 5, 11 Hz, 1H), 4.02 (m, 1H), 3.92 (m, 1H), 3.76 (s, 3H), 3.44 (m, 1H), 3.07 (s, 3H), 3.00 (t, J = 11 Hz, 1H), 1.73−2.16 (m, 3H); cis isomer δ 6.71 (d, J = 12 Hz, 1H), 6.46 (m, 1H), 5.32 (br, 2H), 5.16 (m, 1H), 3.92 (m, 1H), 3.78 (m, 1H), 3.76 (s, 3H), 3.75 (m, 1H), 3.35 (t, J = 12 Hz, 1H), 3.08 (s, 3H), 1.73−2.16 (m, 3H).

(4*R,4a′*S**,10a′*S**)-2-((*E*)-((Dimethylamino)methylene)-amino)-7′-fluoro-1-methyl-5-oxo-1,3′,4′,4a′,5,10a′-hexahydro-1′*H*-spiro[imidazole-4,10′-pyrano[4,3-b]chromen]-8′-yl Trifluoromethanesulfonate (54).** Compound **53** (500 mg, 1.49 mmol) was treated with 48% aq. HBr (7.5 mL, 1.49 mmol) and heated to 100 °C in a sealed vessel for 3 h. The reaction was cooled to rt and treated with DCM (50 mL) and satd. aq. $NaHCO_3$ until slightly basic. The pH was brought to ∼5 by careful addition of aq. 1 N HCl. The biphasic mixture was then concentrated in vacuo, and the residue was triturated with 10% MeOH/DCM. The resulting suspension was filtered, and the filtrate was concentrated to provide a crude oil, which was then stirred with DMF-dimethylacetal (0.90 mL, 7.45 mmol) in DMF (10 mL) at rt for 15 h. The reaction mixture was then concentrated in vacuo to an oil. This oil was subsequently stirred with Et_3N (0.42 mL, 3.0 mmol) and DCM (7 mL) and treated with 1,1,1-trifluoro-*N*-phenyl-*N*-(trifluoromethylsulfonyl)methanesulfonamide (0.799 g, 2.24 mmol). The reaction was stirred for 15 h at rt. The reaction mixture was washed with water and brine and dried over Na_2SO_4. The crude was purified by silica gel chromatography to provide **54** (90 mg, 12% yield, 3 steps). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.59 (s, 1H), 7.17 (m, 1H), 6.74 (m, 1H), 4.98 (td, J = 5, 11 Hz, 1H), 4.05 (m, 1H), 3.85 (m, 1H), 3.46 (m, 1H), 3.18 (s, 3H), 3.17 (s, 3H), 3.07 (s, 3H), 3.01 (t, J = 11 Hz, 1H), 2.26 (m, 1H), 2.15 (m, 1H), 1.82 (m, 1H).

(4*R*,4a′*S*,10a′*S*)-2-Amino-8′-bromo-1-methyl-3′,4′,4a′,10a′-tetrahydro-1′*H*-spiro[imidazole-4,10′-pyrano[4,3-b]chromen]-5(1*H*)-one (55). The title compound was obtained by resolution of the racemate **13** using chiral SFC, performed as follows. System, Thar 350; column, Chiralpak IB, 5 cm × 25 cm, 5 μm; mobile phase A, CO_2; mobile phase B, 80% MeOH with 0.1% NH_4OH, 20% DCM; isocratic conditions, 65% A, 35% B; flow rate, 300 mL/min; backpressure, 100 bar; temperature, 40 °C; UV detection, 220 nm; racemic **13** was dissolved in a 50:35:15 mixture of MeOH/DCM/formic acid, which was loaded on the column in 3 mL injections every 5.5 min. Compound **55** was >98% pure by LC−MS (254 nm) and >98% ee by chiral SFC analysis. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.27 (dd, J = 2, 9 Hz, 1H), 7.01 (d, J = 2 Hz, 1H), 6.76 (d, J = 9 Hz, 1H),

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4.84 (m, 1H), 4.68 (br s, 2H), 4.03 (m, 1H), 3.94 (m, 1H), 3.45 (m, 1H), 3.05 (s, 3H), 3.00 (t, J = 11 Hz, 1H), 2.11 (m, 2H), 1.82 (m, 1H); m/z (APCI-pos) M + 1 = 366, 368.

(4*R,4a′*S**,10a′*S**)-2-Amino-8′-(3-methoxyphenyl)-1-methyl-3′,4′,4a′,10a′-tetrahydro-1′*H*-spiro[imidazole-4,10′-pyrano[4,3-b]chromen]-5(1*H*)-one (56).** Compound **56** was prepared from **13**, as described for **61**, replacing 2-fluoropyridin-3-ylboronic acid with 3-methoxyphenylboronic acid. Crude product was purified by preparative TLC (0.5 mm thickness, R_f = 0.49) eluting with 10% MeOH (containing 7N NH_3)/DCM. Yield: 9 mg (41%). 99% pure by LC−MS (220 nm). ^{1}H NMR (400 MHz, $CDCl_3$ + CD_3OD) δ 7.42 (m, 1H), 7.31 (t, J = 8 Hz, 1H), 7.08 (m, 1H), 7.06 (m, 1H), 6.99 (m, 1H), 6.96 (d, J = 9 Hz, 1H), 6.85 (m, 1H), 4.94 (td, J = 5, 11 Hz, 1H), 4.08 (m, 1H), 3.95 (m, 1H), 3.84 (s, 3H), 3.51 (m, 1H), 3.08 (s, 3H), 3.06 (t, J = 11 Hz, 1H), 2.25 (m, 2H), 1.89 (m, 1H); m/z (APCI-pos) M + 1 = 394.

(4*R,4a′*S**,10a′*S**)-2-Amino-8′-(3-(difluoromethoxy)-phenyl)-1-methyl-3′,4′,4a′,10a′-tetrahydro-1′*H*-spiro-[imidazole-4,10′-pyrano[4,3-b]chromen]-5(1*H*)-one (57).** Compound **57** was prepared from **13**, as described for **61**, replacing 2-fluoropyridin-3-ylboronic acid with 3-(difluoromethoxy)phenylboronic acid. Crude product was purified by preparative TLC (0.5 mm thickness, R_f = 0.49) eluting with 10% MeOH (containing 7 N NH_3)/DCM. Yield: 10 mg (42%), >98% pure by LC−MS (220 nm). ^{1}H NMR (400 MHz, $CDCl_3$ + CD_3OD) δ 7.39 (dd, J = 2, 8 Hz, 1H), 7.37 (t, J = 8 Hz, 1H), 7.31 (m, 1H), 7.20 (m, 1H), 7.06 (d, J = 2 Hz, 1H), 7.05 (m, 1H), 6.97 (d, J = 9 Hz, 1H), 6.56 (t, J = 74 Hz, 1H), 4.94 (td, J = 5, 11 Hz, 1H), 4.06 (m, 1H), 3.96 (m, 1H), 3.50 (m, 1H), 3.08 (s, 3H), 3.05 (t, J = 12 Hz, 1H), 2.27 (m, 2H), 1.89 (m, 1H); m/z (APCI-pos) M + 1 = 430.

3-((4*R,4a′*S**,10a′*S**)-2-Amino-1-methyl-5-oxo-1,3′,4′,4a′,5,10a′-hexahydro-1′*H*-spiro[imidazole-4,10′-pyrano[4,3-b]chromen]-8′-yl)benzonitrile (58).** Compound **58** was prepared from **13**, as described for **61**, replacing 2-fluoropyridin-3-ylboronic acid with 3-cyanophenylboronic acid. Crude product was purified by preparative TLC (0.5 mm thickness, R_f = 0.51) eluting with 10% MeOH (containing 7 N NH_3)/DCM. Yield: 11 mg (50%), 97% pure by LC−MS (220 nm). ^{1}H NMR (400 MHz, $CDCl_3$ + CD_3OD) δ 7.74 (s, 1H), 7.70 (m, 1H), 7.57 (m, 1H), 7.51 (m, 1H), 7.41 (m, 1H), 7.06 (m, 1H), 7.00 (d, J = 9 Hz, 1H), 4.95 (td, J = 5, 11 Hz, 1H), 4.07 (m, 1H), 3.96 (m, 1H), 3.51 (m, 1H), 3.10 (s, 3H), 3.05 (t, J = 11 Hz, 1H), 2.24 (m, 2H), 1.91 (m, 1H); m/z (APCI-pos) M + 1 = 389.

3-((4*R*,4a′*S*,10a′*S*)-2-Amino-1-methyl-5-oxo-1,3′,4′,4a′,5,10a′-hexahydro-1′*H*-spiro[imidazole-4,10′-pyrano[4,3-b]chromen]-8′-yl)benzonitrile (59). Compound **59** was prepared from **55** (700 mg, 1.91 mmol), as described for **61**, replacing 2-fluoropyridin-3-ylboronic acid with 3-cyanophenylboronic acid (421 mg, 2.87 mmol). Yield: 474 mg (63%), >98% pure by LC−MS (220 nm). ^{1}H NMR (400 MHz, $CDCl_3$ + CD_3OD) δ 7.74 (s, 1H), 7.70 (m, 1H), 7.57 (m, 1H), 7.51 (m, 1H), 7.41 (m, 1H), 7.06 (m, 1H), 7.00 (d, J = 9 Hz, 1H), 4.95 (td, J = 5, 11 Hz, 1H), 4.07 (m, 1H), 3.96 (m, 1H), 3.51 (m, 1H), 3.10 (s, 3H), 3.05 (t, J = 11 Hz, 1H), 2.24 (m, 2H), 1.91 (m, 1H); m/z (APCI-pos) M + 1 = 389.

