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Stereoselective Synthesis of Photoreactive Peptidomimetic γ-Secretase Inhibitors

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Abstract: The first asymmetric synthesis of novel, potent photoreactive γ-secretase inhibitors **2** and **3** has been accomplished. Two stereoselective methods for the preparation of lactone **9** are described. Protected benzophenone intermediate **19** is prepared via an aldol-elimination reaction followed by a PtO_2-catalyzed asymmetric hydrogenation. Two routes leading from **19** to compounds **2** and **3** are evaluated. The application of **3** as an activity-based probe has been demonstrated by localizing γ-secretase activity in the plasma membrane of intact cells.

γ-Secretase, which is involved in the neuropathogenesis of Alzheimer's disease, cleaves amyloid precursor protein and is thus an appealing drug target.[1] Moreover, γ-secretase is an extraordinary catalyst because the amide bonds which are cleaved appear to be situated within the hydrophobic environment of a transmembrane domain even though water is required for peptide hydrolysis.[2] Unraveling the intricacies of this enigmatic process is a formidable challenge that will require a novel chemical approach.

Replacement of the scissile peptide bond of the substrate with a hydroxyethylene isostere has proven to be an effective approach in the design of aspartyl protease inhibitors.[3] L-685,458 (**1**),[4] a potent γ-secretase inhibitor containing a hydroxyethylene isostere, has been utilized to study the molecular mechanism of γ-secretase.[5,6] L-685,458 contains P1, P1′, P2, P2′, and P3′ residues which putatively bind to the subsites S1, S1′, S2, S2′, and S3′, respectively, in the active site of γ-secretase (Figure 1). Incorporating a photoreactive group at different positions of L-685,458 should allow identification of the interacting proteins involved in the catalytic machinery, and thus provide a practical approach to understand the architecture of the γ-secretase active site.

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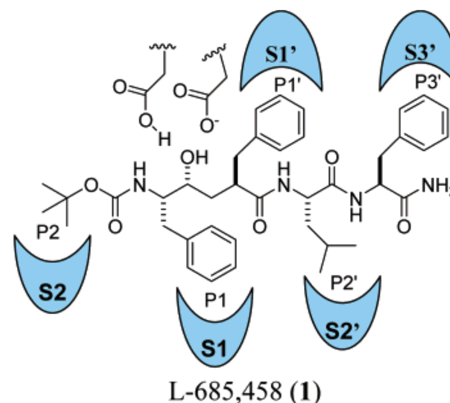
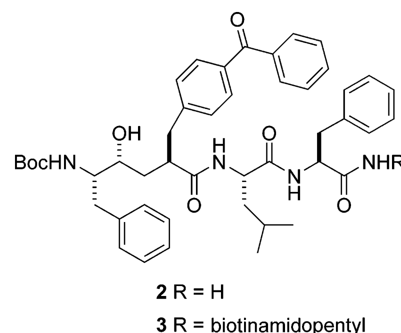


FIGURE 1. Proposed contact regions of the γ-secretase active site (**S2**−**S3′**) with L-685,458. The hydroxyethylene dipeptide isostere is designed to interact with the two catalytic aspartyl residues.

CHART 1



Benzophenones have been widely used as photoprobes due to their ability to insert into accessible C−H bonds of amino acid residues to form photoadducts and their stability.[7] Photoreactive benzophenone groups have been incorporated at positions P2 and P3′ of L-685,458 by peptide coupling reaction with benzoylphenylalanine (BPA).[6] Since the P1′ residue is proximal to the scissile bond (Figure 1), inhibitors that contain a photoreactive group at the P1′ position will be more suitable probes to scrutinize the active site of γ-secretase. However, synthesis of this type of photoreactive dipeptide isostere has not yet been achieved. In the present study, we describe the more challenging asymmetric synthesis of two new analogues of L-685,458 (**2** and **3**, Chart 1) that bear a benzophenone moiety at the P1′ position and demonstrate that they are potentially valuable tools for elucidating the molecular mechanism of γ-secretase. Moreover, the novel Phe-BPA isostere will be useful for the development of activity-based probes for proteomic profiling of aspartyl proteases.

