Difluoro Ketone Peptidomimetics Suggest a Large S1 Pocket for Alzheimer's γ-Secretase: Implications for Inhibitor Design†

Chad L. Moore,‡ Dartha D. Leatherwood,‡ Thekla S. Diehl,§ Dennis J. Selkoe,§ and Michael S. Wolfe*,‡,§

Department of Pharmaceutical Sciences, University of Tennessee, Memphis, Tennessee 38163, and Center for Neurologic Diseases, Brigham and Women's Hospital and Harvard Medical School, Boston, Massachusetts 02115

Received March 3, 2000

The final step in the generation of the amyloid-β protein (Aβ), implicated in the etiology of Alzheimer's disease, is proteolysis within the transmembrane region of the amyloid precursor protein (APP) by γ-secretase. Although considered an important target for therapeutic design, γ-secretase has been neither well-characterized nor definitively identified. Previous studies in our laboratory using substrate-based difluoro ketone and difluoro alcohol transition-state analogue inhibitors suggest that γ-secretase is an aspartyl protease with loose sequence specificity. To further characterize the active site of γ-secretase, we prepared a series of difluoro ketone peptide analogues with varying steric bulkiness in the P1 position and tested the ability of these compounds to inhibit Aβ production in APP-transfected cells. Incorporation of bulky, aliphatic P1 side chains, such as *sec*-butyl or cyclohexylmethyl, led to increased γ-secretase inhibitory potency, suggesting a large S1 pocket to accommodate these substituents and providing further evidence for loose sequence specificity. The cyclohexylmethyl P1 substituent allowed N-terminal truncation to a low-molecular-weight compound (<600 Da) that effectively blocked Aβ production ($IC_{50} \sim 5\ \mu M$). This finding suggests that optimal S1 binding may allow the development of potent inhibitors with ideal pharmaceutical properties. Moreover, a difluoro alcohol analogue with a cyclohexylmethyl P1 substituent was equipotent with its difluoro ketone counterpart, providing strong evidence that γ-secretase is an aspartyl protease. All new analogues inhibited total Aβ and $Aβ_{42}$ production with the same rank order of potency and increased $Aβ_{42}$ production at low concentrations, providing further evidence for distinct γ-secretases that are nevertheless closely similar with respect to active site topology and mechanism.

Introduction

Advances in the understanding of the early biochemical events that lead to Alzheimer's disease (AD) offer the hope of identifying important new targets for drug development.[1] Genetic, biochemical, cellular, and histological studies all point to the 4-kDa amyloid-β protein (Aβ), the major protein component of the characteristic amyloid plaques that litter the AD brain, as a key initiator of the disease process. The highly hydrophobic Aβ can nucleate and form fibrils[2] that are neurotoxic[3,4] and are capable of activating inflammatory processes in the brain.[5,6] Moreover, various genetic factors known to cause or increase the chances of developing AD also affect the formation of Aβ and/or its cerebral deposition.[7]

Among AD-causing genetic mutations are those in the amyloid precursor protein (APP), an integral membrane protein of unknown function that is proteolytically processed to Aβ (Figure 1). Cleavage of APP just outside the single transmembrane region by α- and β-secretases forms soluble versions of APP (α- and β-APP_s) that display neurotrophic activities[8] and membrane-associated 83- and 99-amino acid C-terminal fragments of APP (C83 and C99), respectively. γ-Secretase then

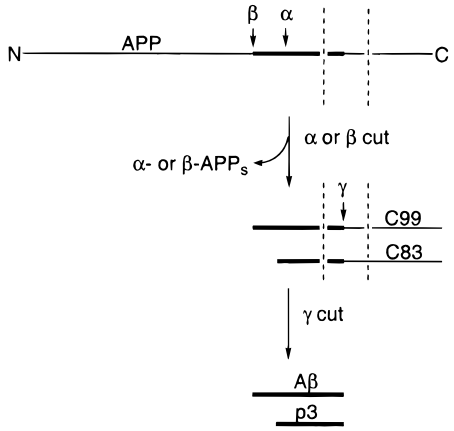


Figure 1. Proteolytic processing of the amyloid-β precursor protein (APP). Dotted lines represent the lipid bilayer.

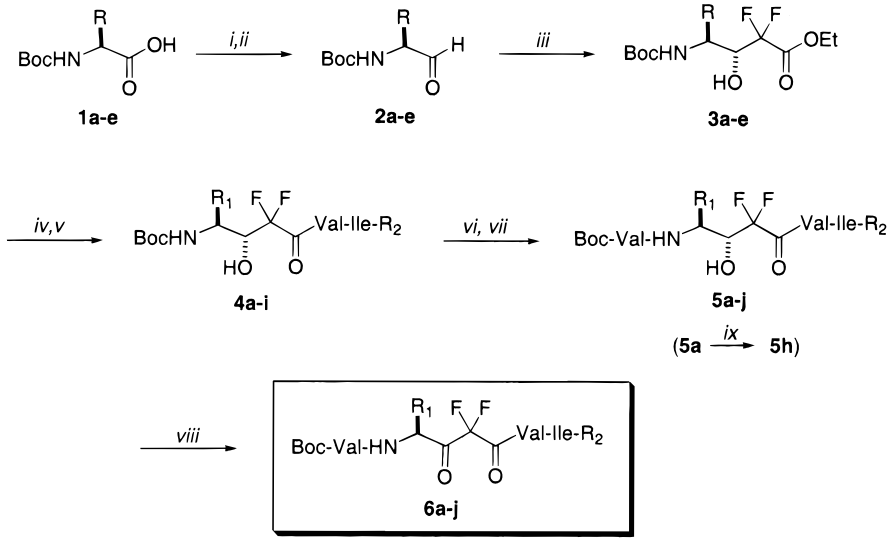
cleaves these fragments in the middle of the transmembrane region to form Aβ (from C99) and p3, a 3-kDa N-terminally truncated Aβ peptide (from C83).[9] The cleavage of γ-secretase is heterogeneous but principally leads to 40- and 42-amino acid peptides, $Aβ_{40}$ and $Aβ_{42}$, in roughly 9:1 proportions. Importantly, all genetic mutations that lead to autosomal dominant familial AD (FAD) lead to increased $Aβ_{42}$ formation.[10,11] Since this longer, more hydrophobic peptide is considerably more prone to nucleation and fibril formation than $Aβ_{40}$ (ref 2) and is the primary Aβ variant of early, diffuse

† This work was supported by NIH Grants NS 37537 (to M.S.W.) and AG 12749 (to D.J.S.).

* To whom correspondence should be addressed. Tel: (617) 525-5511. Fax: (617) 525-5252. E-mail: wolfe@cnd.bwh.harvard.edu.
‡ University of Tennessee.
§ Harvard Medical School.

10.1021/jm000100f CCC: $19.00 © 2000 American Chemical Society
Published on Web 08/22/2000

Scheme 1[a]



[a] (i) MeNHOMe-HCl, EDC, HOBT, *i*-Pr$_2$NEt; (ii) LiAlH$_4$; (iii) Zn, BrF$_2$CCO$_2$Et; (iv) LiOH, MeCN; (v) HATU, *i*-Pr$_2$NEt, DMF, NH$_2$-Val-Ile-R$_2$; (vi) TFA/CH$_2$Cl$_2$; (vii) Boc-Val, EDC, HOBT, *i*-Pr$_2$NEt; (viii) Dess−Martin periodinane; (ix) MeNH$_2$.

presymptomatic amyloid plaques, Aβ_{42} is considered a prime suspect in the etiology of AD.

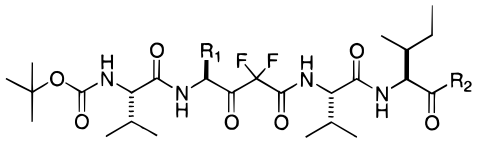
γ-Secretase has not been definitively identified nor well-characterized, and even the issue of whether the formation of Aβ_{40} and Aβ_{42} is due to different γ-secretases is unclear. Inhibitor studies by us and others demonstrated that while Aβ_{40} and Aβ_{42} formation is pharmacologically distinct,[12,13] panels of γ-secretase inhibitors show the same rank order of potency for blocking the formation of the two peptides,[14,15] suggesting that the active site topologies for the responsible enzymes are similar, if not identical. We have shown that substrate-based difluoro ketones and difluoro alcohols block γ-secretase activity in cell-based assays.[14] Because difluoro alcohols mimic the *gem*-diol transition state of aspartyl proteases and are only known to interact with aspartyl proteases,[16−18] these results are consistent with an aspartyl protease mechanism for γ-secretase.[14] Moreover, modeling[14] and mutagenesis studies[19] support a helical conformation of the APP transmembrane domain for the initial interaction with γ-secretase (i.e., a conformation consistent with intramembranous proteolysis). Furthermore, we recently demonstrated that two transmembrane aspartates in presenilin-1 are required for γ-secretase activity.[20] Mutations in the presenilins are responsible for most cases of FAD, and presenilins are known to mediate γ-secretase activity.[21] Thus, presenilins are prime candidates for the elusive γ-secretases.[22]

The difluoro ketone peptidomimetics developed in our laboratory also provided evidence for loose sequence specificity for γ-secretases,[14] corroborating site-directed mutagenesis studies on APP near the γ-secretase cleavage sites.[19,23−25] To further characterize the active site of γ-secretase, we prepared a series of difluoro ketone analogues with hydrophobic substituents varying in steric bulkiness in the P1 position. Here we show that the more sterically demanding substituents increased γ-secretase inhibitory potency, suggesting a large, complementary S1 pocket in the protease. The inhibitory effects of these compounds provide further support for loose sequence specificity and an aspartyl protease mechanism for γ-secretase. The results also suggest that pharmacologically distinct γ-secretases are responsible for producing Aβ_{40} and Aβ_{42} and that these proteases nevertheless possess closely similar active sites.