(4*R,4a′*S**,10a′*S**)-2-Amino-8′-(3-chloro-5-fluorophenyl)-1-methyl-3′,4′,4a′,10a′-tetrahydro-1′*H*-spiro[imidazole-4,10′-pyrano[4,3-b]chromen]-5(1*H*)-one (60).** Compound **60** was repared from **13**, as described for **61**, replacing 2-fluoropyridin-3-ylboronic acid with 3-chloro-5-fluorophenylboronic acid. Crude product was purified by preparative TLC (1 mm thickness, R_f = 0.29) eluting with 5% MeOH/EtOAc. Yield: 19 mg (16%), >98% pure by LC−MS (220 nm). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.37 (dd, J = 2, 8 Hz, 1H), 7.22 (s, 1H), 7.05 (m, 2H), 7.00 (m, 1H), 6.97 (d, J = 9 Hz, 1H), 4.93 (td, J = 5, 11 Hz, 1H), 4.07 (dd, J = 5, 12 Hz, 1H), 3.99 (dd, J = 4, 11 Hz, 1H), 3.48 (td, J = 2, 13 Hz, 1H), 3.13 (s, 3H), 3.04 (t, J = 11 Hz, 1H), 3.03 (br s, 2H), 2.27 (td, J = 4, 11 Hz, 1H), 2.18 (m, 1H), 1.87 (m, 1H); m/z (APCI-pos) M + 1 = 416.

(4*R,4a′*S**,10a′*S**)-2-Amino-8′-(2-fluoropyridin-3-yl)-1-methyl-3′,4′,4a′,10a′-tetrahydro-1′*H*-spiro[imidazole-4,10′-pyrano[4,3-b]chromen]-5(1*H*)-one (61).** A thick walled, glass pressure vessel plus stir bar with **13** (2.2 g, 6.0 mmol), dioxane (30 mL), 2-fluoropyridin-3-ylboronic acid (1.3 g, 9.0 mmol), $Pd[P(Ph)_3]_4$ (0.17 g, 0.15 mmol), and 2 N aq. Na_2CO_3 (9.0 mL, 18 mmol) was charged. The mixture was sparged with N_2 for 15 min, then heated to 90 °C for 1 h with stirring. The reaction mixture was partitioned between EtOAc (50 mL) and water (50 mL). Phases were separated and re-extracted with aqueous EtOAc (2 × 30 mL). The combined organic phases were washed with brine (50 mL), dried ($MgSO_4$), filtered, and concentrated. The crude was taken up in DCM (30 mL) and concentrated again to remove residual solvents from the workup. Then the crude solid was triturated in DCM (10 mL) at rt. The solids were filtered, rinsing with DCM (3 × 5 mL). Compound **61** was obtained as an off-white solid (1.7 g, 74% yield) in 96% purity (HPLC, 220 nm). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.05 (m, 1H), 7.75 (m, 1H), 7.36 (m, 1H), 7.18 (m, 1H), 7.14 (m, 1H), 6.98 (d, J = 9 Hz, 1H), 5.73 (br s, 2H), 4.95 (td, J = 5, 11 Hz, 1H), 4.05 (dd, J = 5, 12 Hz, 1H), 3.98 (dd, J = 4, 11 Hz, 1H), 3.47 (m, 1H), 3.04 (s, 3H), 3.03 (m, 1H), 2.18 (m, 2H), 1.83 (m, 1H); m/z (APCI-pos) M + 1 = 383.

(4*R*,4a′*S*,10a′*S*)-2-Amino-8′-(2-fluoropyridin-3-yl)-1-methyl-3′,4′,4a′,10a′-tetrahydro-1′*H*-spiro[imidazole-4,10′-pyrano[4,3-b]chromen]-5(1*H*)-one (62) and (4*S*,4a′*R*,10a′*R*)-2-Amino-8′-(2-fluoropyridin-3-yl)-1-methyl-3′,4′,4a′,10a′-tetrahydro-1′*H*-spiro[imidazole-4,10′-pyrano[4,3-b]chromen]-5(1*H*)-one (63). The title compounds were obtained by resolution of the racemate **61** using chiral SFC, performed as follows. System, Thar 350; column, Phenomenex Lux Cellulose-2, 3 cm × 25 cm, 5 μm; mobile phase A, CO_2; mobile phase B, MeOH with 0.1% NH_4OH; isocratic conditions, 50% A, 50% B; flow rate, 200 mL/min; backpressure, 100 bar; temperature, 40 °C; UV detection, 220 nm; racemic **61** was dissolved in a 3:1 mixture of MeOH/formic acid, which was loaded on the column in 3 mL injections every 4 min. Compounds **62** and **63** were 98% and 99% chemically pure, respectively (HPLC, 220 nm). Both compounds were >98% ee by chiral SFC analysis. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.05 (m, 1H), 7.75 (m, 1H), 7.36 (m, 1H), 7.18 (m, 1H), 7.14 (m, 1H), 6.98 (d, J = 9 Hz, 1H), 5.73 (br s, 2H), 4.95 (td, J = 5, 11 Hz, 1H), 4.05 (dd, J = 5, 12 Hz, 1H), 3.98 (dd, J = 4, 11 Hz, 1H), 3.47 (m, 1H), 3.04 (s, 3H), 3.03 (m, 1H), 2.18 (m, 2H), 1.83 (m, 1H); m/z (APCI-pos) M + 1 = 383.

(4*R,4a′*S**,10a′*S**)-2-Amino-8′-(5-fluoropyridin-3-yl)-1-methyl-3′,4′,4a′,10a′-tetrahydro-1′*H*-spiro[imidazole-4,10′-pyrano[4,3-b]chromen]-5(1*H*)-one (64).** Compound **64** was prepared from **13**, as described for **61**, replacing 2-fluoropyridin-3-ylboronic acid with 5-fluoropyridin-3-ylboronic acid. Crude product was purified by preparative TLC (0.5 mm thickness, R_f = 0.29) eluting with 10% MeOH (containing 7 N NH_3)/DCM. Yield: 12 mg (55%), 96% pure by LC−MS (220 nm). ^{1}H NMR (400 MHz, $CDCl_3$ + CD_3OD) δ 8.50 (m, 1H), 8.34 (d, J = 3 Hz, 1H), 7.56 (m, 1H), 7.43 (dd, J = 2, 9 Hz, 1H), 7.09 (d, J = 2 Hz, 1H), 7.02 (d, J = 9 Hz, 1H), 4.97 (td, J = td, 1H), 4.09 (m, 1H), 3.96 (m, 1H), 3.51 (m, 1H), 3.10 (s, 3H), 3.05 (t, J = 11 Hz, 1H), 2.27 (m, 2H), 1.90 (m, 1H); m/z (APCI-pos) M + 1 = 383.

(4*R,4a′*S**,10a′*S**)-2-Amino-8′-(5-chloropyridin-3-yl)-1-methyl-3′,4′,4a′,10a′-tetrahydro-1′*H*-spiro[imidazole-4,10′-pyrano[4,3-b]chromen]-5(1*H*)-one (65).** Compound **65** was prepared from **13**, as described for **61**, replacing 2-fluoropyridin-3-ylboronic acid with 5-chloropyridin-3-ylboronic acid. Crude product was purified by preparative TLC (0.5 mm thickness, R_f = 0.50) eluting with 10% MeOH (containing 7 N NH_3)/DCM. Yield: 14 mg (50%), 97% pure by LC−MS (220 nm). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.56 (d, J = 2 Hz, 1H), 8.45 (d, J = 2 Hz, 1H), 7.70 (t, J = 2 Hz, 1H), 7.37 (dd, J = 2, 9H, 1H), 7.06 (d, J = 2 Hz, 1H), 6.99 (d, J = 9 Hz, 1H), 4.91 (td, J = 5, 11 Hz, 1H), 4.77 (br s, 2H), 4.03 (dd, J = 5, 11 Hz, 1H), 3.94 (dd, J = 4, 11 Hz, 1H), 3.46 (m, 1H), 3.08 (s, 3H), 3.02 (m, 1H), 2.13 (m, 2H), 1.82 (m, 1H); m/z (APCI-pos) M + 1 = 399.

(4*R,4a′*S**,10a′*S**)-2-Amino-8′-(5-methoxypyridin-3-yl)-1-methyl-3′,4′,4a′,10a′-tetrahydro-1′*H*-spiro[imidazole-4,10′-pyrano[4,3-b]chromen]-5(1*H*)-one (66).** Compound **66** was prepared from **13**, as described for **61**, replacing 2-fluoropyridin-3-ylboronic acid with 5-methoxypyridin-3-ylboronic acid. Crude product was purified by preparative TLC (0.5 mm thickness, R_f = 0.24) eluting with 10% MeOH (containing 7 N NH_3)/DCM. Yield: 10 mg (45%), >98% pure by LC−MS (220 nm). ^{1}H NMR (400 MHz, $CDCl_3$ + CD_3OD) δ 8.25 (d, J = 2 Hz, 1H), 8.16 (d, J = 3 Hz, 1H), 7.41 (m,

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1H), 7.33 (m, 1H), 7.08 (d, J = 2 Hz, 1H), 7.01 (d, J = 9 Hz, 1H), 4.96 (td, J = 5, 11 Hz, 1H), 4.09 (m, 1H), 3.94 (m, 1H), 3.92 (s, 3H), 3.51 (m, 1H), 3.10 (s, 3H), 3.05 (t, J = 11 Hz, 1H), 2.27 (m, 2H), 1.88 (m, 1H); m/z (APCI-pos) M + 1 = 395.

5-((4*R,4a′*S**,10a′*S**)-2-Amino-1-methyl-5-oxo-1,3′,4′,4a′,5,10a′-hexahydro-1′*H*-spiro[imidazole-4,10′-pyrano[4,3-b]chromen]-8′-yl)nicotinonitrile (67).** Compound **67** was prepared from **13**, as described for **61**, replacing 2-fluoropyridin-3-ylboronic acid with 5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-nicotinonitrile. Crude product was purified by preparative TLC (2 mm thickness, R_f = 0.56) eluting with 10% MeOH (containing 7 N NH_3)/DCM. Yield: 64 mg (59%), 98% pure by LC−MS (220 nm). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.90 (m, 1H), 8.76 (m, 1H), 8.06 (m, 1H), 7.42 (m, 1H), 7.08 (m, 1H), 7.03 (d, J = 9 Hz, 1H), 4.97 (m, 1H), 4.08 (m, 1H), 3.95 (m, 1H), 3.47 (d, J = 12 Hz, 1H), 3.09 (s, 3H), 3.03 (t, J = 12 Hz, 1H), 2.20 (m, 2H), 1.88 (m, 1H); m/z (APCI-pos) M + 1 = 390.

