

## Structure-Based Design, Synthesis, and Memapsin 2 (BACE) Inhibitory Activity of Carbocyclic and Heterocyclic Peptidomimetics

Stephen Hanessian, Hongying Yun, Yihua Hou, Gaoqiang Yang, Malken Bayrakdarian, Eric Therrien, Nicolas Moitessier, Silvio Roggo, Siem Veenstra, Marina Tintelnot-Blomley, Jean-Michel Rondeau, Christian Ostermeier, Andr Strauss, Paul Ramage, Paolo Paganetti, Ulf Neumann, and Claudia Betschart

*J. Med. Chem.*, **2005**, 48 (16), 5175-5190 • DOI: 10.1021/jm050142+ • Publication Date (Web): 15 July 2005

Downloaded from <http://pubs.acs.org> on March 28, 2009

### More About This Article

Additional resources and features associated with this article are available within the HTML version:

- Supporting Information
- Links to the 9 articles that cite this article, as of the time of this article download
- Access to high resolution figures
- Links to articles and content related to this article
- Copyright permission to reproduce figures and/or text from this article

[View the Full Text HTML](#)

## Structure-Based Design, Synthesis, and Memapsin 2 (BACE) Inhibitory Activity of Carbocyclic and Heterocyclic Peptidomimetics

Stephen Hanessian,<sup>\*,†</sup> Hongying Yun,<sup>†</sup> Yihua Hou,<sup>†</sup> Gaoqiang Yang,<sup>†</sup> Malken Bayraktarian,<sup>†</sup> Eric Therrien,<sup>†</sup> Nicolas Moitessier,<sup>§</sup> Silvio Roggo,<sup>‡</sup> Siem Veenstra,<sup>‡</sup> Marina Tintelnot-Blomley,<sup>‡</sup> Jean-Michel Rondeau,<sup>‡</sup> Christian Ostermeier,<sup>‡</sup> André Strauss,<sup>‡</sup> Paul Ramage,<sup>‡</sup> Paolo Paganetti,<sup>‡</sup> Ulf Neumann,<sup>‡</sup> and Claudia Betschart<sup>‡</sup>

Department of Chemistry, Université de Montréal, C. P. 6128, Succursale Centre-Ville, Montréal, P. Q., Canada, H3C 3J7, and Novartis Institutes for BioMedical Research, Novartis Pharma AG, Postfach, CH-4002 Basel, Switzerland

Received February 14, 2005

Molecular modeling based on the X-ray crystal structure of the Tang-Ghosh heptapeptide inhibitor **1** (OM99-2) of BACE led to the design and synthesis of a series of constrained P<sub>1</sub>' analogues. A cyclopentane ring was incorporated in **1** spanning the P<sub>1</sub>' Ala methyl group and the adjacent methylene carbon atom of the chain. Progressive truncation at the P<sub>2</sub>'–P<sub>4</sub>' sites led to a potent truncated analogue **5** with good selectivity over Cathepsin D. Using the same backbone replacement concept, a series of cyclopentane, cyclopentanone, tetrahydrofuran, pyrrolidine, and pyrrolidinone analogues were synthesized with considerable variation at the P and P' sites. The cyclopentanone and 2-pyrrolidinone analogues **45** and **57** showed low nM BACE inhibition. X-ray cocrystal structures of two analogues **5** and **45** revealed excellent convergence with the original inhibitor **1** structure while providing new insights into other interactions which could be exploited for future modifications.