Chemistry

We[26] and others[16,18] have reported the synthesis of key intermediates [*N*-*tert*-butoxycarbonyl]-4-amino-2,2-difluoro-3-hydroxybutanoate esters of general structure **3** (Scheme 1). Originally, we had formed the immediate precursor, Boc amino aldehyde **2**, via Swern oxidation of Boc-protected amino alcohols. We now find it more convenient to generate these amino aldehydes from Boc-protected amino acids via reduction of the *N*,*O*-dimethylhydroxamides (Weinreb amides) according to a published procedure.[27] Formation of the Weinreb amides was accomplished by coupling *N*,*O*-dimethylhydroxylamine to Boc-protected amino acids via EDC and HOBT in 80−90% yields. These efficient couplings also produce small proportions (<10%) of an undesired *N*,*N*-dimethylamide (formed from dimethylamine, an impurity in the *N*,*O*-dimethylhydroxylamine), but this side product can be easily removed by column chromatography. LAH reduction at ambient temperature for 20 min provided the protected α-amino aldehydes in good yields (70−85%) and, as originally reported by Fehrentz et al.,[27] with little or no compromise of the stereocenter. Treatment of these aldehydes with ethyl bromodifluoroacetate and activated zinc (Reformatsky reaction) in refluxing THF leads to stereoselective addition due to chelation control.[16] Each aldehyde except one was converted to a single diastereomer (3*R*,4*S*) of difluoro alcohol **3**. The alanine-derived aldehyde **2a**, with the smaller methyl substituent, gives a 4:1 mixture of diastereomers, with the major diastereomer likewise being 3*R*,4*S*.[26]

Toward target compounds **6a**−**g** and **6j** (Figure 2), the pseudo dipeptide building blocks **3a**−**e** were saponified with 1.05 equiv of NaOH or LiOH in aqueous acetonitrile to provide the free acids, and these were immediately coupled to dipeptidyl esters Val-Ile-OR with HATU and diisopropylethylamine in DMF. Removal of the N-terminal Boc protecting group with 50% TFA in



Compound	R_1	R_2	IC_{50} (μM)
6a	Me	OMe	15
6b	i-Pr	OMe	9
6c	Ph	OMe	9
6d	s-Bu	OMe	6
6e	CHM[a]	OMe	4
6f	Me	OEt	9
6g	Me	OBn	4
6h	Me	NHMe	<100
6i	Me	NMe_2	25
6j	CHM[a]	OBn	6

[a]CHM: cyclohexylmethyl

Figure 2. Structure of target compounds and their IC_{50} values for inhibition of Aβ production in APP-transfected Chinese hamster ovary (CHO) cells. IC_{50} values were determined from results depicted graphically in Figures 3 and 5.

CH_2Cl_2 was followed by coupling with Boc-L-valine using EDC and HOBT. Oxidation of the penultimate difluoro alcohols with Dess−Martin periodinane reagent proceeded in essentially quantitative yields providing the final compounds. Toward *N*-methylamide **6i**, methyl ester **5a** was treated with saturated methylamine in CH_2Cl_2, with the product **5i** precipitating from the mixture as the reaction proceeds. The precipitate was isolated by filtration and oxidized to **6i** as described above. Toward *N*,*N*-dimethylamide **6j**, Boc-Val-Ile-OMe was saponified and coupled to dimethylamine using HATU and diisopropylethylamine in DMF to afford Boc-Val-Ile-NMe_2 in 70% yield. This dipeptide was deprotected with TFA and carried on to the target compound as described above.

Biological Results and Discussion

Effects of P1 Alterations in Difluoro Ketone Peptidomimetics on γ-Secretase Activity. New compounds were tested for their ability to block total Aβ and Aβ_{42} production in cell culture. Chinese hamster ovary (CHO) cells stably transfected with human APP (cell line 7W) were treated with each compound for 4 h, and media were removed for analysis using a double-antibody (sandwich) ELISA as previously described.[14,20] Briefly, stock concentrations of peptide analogue in DMSO were added to media to reach the final concentrations with 1% DMSO. Positive controls contained 1% DMSO only. After 4 h, the medium was removed and microfuged at 6000 rpm for 5 min to pellet loose cells, and the supernatant was stored at −80 °C until the total Aβ- and Aβ_{42}-specific ELISAs were performed. All of the compounds **6a**−**e** effectively blocked Aβ production (Figure 3). Although **6a** is a poor inhibitor at 10 μM, this compound blocks Aβ production ≥65% at 25 μM (data not shown and ref. 14). Compound **6b** inhibited Aβ production with the same potency during a 24-h treatment (data not shown), suggesting that this compound and its P1 variants are sufficiently stable during the standard 4-h treatments used in these studies. Although the potencies of the compounds were somewhat variable between experiments, the rank order of potency within a given experiment consistently showed **6e** > **6d** > **6c** ≥ **6b** > **6a**. Thus, the preference for P1 substituent was cyclohexylmethyl > *sec*-butyl > benzyl ≥ isopropyl > methyl, suggesting a relatively large S1 pocket for γ-secretases. This result was surprising, as we expected to determine the steric boundaries of the S1 pocket with this set of compounds.

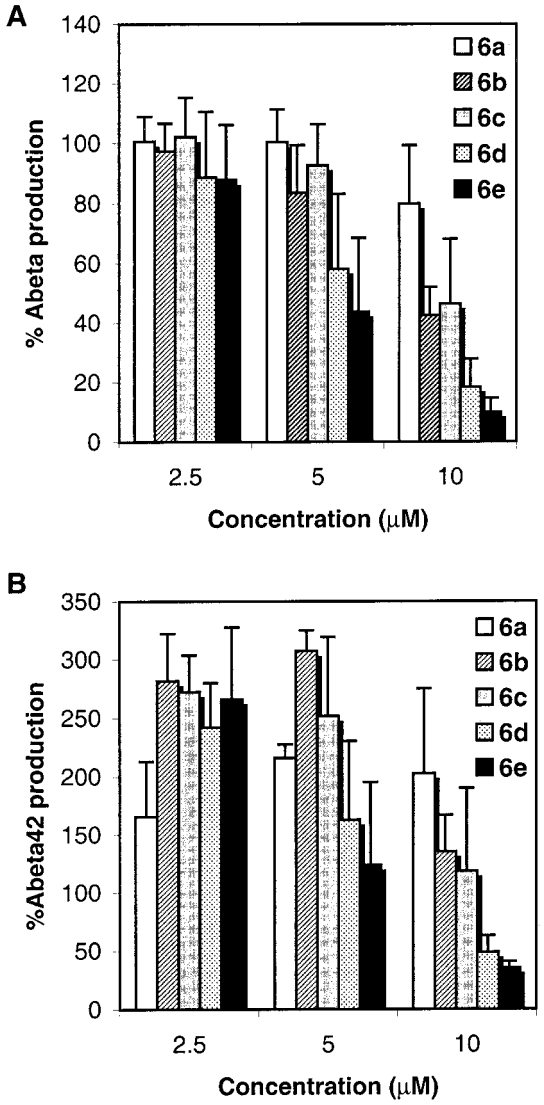


Figure 3. Effects of difluoro ketone peptidomimetics **6a**−**e** on Aβ production as determined by sandwich ELISA: (A) effects of compounds on total Aβ production after 4 h of treatment of CHO cells stably transfected with the 751-amino acid splice variant of human APP (cell line CHO 7W); (B) effects of compounds on Aβ_{42} production in the same cell line. Error bars denote standard deviations (n = 4). For further details concerning cell treatments and ELISAs, see ref 14.

The most active compound in the set, compound **6e**, was further tested for effects on other APP metabolites. After a 4-h treatment with various concentrations of **6e**, 7W cells were harvested and lysed, and the lysate was subjected to immunoprecipitation with antibodies specific to the APP C-terminus. γ-Secretase substrates C99 and C83 increased dramatically (Figure 4) and with a similar dose−response effect as seen for inhibition of Aβ production, indicating that **6e** inhibits Aβ production at the level of γ-secretase. Similar analysis of C99 and C83 levels 4 h after replacement with media alone showed that the inhibition of γ-secretase by this compound is reversible. Radiolabeling 7W cells with [^{35}S]-Met in the presence of **6e** and immunoprecipitating with

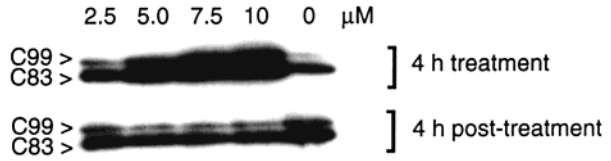


Figure 4. Effects of compound **6e** on γ-secretase substrates C83 and C99. CHO 7W cells were treated with the indicated concentrations of **6e** for 4 h. Medium was removed from an identical set of cells treated in parallel and replaced with compound-free medium for an additional 4 h. The cells were lysed and subjected to immunoprecipitation with antibody C7 to the APP C-terminus. Precipitated proteins were resolved by SDS−PAGE, transferred to poly(vinylidene difluoride), and immunoblotted with monoclonal antibody 13G8 to the APP C-terminus. For further details on the antibodies, immunoprecipitation, and blotting protocols, see ref 20.

specific antibodies demonstrated that α- and β-APP$_s$ levels were unaltered by this compound (data not shown), indicating that α- and β-secretase activities were not inhibited. Thus, despite the recent cloning and identification of β-secretase as a membrane-tethered aspartyl protease,[28−31] the difluoro ketone analogues reported here are apparently selective for γ-secretase.

Interestingly, we also found that a truncated form of **6e**, lacking the N-terminal valine (oxidized **4e**), retained full potency toward inhibiting Aβ production ($IC_{50} \sim 5$ μM). We had previously shown that when R_1 = Me, such a truncation yields an inactive compound.[26] Apparently, installing an optimal R_1 substituent (cyclohexylmethyl) allows removal of the P2 residue. This finding has implications for the design of therapeutic agents that block γ-secretase, since this truncation brings the molecular weight down below 600 Da. Installation of the cyclohexylmethyl group in P1 also led to the identification of a difluoro alcohol (the immediate synthetic precursor to **6e**) equipotent to its difluoro ketone counterpart toward inhibiting total Aβ production. This difluoro alcohol was an order of magnitude more potent than other difluoro alcohols we have previously reported.[14] Since difluoro alcohols are only known to inhibit aspartyl proteases,[16−18] this provides strong evidence that γ-secretase is an aspartyl protease.