(4*R,4a′*S**,10a′*S**)-2-Amino-1-methyl-8′-(pyrimidin-5-yl)-3′,4′,4a′,10a′-tetrahydro-1′*H*-spiro[imidazole-4,10′-pyrano-[4,3-b]chromen]-5(1*H*)-one (68).** Compound **68** was prepared from **13**, as described for **61**, replacing 2-fluoropyridin-3-ylboronic acid with pyrimidin-5-ylboronic acid. Crude product was purified by preparative TLC (0.5 mm thickness, R_f = 0.33) eluting with 10% MeOH (containing 7 N NH_3)/DCM. Yield: 9 mg (44%), >98% pure by LC−MS (220 nm). ^{1}H NMR (400 MHz, $CDCl_3$ + CD_3OD) δ 9.10 (s, 1H), 8.87 (s, 2H), 7.46 (m, 1H), 7.12 (s, 1H), 7.06 (d, J = 9 Hz, 1H), 4.96 (td, J = 5, 11 Hz, 1H), 4.10 (m, 1H), 3.97 (m, 1H), 3.51 (m, 1H), 3.12 (s, 3H), 3.05 (t, J = 11 Hz, 1H), 2.27 (m, 2H), 1.91 (m, 1H); m/z (APCI-pos) M + 1 = 366.

(4R*,4a′S*,10a′S*)-8′-(5-Chloropyridin-3-yl)-3′,4′,4a′,10a′-tetrahydro-1′*H*,5*H*-spiro[oxazole-4,10′-pyrano[4,3-b]-chromen]-2-amine (69). Compound **69** was prepared from **15a**, as described for **61**, replacing 2-fluoropyridin-3-ylboronic acid with 5-chloropyridin-3-ylboronic acid. Crude product was purified by preparative TLC (1 mm thickness, R_f = 0.57) eluting with 10% MeOH (containing 7 N NH_3)/DCM. Yield: 12 mg (41%). ^{1}H NMR (400 MHz, $CDCl_3$ + CD_3OD) δ 8.47 (d, J = 2 Hz, 1H), 8.30 (d, J = 2 Hz, 1H), 7.75 (m, 1H), 7.38 (d, J = 2 Hz, 1H), 7.25 (m, 1H), 6.77 (d, J = 8 Hz, 1H), 4.36 (d, J = 9 Hz, 1H), 3.95 (m, 3H), 3.90 (d, J = 9.0 Hz, 1H), 3.38 (m, 1H), 3.16 (d, J = 11 Hz, 1H), 2.04 (m, 2H), 1.78 (m, 1H); m/z (APCI-pos) M + 1 = 372; 93% pure by LC−MS (220 nm).

(4R*,4a′R*,10a′R*)-8′-(5-Chloropyridin-3-yl)-3′,4′,4a′,10a′-tetrahydro-1′*H*,5*H*-spiro[oxazole-4,10′-pyrano[4,3-b]-chromen]-2-amine (70). Compound **70** was prepared from **15b**, as described for **61**, replacing 2-fluoropyridin-3-ylboronic acid with 5-chloropyridin-3-ylboronic acid. Crude product was purified by preparative TLC (1 mm thickness, R_f = 0.57) eluting with 10% MeOH (containing 7 N NH_3)/DCM. Yield: 9 mg (40%), >98% purity by LC−MS (220 nm). ^{1}H NMR (400 MHz, $CDCl_3$ + CD_3OD) δ 8.49 (d, J = 2 Hz, 1H), 8.36 (d, J = 2 Hz, 1H), 7.74 (t, J = 2 Hz, 1H), 7.29 (m, 2H), 6.85 (d, J = 9 Hz, 1H), 4.54 (d, J = 9 Hz, 1H), 4.32 (d, J = 9 Hz, 1H), 4.17 (td, J = 5, 11 Hz, 1H), 3.97 (m, 2H), 3.44 (m, 1H), 3.30 (d, J = 12 Hz, 1H), 2.09 (m, 1H), 1.85 (m, 2H); m/z (APCI-pos) M + 1 = 372.

(4*R,4a′*S**,10a′*S**)-8′-(2-Fluoropyridin-3-yl)-3′,4′,4a′,10a′-tetrahydro-1′*H*,5*H*-spiro[oxazole-4,10′-pyrano[4,3-b]-chromen]-2-amine (71).** Compound **71** was prepared from **15a**, as described for **61**. Crude product was purified by preparative TLC (0.5 mm thickness, R_f = 0.65) eluting with 10% MeOH (containing 7 N NH_3)/DCM. Yield: 7 mg (36%), 97% pure by LC-MS (220 nm). ^{1}H NMR (400 MHz, $CDCl_3$ + CD_3OD) δ 8.12 (m, 1H), 7.88 (m, 1H), 7.51 (m, 1H), 7.41 (m, 1H), 7.29 (m, 1H), 6.90 (d, J = 9 Hz, 1H), 4.48 (d, J = 9 Hz, 1H), 4.02−4.14 (m, 4H), 3.52 (m, 1H), 3.30 (m, 1H), 2.20 (m, 2H), 1.97 (m, 1H); m/z (APCI-pos) M + 1 = 356.

(4*R,4a′*R**,10a′*R**)-8′-(2-Fluoropyridin-3-yl)-3′,4′,4a′,10a′-tetrahydro-1′*H*,5*H*-spiro[oxazole-4,10′-pyrano[4,3-b]-chromen]-2-amine (72).** Compound **72** was prepared from **15b**, as described for **61**. Crude product was purified by preparative TLC (0.5 mm thickness, R_f = 0.62) eluting with 10% MeOH (containing 7 N NH_3)/DCM. Relative stereochemistry was confirmed from cocrystal structures with BACE1 using the same conditions as described for compound **62**. Yield: 10 mg (67%), >98% purity by LC−MS (220 nm). ^{1}H NMR (400 MHz, $CDCl_3$ + CD_3OD) δ 8.05 (m, 1H), 7.77 (m, 1H), 7.34 (m, 2H), 7.22 (m, 1H), 6.86 (d, J = 8 Hz, 1H), 4.55 (d, J = 9 Hz, 1H), 4.34 (d, J = 9 Hz, 1H), 4.20 (m, 1H), 3.99 (m, 2H), 3.47 (m, 1H), 3.32 (m, 1H), 2.11 (m, 1H), 1.91 (m, 1H), 1.81 (m, 1H); m/z (APCI-pos) M + 1 = 356.

(4*R,4a′*S**,10a′*S**)-8′-(Pyrimidin-5-yl)-3′,4′,4a′,10a′-tetrahydro-1′*H*,5*H*-spiro[oxazole-4,10′-pyrano[4,3-b]chromen]-2-amine (73).** Compound **73** was prepared from **15a**, as described for **61**, replacing 2-fluoropyridin-3-ylboronic acid with pyrimidin-5-ylboronic acid. Crude product was purified by preparative TLC (0.5 mm thickness, R_f = 0.48) eluting with 10% MeOH (containing 7 N NH_3)/DCM. Yield: 7 mg (38%), >98% purity by LC−MS (220 nm). ^{1}H NMR (400 MHz, $CDCl_3$ + CD_3OD) δ 9.03 (s, 1H), 8.84 (s, 2H), 7.47 (m, 1H), 7.34 (m, 1H), 6.88 (d, J = 9 Hz, 1H), 4.42 (d, J = 9 Hz, 1H), 4.03 (m, 3H), 3.97 (d, J = 9 Hz, 1H), 3.44 (m, 1H), 3.23 (m, 1H), 2.12 (m, 2H), 1.88 (m, 1H); m/z (APCI-pos) M + 1 = 339.

(4*R,4a′*R**,10a′*R**)-8′-(Pyrimidin-5-yl)-3′,4′,4a′,10a′-tetrahydro-1′*H*,5*H*-spiro[oxazole-4,10′-pyrano[4,3-b]chromen]-2-amine (74).** Compound **74** was prepared from **15b**, as described for **61**, replacing 2-fluoropyridin-3-ylboronic acid with pyrimidin-5-ylboronic acid. Crude product was purified by preparative TLC (0.5 mm thickness, R_f = 0.51) eluting with 10% MeOH (containing 7 N NH_3)/DCM. Yield: 5 mg (35%), >98% purity by LC−MS (220 nm). ^{1}H NMR (400 MHz, $CDCl_3$ + CD_3OD) δ 9.12 (s, 1H), 8.90 (s, 2H), 7.43 (m, 2H), 7.00 (d, J = 9 Hz, 1H), 4.64 (d, J = 9 Hz, 1H), 4.43 (d, J = 9 Hz, 1H), 4.28 (m, 1H), 4.08 (m, 2H), 3.56 (m, 1H), 3.41 (m, 1H), 2.21 (m, 1H), 2.00 (m, 1H), 1.90 (m, 1H); m/z (APCI-pos) M + 1 = 339.

(4a*R,4′*R**,10a*R**)-8-(5-Chloropyridin-3-yl)-3,4,4a,10a-tetrahydro-1*H*,5′*H*-spiro[pyrano[4,3-b]chromene-10,4′-thiazol]-2′-amine (75).** Compound **75** was prepared from **16**, as described for **61**, replacing 2-fluoropyridin-3-ylboronic acid with 5-chloropyridin-3-ylboronic acid. Crude product was purified by preparative TLC (1 mm thickness, R_f = 0.66) eluting with 10% MeOH (containing 7 N NH_3)/DCM. Diastereomer **75** was repurified by a second preparative TLC (0.5 mm thickness, R_f = 0.45) eluting with 10% MeOH/DCM. The minor diastereomer was not isolated. Yield of **75**: 10 mg (21%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.65 (d, J = 2 Hz, 1H), 8.50 (d, J = 2 Hz, 1H), 7.77 (t, J = 2 Hz, 1H), 7.73 (d, J = 2 Hz, 1H), 7.38 (dd, J = 2, 9 Hz, 1H), 6.95 (d, J = 9 Hz, 1H), 4.63 (br s, 2H), 4.36 (m, 1H), 4.14 (m, 1H), 4.09 (m, 1H), 3.87 (d, J = 12 Hz, 1H), 3.76 (d, J = 12 Hz, 1H), 3.53 (m, 1H), 3.39 (m, 1H), 2.17 (m, 1H), 2.05 (m, 1H), 1.90 (m, 1H); m/z (APCI-pos) M + 1 = 388; 88% purity by LC−MS (220 nm).