### Introduction

A major histopathological hallmark of Alzheimer's disease is extracellular plaques of deposited  $\beta$ -amyloid (A $\beta$ ) in the brain. There is compelling evidence that  $\beta$ -amyloid (A $\beta$ ) plays a prominent role in the pathogenesis of Alzheimer's disease, either as extracellular or intracellular deposits, or as small soluble aggregates.<sup>1</sup> Reducing the production of A $\beta$  or increasing its clearance therefore are attractive strategies for the treatment of Alzheimer's disease.<sup>2–6</sup> The generation of A $\beta$  depends on two consecutive enzymatic steps. The endoproteolytic cleavage of  $\beta$ -amyloid precursor protein (APP) is initiated by the membrane-bound aspartic protease BACE (Asp-2, memapsin 2), which exhibits all the characteristics assigned to the  $\beta$ -secretase activity.<sup>7–11</sup> BACE releases the large ectodomain of APP (APPs $\beta$ ) and produces a membrane-bound C99 fragment. This latter is then the substrate of  $\gamma$ -secretase for the release of A $\beta$ .<sup>12</sup>

BACE is considered as an ideal target for small molecule inhibitors, with the ultimate aim of developing an effective therapeutic agent.<sup>13–16</sup> The first crystal structure of the extracellular domain of BACE complexed with a synthetic heptapeptide inhibitor (**1**) harboring an unnatural hydroxyethylene spacer as a transition state mimic was reported by Hong, Ghosh, and Tang.<sup>17</sup> This disclosure, followed by additional crystal structures of BACE with<sup>18–22</sup> and without<sup>19</sup> complexed inhibitor, as well as studies on the substrate specificity<sup>22–24</sup> provide valuable insights for structure-based design of inhibitors. Indeed, Ghosh and Tang<sup>21,25,26</sup>

have subsequently reported the synthesis of low nM inhibitors with related structures and reduced molecular weights by appropriate truncation and functional modification of the original lead structure. Reports from other laboratories<sup>27</sup> on inhibitors incorporating the hydroxyethylene<sup>28–31</sup> transition state mimetic group followed. Further peptidomimetic isosteres that have successfully been implemented for inhibitors of BACE are statine,<sup>32–34</sup> bis-statine,<sup>35</sup> phenylnorstatine,<sup>36,37</sup> hydroxymethylcarbonyl,<sup>38</sup> and hydroxyethylamine.<sup>39–40</sup>

Clearly, the inhibition of BACE relying on conceptually novel approaches to structure-based design presents a major intellectual and operational challenge. Herein, we report our results on the design and synthesis of novel nM inhibitors of BACE. Molecular modeling based on the X-ray structure of **1** with BACE<sup>17</sup> led us to a subtle modification at the P<sub>1</sub>' Ala position with the introduction of a fused cyclopentane ring (Figure 1). Indeed, an energy minimized CP (cyclopentano) analogue of **1**, in which the methyl group of the P<sub>1</sub>' Ala and the adjacent methylene carbon atom were joined as a constrained entity, was well matched with the X-ray conformation of **1**. Our first objective was to ascertain the viability of the CP as a backbone replacement for a segment of the hydroxyethylene isostere. As a proof of concept, we prepared three analogues, **3–5**, of **1** incorporating a CP ring, with progressive truncation at the P<sub>3</sub>'–P<sub>4</sub>' C-terminus (Figure 1). We also prepared a truncated acyclic analogue **2** as a control for compound **5**.