Because of the P1 differences in the γ-secretase cleavage events leading to Aβ$_{40}$ and Aβ$_{42}$ (Val and Ala, respectively), we expected to find active site differences between the S1 pockets of the enzymes. As we have observed before with related peptidomimetics,[14] the rank order of potencies for inhibition of Aβ$_{40}$ and Aβ$_{42}$ production were essentially the same (compare panels A and B in Figure 3, especially at 10 μM), suggesting that the active site topologies for γ(40)- and γ(42)-secretases are closely similar. (Roughly 90% of total Aβ is Aβ$_{40}$; therefore, reductions in total Aβ reflect reductions in Aβ$_{40}$.) Nevertheless, these related γ-secretase activities are clearly distinct, since Aβ$_{40}$ production is more sensitive to γ-secretase inhibitors than is Aβ$_{42}$ production. Moreover, low concentrations of γ-secretase inhibitors elicit profound increases in Aβ$_{42}$ production. All γ-secretase inhibitors reported to date show these effects, including those in this study (see Figures 3 and 5). Because the ability of compounds to increase Aβ$_{42}$ production closely correlates with their inhibitory potency against γ-secretase activity, we hypothesize that partial inhibition of Aβ$_{40}$ production increases the availability of substrate C99 for Aβ$_{42}$ production. That

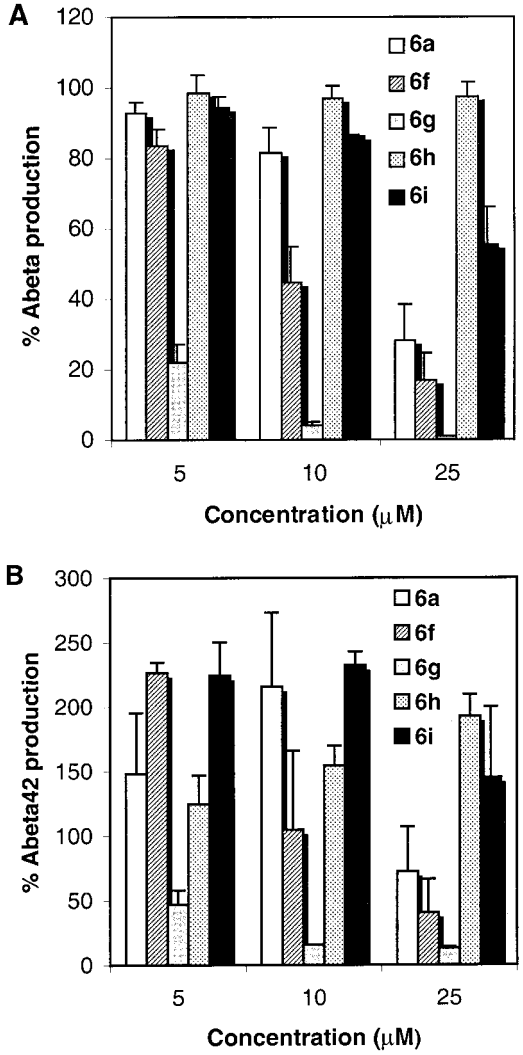


Figure 5. Effects of difluoro ketone peptidomimetics **6a,f−i** on Aβ production as determined by sandwich ELISA: (A) effects of compounds on total Aβ production after 4 h of treatment of CHO 7W cells; (B) effects of compounds on Aβ$_{42}$ production in the same cell line. Error bars denote standard deviations (n = 4).

is, γ(42)-secretase has a higher K_m for substrate than γ(40)-secretase, a conclusion supported by a recent study with peptide aldehyde γ-secretase inhibitors.[32] Another recent study demonstrated that pharmacologically induced increases in Aβ$_{42}$ production can be separated from γ-secretase activity,[15] although the reverse observation, inhibiting γ-secretase activity without enhancing Aβ$_{42}$ production, has not yet been reported. Apparently, increasing C99, either by partially blocking γ(40)-secretase activity or by stabilizing C99 against other catabolic processes, can augment Aβ$_{42}$ production.

Effects of C-Terminal Variations of Difluoro Ketone Peptidomimetics on γ-Secretase Activity. Since methyl esters of peptidomimetics can be rapidly metabolized in cells, we considered C-terminal alterations that might enhance stability in our cell-based assay. We reasoned that increasing steric bulk might hinder cleavage by intracellular esterases, so ethyl and benzyl esters **6f**,**g** were synthesized. Similarly, because amides are more difficult to hydrolyze than esters, *N*-methylamide **6h** was made as well. The dimethylamide **6i**, with the combination of the more stable amide

bond and more sterically demanding tertiary amide, was expected to be virtually resistant to hydrolysis in culture.

Indeed for the esters, increasing steric bulk enhanced activity, with benzyl ester **6g** being 2−3-fold more active than the parent methyl ester **6a**; ethyl ester **6f** possessed intermediate potency (Figures 2 and 5). However, the simple replacement of amide for ester (**6h** vs **6a**) virtually abolished activity, a surprising result since the amide should be more metabolically stable and should be a better mimic of the native substrate. Adding another methyl group to the amide (compound **6i**) regained activity, although this compound was not quite equipotent to the parent methyl ester **6a**. Again, as noted above, the rank order of potency for inhibiting Aβ_{40} and Aβ_{42} production was essentially the same for these C-terminal variants, and the more potent inhibitors increased Aβ_{42} production at subinhibitory concentrations.

Previous studies in our laboratory showed that extension of compounds such as **6a** into the P4′ position with amino esters (Val, Ile, Phe) is well-tolerated.[14] Thus, despite the inactivity of **6h**, a secondary amide after P3′ is acceptable, perhaps as long as a large substituent is added. Addressing the difference in cellular uptake and metabolism would require radiolabeling a series of compounds and careful, quantitative analysis of cell extracts. Although differences in cell uptake and metabolism may explain the observed effects on Aβ inhibition, it is nevertheless clear that γ-secretases can tolerate the addition of bulky substituents after P3′, whether the extension is through an ester or an amide bond.

Since cyclohexylmethyl in the P1 position and benzyl ester at the C-terminus provided the highest potency toward inhibiting γ-secretase activity in our series of difluoro ketone peptidomimetics, we combined these two favorable features into a single compound (**6j**) in the hope that it would prove more active than either compound (**6e** or **6g**) with only one of these substructures. However, this compound displayed potency comparable to **6e**,**g** toward inhibiting total Aβ and Aβ_{42} (data not shown). Apparently, the presence of the cyclohexylmethyl substituent in P1 alters the structure−activity relationship (SAR) vis-á-vis a methyl substituent in P1. This is not an uncommon observation with peptidomimetics, and we have observed this before with our difluoro ketone peptidomimetics. Specifically, a D-Val as P2 reduces activity but is nevertheless tolerated when the P1 substituent is methyl. In contrast, incorporation of D-Val as P2 when P1 contains an isopropyl substituent leads to a compound completely incapable of blocking Aβ production.[14] We should add here that the goal of our project is primarily to use the difluoro ketone peptidomimetics to understand the active sites of the γ-secretases. If our goal were primarily to develop highly potent inhibitors as drug candidates, then clearly a combinatorial approach would be appropriate, especially given the interdependency of different positions of the compounds with respect to SAR.

Summary and Conclusions

Modification of substrate-based difluoro ketone transition-state mimics for γ-secretases led to compounds with enhanced potencies compared to the parent compound **6a**. These inhibitors are useful probes for the γ-secretases, providing evidence for an S1 pocket large enough to accommodate the sterically bulky cyclohexylmethyl substituent. These results largely corroborate the recent report of Hagaki et al., in which bulky P1 residues (Phe, Leu, *t*-BuAla) within short, hydrophobic peptide aldehydes had the best γ-secretase inhibitory activity.[33] However, P1 Val and Ile residues were the least potent in the peptide aldehyde series, whereas in the difluoro ketones reported here, P1 Val and especially Ile substituents are well-tolerated. These differences between the aldehyde and difluoro ketone peptide analogues may be due to different binding orientations or even different sites of action. We have tested one of the difluoro ketone analogues (**6b**) in a cell-free microsomal system and found that it also inhibits Aβ production under these conditions,[34] consistent with the expected direct interaction between these transition-state mimics and γ-secretases. Our studies with these new difluoro ketone peptidomimetics also further support the idea that separate γ-secretases are responsible for the generation of Aβ_{40} and Aβ_{42} but that the active sites for these proteases are closely similar.

Recently, we reported that two conserved transmembrane aspartates in presenilins 1 and 2 (PS1 and PS2) are required for γ-secretase activity, suggesting that the presenilins are the long-sought γ-secretases, novel intramembrane-cleaving aspartyl proteases.[20,35] Knockout of the PS1 gene or mutation of either PS1 transmembrane aspartate lowers total Aβ and Aβ_{42} to similar degrees.[20,21] The parallel drop in total Aβ and Aβ_{42} by knockout of PS1 or expression of aspartate-mutant PS1 is inconsistent with the notion that PS1 is γ(40)-secretase and PS2 is γ(42)-secretase or vice versa. The different activities may be due instead to differences in posttranslational modifications, protein binding partners, subcellular localizations, or stable conformations of a given presenilin. Finally, we have most recently found that an affinity reagent based on the difluoro alcohol of **6d** binds directly and specifically to presenilins, strongly suggesting that presenilins comprise the active site of γ-secretases.[36] Thus, the transition-state analogue peptidomimetics reported here have proven to be very useful probes for γ-secretase, aiding in the characterization and identification of this elusive, intriguing, and medically important protease.

Experimental Section

Boc-protected L-cyclohexylalanine was purchased from NovaBiochem (San Diego, CA). HATU uronium coupling reagent was purchased from Perceptive Biosystems (Warrington, England). Dess−Martin periodinane was either synthesized[37] or purchased from Omega Chemicals (Quebec, Canada). All other reagents and solvents were purchased from Sigma-Aldrich (St. Louis, MO; Milwaukee, WI). ^{1}H and ^{13}C NMR (300 and 74.5 MHz, respectively) were performed on a Bruker ARX-300 FT-NMR spectrometer, and mass spectra were performed on a Bruker Esquire-LC with electrospray ionization source unless otherwise noted. Optical rotations were determined on a Rudolph Autopol III polarimeter, and uncorrected melting points were obtained using a Thomas-Hoover melting point apparatus. Column chromatography was performed with 70−230 mesh silica gel (Aldrich Chemical). Elemental analysis was performed by Desert Analytics (Tucson, AZ).