The synthesis of **2** and **3** began with the preparation of the key hydroxyethylene precursor **9** (Scheme 1), using a modification of the method developed by Nadin,[11] who prepared intermediate epoxide **7** using the Barrish−Polniaszek method.[8] Thus, treatment of *N*-Boc-Phe-OMe (**4**) with CH_2ICl and excess LDA provided ketone **5**, which was reduced by $NaBH_4$ to give chlorohydrin **6** (9:1 dr).[9] Although the two diastereoisomers of **6** were difficult to

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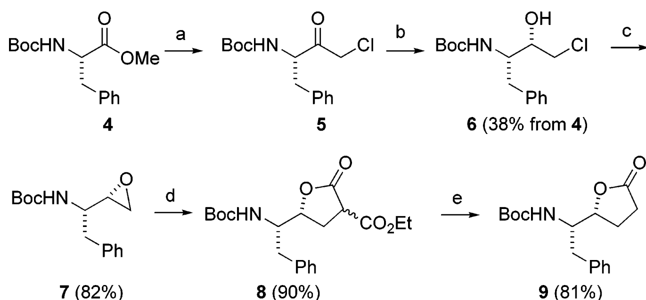
SCHEME 1. Synthesis of Lactone 9[a]



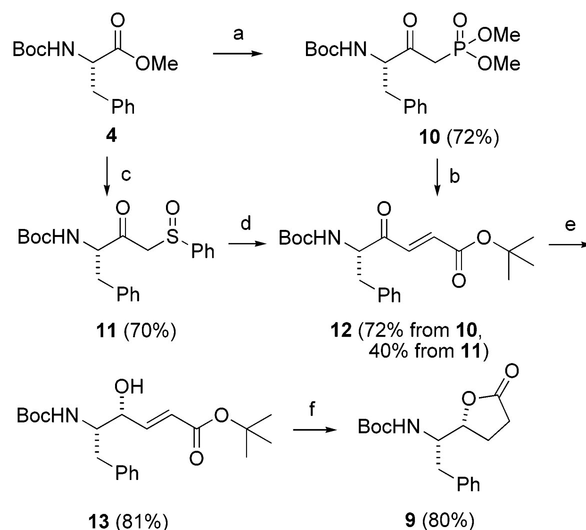
[a] Reagents and conditions: (a) CH_2ICl, LDA, THF, −78 °C; (b) $NaBH_4$, EtOH/toluene, −78 − 0 °C; (c) KOH, EtOH, rt; (d) $CH_2(CO_2Et)_2$, EtONa, EtOH, rt; (e) (i) LiOH/DME−H_2O, 50 °C, 6 h; (ii) toluene, reflux, 8 h.

separate by column chromatography, recrystallization from EtOAc provided the desired diastereomer of **6**, albeit in low yield (38% yield, 99:1 dr). Chlorohydrin **6** was then treated with ethanolic KOH to provide epoxide **7**, which was further purified via recrystallization from hexane (82% yield, >99:1 dr). Treatment of epoxide **7** with the sodium salt of diethyl malonate (3.5 equiv) provided the carbethoxylactone **8** in 90% yield.[10] Hydrolysis of **8** with LiOH in DME−H_2O followed by decarboxylation provided lactone **9** in 81% yield.[11]

In view of the difficulties required to purify **6**, we also developed a second route to lactone **9** that involved two simple steps from **13**: hydrogenation and then acid-mediated lactone formation (Scheme 2). Following the work of Luthman et al.,[12] we prepared ketoester **12** by Horner−Wadsworth−Emmons reaction of the phosphonate **10** with *tert*-butyl glyoxylate (prepared from L-tartaric acid in two steps[12]). Diastereoselective reduction of **12** with LiAlH(O-*t*-Bu)$_3$ in EtOH at −78 °C[9,12] gave **13** in 81% yield (>95:5 dr). Gratifyingly, hydroxy ester **13** was converted to lactone **9** in 80% yield by hydrogenation over 10% Pd/C followed by heating the resulting saturated γ-hydroxy ester in toluene in the presence of HOAc.[16] This alternative route offers a convenient procedure to prepare **9** in good yield.

