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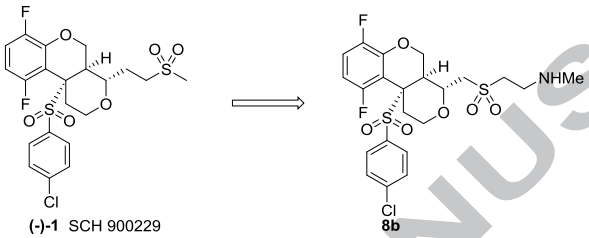

(-)-1 SCH 900229 **8b**

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Design and synthesis of water soluble β-aminosulfone analogues of SCH 900229 as γ-secretase inhibitors

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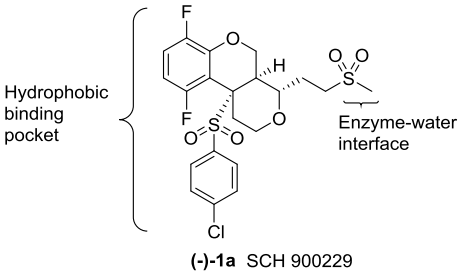
ABSTRACT

In this paper we describe our strategy to improve the aqueous solubility of SCH 900229, a potent PS1-selective γ-secretase inhibitor for the treatment of Alzheimer's disease. Incorporation of ionizable amino groups into the side chain terminal generates water soluble β-aminosulfone analogues of SCH 900229 that maintain robust in vitro potency and in vivo efficacy.

Alzheimer's disease (AD) is a neurodegenerative disease afflicting 36 million people worldwide.[1] The search for a disease modifying treatment for AD has been accelerated, particularly in the last two decades, with newly found genetic evidence supporting the so-called amyloid hypothesis.[2,3] This hypothesis postulates that the β-amyloid (Aβ) peptides play a causative role in the disease. As a result, numerous programs targeting the Aβ pathway have been reported.[4] The two enzymes involved in the Aβ production are β-site amyloid cleaving enzyme 1 (BACE1) and γ-secretase. γ-Secretase is a complex of four distinct proteins: presenilin 1 (PS1) or presenilin 2 (PS2), nicastrin, aph-1α or aph1-β and pen-2. [5] There are thus multiple isoforms of γ-secretase as a result of different combinations of PS1 and PS2 with Aph-1α and Aph-1β. Selective inhibition of γ-secretase isoforms containing PS1 proved to be a promising strategy in the design and development of γ-secretase inhibitors (GSIs) that maintained Aβ-lowering efficacy but reduced mechanism-based Notch-related adverse effects.[6,7] Toward that end, we have designed a series of bis-sulfone analogues as PS1-selective GSIs and advanced the lead compound SCH 900229, [(-)-**1a**, Figure 1] into clinical trials.[8] Extensive structure-activity relationship (SAR) efforts around the frame of SCH 900229 revealed limited tolerance of substituents on the two phenyl rings. By contrast, analogues bearing various sulfone side chains showed robust in vitro potency; however, only selected compounds demonstrated good pharmacokinetics and in vivo efficacy. The two carbon linker is optimal for the sulfone series of analogues.[9]

The clinical candidate SCH 900229 is a non-ionizable molecule with low aqueous solubility (kinetic solubility, Ksol 50 μM) with a BCS classification of II. It is therefore likely that mproving the physicochemical properties of SCH 900229 is necessary for optimum therapeutic performance.



(-)-1a SCH 900229

Figure 1. γ-Secretase inhibitor SCH 900229 and SAR development

In our γ-secretase inhibitor backup program, we aimed to improve upon the aqueous solubility of SCH 900229, while maintaining the basic structure and the favorable in vitro and in vivo properties. The wide tolerance for various side chains in terms of in vitro potency suggested that this region may be a 'sweet spot' for modulating physicochemical properties without compromising the key interactions with the enzyme. As reported previously,[9] analogues bearing either a polar hydroxyl (**1b**, table 1) or carboxylic acid group (**1c**) showed great aqueous solubility (Ksol > 200 μM) but poor in vivo efficacy. We investigated

whether side chains with basic moieties, such as the β- aminosulfone as shown in Figure 2, would make a difference. We reasoned that the basic amine group can be protonated to form salts that generally have a higher aqueous solubility and a more rapid dissolution rate than the free base, while the neutral portion can still allow cell membrane and blood-brain barrier (BBB) permeability. On the other hand, a strong electron-withdrawing sulfone group will keep the basicity of the amine between 7 and 9. This balanced effect is important because excessive basicity combined with large lipophilic groups may cause adverse effects, such as inhibition of the hERG channel or phospholipidosis.[10] Indeed, similar considerations have been demonstrated in the design of launched drugs, including dalfopristin[11] and lapatinib.[12]