General Procedure Toward *N*-Boc-L-amino Aldehydes. To a 0 °C solution of *N*-Boc-protected l-amino acid, triethylamine (TEA; 1.1 equiv), and HATU (1.1 equiv) in dry CH_2Cl_2 (0.5 M amino acid) were added 1.2 equiv of *N*,*O*-dimethylhydroxylamine hydrochloride and 1.2 equiv of TEA, whereupon the reaction was allowed to warm to ambient temperature. After 3 h, the reaction was partitioned between CH_2Cl_2 and 1 N HCl. The organic layer was washed 3× with 1 N HCl, 3× with saturated aqueous $NaHCO_3$, and 3× with brine. The organic layer was dried over Na_2SO_4, filtered, and concentrated to isolate the Weinreb amide. If necessary, chromatography with 2:1 hexane:ethyl acetate was performed to remove any dimethyl amide impurity. To a 0.05 M solution of Weinreb amide in ethyl ether was added 1.25 equiv of LAH. After 20 min, 1.5 equiv of 1 M $KHSO_4$ was added, and the aqueous layer was extracted with ether. The extracts were combined and washed 3× with 1 N HCl, 3× with saturated aqueous $NaHCO_3$, and 3× with brine. The organic layer was dried over $MgSO_4$, and concentrated to give *N*-Boc-protected L-amino aldehyde. The aldehyde was used immediately in the next step without further purification.

(*S*)-(*N*-*tert*-Butoxycarbonyl)-2-aminopropional (2a): 72%; $[\alpha]_D = +33.2°$ ($c = 1$ in CH_2Cl_2) (lit. +36.7°, $c = 1$ in CH_2Cl_2);[27] ^{1}H NMR ($CDCl_3$) δ 1.34 (3H, d $J = 7$ Hz), 1.46 (9H, s), 4.23, (1H, dd $J = 7$ Hz), 5.12 (1H, br), 9.57 (1H, s).

(2*S*)-(*N*-*tert*-Butoxycarbonyl)-2-amino-3-methylbutanal (2b): oil; 70%; $[\alpha]_D = +80.4°$ ($c = 1$ in CH_2Cl_2) (lit. +82.1°, $c = 1$ in CH_2Cl_2);[27] ^{1}H NMR ($CDCl_3$) δ 0.95 (3H, d $J = 7$ Hz), 1.03 (3H, d $J = 10$ Hz), 1.45 (9H, s), 2.27 (1H, m), 4.23 (1H, br m), 5.29 (1H, br), 9.64 (1H, s).

(2*S*)-(*N*-*tert*-Butoxycarbonyl)-2-amino-3-phenylpropional (2c): mp 82−82 °C (lit. 86 °C); 87%; $[\alpha]_D = +41.6°$ ($c = 1.1$ CH_2Cl_2) (lit. +40.4°, $c = 1.0$ CH_2Cl_2);[27] ^{1}H NMR ($CDCl_3$) δ 1.43 (9H, s), 3.11 (2H, m), 4.42 (1H, m), 5.02 (1H, br), 7.27 (5H, m), 9.63 (1H, s).

(2*S*,3*S*)-(*N*-*tert*-Butoxycarbonyl)-2-amino-3(*S*)-methylpentanal (2d): oil; 74%; $[\alpha]_D = +85.0°$ ($c = 1$ in CH_2Cl_2) (lit. +92.8°, $c = 1$ in CH_2Cl_2);[27] ^{1}H NMR ($CDCl_3$) δ 0.92 (3H, d $J = 6$ Hz), 0.98 (3H, app. t $J = 7$ Hz), 1.17−1.29 (2H, m), 1.45 (9H, s), 2.02 (1H, m), 4.25 (1H, m), 5.39 (1H, br), 9.63 (1H, s).

(2*S*)-(*N*-*tert*-Butoxycarbonyl)-2-amino-3-cyclohexylpropional (2e): oil; 82%; $[\alpha]_D = -23°$ ($c = 0.5$ in CH_2Cl_2) (lit. −23.9°, $c = 0.44$ in $CHCl_3$);[38] ^{1}H NMR ($CDCl_3$) δ 0.81−1.30 (6H, br m), 1.45 (9H, s), 1.59−1.87 (7H, br m), 4.26 (1H, br), 5.00 (1H, br), 9.58 (1H, s).

General Procedure Toward Difluoro Alcohols 3. A solution of Boc-amino aldehyde and ethyl bromodifluoroacetate (3 equiv) was added dropwise to a suspension of zinc dust (3 equiv) in dry THF (0.5 mM final concentration of aldehyde). After complete addition, the reaction was brought to reflux for 30 min. The heat source was then removed, and the reaction was allowed to cool to ambient temperature. The mixture was partitioned between 1 M $KHSO_4$ and CH_2Cl_2, and the organic layer was dried over Na_2SO_4, filtered, and concentrated. The product was purified by column chromatography with 20% ethyl acetate in hexanes.

Ethyl 4(*S*)-[(*N*-*tert*-butoxycarbonyl)amino]-2,2-difluoro-3(*R* and *S*)-hydroxypentanoate (3a): yellow-brown oil; 62% (as a 4:1 mixture of diastereomers); ^{1}H NMR spectra was identical to that previously reported by our laboratory.[26]

Ethyl 5-methyl-4(*S*)-[(*N*-*tert*-butoxycarbonyl)amino]-2,2-difluoro-3(*R*)-hydroxyhexanoate (3b): yellow-brown oil; 67%; ^{1}H NMR ($CDCl_3$) δ 0.97 (6H, dd $J = 6.8$ and 6.8 Hz), 1.36 (3H, t $J = 7$ Hz), 1.43 (9H, s), 1.93−2.13 (1H, m), 3.54 (1H, br), 4.20 (2H, m), 4.35 (2H, q $J = 7$ Hz), 5.14 (1H, br d).

Ethyl 5-phenyl-4(*S*)-[(*N*-*tert*-butoxycarbonyl)amino]-2,2-difluoro-3(*R*)-hydroxypentanoate (3c): yellow-brown oil; 87%; crystallized to a white solid from ethyl acetate and hexanes; mp 134−135 °C (lit. 137−138.6 °C[16]); ^{1}H NMR ($CDCl_3$) δ 1.33 (3H, t $J = 7$ Hz), 1.40 (9H, s), 2.90−3.18 (2H, br), 3.89−4.24 (3H, br), 4.32 (2H, q $J = 7$ Hz), 4.78−4.93 (1H, br), 7.26 (5H, m); MS *m*/*z* 396 ($M^+ + Na^+$). Anal. ($C_{18}H_{25}F_2NO_4$) C,H,N.

Ethyl 5(*S*)-methyl-4(*S*)-[(*N*-*tert*-butoxycarbonyl)amino]-2,2-difluoro-3(*R*)-hydroxyheptanoate (3d): yellow-brown oil; 56%; ^{1}H NMR ($CDCl_3$) δ 0.89 (3H, t $J = 7.3$ Hz), 0.94 (3H, d $J = 6.8$ Hz), 1.06−1.19 (1H, m), 1.34 (3H, t $J = 7$ Hz), 1.42 (9H, s), 1.45−1.58 (1H, m), 1.76−1.93 (1H, m), 3.51−3.65 (1H, br), 4.14−4.25 (1H, br), 4.33 (3H, br q $J = 7$ Hz), 4.78−5.01 (1H, br).

Ethyl 5-cyclohexyl-4(*S*)-[(*N*-*tert*-butoxycarbonyl)amino]-2,2-difluoro-3(*R*)-hydroxypentanoate (3e): yellow-brown oil; 64%; crystallized to a white solid from ethyl acetate and hexanes; mp 110.5−111.5 °C (lit. 112−114 °C[16]); ^{1}H NMR ($CDCl_3$) δ 0.81−1.30 (7H, br m), 1.36 (3H, t $J = 7$ Hz), 1.44 (9H, s), 1.59−1.87 (6H, br m), 3.84−4.07 (3H, br), 4.33 (1H, q $J = 7$ Hz), 4.35 (1H, q $J = 7$ Hz), 4.62−4.75 (1H, br); MS *m*/*z* 402 ($M^+ + Na^+$). Anal. ($C_{18}H_{31}F_2NO_4$) C,H,N.

General Procedure Toward Compounds 4. Hydrolysis of **3** to the carboxylic acid was accomplished with 1.05 equiv of 0.25 N NaOH or LiOH in an equal volume of AcN for 2 h. The mixture was extracted with ethyl acetate to remove unhydrolyzed starting material. The aqueous phase was then acidified to pH 2 with 1 N HCl and extracted with 4× with an equal volume of ethyl acetate. The organic layer was dried over Na_2SO_4 and concentrated to afforded the carboxylic acid, which was immediately carried on to the next step. To a 0 °C solution of this product and the appropriate C-terminally protected peptide (1 equiv) in anhydrous DMF under N_2 was added HATU (1.1 equiv) and diisopropylethylamine (DIPEA; 3 equiv). After overnight reaction, the mixture was taken up in EtOAc and washed 3× with 1 N HCl, 3× with saturated $NaHCO_3$ solution, and 3× with brine. The organic layer was dried over Na_2SO_4, filtered, and concentrated. Column chromatography with EtOAc:hexanes 2:1 afforded pure samples which were recrystallized from ether/pentane whenever possible.

[[4(*S*)-[(*N*-*tert*-Butoxycarbonyl)amino]-2,2-difluoro-3(*R*)-hydroxypentanoyl]-L-valyl]-L-isoleucine methyl ester (4a): physical and spectroscopic data for this compound matched that from a previous report from our laboratory.[26]

[[4(*S*)-[(*N*-*tert*-Butoxycarbonyl)amino]-2,2-difluoro-5-methyl-3(*R*)-hydroxyhexanoyl]-L-valyl]-L-isoleucine methyl ester (4b): 67%; mp 128.5−130 °C; ^{1}H NMR ($CDCl_3$) δ 0.89 (6H, two d, both $J = 7$ Hz), 0.99 (14H, m), 1.44 (9H, s), 1.92 (1H, m), 2.11 (1H, m), 2.27 (1H, m), 3.39 (1H, br), 3.73 (3H, s), 3.96 (1H, br d), 4.25 (2H, br dd $J = 9$ and 8 Hz), 4.61 (1H, dd $J = 9$ and 8 Hz), 5.31 (1H, d $J = 9$ Hz), 6.77 (1H, d $J = 9$ Hz), 7.22 (1H, d $J = 9$ Hz). Anal. ($C_{24}H_{43}F_2N_3O_7$) C,H,N.