(4a*R,4′*R**,10a*R**)-8-(2-Fluoropyridin-3-yl)-3,4,4a,10a-tetrahydro-1*H*,5′*H*-spiro[pyrano[4,3-b]chromene-10,4′-thiazol]-2′-amine (76).** Compound **76** was prepared from **16**, as described for **61**. Crude product was purified by preparative TLC (1 mm thickness, R_f = 0.68) eluting with 10% MeOH (containing 7 N NH_3)/DCM. The alternate diastereomer was not isolated. Relative stereochemistry was confirmed from cocrystal structures with BACE1 using the same conditions as described for compound **62**. Yield: 9 mg (21%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.14 (d, J = 5 Hz, 1H), 7.80 (m, 1H), 7.75 (m, 1H), 7.36 (m, 1H), 7.24 (m, 1H), 6.93 (d, J = 9 Hz, 1H), 4.56 (br s, 2H), 4.37 (m, 1H), 4.16 (m, 1H), 4.08 (m, 1H), 3.90 (d, J = 12 Hz, 1H), 3.75 (d, J = 12 Hz, 1H), 3.53 (m, 1H), 3.39 (t, J = 11 Hz, 1H), 2.19 (m, 1H), 2.05 (m, 1H), 1.92 (m, 1H); m/z (APCI-pos) M + 1 = 372; 92% purity by LC−MS (220 nm).

3-((4*R*,4a′*S*,10a′*S*)-2-Amino-4a′-methyl-3′,4′,4a′,10a′-tetrahydro-1′*H*,5*H*-spiro[oxazole-4,10′-pyrano[4,3-b]chromene]-8′-yl)benzonitrile (77). Compound **77** was prepared from **27** as described for **79**, replacing (5-chloro-3-pyridyl)boronic acid with (3-cyano-phenyl)boronic acid. Purification by silica gel provided a mixture of product diastereomers. The mixture was further purified by chiral SFC on a Chiralpak AD (2 × 15 cm) column eluting with 25% MeOH (0.1% NH_4OH)/CO_2 at 100 bar at a flow rate of 70 mL/min. The peaks isolated were analyzed on a Chiralpak AD (50 × 0.46 cm) column eluting with 25% MeOH (0.1% NH_4OH)/CO_2, at 120 bar (flow rate 5 mL/min, 220 nm). From this purification, **77** (peak-3,

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8.0 mg, 8% yield, chemical purity >99%, > 99% ee) was isolated. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.05−8.01 (m, 1H), 7.88 (d, J = 8.0 Hz, 1H), 7.76 (d, J = 7.6 Hz, 1H), 7.64 (t, J = 7.8 Hz, 1H), 7.51 (dd, J = 8.4, 2.4 Hz, 1H), 7.39 (d, J = 2.4 Hz, 1H), 6.84 (d, J = 8.5 Hz, 1H), 6.13 (s, 2H), 4.46 (d, J = 8.9 Hz, 1H), 4.11 (d, J = 9.0 Hz, 1H), 3.92 (dd, J = 11.9, 4.5 Hz, 1H), 3.71 (dd, J = 11.2, 4.3 Hz, 1H), 3.55 (td, J = 11.9, 2.7 Hz, 2H), 2.19 (dd, J = 11.3, 4.2 Hz, 1H), 1.89 (td, J = 12.8, 5.0 Hz, 1H), 1.80 (d, J = 12.5 Hz, 1H), 1.32 (s, 3H); m/z (ESI-pos) M + 1 = 376.2.

(4*R*,4a′*S*,10a′*S*)-8′-(3-Chloro-5-fluorophenyl)-4a′-methyl-3′,4′,4a′,10a′-tetrahydro-1′*H*,5*H*-spiro[oxazole-4,10′-pyrano-[4,3-b]chromen]-2-amine (78). Compound **78** was prepared from 8′-bromo-4a′-methyl-3′,4′,4a′,10a′-tetrahydro-1′*H*,5*H*-spiro[oxazole-4,10′-pyrano[4,3-b]chromen]-2-amine **27**, as described for **79**, replacing (5-chloro-3-pyridyl)boronic acid with (3-chloro-5-fluorophenyl)boronic acid. Purification by silica gel provided a mixture of diastereomers. The mixture was further purified by chiral SFC on a Chiralpak AD (2 × 15 cm) column eluting with 25% MeOH (0.1% NH_4OH)/CO_2 at 100 bar at a flow rate of 70 mL/min. The peaks isolated were analyzed on Chiralpak AD (50 × 0.46 cm) column eluting with 25% MeOH (0.1% NH_4OH)/CO_2, at 120 bar (flow rate 5 mL/min, 220 nm). From the chiral SFC separation, **78** (peak-4, 43 mg, 15% yield, chemical purity >99%, > 99% ee) was isolated. ^{1}H NMR (400 MHz, $(CD_3)_2SO$) δ 7.51 (dd, J = 7, 3 Hz, 1H), 7.42 (ddd, J = 9, 4, 3 Hz, 1H), 7.34 (dd, J = 13, 6 Hz, 2H), 7.26 (s, 1H), 6.83 (d, J = 9 Hz, 1H), 6.13 (s, 2H), 4.44 (d, J = 9 Hz, 1H), 4.06 (d, J = 9 Hz, 1H), 3.92 (dd, J = 12, 4 Hz, 1H), 3.71 (dd, J = 11, 4 Hz, 1H), 3.54 (t, J = 11 Hz, 2H), 2.21 (dd, J = 11, 4 Hz, 1H), 1.96 − 1.83 (m, 1H), 1.80 (d, J = 13 Hz, 1H), 1.32 (s, 3H); m/z (ESI-pos) M + 1 = 403.0.

(4*R,4a′*S**,10a′*S**)-8′-(5-Chloropyridin-3-yl)-4a′-methyl-3′,4′,4a′,10a′-tetrahydro-1′*H*,5*H*-spiro[oxazole-4,10′-pyrano-[4,3-b]chromen]-2-amine (79).** Diastereomers **27** (95 mg, 0.269 mmol), (5-chloro-3-pyridyl)boronic acid (55 mg, 0.350 mmol), Na_2CO_3 (86 mg, 0.807 mmol), and $Pd[P(Ph)_3]_4$ (31.1 mg, 0.0269 mmol) were added as solids to a vial, followed by the simultaneous addition of dioxane (4.6 mL) and degassed water (0.5 mL). The vial was sealed and heated at 90 °C for 4 h. The reaction mixture was then cooled and diluted with DCM and a mixture of brine and NH_4OH solution. The organic layer was dried over Na_2SO_4, filtered, and concentrated. The crude material was purified by silica gel eluting with a linear gradient of 0−6% MeOH/DCM + 1% NH_4OH solution to provide **79** (17.5 mg, 17% yield) as the second eluting UV active fraction. Relative stereochemistry was confirmed from cocrystal structures with BACE1 using the same conditions as described for compound **62**. HPLC purity: 100% (254 nm). ^{1}H NMR (400 MHz, $(CD_3)_2SO$) δ 8.74 (d, J = 2 Hz, 1H), 8.56 (d, J = 2 Hz, 1H), 8.12 (t, J = 2 Hz, 1H), 7.55 (dd, J = 9, 2 Hz, 1H), 7.42 (d, J = 2 Hz, 1H), 6.85 (d, J = 9 Hz, 1H), 6.13 (s, 2H), 4.46 (d, J = 9 Hz, 1H), 4.13 (d, J = 9 Hz, 1H), 3.92 (dd, J = 11, 4 Hz, 1H), 3.71 (dd, J = 11, 4 Hz, 1H), 3.55 (t, J = 11 Hz, 2H), 2.19 (dd, J = 11, 4 Hz, 1H), 1.88 (dd, J = 13, 5 Hz, 1H), 1.80 (d, J = 13 Hz, 1H), 1.32 (s, 3H); m/z (ESI-pos) M + 1 = 386.1.

(4*R,4a′*S**,10a′*S**)-8′-(5-Fluoropyridin-3-yl)-4a′-methyl-3′,4′,4a′,10a′-tetrahydro-1′*H*,5*H*-spiro[oxazole-4,10′-pyrano-[4,3-b]chromen]-2-amine (80).** Compound **80** was prepared from **27** as described for **79**, replacing (5-chloro-3-pyridyl)boronic acid with (5-fluoropyridin-3-yl)boronic acid. Yield: 29.4 mg (30%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.69−8.65 (m, 1H), 8.51 (d, J = 2.7 Hz, 1H), 7.93 (dt, J = 10.5, 2.3 Hz, 1H), 7.54 (dd, J = 8.5, 2.4 Hz, 1H), 7.42 (d, J = 2.4 Hz, 1H), 6.86 (d, J = 8.5 Hz, 1H), 6.13 (s, 2H), 4.46 (d, J = 9.0 Hz, 1H), 4.12 (d, J = 8.9 Hz, 1H), 3.92 (dd, J = 11.7, 4.6 Hz, 1H), 3.71 (dd, J = 11.3, 4.3 Hz, 1H), 3.55 (td, J = 11.6, 3.0 Hz, 2H), 2.19 (dd, J = 11.3, 4.3 Hz, 1H), 1.90 (td, J = 12.6, 5.0 Hz, 1H), 1.80 (d, J = 12.4 Hz, 1H), 1.32 (s, 3H). m/z (ESI-pos) M + 1 = 370.2. HPLC purity: 88% (254 nm).