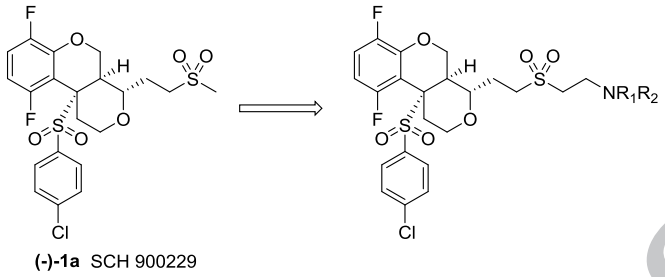
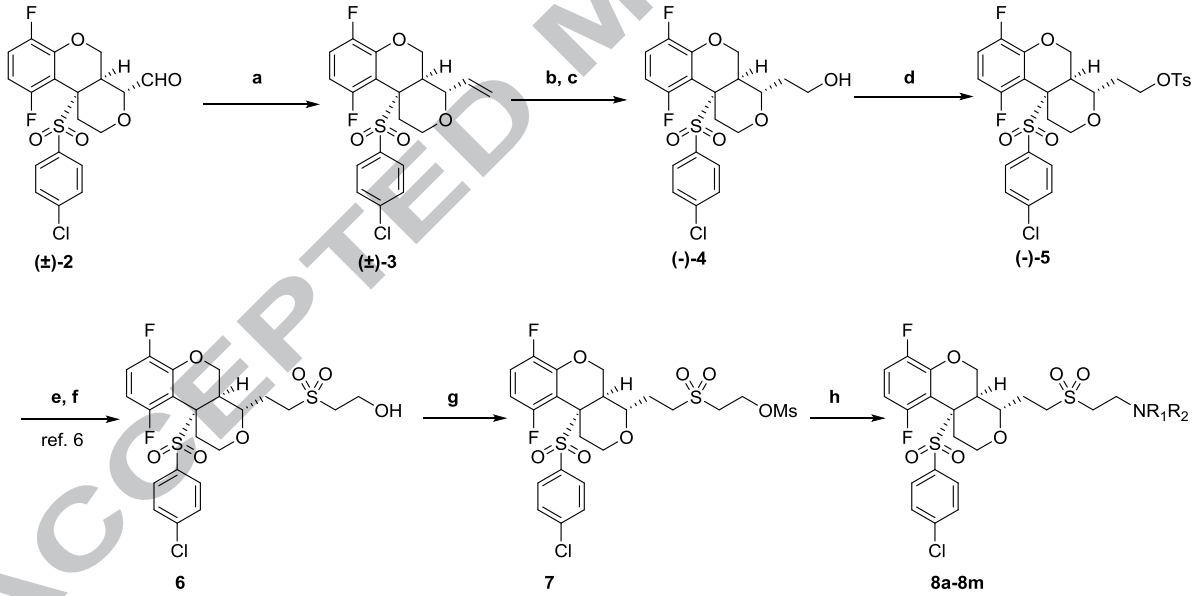