[[4(*S*)-[(*N*-*tert*-Butoxycarbonyl)amino]-2,2-difluoro-5-phenyl-3(*R*)-hydroxypentanoyl]-L-valyl]-L-isoleucine methyl ester (4c): mp 136−138 °C; 64%; ^{1}H NMR ($CDCl_3$) δ 0.93 (6H, m), 1.02 (6H, m), 1.40 (9H, s), 1.90 (1H, m), 2.38 (1H, m), 3.06 (2H, br), 3.76 (3H, s), 4.08 (1H, br), 4.18−4.40 (2H, br), 4.65 (1H, dd $J = 9$ and 8 Hz), 4.79 (1H, d $J = 8$ Hz), 4.95 (1H, br), 6.79 (1H, d $J = 7$ Hz), 6.93 (1H, br), 7.18−7.37 (5H, m); MS *m*/*z* 595 ($M^+ + Na^+$). Anal. ($C_{28}H_{43}F_2N_3O_7 \cdot 3/2H_2O$) C,H,N.

[[4(*S*)-[(*N*-*tert*-Butoxycarbonyl)amino]-2,2-difluoro-5(*S*)-methyl-3(*R*)-hydroxyheptanoyl]-L-valyl]-L-isoleucine methyl ester (4d): 64%; ^{1}H NMR ($CDCl_3$) δ 0.79−1.06 (20H, m), 1.45 (9H, s), 1.78−2.00 (2H, br), 2.25 (1H, m), 3.49 (1H, br), 3.73 (3H, s), 4.21−4.46 (2H, br), 4.59 (1H, dd $J = 5$ and 7 Hz), 5.41 (1H, d $J = 9$ Hz), 7.02 (1H, d $J = 8$ Hz), 7.35 (1H, d $J = 8$ Hz); MS *m*/*z* 560 ($M^+ + Na^+$).

[[4(*S*)-[(*N*-*tert*-Butoxycarbonyl)amino]-2,2-difluoro-5-cyclohexyl-3(*R*)-hydroxypentanoyl]-L-valyl]-L-isoleucine methyl ester (4e): 66%; ^{1}H NMR ($CDCl_3$) δ 0.93 (6H, m), 1.02 (6H, m), 1.23 (6H, br m), 1.46 (9H, s), 1.60−2.00 (8H, br), 2.27 (1H, m), 3.16 (1H, br), 3.75 (3H, s), 4.05 (1H, br), 4.27 (2H, br), 4.61 (1H, dd $J = 9$ and 5 Hz), 5.24 (1H, br d), 6.58 (1H, d $J = 8$ Hz), 7.18 (1H, d $J = 10$ Hz); MS *m*/*z* 600 ($M^+ + Na^+$). Anal. ($C_{28}H_{49}F_2N_3O_7 \cdot 1/2H_2O$) C,H,N.

[[4(*S*)-[(*N*-*tert*-Butoxycarbonyl)amino]-2,2-difluoro-3(*R*)-hydroxypentanoyl]-L-valyl]-L-isoleucine ethyl ester (4f): 40%; ^{1}H NMR ($CDCl_3$) δ 0.90 (6H, m), 1.00 (6H, m), 1.44 (9H, s), 1.90 (1H, m), 2.28 (1H, m), 4.04 (2H, m), 4.17 (2H, m), 4.38 (1H, m), 4.60 (1H, m), 5.15 (1H, br), 7.20 (1H, br), 7.51 (1H, br).

[[4(*S*)-[(*N*-*tert*-Butoxycarbonyl)amino]-2,2-difluoro-3(*R*)-hydroxypentanoyl]-L-valyl]-L-isoleucine benzyl ester (4g): 68%; ^{1}H NMR ($CDCl_3$) δ 0.86 (6H, m), 1.00 (6H, m), 1.30 (3H, d), 1.46 (9H, s), 2.30 (1H, br), 3.71 (1H, br), 4.02 (2H, m), 4.36 (1H, dd), 4.69 (1H, dd), 5.16 (3H, q), 7.09 (1H, br), 7.36 (5H, m), 7.46 (1H, br).

[[4(*S*)-[(*N*-*tert*-Butoxycarbonyl)amino]-2,2-difluoro-3(*R*)-hydroxypentanoyl]-L-valyl]-L-isoleucine dimethylamide (4i): 68%; ^{1}H NMR ($CDCl_3$) δ 0.89 (6H, m), 1.00 (6H, m), 1.36 (3H, d J = 6.7 Hz), 1.46 (9H, s), 1.67 (3H, br), 1.80 (1H, m), 2.06 (1H, s), 2.35 (1H, m), 2.97 (3H, s), 3.15 (3H, s), 4.04 (2H, m), 4.29 (1H, m), 4.87 (1H, dd J = 7.4 Hz), 5.21 (1H, d J = 8.7 Hz), 7.23 (1H, br), 7.35 (1H, br).

[[4(*S*)-[(*N*-*tert*-Butoxycarbonyl)amino]-2,2-difluoro-5-cyclohexyl-3(*R*)-hydroxypentanoyl]-L-valyl]-L-isoleucine benzyl ester (4j): mp 60−61 °C; 84%; ^{1}H NMR ($CDCl_3$) δ 0.80−0.92 (6H, m), 0.93−1.02 (6H, dd J = 6.6 and 6.6 Hz), 1.03−1.35 (11 H, m), 1.44 (9H,s), 1.92 (2 H, m), 2.22 (1H, m), 3.74 (1H, br), 4.04 (1H, dd J = 14 and 13 Hz), 4.24 (1H, dd J = 9 and 8 Hz), 4.64 (1H, dd J = 9 and 9 Hz), 5.11 (1H, d 12 Hz), 5.22 (1H, d 12 Hz), 6.53 (1H, d J = 8 Hz), 7.16 (1H, br), 7.35 (5H, m); MS m/z 677 ($M^+ + Na^+$). Anal. ($C_{34}H_{53}F_2N_3O_7$) C,H,N.

General Method Toward Compounds 5. Compounds of general structure **4** were deprotected using TFA/CH_2Cl_2 1:1 at ambient temperature for 1 h. The TFA/CH_2Cl_2 layer was evaporated, and the residue was partitioned between CH_2Cl_2 and saturated $NaHCO_3$ solution. The aqueous layer was extracted with additional CH_2Cl_2. The organic layers were combined, dried over Na_2SO_4, and concentrated. To a 0.5 M solution of the free amine in CH_2Cl_2 were added *N*-Boc-L-valine (1.25 equiv), HOBT (1.4 equiv) and EDC (1.4 equiv). After overnight reaction, the mixture was diluted with more CH_2Cl_2, washed with 4× saturated $NaHCO_3$ solution, dried over Na_2SO_4, filtered, and concentrated. Column chromatography with either ethyl acetate/hexanes 2:1 or CH_2Cl_2/MeOH 99:1 provided purified products.

[4(*S*)-[[(*N*-*tert*-Butoxycarbonyl)-L-valyl]amino-2,2-difluoro-3(*R*)-hydroxypentanoyl]-L-valyl]-L-isoleucine methyl ester (5a): 81%; mp 142 °C; ^{1}H NMR ($CDCl_3$) δ 1.01 (18H, m aliphatic H, 1.41 (3H, d J = 7.1 Hz), 1.46 (9H, s), 1.92 (2H, m), 2.29 (1H, m), 3.29 (1H, m), 3.59 (1H, m), 3.79 (3H, s), 4.13 (2H, m), 4.70 (1H, dd J = 5.5 and 3.4 Hz), 5.01 (1H, br), 6.30 (1H, br), 6.68 (1H, br), 8.21 (1H, br), 8.36 (1H, br); MS (MALDI-TOF) m/z 617 (M + Na). Anal. ($C_{27}H_{48}F_2N_4O_8 \cdot H_2O$) C,H,N.

[4(*S*)-[[(*N*-*tert*-Butoxycarbonyl)-L-valyl]amino-2,2-difluoro-5-methyl-3(*R*)-hydroxyhexanoyl]-L-valyl]-L-isoleucine methyl ester (5b): 88%; mp 90−91 °C; ^{1}H NMR ($CDCl_3$) δ 0.87−1.11 (25H, m), 1.45 (9H, s), 1.98 (2H, m), 2.27 (1H, m), 2.46 (1H, m), 2.62 (1H, m), 3.73 (1H, br), 3.75 (1H, s), 4.18−4.39 (3H, br), 4.66 (1H, dd J = 8 and 9 Hz), 5.44 (1H, d J = 9 Hz), 6.35 (1H, br), 7.40 (1H, d J = 8 Hz), 8.25 (1H, d J = 7 Hz), 8.46 (1H, d J = 8 Hz); Anal. ($C_{29}H_{52}F_2N_4O_8$) C,H,N.

[4(*S*)-[[(*N*-*tert*-Butoxycarbonyl)-L-valyl]amino-2,2-difluoro-5-phenyl-3(*R*)-hydroxypentanoyl]-L-valyl]-L-isoleucine methyl ester (5c): 72%; mp 159−161 °C; ^{1}H NMR ($CDCl_3$) δ 0.80 (6H, m), 0.91 (6H, m), 1.05 (6H, m), 1.18 (2H, m), 1.45 (9H, s), 2.00 (2H,m), 2.33 (1H, m), 3.20 (2H, br), 3.74 (3H, s), 3.78 (1H, br), 3.90 (1H, br), 4.24 (1H, br), 4.38 (2H, br), 4.63 (1H, m), 4.84 (1H, br), 6.73 (2H, br), 7.21 (5H, m), 7.44 (1H, br); MS m/z 694 ($M^+ + Na^+$). Anal. ($C_{33}H_{52}F_2N_4O_8 \cdot H_2O$) C,H,N.

