Bioorganic & Medicinal Chemistry Letters 19 (2009) 922–925

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Stereo-controlled synthesis of novel photoreactive γ-secretase inhibitors

Guangli Yang [a], Ye Ingrid Yin [b†], Jiong Chun [b‡], Christopher C. Shelton [b], Ouathek Ouerfelli [a], Yue-Ming Li [b,*]

[a] Organic Synthesis Core Facility, Memorial Sloan-Kettering Cancer Center, 1275 York Avenue, New York, NY 10065, USA
[b] Molecular Pharmacology and Chemistry Program, Memorial Sloan-Kettering Cancer Center, 1275 York Avenue, New York, NY 10065, USA

ARTICLE INFO

Article history:
Received 20 September 2008
Revised 25 November 2008
Accepted 26 November 2008
Available online 7 December 2008

Keywords:
Aspartyl proteases
Alzheimer disease
Photoaffinity labeling
Secretase
Dipeptide isostere

ABSTRACT

The stereoselective synthesis of novel photoreactive γ-secretase inhibitors **2** and **3** has been achieved. Key steps of the strategy involve preparation of α-*N*-Boc-epoxide **8** and formation of lactone **14** in a practical and stereo-controlled fashion. Compounds **2** and **3** are potent γ-secretase inhibitors and directly interact with presenilin-1, a catalytic subunit of γ-secretase.

γ-Secretase cleaves the amyloid precursor protein (APP) to generate β-amyloid (Aβ) peptides, which are believed to play a causative role in the pathogenesis of Alzheimer disease (AD).[1] γ-Secretase is an aspartyl protease composed of at least four proteins, including presenilin, nicastrin, APH and Pen2.[2] Genetic and biochemical studies have indicated that presenilin is the catalytic core of γ-secretase[3–5] and as such, familial mutations of presenilin have been associated with early on-set of AD[6] through alteration of the specificity of γ-secretase. Furthermore, γ-secretase represents a novel class of protease that hydrolyzes the scissile bond within the transmembrane domain of substrate.[7,8]

L-685,458 (**1**) (Fig. 1), a potent γ-secretase inhibitor[9] that contains a hydroxyethylene isostere, can be modified into a photoreactive compound by replacing an unsubstituted phenyl with a benzophenone (BP). These substitutions at P2, P1′ and P3′ have been synthesized and utilized to study γ-secretase.[4,10] However, synthesis of a photoreactive dipeptide isostere at the P1 position has not yet been achieved. In this study, we describe the stereo-controlled synthesis of two new analogs of L-685,458 (**2** and **3**, Chart 1) with BPA (benzophenone alanine) at the P1 position and demonstrate that they directly interact with presenilin, the catalytic subunit of γ-secretase. Moreover, this novel BPA-Phe isostere could be useful as a functional unit to synthesize active site directed inhibitors for profiling aspartyl proteases.

The synthesis of **2** and **3** started with the preparation of epoxide **8** using a modified Barrish–Polniaszek's method[11] (Scheme 1). Methylation of Boc-*p*-Bz-Phe-OH (**4**) with $TMSCHN_2$ in methanol[12] provided methyl ester **5**.

However, an attempt that followed the same synthetic route for preparation of Phe-BPA isostere[10] to protect benzophenone **5** as a dioxolane using ethylene glycol, *p*-TsOH and benzene at reflux for 2 days failed to generate any product. Thus, we changed our strategy by reducing ketone to an alcohol. We intended to find conditions that allow for the stereo- and regioselective reduction the ketone of benzophenone. Initially, we treated **5** with $NaBH_4$ at 0 °C[13] with favorable stereoselectivity (85:15 dr) and 70% yield, but this condition also led to the formation of a small amount of reduced methyl ester. However, when we performed the same reaction at −60 °C, we obtained the stereoselective product (85:15 dr) in 86% yield without reducing the methyl ester. Silylation of the resulting secondary alcohol produced **6**, which led to the generation of a chiral center at the benzylic carbon. The config-

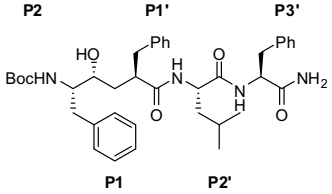
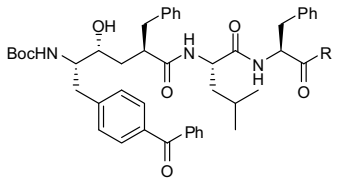


Figure 1. Structure of L-685,458 (**1**). The side chains corresponding to the P and P′ sites are marked.

