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Synthesis and γ-Secretase Activity of APP Substrate-Based Hydroxyethylene Dipeptide Isosteres

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Abstract—Two new APP substrate-based hydroxyethylene isosteres (AT and VI) were prepared and their dipeptide conjugates shown not to inhibit the γ-secretase-mediated formation of either Aβ1-40 or Aβ1-42. The FG isostere and a des-hydroxy hydroxyethylene isostere also gave inactive compounds. Conversely, a number of compounds containing the intact substrate-unrelated Phe-Phe (FF) hydroxyethylene isostere were shown to be potent inhibitors (ED_{50} = 14–732 nM). These results show that the factors governing the substrate-based design of γ-secretase inhibitors are more complicated than first thought.

Alzheimer's Disease (AD) is a progressive, neurodegenerative disease characterized by behavioural disturbance and a general decline in cognitive function. Current therapies rely on the use of cholinesterase inhibitors which temporarily improve cognitive function but do little to address the progression of AD.[1] Clearly there is an unmet clinical need for treatments to arrest or reverse the progression of AD. Many therapeutic strategies to this end have been proposed, including the suppression of amyloid-β peptide (Aβ) formation by inhibition of the proteases that cleave Amyloid-β Precursor Protein (APP).[2] Aβ is formed by initial cleavage of APP by β-secretase to form a membrane-bound C-terminal fragment (C99) (Fig. 1), which is then proteolyzed further by γ-secretase to form Aβ(1-40) or Aβ(1-42). The more aggregable, less-soluble Aβ(1-42) is the main component of the amyloid plaques found in diseased brain tissue, despite comprising only about 10% of total secreted Aβ, and hence compounds selective for the inhibition of Aβ(1-42) production may be of particular interest. The identity of γ-secretase and the role presenilin-1 (PS-1) plays in the γ-secretase-mediated cleavage of APP is currently a subject of much debate. PS-1 may indeed be γ-secretase itself, or a key component of a large proteolytic complex.[3]

Several structurally diverse inhibitors of γ-secretase have recently been reported in the literature. These include peptide aldehydes,[4] statines,[5] fenchylamine sulfonamides,[6] difluoroketones,[7a–c] benzodiazepines,[8] and substituted succinates.[9] We disclosed recently that dipeptide isostere-dipeptide conjugates (e.g., **1**, Fig. 2) are potent inhibitors of γ-secretase.[10] As such, derivatives of **1** have been used to elucidate the molecular target of γ-secretase inhibitors;[11] to investigate the stability of full-length presenilin-1 (PS1-FL)[12] and to investigate the proteolytic processing of the low density lipoprotein receptor-related protein[13] amongst many other studies.

Compound **1**, prepared originally as a minor diastereomer of an HIV protease inhibitor, is an N-terminal hydroxyethylene dipeptide isostere linked to a dipeptide. The hydroxyethylene dipeptide isostere contained in **1** mimics F-F. Since the cleavage of APP by γ-secretase to give Aβ occurs between V-I residues [Aβ(1-40)]

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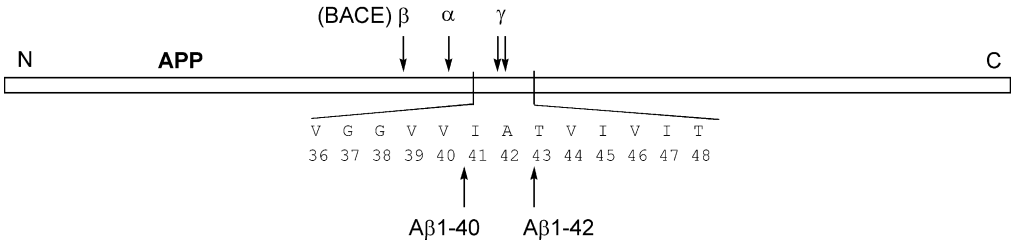
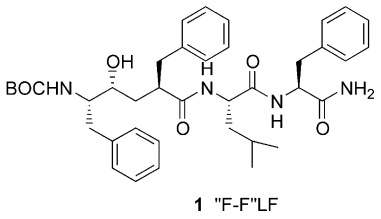


Figure 1. Cleavage of APP by β-, α- and γ-secretases (BACE = β-site APP cleaving enzyme).