### Results and Discussion

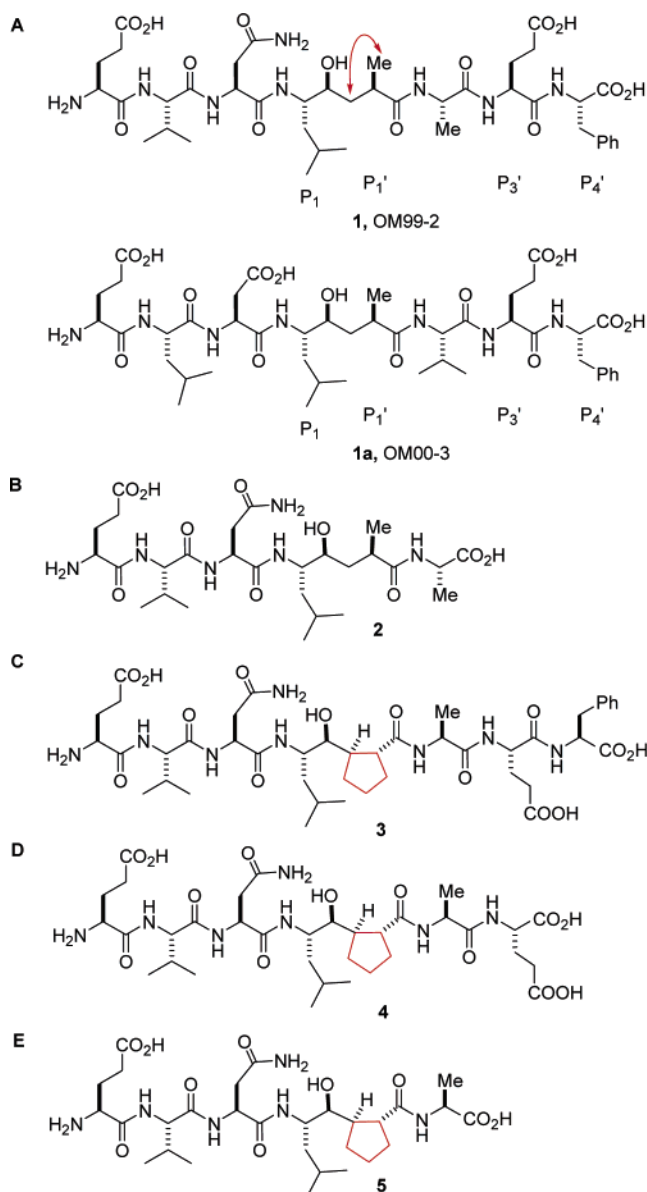
**Chemistry. CP/1 and P' Truncation.** *N*-Boc L-leucinal was converted to the acetylenic ester **6** in analogy to a known procedure<sup>41</sup> albeit in low yields (25–30%). However, using the less basic and more oxophilic cerium methyl propiolate<sup>42</sup> resulted in a significant

\* To whom correspondence should be addressed. Phone: 514-343-6738. Fax: 514-343-5728. E-mail: stephen.hanessian@umontreal.ca.

<sup>†</sup> Université de Montréal.

<sup>§</sup> Present address: McGill University, 801 Sherbrooke St. W., Montréal, Québec H3A 2K6, Canada.

<sup>‡</sup> Novartis Pharma AG.



**Figure 1.** A. Structure of inhibitors **1** and **1a** (OM99-2, OM00-3) and proposed constrained  $P_1'$  region; B.  $P_3'$ ,  $P_4'$  truncated analogue of **1**; C. Constrained CP (cyclopentano) backbone modification of **1**; D, E.  $P_3'$  and  $P_4'$  truncated constrained CP modifications.

improvement, affording **6** in 72% yield as a 5:1 mixture of isomers (Scheme 1). Conversion to the mixed acetal **7** and Lindlar reduction afforded the *cis*-ester **8** which was subjected to Pd-catalyzed cycloannulation with 2-(acetoxymethyl)-3-allyltrimethylsilane **9**, according to Trost's method<sup>43</sup> to give the corresponding *exo*-methylene CP adduct as a mixture of epimers. Treatment with sodium methoxide in MeOH resulted in complete epimerization to the enantiopure *trans*-isomer **10** in excellent overall yield. Oxidative cleavage with sodium meta-periodate and catalytic osmium tetroxide led to the cyclopentanone analogue **11** which was deoxygenated by transformation to the tosylhydrazone and treatment with catecholborane<sup>44</sup> to give the core CP intermediate **12** in excellent overall yield. The structural and configurational confirmation of **12** was ascertained from a single-crystal X-ray analysis.