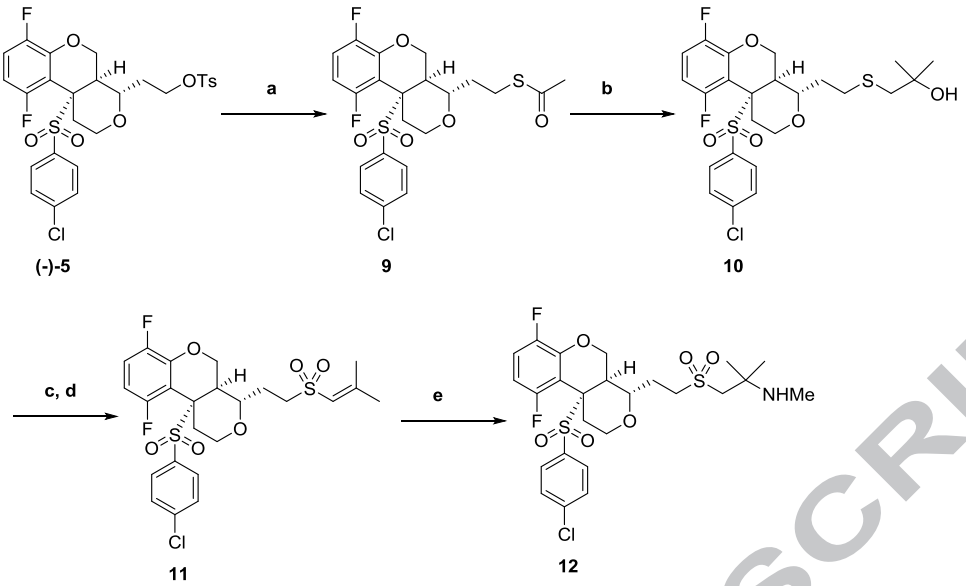
Figure 2. Design of β-aminosulfone analogues

We took advantage of SCH 900229 intermediates to further the SAR exploration. As shown in Scheme 1, the racemic aldehyde **2** was elongated by Wittig olefination to give racemic **3**. Reduction of the terminal alkene with 9-BBN followed by oxidation with H_2O_2 provided the racemic alcohol,[13] which was resolved by chiral HPLC (Chiralcel OJ column) to give the desired enantiomerically pure intermediate **(-)-4**. Compound **4** was converted to its tosylate derivative **5**, and the tosylate was then displaced with hydroxyethylthiol followed by mCPBA oxidation to give the β-hydroxysulfone **6**. The alcohol was derivatized to its mesylate **7**, which was treated with various amines to generate β-aminosulfone analogues **8a-8m**, presumably through β-elimination followed by aza-Michael addition of amines to the vinyl sulfone intermediate.[14]



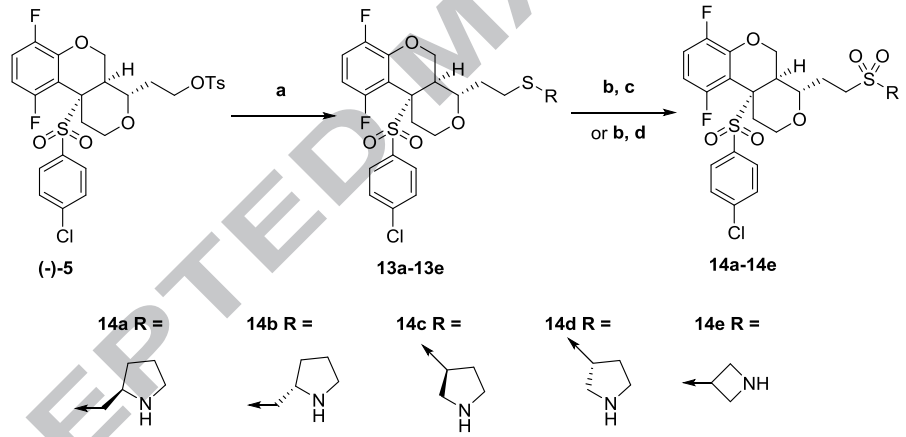
Scheme 1. Reagents and conditions: (a) $Ph_3P^+CH_3Br^-$, n-BuLi, THF, -50 °C to rt, 62%; (b) 9-BBN, THF; H_2O_2, NaOH, 60%; (c) Chiral HPLC separation; (d) TsCl, CH_2Cl_2, 90%; (e) $HOCH_2CH_2SH$, KOH, EtOH; (f) mCPBA, CH_2Cl_2, 80% in 2-step; (g) MsCl, Et_3N, CH_2Cl_2, 94%; (j) R_1R_2NH, DMF, rt, >90%.

The synthesis of the α,α-dimethyl substituted analogue **12** is depicted in Scheme 2. The tosylate **5** was treated with potassium thioacetate to give thioester **9**. Hydrolysis of the thioester with KOMe in methanol followed by addition of 2,2-dimethyloxirane generated alcohol **10** in one-pot. After oxidation with mCPBA, treatment of the tertiary alcohol with mesyl chloride in the presence of triethylamine gave the α,β-unsaturated sulfone **11** directly. Finally, aza-Michael addition reaction between methylamine and **11** gave the β-aminosulfone analogue **12**.