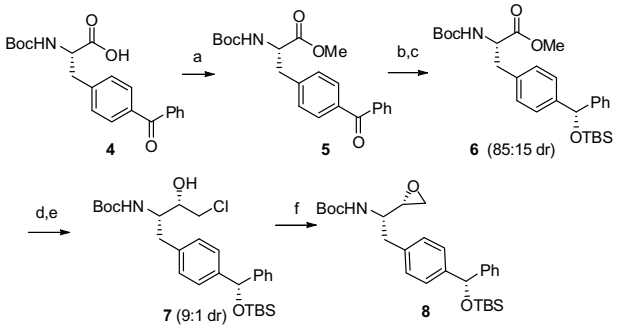
* Corresponding author. Tel.: +1 646 888 2193; fax: +1 646 422 0640.
E-mail address: liy2@mskcc.org (Y.-M. Li).
† Present Address: Wellington Management, Boston, MA, USA.
‡ Present Address: Symrise Inc., 300 North Street, Teterboro, NJ, USA.

0960-894X/$ - see front matter © 2008 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2008.11.117



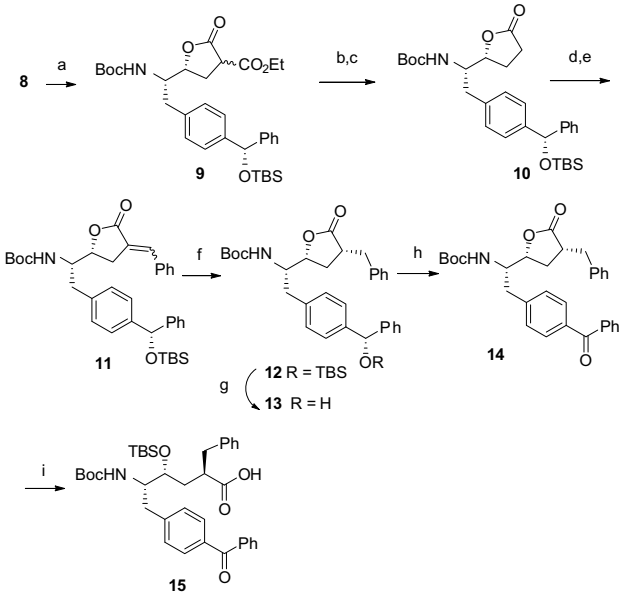
2 R = OMe
3 R = biotinamidopentyl

Chart 1.



Scheme 1. Synthesis of epoxide **8**. Reagents and conditions: (a) $TMSCHN_2$, MeOH, 0 °C to rt, 18 h, 90%; (b) $NaBH_4$, MeOH, −60 °C, 86%; (c) TBSCl, CH_2Cl_2, imidazole, rt, 95%; (d) 4 equiv CH_2ICl, 5 equiv LDA, THF, −78 °C; (e) 4 equiv $NaBH_4$, MeOH, −78–0 °C, 65%; (f) KOH, EtOH, 0 °C to rt, 95%.

uration of **6** is assigned by X-ray crystallographic analysis of intermediate **12** (Scheme 2) as described later in Figure 2. Treatment of methyl ester **7** with excess LDA/CH_2ICl provided an α-chloroketone, which was reduced with $NaBH_4$ to give chlorohydrin **7** (9:1 dr) in favor of the desired stereoisomer as demonstrated by X-ray analysis. The two diastereoisomers of **7** were separated by column chromatography (65% yield of the desired compound, based