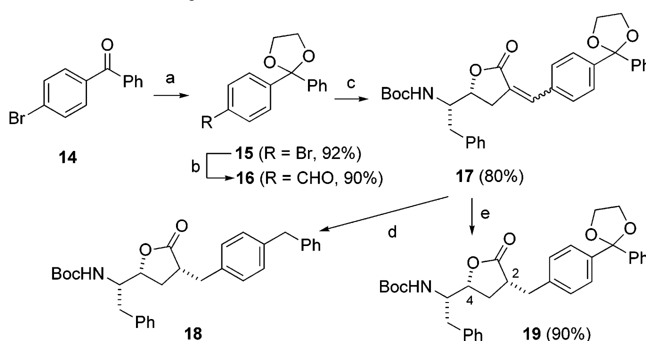
We also explored a new route employing β-ketosulfoxide **11** prepared in 70% yield by condensation of ester **4** with 3 equiv of the carbanion of methyl phenyl sulfoxide

SCHEME 2. Synthesis of Lactone 9 via γ-Keto Ester 12[a]



[a] Reagents and conditions: (a) MeP(O)(OMe)$_2$, LDA, THF, −78 °C; (b) $CHOCO_2$-*t*-Bu, Et_3N, 0 °C; (c) MeS(O)Ph, LDA, −78 °C to room temperature; (d) K_2CO_3, $BrCH_2CO_2$-*t*-Bu, DMF, rt; (e) LiAlH(O-*t*-Bu)$_3$, EtOH, −78 °C; (f) (i) H_2, Pd/C, EtOAc; (ii) toluene, HOAc, reflux.

SCHEME 3. Synthesis of Lactone 19[a]



[a] Reagents and conditions: (a) ethylene glycol, *p*-TsOH, benzene, reflux, 44 h; (b) *n*-BuLi, THF, DMF, −78 °C; (c) (i) **9**, LDA, THF, −78 °C; (ii) Ac_2O, Et_3N, 120 °C; (d) H_2, 10% Pd/C, EtOAc, rt, 24 h; (e) H_2, PtO_2, EtOAc, rt.

at −78 °C.[13] Alkylation of **11** with *tert*-butyl bromoacetate (KOBu-*t*/THF[14]), followed by elimination of PhSOH at 50 °C provided conjugated ketone **12** in 34% yield. Although alkylation in the presence of K_2CO_3/DMF[15] did improve the yield marginally (40%), higher elimination temperatures were of no help. Thus, although giving a lower yield than the previous approach, the new method is shorter and employs commercially available *tert*-butyl bromoacetate.

Conversion of lactone **9** to the protected benzophenone intermediate **19** was then accomplished as shown in Scheme 3. Thus, benzophenone **14** was protected as a dioxolane **15**, then converted to aldehyde **16** by reaction with *n*-BuLi followed by anhydrous DMF.[17] Aldol condensation of lactone **9** with aldehyde **16** followed by dehydration with Ac_2O−Et_3N at 120 °C provided the α,β-unsaturated lactone **17**.[11] Hydrogenation of **17** in the presence of 10% Pd/C[18] (2−3 h) gave a mixture of the desired dioxolane **19** and ketone **24** (Scheme 4). Extended reaction times led to overreduction, with compound **18**

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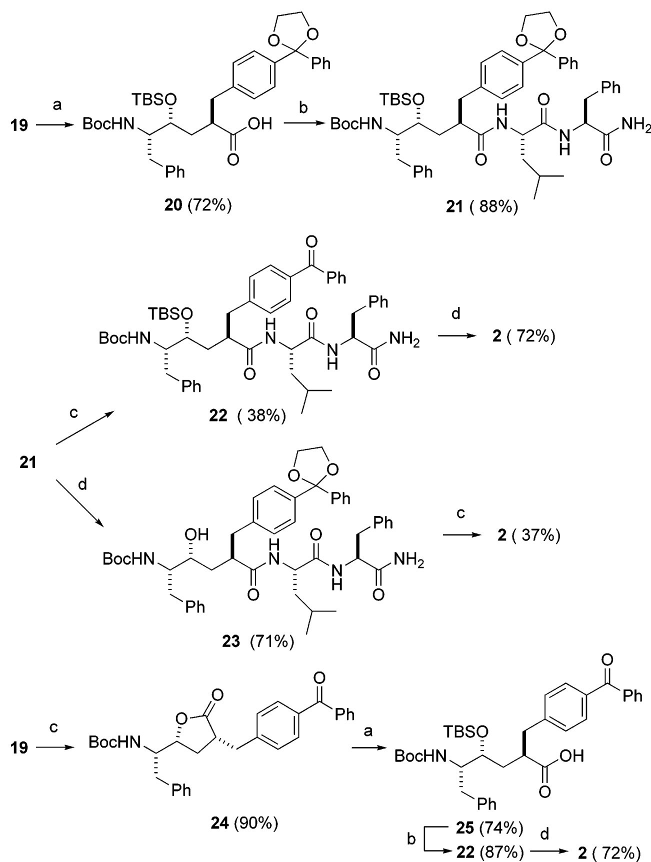
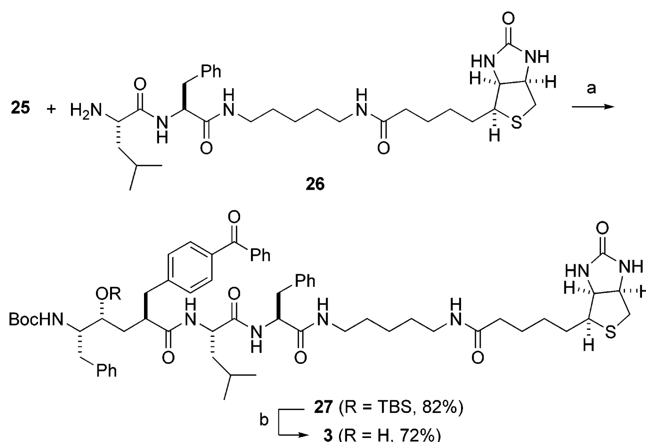
SCHEME 4. Synthesis of Compound 2[a]