1 "F-F"LF

Figure 2.

or A-T residues [Aβ(1-42)] it was reasoned that this was unlikely to be an optimal arrangement for an inhibitor of γ-secretase. Furthermore, by designing inhibitors based around the A42-T43 cleavage site it may be possible to selectively reduce formation of the particularly amyloidogenic Aβ(1-42) species, if two distinct proteases exist. Consequently, substrate-based inhibitors of γ-secretase based on the hydroxyethylene dipeptide isostere-dipeptide motif found in **1** (and in particular 'VI'AT and 'AT'VI) were prepared.[14] In this communication, we report the synthesis and in vitro data for these and related compounds.

The synthesis of the AT (**7**) and VI (**14**) hydroxyethylene dipeptide isosteres is depicted in Scheme 1 and parallels that described for the isostere portion of L-685,458 (**1**),[15] but with additional complications arising from the extra stereocenter present in the β-branched side-chains of amino acids T and I. Starting with the appropriate amino acid **2a**/**b**, protection, Kowalski homologation,[16] stereoselective reduction and base-mediated oxirane-formation resulted in the preparation of epoxides **4a**/**b**. For the preparation of the VI (**14**) analogue, the epoxide was opened with sodium diethyl malonate, resulting in the spontaneous formation of lactone **11**. Hydrolysis and decarboxylation afforded the unsubstituted lactone **12**. The side-chain was introduced by means of an aldol addition-elimination-hydrogenation sequence (direct alkylation gives almost exclusively the unwanted *trans* lactone). Although the hydrogenation step was highly stereoselective for the preparation of the isostere portion of **1**, a mixture of four diastereomers of **13** was obtained probably because of isomerization of the double bond to a β,γ-position prior to saturation. The lactones thus obtained were hydrolyzed and the resulting hydroxy acids carefully silylated to afford silyloxy acid **14**.

The synthesis of the AT isostere **7** was achieved by treatment of epoxide **4b** with sodium acetylacetonate (in refluxing dioxane) to afford **5**. Reduction of the ketone with $NaBH_4$ and silylation gave a complex mixture of β-silyloxy diastereomers, which, after hydrolysis of the lactone and silylation of the remaining hydroxyl group, afforded a mixture of α***R***,β***RS*-7** and α***S***,β***S*-7** that was readily separable by column chromatography. The stereochemistries of the newly-formed chiral centers were rigorously established by chemical transformations and NOE experiments. Thus desilylation of α***S***,β***S*-7** with THF–H_2O–AcOH gave *cis*-lactone **9**, which exhibited a large nOe between the two lactone methine protons that was absent in lactone **8** (obtained by the same procedure from α***R***,β***RS*-7**). Reduction of **9** and acetal formation from the resulting triol gave dioxolane **10**. Through a combination of ^1H–^{13}C NMR experiments, H^a and H^b were identified, and the coupling between them (11.5 Hz) indicated a *trans*-diaxial arrangement. Intriguingly, only three of four possible diastereomers were formed in the reduction step (**6**→**7**). The remaining diastereomer α***S***,β***R*-7** (corresponding to the isomer pattern found in naturally occurring threonine) was prepared by a multistep sequence involving a Mitsunobu inversion (**9**→**11**).