We found it more practical to introduce the  $P_2'$ – $P_4'$  subunits first, then to elongate the chain on the P sites

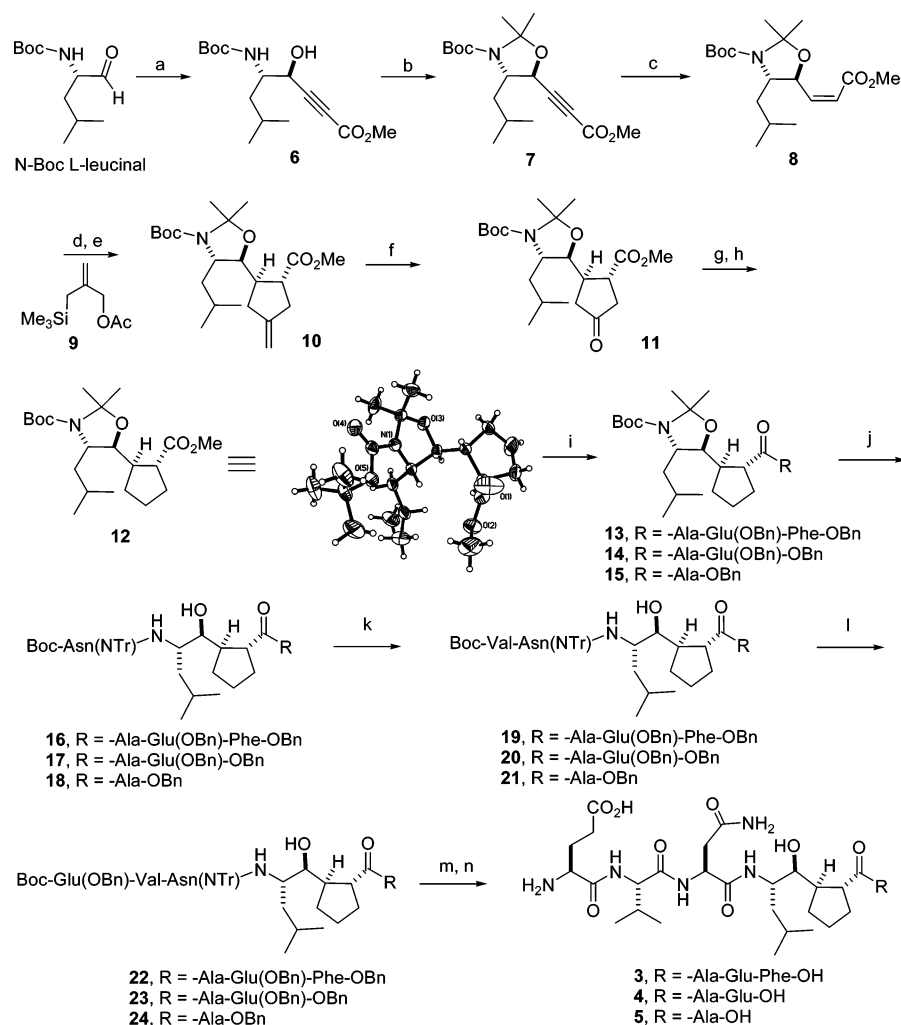
systematically. This protocol of stepwise addition of protected amino acids gave more consistent results and better yields compared to a block coupling with di- or tripeptides. Thus, **12** was hydrolyzed to the carboxylic acid, which was coupled with the respective peptidic precursors to give **13**–**15** as benzyl esters in good to excellent yields. Removal of the *N*-Boc and acetal groups by acid hydrolysis provided the corresponding amino alcohols which were individually coupled with Boc-Asn(*N*Tr)-OH to give **16**–**18**, respectively. Cleavage of the *N*-Boc group and coupling with Boc-Val-OH led to the respective products **19**–**21**, which were further elaborated to the protected full length CP oligopeptides **22**–**24**. Treatment with TFA, followed by catalytic hydrogenation afforded the desired constrained  $P_1'$ – $P_4'$  analogue **3** of **1** as well as its  $P_3'$ – $P_4'$  truncated congeners **4** and **5** (Figure 1, Scheme 1). For comparison of biological data we also prepared the  $P_3'$ – $P_4'$  truncated peptide analogue **2**.<sup>45</sup>