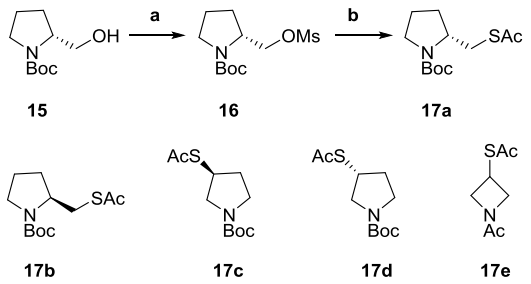
Scheme 2. Reagents and conditions: (a) MeCOSK, EtOH, 80%; (b) KOMe, MeOH, 2,2-dimethylepoxide, 60%; (c) mCPBA, CH_2Cl_2, 91%; (d) MsCl, Et_3N, rt; (e) $MeNH_2$, MeCN, rt.

To synthesize cyclic amine analogues **14a-14e**, a different strategy was adopted as depicted in Scheme 3. The tosylate **5** was treated with various thio intermediates that were generated in-situ from appropriate thioester precursors **17a-17e** to provide sulfides **13a-13e**. Oxidation of the sulfides with mCPBA and deprotection of N-Boc with TFA or N-Ac with KOH furnished the targets **14a-14e**.



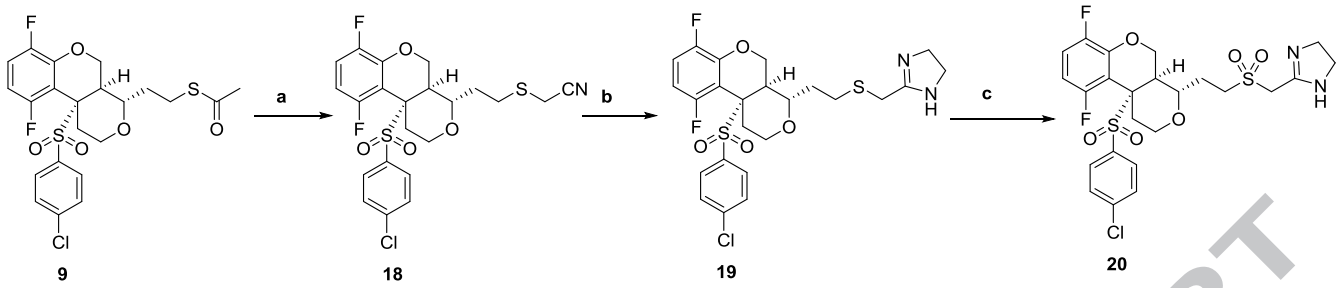
Scheme 3. Reagents and conditions: (a) Thioesters **17a-17e**, KOMe, MeOH, > 50%; (b) mCPBA, CH_2Cl_2; > 90% (c) TFA, CH_2Cl_2, > 90%; (d) KOH, EtOH, reflux, 77%.

The required thioester precursors **17a-17d** were prepared from commercially available primary and secondary alcohols, as exemplified by compound **17a** in Scheme 4. Compound **17e** was prepared according to a literature procedure.[15]



Scheme 4. Reagents and conditions: (a) $MeSO_2Cl$, Pyridine, CH_2Cl_2; (b) MeCOSK, DMF, 100 ^oC, 83% in 2-step.

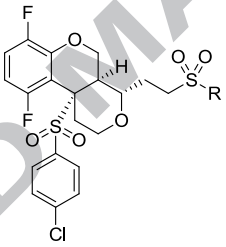
The amidine analogue **20** was synthesized from the intermediate **9** as shown in Scheme 5. As described previously, hydrolysis followed by in-situ alkylation provided nitrile **18**. Treatment of the nitrile with ethylenediamine in the presence of sulfur gave amidine **19**.[16] Finally, mCPBA oxidation under acidic conditions furnished the sulfone **20** in good yield.

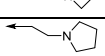
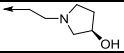
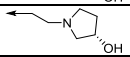
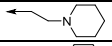
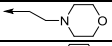
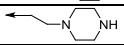
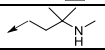


Scheme 5. Reagents and conditions: (a) $BrCH_2CN$, KOMe, MeOH, ~ 70%; (b) S, $H_2NCH_2CH_2NH_2$; (c) mCPBA, TFA, CH_2Cl_2, 40% in 2-step.