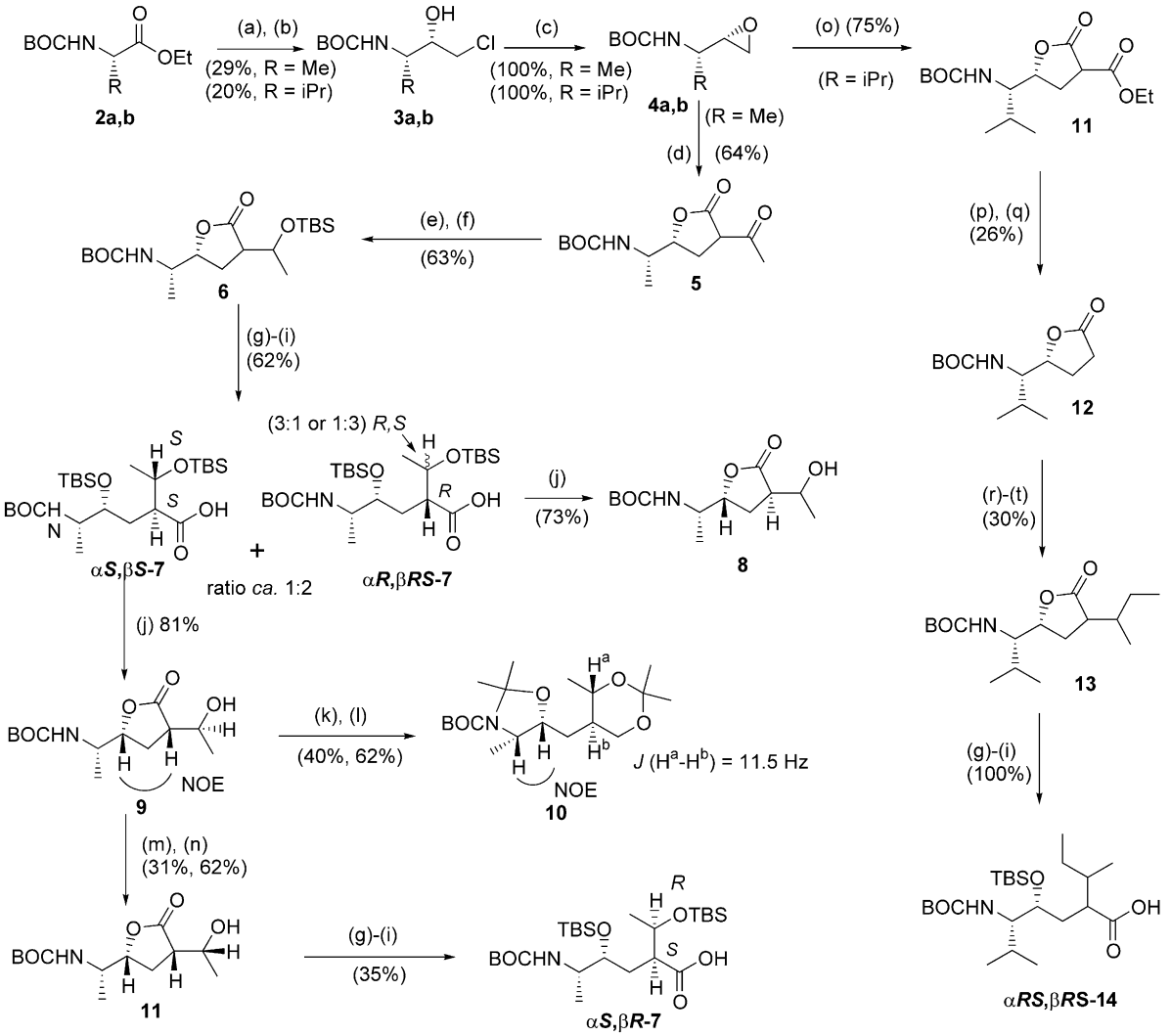
The isosteres **7**, **14**, FF[15] and FG[17] were then coupled to a range of dipeptides under standard EDC/HOBT conditions (Table 1), and the hydroxyl group(s) revealed by desilylation with TBAF (where necessary) to afford amides **1**[13] and **15**–**21** in good yields following purification by column chromatography.