(4*R,4a′*S**,10a′*S**)-8′-(2-Fluoropyridin-3-yl)-4a′-methyl-3′,4′,4a′,10a′-tetrahydro-1′*H*,5*H*-spiro[oxazole-4,10′-pyrano-[4,3-b]chromen]-2-amine (81).** Compound **81** was prepared from **27** as described for **79**, replacing (5-chloro-3-pyridyl)boronic acid with (2-fluoropyridin-3-yl)boronic acid. Yield: 23.0 mg (20%). HPLC purity: 99% (254 nm). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.20−8.15 (m, 1H), 8.02 (ddd, J = 10.0, 7.5, 1.9 Hz, 1H), 7.44 (ddd, J = 7.0, 4.7, 1.9 Hz, 1H), 7.39 (dt, J = 8.5, 2.0 Hz, 1H), 7.34−7.27 (m, 1H), 6.85 (d, 1H), 6.13 (s, 2H), 4.44 (d, J = 8.9 Hz, 1H), 4.04 (d, J = 8.9 Hz, 1H), 3.92 (dd, J = 11.8, 4.5 Hz, 1H), 3.71 (dd, J = 11.3, 4.3 Hz, 1H), 3.61−3.49 (m, 2H), 2.22 (dd, J = 11.3, 4.2 Hz, 1H), 1.90 (td, J = 12.8, 5.2 Hz, 1H), 1.84−1.73 (m, 1H), 1.32 (s, 3H). m/z (ESI-pos) M + 1 = 370.1.

(4*R,4a′*S**,10a′*S**)-4a′-Methyl-8′-(pyrimidin-5-yl)-3′,4′,4a′,10a′-tetrahydro-1′*H*,5*H*-spiro[oxazole-4,10′-pyrano-[4,3-b]chromen]-2-amine (82).** Compound **82** was prepared from **27** as described for **79**, replacing (5-chloro-3-pyridyl)boronic acid with pyrimidin-5-ylboronic acid. Yield: 18.3 mg (18%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.13 (s, 1H), 9.02 (s, 2H), 7.57 (dd, J = 8.4, 2.4 Hz, 1H), 7.44 (d, J = 2.3 Hz, 1H), 6.89 (d, J = 8.4 Hz, 1H), 6.13 (s, 2H), 4.46 (d, J = 8.9 Hz, 1H), 4.12 (d, J = 8.9 Hz, 1H), 3.92 (dd, J = 11.7, 4.7 Hz, 1H), 3.71 (dd, J = 11.3, 4.3 Hz, 1H), 3.55 (td, J = 10.8, 10.1, 2.4 Hz, 2H), 2.20 (dd, J = 11.3, 4.3 Hz, 1H), 1.90 (td, J = 12.7, 5.1 Hz, 1H), 1.81 (d, J = 12.4 Hz, 1H), 1.33 (s, 3H). m/z (ESI-pos) M + 1 = 353.1. HPLC purity: 87% (254 nm).

(1′*R,4*R**,4a′*R**,10a′*R**)-2-Amino-8′-(2-fluoropyridin-3-yl)-1,1′-dimethyl-3′,4′,4a′,10a′-tetrahydro-1′*H*-spiro[imidazole-4,10′-pyrano[4,3-b]chromen]-5(1*H*)-one (83).** Compound **83** was prepared from **44** in three steps using reaction conditions described for the synthesis of compounds **12**, **13**, and **61**. Crude **83** was purified by preparative TLC (2 mm thickness, R_f = 0.44) eluting with 10% MeOH (containing 7 N NH_3)/DCM. Then the resulting product was triturated with a minimum amount of DCM and filtered. Relative stereochemistry was confirmed from cocrystal structures with BACE1 using the same conditions as described for compound **62**. Yield: 23 mg (7%, 3 steps). ^{1}H NMR (400 MHz, $CDCl_3$ + CD_3OD) δ 8.11 (m, 1H), 7.78 (m, 1H), 7.37 (m, 1H), 7.25 (m, 1H), 6.97 (d, J = 9 Hz, 1H), 6.93 (m, 1H), 4.18 (m, 1H), 4.02 (m, 1H), 3.55 (m, 1H), 3.46 (m, 1H), 3.18 (s, 3H), 2.25 (m, 2H), 2.00 (m, 1H), 1.03 (d, J = 6 Hz, 3H); m/z (APCI-pos) M + 1 = 397; 94% purity by LC−MS (220 nm).

(1′*R,4*R**,4a′*R**,10a′*R**)-8′-(2-Fluoropyridin-3-yl)-1′-methyl-3′,4′,4a′,10a′-tetrahydro-1′*H*,5*H*-spiro[oxazole-4,10′-pyrano-[4,3-b]chromen]-2-amine (84).** Compound **84** was prepared from **43** in three steps using reaction conditions described for the synthesis of compounds **14**, **15**, and **61**. Diastereomers were partially separated by preparative TLC (1 mm thickness) eluting with 10% MeOH/DCM. Then **84** was repurified by preparative TLC (1 mm thickness) eluting with 7.5% MeOH (containing 7 N NH_3)/DCM. Relative stereochemistry was confirmed from cocrystal structures with BACE1 using the same conditions as described for compound **62**. Yield: 13 mg (6%, 3 steps). ^{1}H NMR (400 MHz, $CDCl_3$ + CD_3OD) δ 8.14 (m, 1H), 8.13 (m, 1H), 7.86 (m, 1H), 7.38 (m, 1H), 7.31 (m, 1H), 6.92 (d, J = 8 Hz, 1H), 4.56 (d, J = 9 Hz, 1H), 4.51 (d, J = 9 Hz, 1H), 4.07 (m, 2H), 3.59 (m, 2H), 2.19 (m, 1H), 1.95 (m, 1H), 1.76 (t, J = 10 Hz, 1H), 1.35 (d, J = 6 Hz, 3H); m/z (APCI-pos) M + 1 = 370; 91% purity by LC−MS (220 nm).

(1*R,4a*R**,4′*R**,10a*R**)-8-(2-Fluoropyridin-3-yl)-1-methyl-3,4,4a,10a-tetrahydro-1*H*,5′*H*-spiro[pyrano[4,3-b]chromene-10,4′-thiazol]-2′-amine (85).** Compound **85** was prepared from **43** in three steps using reaction conditions described for the synthesis of compounds **14**, **16**, and **61**. Diastereomers were separated by preparative TLC (2 mm thickness) eluting with 10% MeOH/DCM + 1% HOAc. Compound **85** was then triturated with 1:1 DCM/Et_2O and filtered. Relative stereochemistry was confirmed from cocrystal structures with BACE1 using the same conditions as described for compound **62**. Yield: 53 mg (8%, 3 steps), >98% purity by LC−MS (220 nm). ^{1}H NMR ($CDCl_3$ + CD_3OD) δ 8.13 (m, 1H), 7.87 (m, 1H), 7.81 (s, 1H), 7.34 (m, 2H), 6.93 (d, J = 8 Hz, 1H), 4.14 (m, 1H), 4.04 (m, 1H), 3.75 (m, 2H), 3.61 (m, 1H), 3.53 (m, 1H), 2.17 (m, 1H), 1.95 (m, 1H), 1.82 (m, 1H), 1.43 (d, J = 5 Hz, 3H); m/z (APCI-pos) M + 1 = 386.

3-((4*R,4a′*S**,10a′*S**)-2-Amino-1,10a′-dimethyl-5-oxo-1,3′,4′,4a′,5,10a′-hexahydro-1′*H*-spiro[imidazole-4,10′-pyrano[4,3-b]chromen]-8′-yl)benzonitrile (86).** Compound **86** was prepared from **45** using reaction conditions described for the synthesis of compounds **10**−**13** and **61**, and replacing 2-fluoropyridin-

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3-ylboronic acid with (3-cyanophenyl)boronic acid. Crude **86** was purified by preparative TLC (2 mm thickness, R_f = 0.47), eluting with 10% MeOH (containing 7 N NH_3)/DCM. Yield: 39 mg (12%, 5 steps). ^{1}H NMR (400 MHz, $CDCl_3$ + CD_3OD) δ 7.75 (s, 1H), 7.72 (d, J = 8 Hz, 1H), 7.56 (m, 1H), 7.50 (t, J = 8 Hz, 1H), 7.42 (m, 1H), 7.08 (m, 1H), 7.00 (d, J = 9 Hz, 1H), 5.29 (m, 1H), 4.07 (m, 1H), 3.60 (d, J = 11 Hz, 1H), 3.48 (t, J = 12 Hz, 1H), 3.13 (m, 1H), 3.09 (s, 3H), 2.10 (m, 1H), 1.94 (m, 1H), 1.19 (s, 3H); m/z (APCI-pos) M + 1 = 403; 93% purity by LC−MS (220 nm).

(4*R,4a′*S**,10a′*S**)-2-Amino-8′-(2-fluoropyridin-3-yl)-1,10a′-dimethyl-3′,4′,4a′,10a′-tetrahydro-1′*H*-spiro[imidazole-4,10′-pyrano[4,3-b]chromen]-5(1*H*)-one (87).** Compound **87** was prepared from **45** using reaction conditions described for the synthesis of compounds **10**−**13** and **61**. Crude **87** was purified by preparative TLC (2 mm thickness, R_f = 0.46), eluting with 10% MeOH (containing 7 N NH_3)/DCM. Relative stereochemistry was confirmed from cocrystal structures with BACE1 using the same conditions as described for compound **62**. Yield: 46 mg (14%, 5 steps). ^{1}H NMR (400 MHz, $CDCl_3$ + CD_3OD) δ 8.11 (m, 1H), 7.84 (t, J = 8 Hz, 1H), 7.43 (m, 1H), 7.29 (m, 1H), 7.11 (m, 1H), 7.00 (d, J = 9 Hz, 1H), 5.29 (m, 1H), 4.10 (m, 1H), 3.60 (d, J = 11 Hz, 1H), 3.49 (t, J = 12 Hz, 1H), 3.12 (d, J = 11 Hz, 1H), 3.07 (s, 3H), 2.14 (m, 1H), 1.98 (m, 1H), 1.19 (s, 3H); m/z (APCI-pos) M + 1 = 397; 91% purity by LC−MS (220 nm).