Analogues **8a-8m, 12, 14a-14e,** and **20** were all enantiomerically pure and converted to their corresponding HCl salt form for all the tests. They were first screened in the membrane-based *in vitro* γ-secretase assay and in the PS2/PS1 ratio assay.[17,6] Selected analogues were then examined for their in vivo activity in mice by measuring Aβ reduction in plasma and brain after a single 10 mg/kg oral dose.[18] As summarized in Table 1, all the β-aminosulfone analogues retained the excellent *in vitro* potency of SCH 900229 [**(-)-1a]**, confirming the notion that this region of the molecule does not interact with the enzyme directly. The primary, secondary and tertiary amine analogue **8a-8f** also display selectivity for PS1 (PS2/PS1 ratios ranging from 18-35). Cyclic amines in various rings, such as **14a-14e**, still maintained good potency and PS1 selectivity. Blocking the α-position of **8b** with gem-dimethyl was well tolerated (**12**). Interestingly, the amidine analogue **20** also has good *in vitro* potency, with an IC_{50} of 5.9 nM. Several compounds also reduced plasma and/or cortex Aβ40 to varying degrees.

Table 1 Right-hand side chain sulfone substitution SAR data.



Compound	R	Aβ40 IC_{50} (nM)[a,b]	PS2/PS1[c]	In vivo Aβ40 (%)[d]	
		membrane		plasma	cortex
1a	Me	1.3	25	-89	-78
1b	$(CH_2)_2OH$	1.0		-42	-46
1c	CH_2COOH	2.7	22	+47	
8a	$CH_2CH_2NH_2$	0.8	35	-54	-52
8b	CH_2CH_2NHMe	4.1	25	-44	-61
8c	CH_2CH_2NHEt	0.9	18		
8d	$CH_2CH_2NH^iPr$	0.5	30		
8e	$CH_2CH_2NH^cBu$	2.0			
8f	$CH_2CH_2NMe_2$	1.7	20	-21	-45
8g		1.6	17		
8h		1.1	13		
8i		0.6	12		
8j		0.8	12		
8k		1.3	9		
8l		3.7	10		
8m		0.8	10	-95	
12		2.2		-51	-33

14a		1.0	23	-71	-42
14b		0.7	14	-57	-48
14c		1.4	30	-82	-56
14d		5.1	21	-90	-73
14e		1.3		-15	
20		5.9		-86	

[a] The IC_{50} data are an average of at least two measurements. The standard deviations were within 20%. [b] Determined in $HEK^{Swe\text{-}Lon}$ 293 cells, see ref. 17. [c] Selectivity for PS1 vs. PS2 was determined as a ratio of IC_{50} values obtained in an in vitro reconstituted PS1 and PS2 γ-secretase system (n =2), see ref. 6. [d] Measured at 3 h after an oral dose of 10 mg/kg administered to,B6C3F1 mice; (-) indicates lowering of Aβ40, (+) indicates an increase of Aβ40, see ref. 18.

As exhibited in Table 2, HCl salts of most of the primary and secondary amine analogues, such as **8a-8c** and **14a-14e,** displayed improved aqueous solubility (Ksol > 200 μM) compared to SCH 900229 (50 μM). Tertiary amine analogues generally did not improve the kinetic solubility, as exemplified by analogue **8f**. Compound **12** had diminished kinetic solubility (20 μM), probably due to its reduced hydration capacity. Surprisingly, the amidine analogue 20 also had poor kinetic solubility despite its high nitrogen count. The pKa of compound **8b** was determined to be 7.7. At physiological pH of 7.4, it was estimated that two-thirds of **8b** will exist in protonated form and the other one-third as the free base.