[4(*S*)-[[(*N*-*tert*-Butoxycarbonyl)-L-valyl]amino-2,2-difluoro-5(*S*)-methyl-3(*R*)-hydroxyheptanoyl]-L-valyl]-L-isoleucine methyl ester (5d): 74%; mp 153−155 °C; ^{1}H NMR ($CDCl_3$) δ 0.77−1.29 (24 H, m), 1.46 (9H, s), 1.67 (1H, m), 1.90 (3H, br), 2.26 (3H, br), 2.65 (1H, br), 3.67 (1H, dd J = 9 and 9 Hz), 3.78 (3H, s), 4.11 (1H, dd J = 9 and 9 Hz), 4.36 (2H, br), 4.66 (1H, dd J = 8 and 9 Hz), 5.07 (1H, d J = 9 Hz), 6.42 (1H, d J = 11 Hz), 6.71 (1H, d J = 9 Hz), 8.22 (1H, d J = 6 Hz), 8.42 (1H, d J = 8 Hz); MS m/z 660 ($M^+ + Na^+$). Anal. ($C_{30}H_{54}F_2N_4O_8$) C,H,N.

[4(*S*)-[[(*N*-*tert*-Butoxycarbonyl)-L-valyl]amino-2,2-difluoro-5-cyclohexyl-3(*R*)-hydroxypentanoyl]-L-valyl]-L-isoleucine methyl ester (5e): 88%; mp 164−165 °C; ^{1}H NMR ($CDCl_3$) δ 0.78−1.27 (26H, m), 1.46 (9H, s), 1.61−2.01 (9H, m), 2.28 (1H, m), 3.20 (1H, br), 3.60 (1H, dd J = 9 and 9 Hz), 3.78 (3H, s), 4.12 (2H, br dd J = 9 and 9 Hz), 4.64 (1H, dd J = 9 and 9 Hz), 5.04 (1H, d J = 9 Hz), 6.33 (1H, d J = 11 Hz), 6.66 (1H, d J = 9 Hz), 8.21 (1H, d J = 7 Hz), 8.34 (1H, d J = 8 Hz); MS m/z 700 ($M^+ + Na^+$). Anal. ($C_{33}H_{58}F_2N_4O_8$) C,H,N.

[4(*S*)-[[(*N*-*tert*-Butoxycarbonyl)-L-valyl]amino-2,2-difluoro-3(*R*)-hydroxypentanoyl]-L-valyl]-L-isoleucine ethyl ester (5f): 87%; ^{1}H NMR ($CDCl_3$) δ 1.02 (18 H, m), 1.32 (2 H,m), 1.41 (3H, d J = 7.0 Hz), 1.45 (9H, s), 1.90 (1H, m), 2.26 (1H, m), 3.28 (1H, m), 3.60 (1H, dd J = 9.0 Hz), 4.11 (1H, m), 4.25 (2H, m), 4.67 (1H, dd J = 9.0 Hz), 5.03 (1H, d J = 9.0 Hz), 6.33 (1H, d J = 10.9 Hz), 6.67 (1H, d J = 8.9 Hz), 8.26 (1H, d J = 6.3 Hz), 8.39 (1H, d J = 6.7 Hz).

[4(*S*)-[[(*N*-*tert*-Butoxycarbonyl)-L-valyl]amino-2,2-difluoro-3(*R*)-hydroxypentanoyl]-L-valyl]-L-isoleucine benzyl ester (5g): 84%; ^{1}H NMR ($CDCl_3$) δ 0.94 (16H, m), 1.09 (2H, d J = 6.5 Hz), 1.39 (3H, d J = 7.0 Hz), 1.45 (9H, s), 1.91 (3H, br), 2.28 (1H, br), 3.26 (1H, br), 3.58 (1H, t), 4.08 (2H, m), 4.74 (1H, dd), 5.03 (1H, d), 5.20 (2H, q J = 15 Hz), 6.29 (1H, br), 6.66 (1H, d), 7.38 (5H, m), 8.18 (1H, d), 8.37 (1H, d).

[4(*S*)-[[(*N*-*tert*-Butoxycarbonyl)-L-valyl]amino-2,2-difluoro-3(*R*)-hydroxypentanoyl]-L-valyl]-L-isoleucine Methylamide (5h). To a 0 °C solution of methyl ester **5a** (85 mg, 0.143 mmol) in 16 mL MeOH was bubbled in methylamine to saturation. After overnight reaction at ambient temperature, **5h** had precipitated from the solution and was isolated by filtration: 80%; ^{1}H NMR ($CDCl_3$) δ 0.96 (18 H, m), 1.85 (2H, m), 1.18 (2H, m), 1.40 (3H, d J = 7 Hz), 1.47 (9H, s), 2.21 (1H, m), 2.84 (3H, d J = 4.7 Hz), 3.38 (1 H, br), 3.83 (1H, m), 4.18 (3H, m), 5.07 (1H, d J = 8.7 Hz), 5.90 (1H, br), 6.41 (1H, br), 7.31 (1H, br), 7.94 (1H, br), 8.48 (1H, br).

[4(*S*)-[[(*N*-*tert*-Butoxycarbonyl)-L-valyl]amino-2,2-difluoro-3-hydroxypentanoyl]-L-valyl]-L-isoleucine dimethylamide (5i): 29% (purified by filtration from CH_2Cl_2); ^{1}H NMR ($CDCl_3$) δ 0.90 (8H, m), 1.02 (12H, m), 1.39 (3H, d J = 7.0 Hz), 1.46 (9H, s), 1.83 (3H, m), 2.06 (1H, s), 2.20 (1H, m), 2.98 (3H, s), 3.22 (3H, s), 3.62 (1H, m), 3.91 (1H, dd J = 9.4 Hz), 4.28 (1H, dd J = 9.2 Hz), 4.81 (1H, dd J = 8.3 Hz), 5.07 (1H, d J = 9.1 Hz), 5.32, 6.59 (1 H, d J = 11.1 Hz), 7.35 (1H, d J = 8.5 Hz), 7.96 (1H, d J = 8.3 Hz), 8.78 (1H, d J = 5.8 Hz).

[4(*S*)-[[(*N*-*tert*-Butoxycarbonyl)-L-valyl]amino-2,2-difluoro-5-cyclohexyl-3(*R*)-hydroxypentanoyl]-L-valyl]-L-isoleucine benzyl ester (5j): mp 140−141 °C; 87%; ^{1}H NMR ($CDCl_3$) δ 0.75−1.04 (26H, m), 1.42 (9H, s), 1.59−1.77 (7H, m), 1.85 (3H, br), 2.21 (1H, br), 3.15 (1H, br), 3.55 (1H, dd J = 9 and 9 Hz), 4.09 (2H, br), 4.64 (1H, dd J = 9 and 9 Hz), 5.00 (1H, d J = 9 Hz), 5.13 (1H, m), 5.21 (1H, m), 6.28 (1H, d J = 11 Hz), 6.57 (1H, d J = 9 Hz), 7.34 (5H, m), 8.17 (1H, d J = 7 Hz), 8.35 (1H, d J = 7 Hz); MS m/z 776 ($M^+ + Na^+$). Anal. ($C_{39}H_{62}F_2N_4O_8$) C,H,N.

General Procedure for Oxidation of Difluoro Alcohols 5 to Difluoro Ketones 6. To a stirred solution of difluoro alcohol **5** in CH_2Cl_2 (10 mM) was added 7 equiv of Dess−Martin periodinane. After 3 h, the reaction mixture was added to $Na_2S_2O_3$ (49 equiv based on **5**) in saturated $NaHCO_3$ solution. After an additional 10 min, the mixture was partitioned between Et_2O and saturated $NaHCO_3$ solution. The organic layer was washed several times with saturated aqueous $NaHCO_3$, dried over $MgSO_4$, filtered, and concentrated to give difluoro ketones **6**. Further purification was usually not necessary. However, if needed, purification was accomplished by either recrystallization from ether/pentane or column chromatography with ethyl acetate/hexanes 2:1 or CH_2Cl_2/MeOH 99:1.

[4(*S*)-[[(*N*-*tert*-Butoxycarbonyl)-L-valyl]amino-2,2-difluoro-3-oxopentanoyl]-L-valyl]-L-isoleucine methyl ester (6a): 86%; mp 75 °C; ^{1}H NMR ($CDCl_3$) δ 0.85 (18 H, m), 1.26 (3H, d J = 7.1 Hz), 1.38 (9H, s), 1.78 (1H, m), 1.90 (1H, m), 2.07 (1H, m), 3.62 (3H, s), 3.82 (1H, dd), 4.22 (1H, m), 4.72

(2H, m), 6.67 (1H, br), 8.36 (1H, br), 8.52 (1H, br), 8.86 (1H, br); MS (MALDI-TOF) *m/z* 614 (M + Na). Anal. ($C_{27}H_{46}F_2N_4O_8$·1/2EtOAc) C,H,N.

[4(*S*)-[[(*N*-*tert*-Butoxycarbonyl)-L-valyl]amino-2,2-difluoro-5-methyl-3-oxohexanoyl]-L-valyl]-L-isoleucine methyl ester (6b): 88%; mp 81−84 °C; ^{1}H NMR ($CDCl_3$) δ 0.85−1.08 (24H, m), 1.14−1.31 (2H, m), 1.46 (9H, s), 1.94 (1H, m), 2.09 (1H, m), 2.22 (1H, m), 2.47 (1H, m), 3.77 (3H, s), 3.91 (1H, dd J = 9 and 9 Hz), 4.24 (1H, dd J = 7.5 and 8 Hz), 4.62 (1H, dd J = 8.5 and 9 Hz), 4.72 (1H, br), 5.06 (1H, br d J = 6 Hz), 6.34 (1H, br d J = 8 Hz), 6.85 (1H, br), 7.59 (1H, br); MS (MALDI-TOF) *m/z* 622.451 ($M^+ + Na^+$). Anal. ($C_{29}H_{50}F_2N_4O_8$) C,H,N.

[4(*S*)-[[(*N*-*tert*-Butoxycarbonyl)-L-valyl]amino-2,2-difluoro-5-phenyl-3-oxohexanoyl]-L-valyl]-L-isoleucine methyl ester (6c): 88%; mp 160−161 °C; ^{1}H NMR (DMSO + D_2O) δ 0.48 (3H, d J = 6.6 Hz), 0.57 (3H, d J = 6.6 Hz), 0.75−0.87 (18H, m), 1.31 (9H, s), 1.72 (2H, m), 1.99 (1H, m), 3.10 (2H, m), 3.58 (3H, s), 4.08 (1H, d J = 6.3 Hz), 4.30 (2H, m), 7.08−7.21 (5H, m). Anal. ($C_{33}H_{50}F_2N_4O_8$·1/2H_2O) C,H,N.