(4*R,4a′*S**,10a′*S**)-2-Amino-1,10a′-dimethyl-8′-(pyrimidin-5-yl)-3′,4′,4a′,10a′-tetrahydro-1′*H*-spiro[imidazole-4,10′-pyrano[4,3-b]chromen]-5(1*H*)-one (88).** Compound **88** was prepared from **45** using reaction conditions described for the synthesis of compounds **10**−**13** and **61**, and replacing 2-fluoropyridin-3-ylboronic acid with pyrimidin-5-ylboronic acid. Crude **88** was purified by preparative TLC (2 mm thickness, R_f = 0.29), eluting with 10% MeOH (containing 7N NH_3)/DCM. Yield: 31 mg (12%, 5 steps), 89% purity by LC−MS (220 nm). ^{1}H NMR (400 MHz, $CDCl_3$ + CD_3OD) δ 9.00 (s, 1H), 8.78 (s, 2H), 7.38 (m, 1H), 7.03 (m, 1H), 6.98 (m, 1H), 5.25 (m, 1H), 3.0−4.2 (m, 4H), 3.01 (s, 3H), 2.07 (m, 1H), 1.90 (m, 1H), 1.10 (s, 3H); m/z (APCI-pos) M + 1 = 380.

(4*R,4a′*R**,10a′*R**)-8′-(2-Fluoropyridin-3-yl)-10a′-methyl-3′,4′,4a′,10a′-tetrahydro-1′*H*,5*H*-spiro[oxazole-4,10′-pyrano-[4,3-b]chromen]-2-amine (89).** Compound **89** was prepared from **45** using reaction conditions described for the synthesis of compounds **14**, **15**, and **61**. Crude **89** was purified by preparative TLC (2 mm thickness, R_f = 0.71), eluting with 10% MeOH (containing 7 N NH_3)/DCM. Relative stereochemistry was confirmed from cocrystal structures with BACE1 using the same conditions as described for compound **62**. Yield: 37 mg (20%, 3 steps). ^{1}H NMR (400 MHz, $CDCl_3$ + CD_3OD) δ 8.13 (m, 1H), 7.87 (m, 1H), 7.46 (m, 1H), 7.39 (m, 1H), 7.30 (m, 1H), 6.94 (m, 1H), 4.64 (m, 1H), 4.51 (m, 1H), 4.43 (m, 1H), 3.67 (m, 1H), 3.3−3.6 (m, 3H), 2.06 (m, 1H), 1.97 (m, 1H), 1.00 (s, 3H); m/z (APCI-pos) M + 1 = 370; 94% purity by LC−MS (220 nm).

(4*R,4a′*S**,10a′*S**)-2-Amino-8′-(3-(difluoromethoxy)-phenyl)-7′-fluoro-1-methyl-3′,4′,4a′,10a′-tetrahydro-1′*H*-spiro[imidazole-4,10′-pyrano[4,3-b]chromen]-5(1*H*)-one (90).** Compound **90** was prepared from **54**, as described for compound **61**, replacing 2-fluoropyridin-3-ylboronic acid with (3-(difluoromethoxy)phenyl)boronic acid. The crude was purified by silica gel chromatography (gradient: 0−100% (80% EtOAc/19% MeOH/1% Et_3N)/EtOAc). Yield: 2.3 mg (9%), 95% purity by LC−MS (254 nm). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.38 (m, 1H), 7.31−7.25 (m, 1H), 7.19 (m, 1H), 7.09 (m, 1H), 6.94 (m, 1H), 6.72 (m, 1H), 6.54 (t, J = 74 Hz, 1H), 4.96 (m, 1H), 4.10−3.91 (m, 2H), 3.48 (m, 1H), 3.08 (s, 3H), 3.04 (m, 1H), 2.23−2.11 (m, 2H), 1.91−1.78 (m, 1H); m/z (APCI-pos) M + 1 = 448.

(4*R,4a′*S**,10a′*S**)-2-Amino-7′-fluoro-8′-(2-fluoropyridin-3-yl)-1-methyl-3′,4′,4a′,10a′-tetrahydro-1′*H*-spiro[imidazole-4,10′-pyrano[4,3-b]chromen]-5(1*H*)-one (91).** Compound **91** was prepared from **54**, as described for compound **61**. The crude was purified by silica gel chromatography (gradient: 0−100% (80% EtOAc/19% MeOH/1% Et_3N)/EtOAc). Yield: 2.5 mg (11%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.16 (s, 1H), 7.77 (m, 1H), 7.22 (m, 1H), 6.97 (m, 1H), 6.73 (m, 1H), 4.98 (m, 1H), 4.10−3.92 (m, 2H), 3.48 (m, 1H), 3.14−3.02 (m, 1H), 3.07 (s, 3H), 2.28−2.10 (m, 2H), 1.92−1.77 (m, 1H); m/z (APCI-pos) M + 1 = 401; 90% purity by LC−MS (254 nm).

(4*R*,4a′*S*,10a′*S*)-8′-(5-Chloropyridin-3-yl)-7′-fluoro-4a′-methyl-3′,4′,4a′,10a′-tetrahydro-1′*H*,5*H*-spiro[oxazole-4,10′-pyrano[4,3-b]chromen]-2-amine (92). Compound **92** was prepared from **28** using reaction conditions described for the synthesis of compounds **19**−**27** and **79**. Crude **92** (mixture of diastereomers) was purified by silica gel eluting with a linear gradient of 0−6% DCM/MeOH + 1% NH_4OH to provide 8′-(5-chloropyridin-3-yl)-7′-fluoro-4a′-methyl-3′,4′,4a′,10a′-tetrahydro-1′*H*,5*H*-spiro[oxazole-4,10′-pyrano[4,3-b]chromen]-2-amine (42 mg) as a 2:1 mixture of diastereomers. This material was further purified by chiral SFC on a Chiralpak AD (2 × 15 cm) column eluting with 25% MeOH (0.1% NH_4OH)/CO_2 at 100 bar at a flow rate of 70 mL/min. The peaks isolated were analyzed on Chiralpak AD (50 × 0.46 cm) column eluting with 25% MeOH (0.1% NH_4OH)/CO_2, at 120 bar (flow rate 5 mL/min, 220 nm). Compound **92** (peak-3, 16 mg, 0.1% yield for 10 steps, chemical purity >99%, >99% ee) was isolated. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.69−8.59 (m, 2H), 8.05 (s, 1H), 7.26 (d, J = 8.8 Hz, 1H), 6.77 (d, J = 12.0 Hz, 1H), 6.13 (s, 2H), 4.45 (d, J = 8.9 Hz, 1H), 4.12 (d, J = 8.9 Hz, 1H), 3.92 (dd, J = 12.2, 4.4 Hz, 1H), 3.70 (dd, J = 11.4, 4.4 Hz, 1H), 3.60−3.48 (m, 2H), 2.18 (dd, J = 11.3, 4.3 Hz, 1H), 1.90 (td, J = 12.5, 4.9 Hz, 1H), 1.81 (d, J = 12.5 Hz, 1H), 1.34 (s, 3H). m/z (ESI-pos) M + 1 = 404.1. HPLC purity = 100% (254 nm).

(4*R*,4a′*S*,10a′*S*)-7′-Fluoro-8′-(2-fluoropyridin-3-yl)-4a′-methyl-3′,4′,4a′,10a′-tetrahydro-1′*H*,5*H*-spiro[oxazole-4,10′-pyrano[4,3-b]chromen]-2-amine (93). Compound **93** was prepared from **28** using reaction conditions described for the synthesis of compounds **19**−**27** and **79**, and replacing (5-chloropyridin-3-yl)boronic acid with (2-fluoropyridin-3-yl)boronic acid. Crude **93** (mixture of diastereomers) was purified as described for compound **92**. Compound **93** (17 mg, 0.1% yield for 10 steps, chemical purity >99%, >99% ee) was isolated. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.27 (d, J = 5.1 Hz, 1H), 8.06−7.96 (m, 1H), 7.46 (ddd, J = 7.1, 4.8, 1.8 Hz, 1H), 7.17 (d, J = 8.4 Hz, 1H), 6.77 (d, J = 11.3 Hz, 1H), 6.08 (s, 2H), 4.30 (s, 2H), 3.92 (dd, J = 11.9, 4.9 Hz, 1H), 3.85 (dd, J = 11.7, 4.0 Hz, 1H), 3.58 (td, J = 12.4, 2.6 Hz, 1H), 3.32 (dd, J = 14.1, 8.4 Hz, 1H), 2.11 (dd, J = 10.9, 4.0 Hz, 1H), 1.87 (dd, J = 12.7, 5.1 Hz, 1H), 1.79 (d, J = 12.7 Hz, 1H), 1.49 (s, 3H); m/z (ESI-pos) M + 1 = 388.1.

X-ray Crystal Structure. Human BACE1 (residues 57−453) was expressed in *E. coli* with a noncleavable 6-His tag at the C-terminus. The protein was refolded and purified in a manner similar to that from Tomasselli et al.[31] Briefly, cells were lysed using mechanical disruption and inclusion bodies recovered by differential centrifugation. Inclusion body proteins were solubilized in urea and refolded by dilution into water. Following a 3 week refold period, BACE protein was recovered by Q-Sepharose anion-exchange chromatography. Fractions containing active protein were pooled and further purified over Source-Q anion exchange and Sephadex 200 size-exclusion columns. Purified BACE protein was concentrated to 10 mg/mL and frozen for crystallographic studies. Crystals were grown by hanging drop vapor diffusion with a precipitant of 20% PEG200, 0.1 M sodium acetate, pH 4.5−5.6, and 0−2 mM cobalt chloride. Crystals grew in space group C2221 in approximately 1 week. Crystals were then transferred to a soaking solution (27−30% PEG200, 0.1 M NaOAc, pH 5.0−5.6, 0−2 mM $CoCl_2$, and 10% DMSO) with compound at a final concentration of 1 mM. Crystals were soaked for 18−24 h and either flash-frozen in liquid nitrogen or mounted directly for data collection. The soaking solution also served as a cryprotectant for data collection at 100 K.