Scheme 2. Synthesis of acid **15**. Reagents and conditions: (a) $CH_2(CO_2Et)_2$, NaOEt, EtOH, rt, 70%; (b) LiOH/DME–H_2O, 50 °C, 6 h; (c) toluene, reflux, 8 h, 60%; (d) LDA, PhCHO, THF, −78 °C; (e) Ac_2O, Et_3N, 120 °C, 80% for 2 steps; (f) H_2, 10% Pd/C, EtOAc, rt, 6 h, 90%; (g) HF·Py, THF, 18 h, 83%; (h) MnO_2, CH_2Cl_2, 18 h, 76%; (i) ia—LiOH/DME–H_2O, rt; ib—TBSCl, imidazole, DMF, rt; ic—MeOH, 79% for 3 steps.

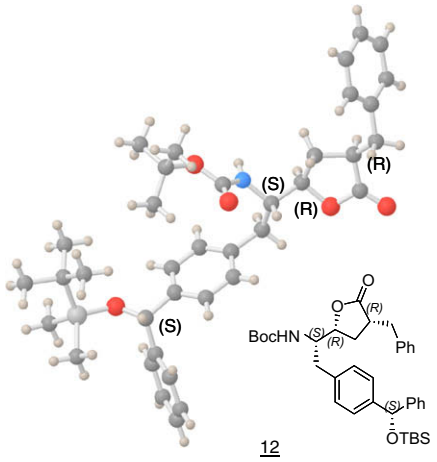
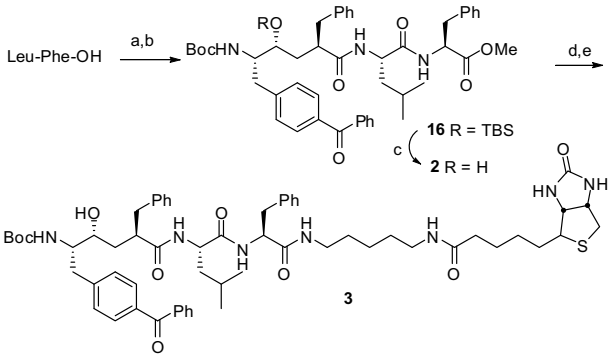


Figure 2. X-ray crystallographic structure of **12**: $C_{37}H_{49}NSiO_5$, M 615.86, orthorhombic $P2_12_12_1$ (No. 19), a = 5.9239(12) Å, b = 12.923(3) Å, c = 46.419(9) Å, V = 3553.0(12) Å^3, D_c (Z = 4) = 1.151 g/cm^3, T = 100 K, μ = 0.106 cm^{-1}. The final R value is 0.2116 for 3442 independent reflections with $I > 2\sigma I$ and 398 parameters. (The crystal structure of **12** has been deposited at the Cambridge Crystallographic Data Centre with the deposition number: CCDC 710680.)

on recovered starting material **6**). Cyclization of chlorohydrin **7** produced epoxide **8** in 95% yield.[14]

Treatment of epoxide **8** with the sodium salt of diethyl malonate directly provided lactone **9** as a mixture of stereoisomers (Scheme 2).[15] Hydrolysis of **9** with aqueous LiOH, followed by decarboxylation gave lactone **10** in 60% yield. Aldol condensation of **10** with benzaldehyde followed by dehydration with acetic anhydride-triethylamine at 120 °C gave the α,β-unsaturated lactone **11** in 80% yield.[16] Hydrogenation of **11** with 10% Pd/C (1 atm, 6 h) provided lactone **12** as the sole product. The assignment of three chiral centers, as indicated in Scheme 2, was confirmed by the X-ray crystallographic analysis of **12** (Fig. 2). Removal of the silyl group in lactone **12** with n-Bu_4NF (TBAF) led to epimerization at the α-lactone position, perhaps due to the basicity of the TBAF reagent. However, we were able to find that treatment of **12** with pyridine/HF overnight successfully removed the silyl protecting group to give **13** without any epimerization.[17] Oxidation of the benzylic alcohol with MnO_2[18] gave benzophenone **14** in 76% yield.[19] Hydrolysis of lactone **14** with LiOH and silylation of the resulting hydroxy acid produced **15** in 79% yield.