[4(*S*)-[[(*N*-*tert*-Butoxycarbonyl)-L-valyl]amino-2,2-difluoro-5(*S*)-methyl-3-oxoheptanoyl]-L-valyl]-L-isoleucine methyl ester (6d): 90%; mp 141−142 °C; ^{1}H NMR (DMSO) δ 0.77−0.87 (24H, m), 1.11−1.29 (2H, m), 1.36 (9H, s), 1.69−2.12 (6H, m), 3.85 (3H, s), 3.85 (1H, dd), 4.20 (2H, m), 4.84 (1H, dd), 6.72 (1H, d), 8.17 (1H, d), 8.33 (1H, d), 8.82 (1H, d); MS *m/z* 658 ($M^+ + Na^+$). Anal. ($C_{30}H_{52}F_2N_4O_8$) C, H, N.

[4(*S*)-[[(*N*-*tert*-Butoxycarbonyl)-L-valyl]amino-2,2-difluoro-5-cyclohexyl-3-oxohexanoyl]-L-valyl]-L-isoleucine methyl ester (6e): 95%; mp 95−97 °C; ^{1}H NMR ($CDCl_3$) δ 0.81−1.10 (20H, m), 1.11−1.26 (4H, m), 1.45 (9H, s), 1.55−1.81 (7H, m), 1.82−2.08 (4H, m), 2.26 (1H, m), 3.83 (3H, s), 3.90 (1H, dd J = 9 Hz, and 9 Hz), 4.09 (1H, dd J = 9 Hz, and 7.5 Hz), 4.23 (1H, br), 4.70 (1H, dd J = 9 Hz, and 9 Hz), 5.08 (1H, d J = 8.5 Hz), 6.47 (1H, d J = 9 Hz), 7.54 (1H, d J = 6 Hz), 8.24 (1H, br); MS *m/z* 698 ($M^+ + Na^+$). Anal. ($C_{33}H_{56}F_2N_4O_8$·H_2O) C,H,N.

[4(*S*)-[[(*N*-*tert*-Butoxycarbonyl)-L-valyl]amino-2,2-difluoro-3-oxopentanoyl]-L-valyl]-L-isoleucine ethyl ester (6f): 85%; mp 142−144 °C; ^{1}H NMR (DMSO) δ 0.85 (18H, m), 1.17 (2H, t J = 7.0 Hz), 1.26 (3H, d J = 7.1 Hz), 1.38 (9H, s), 1.79 (1H, m), 1.91 (1H, m), 2.04 (1H, m), 3.82 (1H, m), 4.09 (2H, m), 4.21 (2H, m), 4.72 (1H, m), 6.68 (1H, d J = 9.0 Hz), 8.34 (1H, d J = 7.7 Hz), 8.54 (1H, br), 8.87 (1H, d 7.5 Hz); MS 608 *m/z* (M + H^+). Anal. ($C_{28}H_{48}F_2N_4O_8$) C,H,N.

[4(*S*)-[[(*N*-*tert*-Butoxycarbonyl)-L-valyl]amino-2,2-difluoro-3-oxopentanoyl]-L-valyl]-L-isoleucine benzyl ester (6g): 97%; mp 155−158 °C; ^{1}H NMR (DMSO) δ 0.80 (16H, m), 1.17 (1H, m), 1.26 (3H, d J = 7 Hz), 1.38 (9H, s), 1.85 (2H, m), 2.05 (1H, m), 3.82 (1H, dd J = 7.5 and 8.5 Hz), 4.25 (2H, m), 4.72 (1H, dd J = 6 and 7 Hz), 5.12 (2 H, s), 6.68 (1H, d J = 9 Hz), 7.35 (5H, m), 8.39 (1H, d J = 8 Hz), 8.53 (1H, d J = 5 Hz), 8.82 (1H, d J = 7 Hz); MS 670 *m/z* (M + H^+). Anal. ($C_{33}H_{50}F_2N_4O_8$) C,H,N.

[4(*S*)-[[(*N*-*tert*-Butoxycarbonyl)-L-valyl]amino-2,2-difluoro-3-oxopentanoyl]-L-valyl]-L-isoleucine methylamide (6h): 69%; mp 188−190 °C dec; ^{1}H NMR (DMSO) δ 0.76−0.85 (18H, m), 1.03−1.20 (4H, br m), 1.23 (3H, d J = 5.5 Hz), 1.36 (9H, s), 1.52−1.75 (2H, m), 2.55 (3H, d J = 4.3 Hz), 3.76 (1H, dd J = 7.3 and 8.2 Hz), 3.93−4.21 (2H, m), 4.31(1H, dd J = 7.9 and 6.9 Hz), 6.76 (1H, br), 7.8−8.2 (3H, br m). Anal. ($C_{27}H_{47}F_2N_5O_7$·1.25MeOH) C,H,N.

[4(*S*)-[[(*N*-*tert*-Butoxycarbonyl)-L-valyl]amino-2,2-difluoro-3-oxopentanoyl]-L-valyl]-L-isoleucine dimethylamide (6i): 89%; mp 95−97 °C; ^{1}H NMR (DMSO) (as a mixture of ketone and hydrate) δ 0.82 (17H, m), 1.09 (2H, m), 1.23 (3H, d J = 7.1 Hz), 1.37 (9H, s), 1.48 (1H, m), 1.76 (1H, br), 1.97 (2H, m), 2.83 (3H, s), 3.07 (3H, s), 3.80 (1H, m), 4.14 (1H, m), 4.31 (1H, m), 4.56 (1H, m), 4.69 (1H, m), 6.71 (1H, d J = 8.7 Hz), 6.79 (1H, m), 6.94 (1H, m), 7.55 (1H, d J = 8.2 Hz), 7.94 (1H, d J = 9.0 Hz), 8.18 (1H, d J = 8.5 Hz), 8.25 (1H, d J = 8.5 Hz), 8.55 (1H, d J = 4.7 Hz), 8.83 (1H, d J = 8.7 Hz); MS 606 *m/z* ($M + H^+$). Anal. ($C_{28}H_{49}F_2N_5O_7$·1.5MeOH) C,H,N.

[4(*S*)-[[(*N*-*tert*-Butoxycarbonyl)-L-valyl]amino-2,2-difluoro-5-cyclohexyl-3-oxohexanoyl]-L-valyl]-L-isoleucine benzyl ester (6j): 89%; mp 150−152 °C; ^{1}H NMR ($CDCl_3$) δ 0.81−1.10 (20H, m), 1.11−1.26 (4H, m), 1.45 (9H, s), 1.55−1.81 (7H, m), 1.82−2.08 (4H, m), 2.26 (1H, m), 3.90 (1H, dd J = 9 Hz, and 9 Hz), 4.09 (1H, dd J = 9 Hz, and 7.5 Hz), 4.23 (1H, br), 4.70 (1H, dd J = 9 and 9 Hz), 5.08 (1H, d J = 8.5 Hz), 5.15 (1H, d J = 12 Hz), 5.25 (1H, d J = 12 Hz), 6.47 (1H, d J = 9 Hz), 7.37 (5H, m), 7.54 (1H, d J = 6 Hz), 8.24 (1H, br); MS 774 *m/z* ($M^+ + Na^+$). Anal. ($C_{39}H_{60}F_2N_4O_8$·H_2O) C,H,N.

[4(*S*)-[[(*N*-*tert*-Butoxycarbonyl)amino]-2,2-difluoro-5-cyclohexyl-3-oxohexanoyl]-L-valyl]-L-isoleucine methyl ester (7): obtained according to general method from **4e**; hygroscopic oil; 78%; ^{1}H NMR ($CDCl_3$) δ 0.84−1.03 (14H, m), 1.10−1.36 (6H,br m), 1.43 (9H, s), 1.56−1.98 (8H, br m), 2.16 (1H, m), 3.74 (3H, s), 4.32 (1H, dd J = 7.5 and 7.8 Hz), 4.59 (1H, dd J = 8.2 and 8.4 Hz), 4.95 (1H, br), 5.4 (1H, br), 6.47 (1H, d J = 8.4 Hz), 7.32 (1H, d J = 8.4 Hz); MS 598 *m/z* (M + Na^+). Anal. ($C_{28}H_{47}F_2N_3O_7$·H_2O) C,H,N.

Acknowledgment. We thank Talat Rahmati for expert technical support with the ELISAs.

References

(1) Moore, C. L.; Wolfe, M. S. Inhibition of β-amyloid formation as a therapeutic strategy. *Exp. Opin. Ther. Patents* **1999**, *9*, 135−46.
(2) Jarrett, J. T.; Berger, E. P.; Lansbury, P. T., Jr. The carboxy terminus of the beta amyloid protein is critical for the seeding of amyloid formation: implications for the pathogenesis of Alzheimer's disease. *Biochemistry* **1993**, *32*, 4693−7.
(3) Pike, C. J.; Burdick, D.; Walencewicz, A. J.; Glabe, C. G.; Cotman, C. W. Neurodegeneration induced by beta-amyloid peptides in vitro: the role of peptide assembly state. *J. Neurosci.* **1993**, *13*, 1676−87.
(4) Lorenzo, A.; Yankner, B. A. Beta-amyloid neurotoxicity requires fibril formation and is inhibited by congo red. *Proc. Natl. Acad. Sci. U.S.A.* **1994**, *91*, 12243−7.
(5) Meda, L.; Cassatella, M. A.; Szendrei, G. I.; Otvos, L., Jr.; Baron, P.; Villalba, M.; Ferrari, D.; Rossi, F. Activation of microglial cells by beta-amyloid protein and interferon-gamma. *Nature* **1995**, *374*, 647−50.
(6) El Khoury, J.; Hickman, S. E.; Thomas, C. A.; Cao, L.; Silverstein, S. C.; Loike, J. D. Scavenger receptor-mediated adhesion of microglia to beta-amyloid fibrils. *Nature* **1996**, *382*, 716−9.
(7) Selkoe, D. J. Translating cell biology into therapeutic advances in Alzheimer's disease. *Nature* **1999**, *399*, A23−31.
(8) Meziane, H.; Dodart, J. C.; Mathis, C.; Little, S.; Clemens, J.; Paul, S. M.; Ungerer, A. Memory-enhancing effects of secreted forms of the beta-amyloid precursor protein in normal and amnestic mice. *Proc. Natl. Acad. Sci. U.S.A.* **1998**, *95*, 12683−8.
(9) Selkoe, D. J. Cell biology of the amyloid beta-protein precursor and the mechanism of Alzheimer's disease. *Annu. Rev. Cell Biol.* **1994**, *10*, 373−403.
(10) Selkoe, D. J. Alzheimer's disease: Genotypes, phenotypes, and treatments. *Science* **1997**, *275*, 630−1.
(11) Hardy, J. The Alzheimer family of diseases: many etiologies, one pathogenesis? *Proc. Natl. Acad. Sci. U.S.A.* **1997**, *94*, 2095−7.
(12) Citron, M.; Diehl, T. S.; Gordon, G.; Biere, A. L.; Seubert, P.; Selkoe, D. J. Evidence that the 42- and 40-amino acid forms of amyloid beta protein are generated from the beta-amyloid precursor protein by different protease activities. *Proc. Natl. Acad. Sci. U.S.A.* **1996**, *93*, 13170−5.
(13) Klafki, H.; Abramowski, D.; Swoboda, R.; Paganetti, P. A.; Staufenbiel, M. The carboxyl termini of beta-amyloid peptides 1−40 and 1−42 are generated by distinct gamma-secretase activities. *J. Biol. Chem.* **1996**, *271*, 28655−9.
(14) Wolfe, M. S.; Xia, W.; Moore, C. L.; Leatherwood, D. D.; Ostaszewski, B.; Donkor, I. O.; Selkoe, D. J. Peptidomimetic probes and molecular modeling suggest Alzheimer's γ-secretases are intramembrane-cleaving aspartyl proteases. *Biochemistry* **1999**, *38*, 4720−7.
(15) Durkin, J. T.; Murthy, S.; Husten, E. J.; Trusko, S. P.; Savage, M. J.; Rotella, D. P.; Greenberg, B. D.; Siman, R. Rank-order of potencies for inhibition of the secretion of abeta40 and abeta42 suggests that both are generated by a single gamma-secretase. *J. Biol. Chem.* **1999**, *274*, 20499−504.