Diffraction data were collected on a Rigaku FR-E generator and Raxis IV++ detector (Rigaku Inc., Woodlands, TX). Data were processed in Moslfm,[32] and the initial structure was solved by molecular replacement from pdb code 1FNK using Molrep. All crystal structures were refined in Refmac, all three of these programs being part of the CCP4 suite (Winn et al., 2011).[33] Model building was performed in Coot.[34]

BACE1 Enzyme Assay. The activity of purified recombinant human BACE1, expressed in CHO cells, was determined using a

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custom synthesized biotinylated peptide based upon sAPP. Uncleaved substrate was detected via HTRF whereby europium-labeled anti-Aβ (amino acids 1−17, clone 6E10) was combined with D2-labeled streptavidin. Specifically, 20 nM enzyme was incubated with 150 nM peptide (Biotin-Lys-Thr-Glu-Glu-Ile-Ser-Glu-Val-Asn-Leu-Asp-Ala-Glu-Phe-Arg-His-Asp-Ser-Gly-Tyr-Glu-Val-His-His-Gln-Lys-Leu, representing P10−P17′) for 6 h at 22 °C in an assay buffer consisting of 50 mM sodium acetate (pH 4.4) and 0.1% CHAPS. In IC_{50} studies, compounds were serially diluted (1:2.5) in DMSO (11 points); compound and enzyme were preincubated for 15 min at room temperature and the enzymatic reaction was initiated with peptide. The detection solution consisted of 43 nM streptavidin-D2 and 1.1 nM antibody in 200 mM Tris (pH 8.0), 20 mM EDTA (pH 8.0), 0.1% BSA, and 0.8 M KF. Following an incubation for 2 h at 22 °C, the samples were excited at 320 nm and the emission ratio of 665 nm/615 nm was determined.

Cathepsin D Enzyme Assay. The catalytic activity of Cathepsin D, purified from human spleen (Calbiochem), was assessed in a FRET peptide substrate assay using 50 mM sodium acetate (pH 4.4) and 0.1% CHAPS. In IC_{50} studies, compounds were serially diluted (1:2.5) in DMSO (11 points). The substrate (Anaspec catalog # 72097) contains a 5-FAM/QXL 520 pair whereby cleavage results in an increase of 5-FAM fluorescence that is measured using an excitation of 485 nm and an emission of 535 nm. The fluorescence was monitored continuously for one hour at room temperature and initial rates were calculated from the fluorescence increase. The final concentrations were 10 nM enzyme and 2 μM substrate.

Cell-Based Assay. Human embryonic kidney cells (HEK293) stably expressing APP_{wt} were plated at a density of 35K cells/well in 96-well collagen coated plates (BD-Biosciences). The cells were cultivated overnight at 37 °C and 5% CO_2 in DMEM supplemented with 10% FBS. The following day the media was changed, and the cells were incubated for 48 h with test compounds at concentrations ranging from 0.0008 to 16 μM. Following incubation with the test compounds, the conditioned medium was collected, and the $Aβ_{1-40}$ levels were determined using an HTRF assay (CisBio). The IC_{50} was calculated from the percent of control $Aβ_{40}$ as a function of the concentration of the test compound. The HTRF to detect $Aβ_{1-40}$ was performed in 384-well microtiter plates (Corning). The antibody pair that was used to detect $Aβ_{1-40}$ from cell supernatants was a pair of monoclonal antibodies, one labeled with Cryptate and one labeled with XL655 as supplied by the manufacturer (CisBio). Conditioned medium was incubated with antibody pair overnight at 4 °C. The plates were measured for timeresolved fluorescence on a Victor2 plate reader (Wallac).

Permeability and Efflux Assays. Both LLC-PK1 and MDR1 transfected LLC-PK1 cells were cultured and plated according to manufacturer's recommendations with the exception that the passage media contained only 2% fetal bovine serum so as to extend passage time out to seven days. Both positive and negative controls were used to assess functionality of P-gp efflux in the assay. Stock solutions for assay controls and the test article were prepared in DMSO for final test concentrations of 10 and 1 μM, respectively. Final organic concentration in the assay was 1%. All dosing solutions contained 10 μM lucifer yellow to monitor LLC-PK1 cell monolayer integrity. For the apical to basolateral determination (A to B), 75 μL of the test article in transport buffer were added to the apical side of the individual transwells and 250 μL of basolateral media, without compound or lucifer yellow, were added to each well. For the basolateral to apical determination (B to A), 250 μL of test article in transport buffer were added to each well and 75 μL transport buffer, without compound or lucifer yellow, were added to each transwell. All tests were performed in triplicate, and each compound was tested for both apical to basolateral and basolateral to apical transport. Propranolol, a high permeability compound (P_{app} AB > 8×10^{-6} cm/s), sulfasalazine, a medium permeability compound (P_{app} > 2×10^{-6} cm/s; P_{app} < 8×10^{-6} cm/s), and cefuroximine, a low permeability compound (P_{app} AB < 2×10^{-6} cm/s), were used as negative controls for P-gp efflux (ER < 2). Digoxin, a medium permeability compound, and quinidine, a high permeability compound, were used as positive controls for P-gp efflux (ER > 2). The plates were incubated for 2 h on a Lab-line Instruments Titer Orbital Shaker (VWR, West Chester, PA) at 50 rpm and 37 °C with 5% CO_2. All culture plates were removed from the incubator, and 50 μL of media were removed from the apical and basolateral portion of each well and added to 150 μL of 1 μM labetalol in 2:1 CH_3CN: H_2O, v/v. The plates were read using a Molecular Devices (Sunnyvale, CA) Gemini Fluorometer to evaluate the lucifer yellow concentrations at excitation/emission wavelengths of 425/535 nm. These values were accepted when found to be below 5% for apical to basolateral and basolateral to apical flux across the MDR1 transfected LLC-PK1 cell monolayers. The plates were sealed, and the contents of each well analyzed by LC−MS/MS. The compound concentrations were determined from the ratio of the peak areas of the compound to the internal standard (labetalol) in comparison to the dosing solution. The LC−MS/MS system comprised an HTS-PAL autosampler (Leap Technologies, Carrboro, NC), an HP1200 HPLC (Agilent, Palo Alto, CA), and an MDS Sciex 4000 Q Trap system (Applied Biosystems, Foster City, CA). Chromatographic separation of the analyte and internal standard was achieved at rt using a C18 column (Kinetics, 50 × 300 mm, 2.6 μm particle size, Phenomenex, Torrance, CA) in conjunction with gradient conditions using mobile phases A (water containing 1% i-PrOH and 0.1% formic acid) and B (0.1% formic acid in CH_3CN). The total run time, including re-equilibration time, for a single injection was 1.2 min. Mass spectrometric detection of the analytes was accomplished using the ion spray positive mode. Analyte responses were measured by multiple reaction monitoring (MRM) of transitions unique to each compound (the protonated precursor ion and selected product ions for each test article and m/z 329 to m/z 162 for labetalol, the internal standard).

Plasma Protein and Brain Tissue Binding. Aliquots of undiluted plasma or diluted brain samples (three volumes of 0.1 M sodium phosphate buffer, pH 7.4) from each animal species were spiked with test compound in DMSO to give final concentrations of 5 μM (0.1% final DMSO) test article. Rapid equilibrium dialysis (RED) inserts were prepared in triplicate by adding 500 μL of PBS for plasma protein binding or 0.1 M sodium phosphate for brain tissue binding to one chamber and 300 μL of spiked plasma or brain homogenate to the opposing chamber. The RED units (inserts and bases) were sealed and incubated at 37 °C on a plate shaker at 300 rpm for 6 h to equilibrate the samples. One hundred microliters of each spiked matrix sample and respective buffer were transferred to a 96-deep well plate for sample extraction. The matrix samples were diluted with 100 μL of either PBS or 0.1 M sodium phosphate buffer, and the buffer samples were diluted with 100 μL of either brain tissue homogenate or plasma to yield identical matrixes between buffer and nonbuffer samples. Plasma or brain proteins were precipitated with 800 μL of CH_3CN spiked with 0.2 μM final concentration (0.02% DMSO) of labetalol, the internal standard. The plates were spun in a centrifuge for 10 min at 3000*g*. One hundred microliters of the supernatant were transferred into a shallow 96-well plate and diluted 1:1 (v/v) with HPLC grade water and sealed for LC−MS/MS analysis (method described below).

Liver Microsomal Incubations. A 100 mM potassium phosphate assay buffer solution (KPB) was prepared as follows. Both KH_2PO_4 and K_2HPO_4 were dissolved separately in reagent grade water resulting in a final concentration of 100 mM. A 75:25 mixture v/v of K_2HPO_4/KH_2PO_4 was prepared, and the pH of the solution was adjusted to 7.4 using diluted HCl or diluted NaOH solutions. A stock solution of the test article(s) was prepared at 10 mM (active compound) in DMSO. The stock solution was diluted immediately before use to 2.5 μM using the KPB solution to create the working standard. All test compounds were completely soluble by visual inspection at rt. The NADPH-regenerating solution (NRS) was prepared on the day of analysis by diluting one volume of 17 mg/mL NADP+ with one volume of 78 mg/mL glucose-6-phosphate (both prepared in KPB, pH 7.4) and 7.9 volumes of 20 mM $MgCl_2$. The final concentrations of NADP+ and glucose-6-phosphate were 1.7 and 7.8 mg/mL, respectively. Immediately prior to use, the NRS was activated by the addition of 10 μL of glucose-6-phosphate dehydrogenase (150 units/mL in KPB, pH 7.4) per mL of NRS stock solution. Liver microsomes were diluted to 2.5

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mg protein/mL using KPB. For each test article or positive control (i.e., dextromethorphan, diazepam, diltiazem, phenacetin, tolbutamide, and verapamil), 20 μL of 2.5 μM working standard solution of test compound and 20 μL of microsomes (2.5 mg protein/mL) were added to each well of a 96-well polypropylene plate (Costar, VWR, West Chester, PA) in duplicate. The plates were placed in an incubator at 37 °C for 5 min before adding the start solution. A 10 μL aliquot of the NRS solution was added to each original well to initiate metabolism. The concentration of the test compound during incubation was 1 μM. One incubation plate was prepared for each time point (i.e., 0 and 20 min). Incubations were conducted at 37 °C and 100% relative humidity. At each time point, the appropriate incubation plate was removed from the incubator and a solution containing internal standard (150 μL, 0.25 μM labetalol in 60% CH_3CN) was added to each well. The plate was immediately spun in a centrifuge at 2095*g* for 7 min at rt using an Allegra benchtop centrifuge (Beckman Coulter, Fullerton, CA). A 200 μL aliquot of the supernatant was transferred from each well to a 96-well shallow plate (Costar). The plates were sealed using reusable plate mats. Analysis was performed as described below.