**$P_2$ – $P_4$  Modification and  $P'$  Truncation of CP/1.** We pursued a new series of truncated analogues maintaining the CP constraint and the  $P_2'$  Ala-OH terminus, while changing the  $P_2$  Asn residue to Met. Tang and Ghosh<sup>26</sup> have shown that this replacement in a shortened analogue of **1** had a small beneficial effect, possibly also diminishing the hydrophilic nature of the original inhibitor.

Thus, the common CP intermediate **10** was transformed to the corresponding Ala-OCH<sub>2</sub>CCl<sub>3</sub> ester **25**, followed by liberation of the amine and coupling with Boc-Met-OH to give **28** (Scheme 2). Further chain elongation by coupling with Ac-Leu-OH afforded **31** and eventually the free acid **34**. The keto and deoxy CP analogues **35** and **36** were prepared following the same protocol starting with **11** and **12** respectively as shown in Scheme 2. Sequential single amino acid coupling was found to minimize racemization compared to alternative dipeptide coupling strategies which required longer reaction times at room temperature. To assess the importance of the  $P_2'$  amino acid residue, we also synthesized the CP Val-OH analogue **40** (Scheme 2).

Our intention was also to diminish the peptidic character of the CP series. Toward this end, we replaced the  $P_3'$ – $P_4'$  Glu-Phe residues by a simple butyramide moiety, while maintaining the new prototypical CP structures represented by **45**, **46**, and **47** (Scheme 3). The intermediate **11** was transformed to the corresponding Val-butylamide derivative **41**. Cleavage of the *N*-Boc and acetal groups and coupling with Boc-Met-OH gave **43** which was cleaved to the amine and further extended with Ac-LeuOH to give the intended oxo-CP target **45**. Following a similar procedure, the CP intermediate **12** was elaborated via **42** and **44** to give the  $P_2'$  Ala butyramide analogue **46**. The CP Val butyramide **47** was prepared from precursor **40**. To assess the importance of the  $P_2'$  amino acid residues in this series, and the implication of a constrained CP subunit, we also prepared the truncated *n*-butyramide analogue **50**, as well as the acyclic analogue **51**<sup>45</sup> (Scheme 3).

In an effort to probe the carbocyclic CP space in the  $P'$  and  $P_2$ – $P_3$  truncated analogues shown in Scheme 3, we also prepared four heterocyclic variants.<sup>45</sup> Thus, the pyrrolidine **53** and lactam analogues **55** and **57** as well as tetrahydrofuran analogue **59** were prepared by