Esterification of Leu-Phe-OH with TMSCl in MeOH,[20] followed by coupling of the resulting amine with acid **15**, and deprotection of the resulting silyl ether with TBAF, produced the desired com-



Scheme 3. Synthesis of compounds **2** and **3**. Reagents and conditions: (a) TMSCl, MeOH, 0 °C to rt, 18 h, 80%; (b) **15**, EDC, HOBt, i-Pr_2NEt, DMF, 57%; (c) n-Bu_4NF, THF, rt, 85%; (d) LiOH, THF/H_2O, rt, 90%; (e) 5-(biotinamido)pentylamine, EDC, HOBt, DMF, rt, 50%.

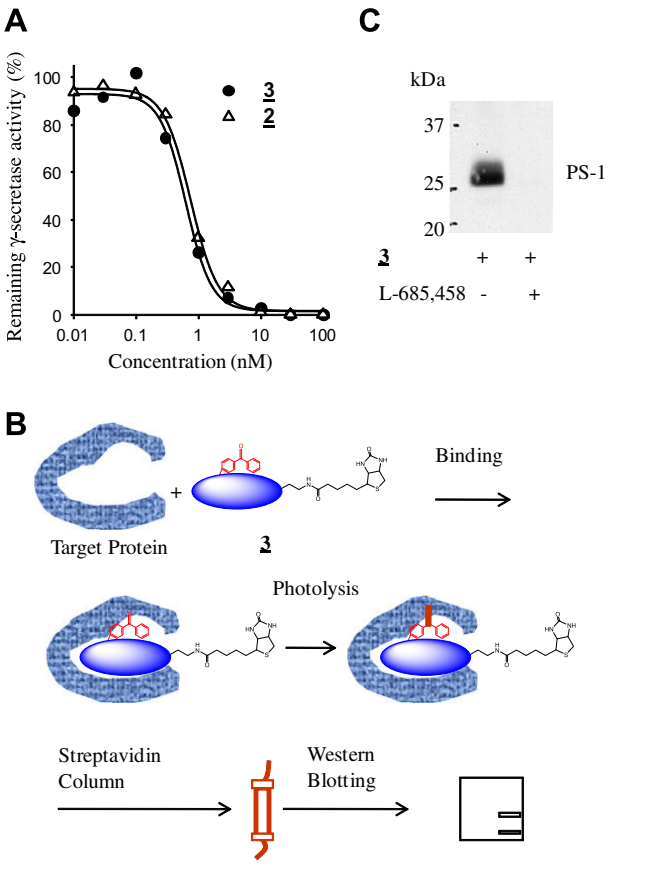


Figure 3. Both **2** and **3** are potent γ-secretase inhibitors that directly bind to presenilin-1. (A) Inhibitory potencies of compounds **2** and **3** against γ-secretase. (B) Scheme of photoaffinity labeling procedure. After photo-crosslinking, the biotinylated proteins were captured, eluted and analyzed by Western analysis. (C) Analysis of photolabeled proteins. The photo-crosslinked proteins were resolved by SDS–PAGE and probed with PS-1-NTF (N-terminal fragment) antibody.

pound **2** in reasonable yield (Scheme 3).[21] In order to facilitate the purification of the labeled proteins or fragments thereof, biotinylated compound **3** was prepared (Scheme 3). Mild saponification of the methyl ester in **2** led to the corresponding carboxylic acid, which was coupled with 5-(biotinamido)pentylamine in the presence of EDC and HOBt and resulted in compound **3**.