Table 2. in vitro and in vivo ADME profiles

Compound	**Ksol [a] (μM)**	**CYP3A4[b] IC_{50} (μM) Co, Pre[c]**	**Hep Cl[d] μL/min/10^6 cells**				**AUC_{6h} (nM.h)[e]**	**B/P[f]**
			human	**rat**	**dog**	**monkey**		
8a	**200**	**15.1/2.8**	**8.5**	**12.1**	**6.4**	**20.3**	**582**	
8b	**200**	**>20/ 8.4**	**17.3**	**22.3**	**11.5**	**40.5**	**532**	**0.4**
8c	**200**	**>20/ 1.4**	**15.1**	**29.1**	**10.8**	**44.6**	**1355**	**0.4**
8f	**50**	**>20/ 9.0**	**57.2**	**66.7**	**56.5**	**65.0**	**blq**	
8m	**200**	**>20/>20**	**5.3**	**6.1**	**7.7**	**12.7**	**blq**	
12	**>20**	**>20/>20**	**20.4**	**25.8**	**23.5**	**55.1**	**477**	**1.0**
14a	**200**	**>20/ 1.0**		**12.8**	**13.6**	**28.0**	**2523**	**0.4**
14b	**200**	**>20/ 1.0**	**5.6**	**15.2**	**12.5**	**26.7**	**674**	**0.6**
14c	**200**	**2.0/ 1.0**	**34.2**	**18.9**	**21.1**	**60.5**	**1021**	**0.3**
14d	**200**	**6.1/ 3.7**	**14.5**	**13.9**	**17.9**	**38.8**	**1174**	**2.0**
20	**20**	**13.2/1.0**	**13.7**	**25.9**	**20.1**	**32.0**	**57**	

[a] The upper limit of the assay for kinetic solubility (Ksol) is 200 mM. [b]There was no detectable inhibition of CYP2C9 and CYP 2D6 (IC_{50}>20 μM). [c]Data for the preincubation (Pre) experiment was collected 30 min after addition of the compound to the assay system, for details on the co-incubation (Co) experiment, see ref. 19. [d]See ref. 20. [e]AUC was calculated over a 6h period after an oral dose of 10 mg/kg (n = 2), blq: below the limit of quantification, see ref. 20. [f] B/P, brain/plasma ratio at the 6h time point.

In order to prioritize the analogues, other *in vitro* and *in vivo* ADME characteristics were also taken into account, including human CYP inhibition, hepatocyte clearance in several species, and rat pharmacokinetics. As shown in Table 2, primary amine **8a** showed some CYP3A4 inhibition; while the secondary amine **8b** had less CYP3A4 inhibition. Other amines **8c**, **8f**, **14a-14d** and amidine **20** also showed more or less CYP3A4 liability when the compounds were preincubated. Compared to primary and secondary amine analogues **8a-8c**, tertiary amine such as **8f** showed higher hepatocyte clearance across species and no plasma exposure in rats. The pyrazine analogue **8m** displayed low hepatocyte clearance but poor pharmacokinetics in rats, presumably due to its high polarity. Compound **12**, with α,α-dimethyl groups, did not show improvement of hepatocyte clearance compared to compound **8b**. Cyclic secondary amine analogues **14a-14d** displayed reasonably good plasma exposure (AUC_{6h}) and brain penetration (B/P), consistent with their robust Aβ lowering in mice.

On the basis of overall profiles, analogue **8b** was thus selected for further studies. In a pharmacokinetic study in rats, **8b** showed neither N-demethylation nor β-elimination of the amine. It showed weak activity in the IonWorks assay for hERG activity: 1% inhibition at 1 μM and 21% inhibition at 10 μM.[21] Compound **8b** demonstrated robust in vivo potency in a time course study in mice, lowering Aβ in plasma by -93% (6h), -35% (12h), and +50% (24h) after oral administration of a dose of 30 mg/kg. In brain, Aβ levels were also reduced by -73% (6h), -66% (12h) and -37% (24h), indicating good brain penetration.

In summary, we have designed a series of β-aminosulfone analogues to improve the aqueous solubility of SCH 900229. The syntheses are straightforward and efficient from the readily available SCH 900229 intermediates. All the analogues showed robust in vitro potency and selected compounds also demonstrated good to excellent in vivo efficacy. Acceptable CYP inhibition, hepatocyte clearance and pharmacokinetic profiles were identified with selected compounds. As expected, the β-aminosulfone analogues have higher aqueous solubility, probably due to salt formation. The design strategy described here may well find applications in other medicinal chemistry optimization programs.

Acknowledgments

The authors would like to Dr. Jennifer Albaneze-Walker for providing a large quantity of key intermediate **2** and Dr. Malcolm MacCoss for his support of the GSI backup program.

Abbreviations

AD, Alzheimer's disease; Aβ, β-amyloid; PS1, presenilin-1; PS2, presenilin-2; APP, amyloid precursor protein; GSI, γ-secretase inhibitor; SAR, structure-activity relationship, ADME, absorption, distribution, metabolism, and excretion; AUC, area under curve; mCPBA, *m*-chloroperbenzoic acid.

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