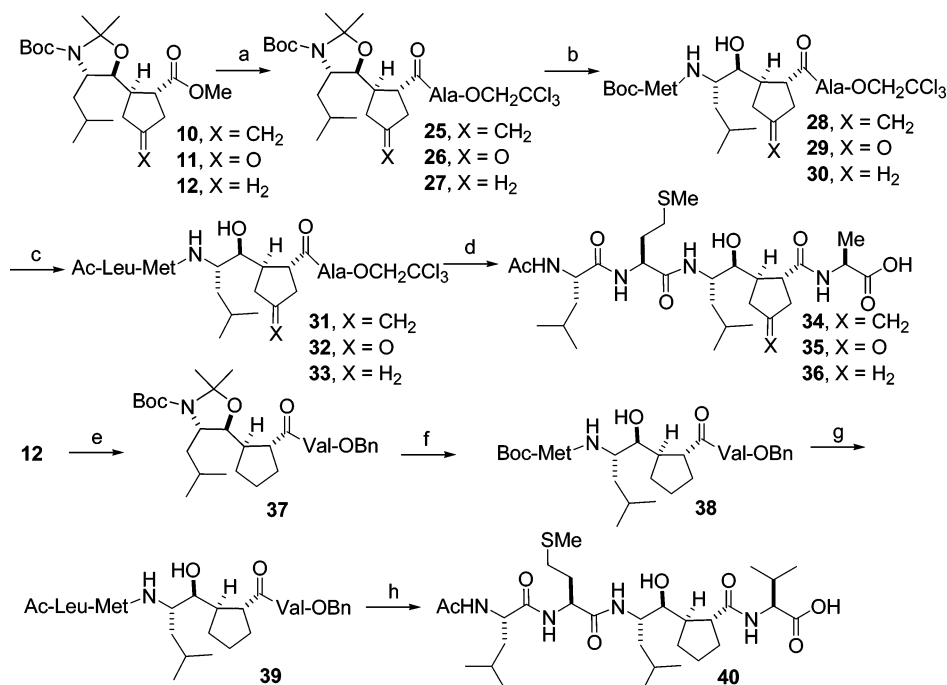
Scheme 1<sup>a</sup>

(a) i: LDA, methyl propiolate, THF,  $-78\text{ }^{\circ}\text{C}$ ; ii:  $\text{CeCl}_3$ , THF,  $-78\text{ }^{\circ}\text{C}$ ; iii: *N*-Boc-L-leucinal,  $-78\text{ }^{\circ}\text{C}$ , 72%; (b) dimethoxypropane, TsOH,  $\text{Me}_2\text{CO}$ , 64% (**6**, major isomer); (c)  $\text{H}_2$ , Lindlar's catalyst, benzene, 99%; (d) toluene,  $\text{Pd}(\text{OAc})_2$ ,  $\text{P}(\text{O}i\text{Pr})_3$ ,  $85\text{ }^{\circ}\text{C}$ , 90%; (e) NaOMe, MeOH, 99%; (f)  $\text{NaIO}_4$ ,  $\text{OsO}_4$  cat., THF/ $\text{H}_2\text{O}$ , 90%; (g)  $\text{H}_2\text{N-NHTs}$ ,  $\text{Na}_2\text{SO}_4$ , MeOH, 99%; (h) catecholborane,  $\text{CHCl}_3$ , then  $\text{NaOAc}\cdot\text{H}_2\text{O}$ , 68%; (i) i: KOH, THF/ $\text{H}_2\text{O}$ ; ii: PyBOP, DIPEA,  $\text{CH}_2\text{Cl}_2$ , H-Ala-Glu(OBn)-Phe-OBn, 57% (**13**, two steps), H-Ala-Glu(OBn)-OBn, 76% (**14**, two steps), H-Ala-OBn, 99% (**15**, two steps); (j) i: TFA,  $\text{CH}_2\text{Cl}_2$ , then satd  $\text{NaHCO}_3$ ; ii: PyBOP, DIPEA,  $\text{CH}_2\text{Cl}_2$ , Boc-Asn(NTr)-OH, 74% (**16**, two steps), 66% (**17**, two steps), 67% (**18**, two steps); (k) i: HCl, then satd  $\text{NaHCO}_3$ ; ii: PyBOP, DIPEA,  $\text{CH}_2\text{Cl}_2$ , Boc-Val-OH, 59% (**19**, two steps), 85% (**20**, two steps), 78% (**21**, two steps); (l) i: HCl, then satd  $\text{NaHCO}_3$ ; ii: PyBOP, DIPEA,  $\text{CH}_2\text{Cl}_2$ , Boc-Glu(OBn)-OH, 77% (**22**, two steps), 56% (**23**, two steps), 78% (**24**, two steps); (m) TFA,  $\text{CH}_2\text{Cl}_2$ ; (n)  $\text{H}_2$ , Pd/C, MeOH, 30% (**3**, two steps), 54% (**4**, two steps), 60% (**5**, two steps).