[a] Reagents and conditions: (a) (i) LiOH/DME-H_2O, rt; (ii) TBSCl, imidazole, DMF, rt, (iii) MeOH; (b) Leu-Phe-NH_2, HOBt, EDC, *i*-Pr_2NEt, DMF, rt; (c) (i) CF_3CO_2H, (ii) Boc_2O, Et_3N; (d) *n*-Bu_4NF, THF, rt.

SCHEME 5. Synthesis of Compound 3[a]



[a] Reagents and conditions: (a) HOBt, EDC, DMF, rt; (b) *n*-Bu_4NF, THF, rt.

formed as the major product. Attempted hydrogenation with Wilkinson's catalyst [$RhCl(PPh_3)_3$],[19] led to a sluggish reaction and unsatisfactory yields. However, we were gratified to find that hydrogenation with Adam's catalyst was quite efficient and diastereoselective (>96:4 dr) and no reduction of the diphenyl ketal group was observed.[20] The desired product **19** was obtained in 90% yield, and trace amounts of the diastereoisomer of **19** were separated by column chromatography. The configuration of the new stereogenic center was confirmed by an observed NOE between H-2 and H-4 of lactone **19**.

Incorporation of this protected benzophenone intermediate in peptidomimetic γ-secretase inhibitors is shown in Scheme 4. Hydrolysis of lactone **19** with LiOH in DME−H_2O and treatment of the resulting hydroxy acid with excess TBSCl followed by selective desilylation of the acylsiloxy moiety (MeOH) provided acid **20** in 72% yield. Peptide coupling of **20** with Leu-Phe-NH_2 gave **21** in 88% yield. Attempted removal of the ethylene glycol protecting group with TsOH/acetone[21] proved sluggish. However, the ethylene glycol protecting group could be removed by stirring with CF_3CO_2H (TFA) overnight at room temperature with concomitant cleavage of the Boc group. The TBS group remained intact under these conditions. Protection of the resulting amine (not shown) with di-*tert*-butyl dicarbonate (Boc_2O) gave a disappointing 38% yield of the Boc protected amine **22**. Deprotection of the silyl ether of **22** with TBAF gave the final product **2** in 72% yield. Essentially the same result was obtained when we reversed the sequence of these reactions: deprotection of **21** with TBAF provided **23** in 71% yield, then simultaneous removal of the ethylene glycol and Boc groups with TFA, and finally treatment of the resulting amine with Boc_2O provided **2** in 37% yield. Since removal of the ethylene glycol protecting group with acid after coupling with Leu-Phe-NH_2 resulted in decomposition byproducts and a low yield of **2**, we examined removal of the ethylene glycol protecting group from **19**, prior to lactone hydrolysis and coupling with Leu-Phe-NH_2. Attempted CBr_4/PPh_3[22] deprotection was ineffective. However, as before, TFA removed both the ethylene glycol and Boc groups. Reprotection of the resulting amine with Boc_2O provided lactone **24** in 90% overall yield from **19**. Thus, TFA provides an effective way to remove the dioxolane protecting group from benzophenone. Hydrolysis of lactone **24** with LiOH/DME−H_2O and treatment of the resulting hydroxy acid with excess TBSCl followed by selective desilylation (MeOH) provided acid **25** in 74% yield. Peptide coupling of **25** with Leu-Phe-NH_2 gave **22** in 87% yield. Deprotection of **22** with TBAF provided **2** in 72% yield. Thus deprotection of the ethylene glycol protecting group at an early stage proved to be a much more effective approach.