Hepatocyte Incubations. A stock solution of the test article(s) was prepared at 10 mM (active compound) in DMSO. The in vitro stability of each test article or positive control was assessed in the presence of hepatocytes as follows. Cryopreserved hepatocytes were thawed, isolated from shipping media, and diluted to a density of 1×10^6 viable cells/mL, according to the supplier's guidelines, using Dulbecco's Modified Eagle Medium, 1×, high glucose (DMEM, Invitrogen, Carlsbad, CA). Viability was determined by trypan blue exclusion using a hemocytometer (3500 Hausser, VWR, West Chester, PA). The 10 mM stock solution of test article(s) or control compound was diluted to 2 μM using supplemented DMEM to create the working standard. A 20 μL aliquot of test compound or control (antipyrine, diazepam, diltiazem, lorazepam, propranolol, verapamil, and 7-ethyl-10-hydroxycamptothecin [SN-38]) was added to each test well of a 96-well polypropylene plate (Costar, VWR, West Chester, PA) immediately followed by the addition of 20 μL of the hepatocyte suspension. One incubation plate was prepared for each time point (i.e., 0, 60, and 120 min) with samples being prepared in duplicate. Incubations were conducted at 37 °C and 100% relative humidity. At each time point, the appropriate incubation plate was removed from the incubator and a solution containing internal standard (200 μL, 0.25 μM labetalol in 60% CH_3CN) was added to each well. The plate was mixed at 700 rpm for 1 min on a plate shaker (IKA MTS 2/4 Digital Microtiter Shaker, VWR) and immediately spun in a centrifuge at 2095*g* for 10 min at rt using an Allegra benchtop centrifuge (Beckman Coulter, Fullerton, CA). A 200 μL aliquot of the supernatant was transferred from each well to a 96-well shallow plate (Costar). The plates were sealed using reusable plate mats.

Analytical Quantitation for Microsomal/Hepatocyte Stability and Tissue Binding Assays. The LC−MS/MS system comprised an HTS-PAL autosampler (Leap Technologies, Carrboro, NC), an HP1200 HPLC (Agilent, Palo Alto, CA), and an API4000 triple quadrupole mass spectrometer (PE Sciex, a division of Applied Biosystems, Foster City, CA). Chromatographic separation of the analyte and internal standard was achieved at rt using a C18 column (Kinetex, 30 × 3.0 mm, 2.6 μm particle size, Phenomenex, Torrance, CA) in conjunction with gradient conditions using mobile phases A (aqueous 0.1% formic acid with 1% i-PrOH) and B (0.1% formic acid in CH_3CN). The total run time, including re-equilibration, for a single injection was 2 min. Mass spectrometric detection of the analytes was accomplished using the ESI+ ionization mode. Ion current was optimized during infusion of a stock solution of each test article. Analyte responses were measured by multiple reaction monitoring (MRM) of transitions unique to each compound. Data were acquired, and peak areas were calculated for test compounds and the internal standard using Analyst 1.5.1 software (Sciex). For the liver microsomal and hepatocyte stability assessments, peak area tables were exported to BioAssay Enterprise (CambridgeSoft, Cambridge, MA), where the average analyte to internal standard peak area ratios were used to calculate percent remaining (%REM), half-life ($t_{1/2}$), predicted hepatic clearance (CL_{hep}), and predicted hepatic extraction ratio (ER).

Pharmacokinetic Studies. Animals and Drug Treatment: Male CD-1 mice (4−6 weeks old, 25−35 g) and Male Hartley guinea pigs (surgically implanted jugular cannulas, 6−8 weeks old, 250−300 g) were obtained from Charles River (Portage, MI, and Kingston, NY, respectively). The cannulae were exteriorized at the back of the neck, and each animal was placed singly in a metabolic cage. Male Sprague−Dawley rats (6−8 weeks old, 250−300 g) were obtained from Harlan (Indianapolis, IN). All the animals were housed at controlled temperature and humidity in an alternating 12 h light and dark cycle with free access to food and water, in accordance with Array BioPharma Institutional Animal Care and Use Committee guidelines and in harmony with the Guide for Laboratory Animal Care and Use. The guinea pigs were housed individually according to NIH guidelines. Mice (3 per group, per time-point) received a 3 mg/kg dose of drug (5 mL/kg) intravenously or 10 mg/kg dose (10 mL/kg) orally. Rats (3 per group) received a 3 mg/kg dose of compound intravenously or 60 mg/kg dose orally. Guinea pigs (4 per group) received a 3 mg/kg dose (1 mL/kg) of compound intravenously via cannula or 60 mg/kg dose (5 mL/kg) orally. Plasma collection: Mice were euthanized by CO_2 inhalation, rats and guinea pigs were anesthetized by isoflurane at designated times between 1 min and 24 h postdose. The whole blood samples were drawn via cardiac puncture (mice), tail vein (rats), and jugular vein cannula (guinea pigs) in tubes containing 1.5% EDTA. The blood samples were centrifuged at 14000 rpm for 10 min; plasma was decanted and stored at −20 °C before drug concentration analysis. In guinea pigs, after each blood draw, the cannulas were flushed with heparinized saline (volume equal to the cannula volume). At termination of the study, guinea pigs were euthanized by euthasol (250 mg/kg) via intraperitoneal injection. Brain collection: The orally dosed mice, postmortem at 30 min to 8 h postdose, were perfused with 5 mL sterile saline. Brains were harvested, and the tissue weights were recorded. The brains were snap frozen immediately in liquid nitrogen and stored at −80 °C until determination of drug concentration in brain samples.

Rat and Guinea Pig Pharmacodynamic Studies. Animals and Drug Treatment: Male Sprague−Dawley rats and male Hartley guinea pigs (6−8 weeks old, 250−350 g), were obtained from Harlan (Indianapolis, IN) and Charles River Laboratories (Kingston, NY), respectively. The animals were housed in groups of 3, at controlled temperature and humidity in an alternating 12 h light and dark cycle with free access to food and water, in accordance with Array BioPharma Institutional Animal Care and Use Committee guidelines and in harmony with the Guide for Laboratory Animal Care and Use. Rats (8 per group) received orally either vehicle (1% carboxymethyl cellulose/0.5% Tween 80) or **62** (60 mg/kg) at a volume of 4 mL/kg. Guinea pigs (6−8 per group) received orally either vehicle (a) 1% carboxymethyl cellulose/0.5% Tween 80 or (b) 40% PEG400/10% ethanol/50% water or drugs: **62** (30, 10, and 60 mg/kg) or **59** (60 mg/kg) at a volume of 4−5 mL/kg. Plasma, CSF, and brain collection: At 1, 3, 5, 8, and 12 h after receiving the vehicle or drug dose, the rats were euthanized by CO_2 inhalation, and guinea pigs were euthanized by euthasol (250 mg/kg) via intraperitoneal injection. The whole blood samples were collected postmortem via cardiac puncture, in tubes containing 1.5% EDTA, centrifuged at 14000 rpm for 10 min. The plasma was stored at −20 °C until drug concentration analysis. The CSF samples were drawn postmortem via cisterna magna puncture through a 25 gauge needle (Becton Dickinson Franklin Lakes, NJ), into tubes containing 50 μL of 10% BSA in water. The CSF samples were snap frozen in liquid nitrogen and stored at −80 °C until Aβ 40 assay. Sample weights were recorded to determine the actual volume of CSF collected. The postmortem animals were perfused with 20 mL of sterile saline and brain hemispheres were dissected (brain stem and cerebellum were not collected), halved longitudinally, weighed, and placed only left half of the brain in a 15 mL conical tube and snap frozen in liquid nitrogen. All tissues were stored at −80 °C until drug concentration determination. CSF Aβ 40 assay: CSF Aβ 40 concentration in rat and guinea pig CSF samples was analyzed by MSD 96-Well MULTI-ARRAY Human/Rodent (4G8)

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Aβ 40 Ultra-Sensitive Assay (rat) or Human (6E10) Aβ 40 Ultra-Sensitive Kit (Meso Scale Discovery, Gaithersburg, MD). The 96- well MULTI-SPOT Aβ 40 peptide plate was incubated with 1% BSA solution at room temperature with shaking for 1 h and washed as per manufacturer's instructions with Tris wash buffer (Tris wash buffer in deionized water). The detection antibody solution (25 μL/well, sulfo-tag 4G8 in 1% BSA) and CSF samples or standards (25 μL/well) were added to the plate for incubation at room temperature for 2 h. The plate was washed three times before the addition of MSD read buffer and was read on Sector Imager 2400 (Meso Scale Discovery, Gaithersburg, MD) immediately after. The Aβ 40 levels in neat CSF samples were then determined using a standard curve, values were obtained as pg/mL, and the results were expressed as mean Aβ 40 reduction percentage (%) of control samples from vehicle-treated animals.

Cyno Pharmacodynamic Studies. A single oral dose of compound at 100 mg/kg was given to 10 male cynomolgus monkeys (3−5 year, 2−3 kg) at Maccine. The study utilized a crossover design: n = 10 vehicle, n = 10 at 100 mg/kg, with an 18−20 days washout period between vehicle and treatment. Baseline was established 216−24 h before dosing. PK was determined for parent drug concentration in plasma and CSF, and PD was determined by measuring $Aβ_{1-40}$ levels in plasma and CSF. Full details are published elsewhere.[30]

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Figure S1 and experimental details for compound **94**. This material is available free of charge via the Internet at http://pubs.acs.org.

Accession Codes

The PDB accession number for the coordinates of the cocrystal structure of BACE1 + compound **62** is 4N00.

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Notes

The authors declare no competing financial interest.

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■ ABBREVIATIONS USED

BLQ, below the level of quantitation; IVIVC, in vitro−in vivo correlation; P_{app} AB, apparent cell permeability in the apical to basolateral direction; APCI, atmospheric-pressure chemical ionization; CHO, Chinese hamster ovary; HTRF, homogeneous time-resolved fluorescence; 5-FAM, 5-carboxyfluorescein; DMEM, Dulbecco's modified eagle medium; LLC-PK1, pig kidney epithelial cells; RED, rapid equilibrium dialysis; ER, extraction ratio; KPB, potassium phosphate buffer; NRS, NADPH-regenerating solution; PDB, protein data bank; tPSA, total polar surface area; S_NAr, nucleophilic aromatic substitution

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