We next examined the biological activities of **2** and **3**. First, we determined their inhibitory potency against γ-secretase using an in vitro assay.[22] The IC_{50} values of **2** and **3** are 0.7 and 0.6 nM, respectively (Fig. 3A), which is similar to the parent compound, L-685,458 (**1**). These findings have demonstrated that incorporating BPA into the P1 position and attaching a biotin tag at the C-terminus do not affect their potency for inhibition of γ-secretase. Second, we tested whether **3** was capable of photo-crosslinking to γ-secretase. HeLa cell membranes were incubated with **3** at a final concentration of 10 nM in the absence and the presence of 2 μM of L-685,458 for 2.5 h. Then samples were irradiated with UV light (>350 nm) and the labeled proteins were solubilized and isolated with streptavidin beads.[4]

The biotinylated proteins were eluted and analyzed by Western blotting with antibodies against presenilin-1 (PS-1). Inhibitor **3** directly photolabels PS-1 (Fig. 3C). Moreover, an excess of L-685,458 is able to block photoinsertion of this probe into presenilin-1. Taken together, these results have demonstrated that compounds **2** and **3** are potent γ-secretase inhibitors that can specifically label the catalytic core of γ-secretase. Therefore, compounds **2** and **3** should be valuable probes for mapping the active site of γ-secretase.

Acknowledgments

This work was supported by NIH Grant AG026660 (Y.M.L.), the Alzheimer's Association (Zenith Fellows Award to Y.M.L.), Mr. William H. Goodwin and Mrs. Alice Goodwin and the Commonwealth Foundation for Cancer Research, the Experimental Therapeutics Center of MSKCC, and the William Randolph Hearst Fund in Experimental Therapeutics. C.C.S. is supported by an NIH NRSA predoctoral fellowship 5F31NS053218-02. We thank Dr. George Sukenick, Ms. Sylvi Rusli (NMR Core Facility, Sloan-Kettering Institute) for mass spectral analyses and Dr. Louis Todaro (Hunter College, New York) for X-ray structure analyses.