Attachment of a biotin group to compound **2** can facilitate the isolation and identification of the labeled proteins or fragments. Thus we also prepared **3** as shown in Scheme 5. A peptide-coupling reaction of Boc-Leu-Phe-OH with 5-(biotinamido)pentylamine followed by TFA deprotection provided biotin-linker **26**. Coupling of acid

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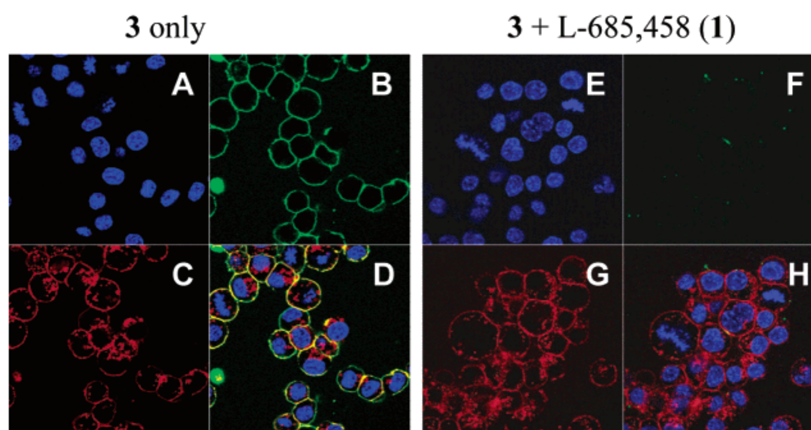

FIGURE 2. Intact cell photolabeling of γ-secretase with **3: (**A, E) DAPI; (B, F) anti-biotin-FITC; (C, G) WGA-Rhodamine; (D, H) merge A−B−C and E−F−G, respectively.

25 with **26** gave adduct **27** in 82% yield. Deprotection of **27** with TBAF provided the final product **3** in 72% yield.

Importantly, **2** and **3** displayed high potency, similar to that of L-685,458, in inhibiting γ-secretase activity. The IC_{50} values of the three compounds were 0.28 nM for **2**, 0.69 nM for **3**, and 0.30 nM for L-685,458. Thus, we have demonstrated that derivatization of L-685,458 by adding a benzophenone group at the P1′ position and by attaching a biotin moiety at the *C*-terminus does not alter the inhibitory ability against γ-secretase. Therefore, compounds **2** and **3** should serve as excellent photolabeling reagents for identifying and mapping the active site of the γ-secretase complex.

Since γ-secretase is a multiple protein complex, defining its activity in the plasma membrane of living cells has been a challenging task.[23] Since compounds **2** and **3** are transition state analogue inhibitors that only bind to the active γ-secretase, they are valuable probes to determine the site of γ-secretase in the cells. We developed an intact cell photolabling technique using probe **3**. After photoactivation, the biotinylated probe **3** was covalently attached to γ-secretase that resides in the plasma membrane of cells. The biotinylated γ-secretase was then detected by an anti-biotin antibody conjugated with FITC (green, Figure 2, panels B and F). The cells were also costained with Rhodamine-conjugated Wheat Germ Agglutinin (WGA) that labels proteins containing *N*-acetylglucosamine (Red, Figure 2, panels C and G) as a cell surface marker. The colocalization (yellow, Figure 2, panel D) of the anti-biotin/γ-secretase and WGA indicated that compound **3** labels target proteins in the plasma membrane. Moreover, the photoinsertion of compound **3** into the target proteins in the plasma membrane was blocked by L-685,458, which demonstrated the specificity of binding of probe **3** to γ-secretase. Our research clearly established that active γ-secretase is presented in the cell surface, which has not been reported previously. These inhibitors are being utilized to elucidate the molecular mechanism of γ-secretase. These studies will facilitate the development of novel therapeutic agents for treatment of Alzheimer's disease.

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Supporting Information Available: Experimental procedures and characterization data for all new compounds; copies of ^{1}H and ^{13}C NMR spectra for compounds **2**, **3**, **19**, and **21**−**24**. This material is available free of charge via the Internet at http://pubs.acs.org.

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