The ED_{50} values for inhibition of Aβ(1-40) and Aβ(1-42) formation by the various inhibitors are summarized in Table 1. As reported previously, compound **1**, which contains the FF isostere, is a potent inhibitor of both Aβ(1-40) and Aβ(1-42) formation with ED_{50} values of 36 and 14 nM, respectively. Compound **17**, which is based on the sequence of APP flanking the 1-40 cleavage site, and in which the FF isostere has been replaced with a VI isostere, had no effect on Aβ production at

Table 1. Isostere + Xaa_1-Xaa_2-$CONH_2$ $\xrightarrow[2.\ TBAF\ (60–88\%)]{1.\ EDC,\ HOBT,\ DMF}$ Amide

Isostere	Xaa_1	Xaa_2	Amide	ED_{50} Cells Aβ(1-40) (nM)[a]	ED_{50} Cells Aβ(1-42) (nM)[a]
F-F	Leu	Phe	**1**	36	14
F-F	Val	Ile	**15**	32	35
F-F	Ala	Thr	**16**	732	230
V-I (**14**)	Ala	Thr	**17**	> 10,000	> 10,000
A-T (α***R***,β***S*-7**)	Val	Ile	**18**	> 10,000	> 10,000
A-T (α***S***,β***RS*-7**)	Val	Ile	**19**	> 10,000	> 10,000
A-T (α***R***,β***R*-7**)	Val	Ile	**20**	> 10,000	> 10,000
F-G	Leu	Phe	**21**	> 10,000	> 10,000

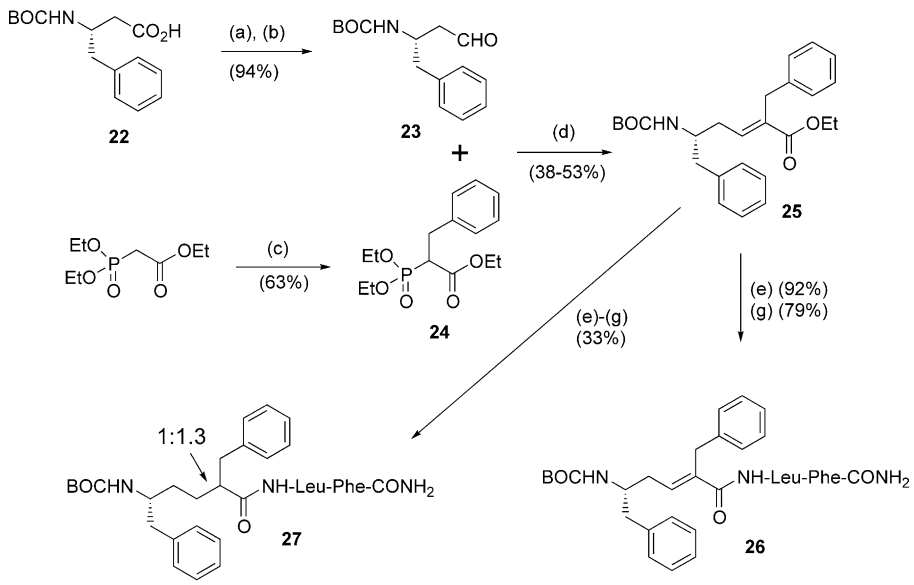
[a]Measured as described in ref 10; all values are the mean of at least two measurements.