References and notes

1. Hardy, J.; Allsop, D. *Trends Pharmacol. Sci.* **1991**, *12*, 383.
2. De Strooper, B. *Neuron* **2003**, *38*, 9.
3. Wolfe, M. S.; Xia, W.; Ostaszewski, B. L.; Diehl, T. S.; Kimberly, W. T.; Selkoe, D. J. *Nature* **1999**, *398*, 513.
4. Li, Y. M.; Xu, M.; Lai, M. T.; Huang, Q.; Castro, J. L.; DiMuzio-Mower, J.; Harrison, T.; Lellis, C.; Nadin, A.; Neduvelil, J. G.; Register, R. B.; Sardana, M. K.; Shearman, M. S.; Smith, A. L.; Shi, X. P.; Yin, K. C.; Shafer, J. A.; Gardell, S. J. *Nature* **2000**, *405*, 689.
5. 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. *Nat. Cell Biol.* **2000**, *2*, 428.
6. Sherrington, R.; Rogaev, E. I.; Liang, Y.; Rogaeva, E. A.; Levesque, G.; Ikeda, M.; Chi, H.; Lin, C.; Li, G.; Holman, K., et al *Nature* **1995**, *375*, 754.
7. Wolfe, M. S.; Kopan, R. *Science* **2004**, *305*, 1119.
8. Brown, M. S.; Ye, J.; Rawson, R. B.; Goldstein, J. L. *Cell* **2000**, *100*, 391.
9. Shearman, M. S.; Beher, D.; Clarke, E. E.; Lewis, H. D.; Harrison, T.; Hunt, P.; Nadin, A.; Smith, A. L.; Stevenson, G.; Castro, J. L. *Biochemistry* **2000**, *39*, 8698.
10. Chun, J.; Yin, Y. I.; Yang, G.; Tarassishin, L.; Li, Y. M. *J. Org. Chem.* **2004**, *69*, 7344.
11. Chen, P.; Cheng, P. T. W.; Spergel, S. H.; Zahler, R.; Wang, X. B.; Thottathil, J.; Barrish, J. C.; Polniaszek, R. P. *Tetrahedron Lett.* **1997**, *38*, 3175.
12. Chai, W.; Murray, W. V. *Tetrahedron Lett.* **1999**, *40*, 7185.
13. Orito, K.; Sato, S.; Suginome, H. *J. Chem. Soc., Perkin Trans. 1* **1995**, *1*, 63.
14. *Preparion of **8**:* *tert*-Butyl (*S*)-2-(4-(*S*)-*tert*-butyldimethylsilyloxy phenylmethylphenyl)-1-((*S*)-oxiran-2-yl)ethylcarbamate **(8)**. To an ice-cold solution of **7** (1.25 g, 2.41 mmol) in EtOH (30 mL) was added KOH (163 mg, 2.9 mmol), and the reaction mixture was stirred at rt for 2 h. The reaction mixture was concentrated under reduced pressure, and the residue was partitioned between EtOAc (200 mL) and H_2O (50 mL). The organic layer was washed with saturated NH_4Cl solution, H_2O, and brine, dried with Na_2SO_4, and concentrated under reduced pressure. Purification of the residue by column chromatography (20% EtOAc in hexane) gave **8** (1.11 g, 95%) as a yellow syrup: $[\alpha]_D^{25}$ 9.1 (*c* 1.0, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$): 7.38 (d, *J* = 7.4 Hz, 2H), 7.33 (m, 4H), 7.22 (t, *J* = 7.1 Hz, 1H), 7.17 (d, *J* = 8.0 Hz, 2H), 5.75 (s, 1H), 4.47 (br s, 1H), 3.70 (br s, 1H), 3.93 (m, 2H), 2.80 (m, 3H), 1.38 (s, 9H), 0.94 (s, 9H), 0.01 (s, 3H), 0.00 (s, 3H); ^{13}C NMR: 155.2, 145.1, 143.7, 135.3, 129.3, 128.1, 126.9, 126.5, 126.2, 79.5, 76.4, 53.2, 46.8, 37.2, 28.2, 25.8, 18.3, −4.8; EIMS: 506.3 [M+Na$^+$], HRMS (ESI) Calcd for $C_{28}H_{41}NSiO_4Na$: 506.2703, found 506.2698.