(16) Thaisrivongs, S.; Pals, D. T.; Kati, W. M.; Turner, S. R.; Thomasco, L. M.; Watt, W. Design and synthesis of potent and specific renin inhibitors containing difluorostatine, difluorostatone, and related analogues. *J. Med. Chem.* **1986**, *29*, 2080−7.
(17) James, M. N.; Sielecki, A. R.; Hayakawa, K.; Gelb, M. H. Crystallographic analysis of transition state mimics bound to penicillopepsin: difluorostatine- and difluorostatone-containing peptides. *Biochemistry* **1992**, *31*, 3872−86.
(18) Doherty, A. M.; Sircar, I.; Kornberg, B. E.; Quin, J. d.; Winters, R. T.; Kaltenbronn, J. S.; Taylor, M. D.; Batley, B. L.; Rapundalo, S. R.; Ryan, M. J.; et al. Design and synthesis of potent, selective, and orally active fluorine-containing renin inhibitors. *J. Med. Chem.* **1992**, *35*, 2−14.
(19) Lichtenthaler, S. F.; Wang, R.; Grimm, H.; Uljon, S. N.; Masters, C. L.; Beyreuther, K. Mechanism of the cleavage specificity of Alzheimer's disease gamma- secretase identified by phenylalanine-scanning mutagenesis of the transmembrane domain of the amyloid precursor protein. *Proc. Natl. Acad. Sci. U.S.A.* **1999**, *96*, 3053−8.
(20) Wolfe, M. S.; Xia, W.; Ostaszewski, B. L.; Diehl, T. S.; Kimberly, W. T.; Selkoe, D. J. Two transmembrane aspartates in presenilin-1 required for presenilin endoproteolysis and γ-secretase activity. *Nature* **1999**, *398*, 513−7.
(21) De Strooper, B.; Saftig, P.; Craessaerts, K.; Vanderstichele, H.; Guhde, G.; Annaert, W.; Von Figura, K.; Van Leuven, F. Deficiency of presenilin-1 inhibits the normal cleavage of amyloid precursor protein. *Nature* **1998**, *391*, 387−90.
(22) Wolfe, M. S.; De Los Angeles, J.; Miller, D. D.; Xia, W.; Selkoe, D. J. Are presenilins intramembrane-cleaving proteases? Implications for the molecular mechanism of Alzheimer's disease. *Biochemistry* **1999**, *38*, 11223−30.
(23) Tischer, E.; Cordell, B. Beta-amyloid precursor protein. Location of transmembrane domain and specificity of gamma-secretase cleavage. *J. Biol. Chem.* **1996**, *271*, 21914−9.
(24) Maruyama, K.; Tomita, T.; Shinozaki, K.; Kume, H.; Asada, H.; Saido, T. C.; Ishiura, S.; Iwatsubo, T.; Obata, K. Familial Alzheimer's disease-linked mutations at Val717 of amyloid precursor protein are specific for the increased secretion of A beta 42(43). *Biochem. Biophys. Res. Commun.* **1996**, *227*, 730−5.
(25) Lichtenthaler, S. F.; Ida, N.; Multhaup, G.; Masters, C. L.; Beyreuther, K. Mutations in the transmembrane domain of APP altering gamma-secretase specificity. *Biochemistry* **1997**, *36*, 15396−403.
(26) Wolfe, M. S.; Citron, M.; Diehl, T. S.; Xia, W.; Donkor, I. O.; Selkoe, D. J. A substrate-based difluoro ketone selectively inhibits Alzheimer's γ-secretase activity. *J. Med. Chem.* **1998**, *41*, 6−9.
(27) Fehrentz, J.-A.; Castro, B. An efficient synthesis of optically active α-(*tert*-butoxycarbonylamino)-aldehydes from α-amino acids. *Synthesis* **1983**, 676−8.
(28) Vassar, R.; Bennett, B. D.; Babu-Khan, S.; Kahn, S.; Mendiaz, E. A.; Denis, P.; Teplow, D. B.; Ross, S.; Amarante, P.; Loeloff, R.; Luo, Y.; Fisher, S.; Fuller, J.; Edenson, S.; Lile, J.; Jarosinski, M. A.; Biere, A. L.; Curran, E.; Burgess, T.; Louis, J. C.; Collins, F.; Treanor, J.; Rogers, G.; Citron, M. beta-Secretase Cleavage of Alzheimer's Amyloid Precursor Protein by the Transmembrane Aspartic Protease BACE. *Science* **1999**, *286*, 735−41.
(29) Sinha, S.; Anderson, J. P.; Barbour, R.; Basi, G. S.; Caccavello, R.; Davis, D.; Doan, M.; Dovey, H. F.; Frigon, N.; Hong, J.; Jacobson-Croak, K.; Jewett, N.; Keim, P.; Knops, J.; Lieberburg, I.; Power, M.; Tan, H.; Tatsuno, G.; Tung, J.; Schenk, D.; Seubert, P.; Suomensaari, S. M.; Wang, S.; Walker, D.; John, V. Purification and cloning of amyloid precursor protein beta-secretase from human brain. *Nature* **1999**, *402*, 537−40.
(30) Yan, R.; Bienkowski, M. J.; Shuck, M. E.; Miao, H.; Tory, M. C.; Pauley, A. M.; Brashier, J. R.; Stratman, N. C.; Mathews, W. R.; Buhl, A. E.; Carter, D. B.; Tomasselli, A. G.; Parodi, L. A.; Heinrikson, R. L.; Gurney, M. E. Membrane-anchored aspartyl protease with Alzheimer's disease beta-secretase activity. *Nature* **1999**, *402*, 533−7.
(31) Hussain, I.; Powell, D.; Howlett, D. R.; Tew, D. G.; Meek, T. D.; Chapman, C.; Gloger, I. S.; Murphy, K. E.; Southan, C. D.; Ryan, D. M.; Smith, T. S.; Simmons, D. L.; Walsh, F. S.; Dingwall, C.; Christie, G. Identification of a Novel Aspartic Protease (Asp 2) as beta-Secretase. *Mol. Cell Neurosci.* **1999**, *14*, 419−27.
(32) Zhang, L.; Song, L.; Parker, E. M. Calpain inhibitor I increases beta-amyloid peptide production by inhibiting the degradation of the substrate of gamma-secretase. Evidence that substrate availability limits beta-amyloid peptide production. *J. Biol. Chem.* **1999**, *274*, 8966−72.
(33) Higaki, J. N.; Chakravarty, S.; Bryant, C. M.; Cowart, L. R.; Harden, P.; Scardina, J. M.; Mavunkel, B.; Luedtke, G. R.; Cordell, B. A combinatorial approach to the identification of dipeptide aldehyde inhibitors of beta-amyloid production. *J. Med. Chem.* **1999**, *42*, 3889−98.
(34) Xia, W.; Ostaszewski, B. L.; Kimberly, W. T.; Rahmati, T.; Moore, C. L.; Wolfe, M. S.; Selkoe, D. J. FAD Mutations in presenilin-1 or amyloid precursor protein decrease the efficacy of a γ-secretase inhibitor: a direct involvement of PS1 in the γ-secretase cleavage complex. *Neurobiol. Disease*, in press.
(35) Kimberly, W. T.; Xia, W.; Rahmati, T.; Wolfe, M. S.; Selkoe, D. J. The transmembrane aspartates in presenilin 1 and 2 are obligatory for gamma-secretase activity and amyloid beta-protein generation. *J. Biol. Chem.* **2000**, *275*, 3173−8.
(36) Esler, W. P.; Kimberly, W. T.; Ostaszewski, B. L.; Diehl, T. S.; Moore, C. L.; Tsai, J.-Y.; Rahmati, T.; Xia, W.; Selkoe, D. J.; Wolfe, M. S. Transition-state analogue inhibitors of γ-secretase bind directly to presenilin-1. *Nat. Cell Biol.* **2000**, *2*, 428−34.
(37) Ireland, R. E.; Liu, L. An improved procedure for the preparation of the Dess−Martin periodinane. *J. Org. Chem.* **1993**, *58*, 2899.
(38) Dondoni, A.; Merchan, F. L.; Merino, P.; Rojo, I.; Tejero, T. Alkylation of camphor and pinanone imines of 2-(aminomethyl)-thiazole. Enantioselective synthesis of 2-(1-aminoalkyl)thiazoles. *Synthesis* **1996**, 641−6.

JM000100F