Scheme 1. Reagents and conditions: (a) ICH_2Cl, LDA, THF, −90 °C; (b) $NaBH_4$, toluene–EtOH, −78 °C→rt; (c) KOH, EtOH, rt; (d) NaH, dioxane, ethyl acetoacetate, reflux, 16 h; (e) $NaBH_4$, EtOH, 0 °C; (f) TBSCl, imidazole, DMF, rt; (g) $LiOH.H_2O$, dioxane–H_2O, rt, 5 h; (h) TBSCl, imidazole, DMF, rt, 16 h; (i) MeOH, rt, 2 h; (j) THF–H_2O–AcOH, rt→50 °C, 24–48 h; (k) $LiAlH_4$, THF, −78 °C→rt; (l) PPTS, 2,2-dimethoxypropane, benzene, reflux, 1 h; (m) 3,5-dinitrobenzoic acid, dimethyl azodicarboxylate, PPh_3, toluene-DCM, rt, 1 week; (n) KOH, MeOH–THF, 0 °C; (o) NaOEt, EtOH, diethyl malonate, reflux, 16 h; (p) $LiOH{\cdot}H_2O$, H_2O–DME, 50 °C, 5 h; (q) toluene, reflux, 8 h; (r) LDA, 2-butanone, THF, −78 °C; (s) Et_3N, Ac_2O, 150 °C, 0.5 h; (t) H_2, Pd/C, EtOH, 4 h.

concentrations up to 10 μM. Similarly, compounds **18**–**20**, which mimic the APP sequence flanking the 1-42 cleavage site of APP, and in which the FF isostere has been replaced with various stereoisomers of the AT isostere, also had no effect on Aβ production at concentrations up to 10 μM. In order to check that the loss of activity seen with **17**–**20** was due to the change in the isostere portion of the molecules and not to the C-terminal dipeptide, the VI and AT dipeptides were coupled to the FF isostere to give inhibitors **15** and **16** respectively. It can be seen from Table 1 that **15** is equipotent with **1** (ED_{50} 32 nM), and that **16**, whilst less potent than **1** (ED_{50} = 732 nM), is still a moderately potent γ-secretase inhibitor. It was therefore concluded from this data that the loss of potency seen with compounds **17**–**20** is due to replacement of the benzyl residues on the FF isostere. It is interesting to note that if one of these benzyl groups is removed, as in compound **21** (F-G isostere), again potency is abolished up to concentrations of 10 μM.[18]

These results led us to re-examine the hypothesis that **1** was functioning as a direct transition-state analogue of the APP (1-40) and (1-42) cleavage sites. Towards this end, compounds **26** and **27**, two des-hydroxy analogues of L-685,458, were prepared following the general synthetic strategy reported by Martinez and co-workers (Scheme 2).[19] Treatment of the Weinreb amide of commercially available BOC-L-β-homophenylalanine (**22**) with $LiAlH_4$ gave aldehyde **23**, which was coupled immediately with phosphonate **24** in a Horner–Emmons reaction to give alkene **25**, as approximately a 10:1 mixture of *E*/*Z* isomers. The stereochemistry was established for the minor isomer by observation of an nOe between the alkene proton and the adjacent benzylic methylene protons. Standard hydrolysis,



Scheme 2. Reagents and conditions: (a) HNMeOMe·HCl, EDC, HOBT, Et_3N, DMF, rt, 2 days; (b) $LiAlH_4$, THF, 0 °C; (c) NaH, BnBr, THF, rt, 2 days; (d) NaH, DME, 0 °C→rt, 10 min; (e) $LiOH·H_2O$, H_2O–dioxane, rt, 16 h; (f) H_2, Pd/C, EtOH, rt, 16 h; (g) Leu-Phe-NH_2, EDC, HOBT, DMF, rt, 16 h.

hydrogenation and coupling reactions then gave **26** and **27**.