15. Evans, B. E.; Rittle, K. E.; Homnick, C. F.; Springer, J. P.; Hirshfield, J.; Veber, D. F. *J. Org. Chem.* **1985**, *50*, 4615.
16. Nadin, A.; Lopez, J. M. S.; Neduvelil, J. G.; Thomas, S. R. *Tetrahedron* **2001**, *57*, 1861.
17. *Preparation of **13**:* *tert*-Butyl (*S*)-1-((2*R*,4*R*)-4-benzyl-5-oxotetrahydrofuran-2-yl)-2-(4-(*S*)-hydroxyphenyl methylphenyl) ethyl carbamate **(13)**. A solution of silyl ether **12** (390 mg, 0.63 mmol) in THF (10 mL) was transferred to a polyethylene vial and Py·HF (70% HF, 1.5 mL) was dropwise added at 0 °C. The reaction mixture was stirred at rt overnight. The reaction mixture was quenched with saturated $NaHCO_3$ solution, and extracted with EtOAc (3× 50 mL). The combined organic layer was washed with brine, dried with Na_2SO_4. The residue was purified by column chromatography (50% EtOAc in hexane) to give **13** (264 mg, 83%) as a white solid: mp 59–60 °C; $[\alpha]_D^{25}$ −58.8 (*c* 0.7, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$): 7.35–7.21 (m, 10 H), 7.15 (d, *J* = 7.2 Hz, 2 H), 7.10 (d, *J* = 7.9 Hz, 2H), 5.77 (d, *J* = 2.7 Hz, 1H), 4.43 (br, 1H), 4.09 (br, 1H), 3.85 (br, 1H), 3.24 (dd, *J* = 4.0, 13.8 Hz, 1H), 2.87–2.68 (m, 4H), 2.63 (d, *J* = 3.3 Hz, 1H, OH), 2.18 (m, 1H), 1.75 (m, 1H), 1.31 (s, 9H); ^{13}C NMR: 177.6, 155.3, 143.9, 142.5, 138.4, 135.9, 129.6, 128.8, 128.7, 128.5, 128.4, 127.5, 127.3, 127.2, 126.8, 126.7, 126.5, 79.8, 78.8, 75.9, 54.3, 43.0, 36.1, 31.6, 28.2; EIMS: 524.1 [M+Na$^+$], HRMS (ESI) Calcd for $C_{31}H_{35}NO_5Na$: 524.2413, found 524.2402.
18. Fauq, A. H.; Cherif-Ziani, C.; Richelson, E. *Tetrahedron: Asymmetry* **1998**, *9*, 2333.
19. *Preparation of **14**:* *tert*-Butyl (*S*)-2-(4-benzoylphenyl)-1-((2*R*,4*R*)-4-benzyl-5-oxotetrahydrofuran-2-yl) ethyl carbamate **(14)**. To an ice-cold solution of **13** (240 mg, 0.478 mmol) in CH_2Cl_2 (10 mL) was added MnO_2 (415 mg, 4.78 mmol). The suspension was stirred at rt overnight. The reaction mixture was filtered through Celite, washed with EtOAc. The combined organic layer was concentrated, the residue was purified by column chromatography (40% EtOAc in hexane) to give **14** (185 mg, 76%) as a white solid: mp 59–60 °C; $[\alpha]_D^{25}$ −68.7 (*c* 0.7, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$): 7.74 (dd, *J* = 7.2, 11.9 Hz, 4H), 7.57 (t, *J* = 7.5 Hz, 1H), 7.45 (t, *J* = 7.7 Hz, 2H), 7.28 (m, 4 H), 7.17 (m, 3H), 4.68 (d, *J* = 9.4 Hz, 1H), 4.32 (br, 1H), 3.93 (br, 1H), 3.26 (dd, *J* = 4.1, 13.9 Hz, 1H),

3.03–2.73 (4H), 2.30 (m, 1H), 1.86 (m, 1H), 1.34 (s, 9H); ^{13}C NMR: 196.4, 177.6, 155.3, 142.3, 138.4, 137.7, 136.1, 132.5, 130.4, 130.0, 129.5, 128.9, 128.8, 128.4, 126.9, 80.0, 79.2, 54.6, 42.5, 36.5, 36.2, 31.4, 28.3; EIMS: 522.2 [M+Na$^+$], HRMS (ESI) Calcd for $C_{31}H_{33}NO_5Na$: 522.2256, found 522.2250.

20. Kokotos, G.; Padrón, J. M.; Martín, T.; Gibbons, W. A.; Martín, V. S. *J. Org. Chem.* **1998**, *63*, 3741.
21. *Preparation of* ***2***: {1*S*-Benzoyl-phenyl-4*R*-[1-(1*S*-methyl-oxycarbonyl-2-phenyl-ethylcarbamoyl)-3-(1*S*)-methyl-butylcarbamoyl]-2*R*-hydroxy-5-phenyl-pentyl}-carbamic acid *tert*-butyl ester (**2**). A solution of **15** (20 mg, 0.0316 mmol), Leu-Phe-OMe (16 mg, 0.054 mmol), 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (11 mg, 0.057 mmol) and 1-hydroxylbenzotriazole (8 mg, 0.057 mmol) in DMF (2 mL) was stirred at rt. The reaction mixture was diluted with EtOAc, and the organic layer was washed with aqueous citric acid, saturated $NaHCO_3$ solution, and brine, dried with Na_2SO_4, and concentrated under reduced pressure. Purification of the residue by column chromatography (50% EtOAc in hexane) gave **16** (20 mg, 70%) as a white foam: ^{1}H NMR (400 MHz, $CDCl_3$): 7.76 (d, *J* = 7.1 Hz, 2H), 7.68 (d, *J* = 8.0 Hz, 2H), 7.59 (t, *J* = 7.4 Hz, 1H), 7.48 (d, *J* = 7.7 Hz, 2H), 7.27 (m, 4H), 7.20 (m, 4H), 7.11 (d, *J* = 7.0 Hz, 2H), 6.96 (d, *J* = 8.0 Hz, 2H), 6.49 (t, *J* = 8.05 Hz, 2H), 4.78 (m, 1H), 4.55 (d, *J* = 8.0 Hz, 1H), 4.43 (m, 1H), 3.84 (m, 1H), 3.69 (s, 3H), 3.65 (m, 1H), 3.12–3.00 (m, 2H), 2.65 (dd, *J* = 5.7, 13.6 Hz, 1H), 2.59 (m, 1H), 2.45 (m, 1H), 1.87 (m, 1H), 1.79–1.58 (m, 3H), 1.37 (s, 9H), 0.91 (s, 9H), 0.86 (dd, *J* = 6.7, 11.1 Hz, 6H), 0.07 (s, 3H), 0.06 (s, 3H); ^{13}C NMR: 196.6, 174.9, 171.9, 171.4, 155.7, 144.2, 139.5, 137.9, 136.1, 135.9, 132.4, 130.5, 130.2, 129.5, 129.4, 129.2, 128.8, 128.8, 128.5, 127.3, 126.7, 79.9, 72.5, 54.9, 53.5, 52.5, 51.9, 45.5, 40.8, 38.9, 38.1, 36.0, 33.9, 28.6, 26.1, 25.0, 22.7, 22.6, 18.2, −4.2, −4.7; EIMS: 928.2 [M+Na$^+$], Calcd for $C_{53}H_{71}N_3O_8Si$: 905.50.To an ice-cold solution of **16** (20 mg, 0.022 mmol) in THF (2 mL) was added a solution of TBAF in THF (1.0 M, 0.2 mL). The reaction mixture was stirred at rt overnight. The reaction mixture was diluted with EtOAc and washed with citric acid and brine, dried with Na_2SO_4, and concentrated under reduced pressure. Purification of the residue by column chromatography (5% MeOH in CH_2Cl_2) gave **2** (17 mg, 85%) as a white solid: mp 147–148 °C; $[\alpha]_D^{25}$ −12.1 (*c* 0.7, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$, a few drop of d_4-MeOH): 7.77 (d, *J* = 7.3 Hz, 2H), 7.72 (d, *J* = 8.1 Hz, 2H), 7.58 (t, *J* = 7.4 Hz, 1H), 7.47 (t, *J* = 7.7 Hz, 2H), 7.33–7.17 (m, 6H), 7.13 (t, *J* = 7.5 Hz, 2H), 6.69 (d, *J* = 7.1 Hz, 1H), 6.17 (d, *J* = 7.2 Hz, 1H), 4.78 (q, *J* = 7.3 Hz, 1H), 4.71 (d, *J* = 8.5 Hz, 1H), 4.26 (q, *J* = 7.3 Hz, 1H), 3.70 (m+s, 6H), 3.09 (m, 2H), 2.93 (dd, *J* = 7.2, 13.5 Hz, 1H), 2.86–2.62 (m, 4H), 1.83 (m, 1H), 1.74 (m, 1H), 1.56 (m, 1H), 1.46 (m, 2H), 1.39 (s, 9H), 0.84 (dd, *J* = 6.3, 8.5 Hz, 6H); ^{13}C NMR: 196.6, 175.8, 172.2, 171.8, 156.2, 143.6, 139.0, 137.9, 136.0, 135.9, 132.5, 130.5, 130.1, 129.5, 129.16, 129.0, 128.8, 128.8, 128.7, 128.4, 127.3, 126.8, 79.9, 73.3, 56.6, 53.5, 52.5, 47.0, 40.8, 39.3, 38.1, 35.7, 35.5, 29.9, 28.4, 26.0, 24.8, 23.0, 22.1; HRMS (ESI) Calcd for $C_{47}H_{58}N_3O_8$: 792.4224, found 792.4229.
22. Li, Y. M.; Lai, M. T.; Xu, M.; Huang, Q.; DiMuzio-Mower, J.; Sardana, M. K.; Shi, X. P.; Yin, K. C.; Shafer, J. A.; Gardell, S. J. *Proc. Natl. Acad. Sci. U.S.A.* **2000**, *97*, 6138.