The ED_{50} values for inhibition of Aβ(1-40) and Aβ(1-42) formation by **26** and **27** were both determined to be >10 μM, indicating that the hydroxyl group of **1** is important for binding. This is in accord with the classical model of **1** functioning as a transition-state mimic, although we cannot rule out the alternative possibility that this is due to a more subtle conformational effect. It is interesting to speculate as to why the APP sequence mimetics **17**–**21** are not potent γ-secretase inhibitors compared to the FF containing molecules **1**,**15**–**16**. One possibility may be that **1** is not functioning as a direct aspartyl protease transition state mimic but rather is interacting with another part of the γ-secretase/PS-1 complex. Another possibility may be that γ-secretase/PS-1 has evolved to process other proteins as a primary function and that APP processing is a secondary event; APP may therefore not be an optimal substrate and **1** may have a structure closer to that of an undiscovered natural substrate. Indeed, other attempts to design substrate-based γ-secretase inhibitors have so far only led to compounds with weak inhibitory potency,[7a–c] whereas a (hydroxyethyl)urea analogue of compound **1** (which contains the same array of BOC group, two benzyl groups, a hydroxyl group and a dipeptide) is reasonably active ($IC_{50} = 300$ nM).[7d] Interestingly, other presenilin-dependent protease activities also occur between non-aromatic residues: the cleavage of ErbB4, a type I membrane receptor tyrosine kinase, is known to occur at an A-V site;[20] the S3 cleavage of Notch 1 occurs between G-V;[21] and an additional presenilin-dependent cleavage of APP has been discovered at an L-V site.[22] All of these three sites are located 3-4 residues inside the transmembrane region from the cytoplasmic side, suggesting that intramembranal positioning is possibly an additional, and perhaps more important, determinant for proteolysis. Indeed, Lichtenthaler and co-workers have recently shown that γ-secretase cleavage of C99 displays no sequence specificity, the major cleavage site determinant being the length of the C99 transmembrane domain.[23]

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24. All new compounds exhibited satisfactory spectroscopic and/or analytical properties. Selected data: Data for **19**: ^{1}H NMR (400 MHz, DMSO) (3:1 mixture of diastereomers) 7.77 (0.25H, d, $J=8.9$ Hz), 7.72 (0.75H, d, $J=8.9$ Hz), 7.58 (1H, d, $J=9.0$ Hz), 7.42 (1H, s), 7.03 (1H, s), 6.37–6.22 (1H, m), 4.6–4.5 (2H, m), 4.23–4.14 (2H, m), 3.72–3.56 (1H, m), 3.2–3.1 (2H, m), 2.03–1.97 (1H, m), 1.67–0.74 (33H, m). HPLC retention time: 3.737 min (minor peak), 3.867 min (major peak) 65% mobile phase: MeCN–pH 3.0 phosphate buffer (65%/35%), stationary phase: ACE 3C8-A1645; 1.0 mL/min; 210 nm detection. Data for **18**: ^{1}H NMR (360 MHz, DMSO) 7.82 (1H, d, $J=8.2$ Hz), 7.57 (1H, d, $J=9.0$ Hz), 7.22 (1H, s), 7.02 (1H, s), 6.32 (1H, d, $J=8.0$ Hz), 4.83 (1H, d, $J=5.2$ Hz), 4.48 (1H, d, $J=5.1$ Hz), 4.15–4.08 (2H, m), 3.75–3.63 (1H, m), 3.44–3.32 (2H, m), 2.41–2.36 (1H, m), 2.12–2.07 (1H, m), 1.73–1.68 (1H, m), 1.6–1.5 (2H, m), 1.37 (9H, s), 1.08 (3H, d, $J=6.2$ Hz), 0.96 (3H, d, $J=6.4$ Hz), 0.87–0.78 (14H, m). HPLC retention time: 5.087 min (conditions as for **19**). Data for **20**: ^{1}H NMR (400 MHz, DMSO) 7.80 (1H, d), 7.62 (1H, d), 7.27 (1H, s), 7.01 (1H, s), 6.29 (1H, d), 4.74 (1H, d), 4.49 (1H, d), 4.18–4.07 (2H, m), 3.69–3.58 (1H, m), 3.5–3.35 (2H, m), 2.41–2.35 (1H, m), 2.04–1.96 (1H, m), 1.77–1.5 (3H, m), 1.37 (9H, m), 1.02–0.80 (20H, m). HPLC retention time: 3.824 min (conditions as for **19**).