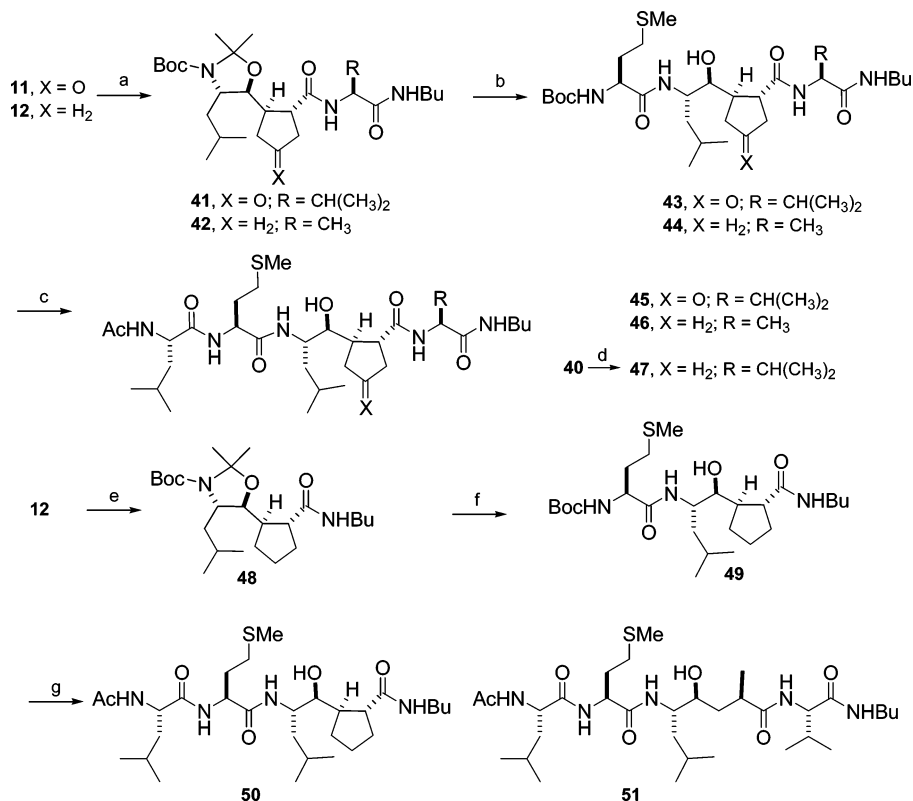
standard coupling with the appropriate amino acid partners as described for the CP analogues (Scheme 4).

**Biological Data and Structure-Activity Relationships. Truncation of the P' Side.** The suitability of the CP moiety as a rigid dipeptide isostere of the known inhibitor **1** and its P'-truncated variants was validated since analogues **3–5** maintained substantial inhibitory activity as shown in Table 1. The full length analogue **3** has an  $\text{IC}_{50}$  of 16 nM, about an order of magnitude weaker than **1**, but, remarkably, the P<sub>4</sub>'-P<sub>3</sub>' truncated CP compound **5** is only 3-fold less potent than its counterpart **2**. While the activity on BACE hardly changed with stepwise elimination of amino acids from the P' side, the Cathepsin D (CathD) activity decreased sharply. Hence, a substantial increase in selectivity toward BACE is afforded by the combination of the P' truncation with the CP modification, while maintaining decent potency. The binding of the P<sub>3</sub>' Glu and P<sub>4</sub>' Phe groups to BACE has been shown to be affected by the nature of the P<sub>2</sub>' residue. In the OM00-3

(P<sub>2</sub>' = Val) crystal structure<sup>18</sup> the P<sub>3</sub>' Glu and P<sub>4</sub>' Phe groups make clearly defined interactions with the enzyme. In comparison, these residues are not well defined in the OM99-2 (P<sub>2</sub>' = Ala) complex, suggesting that they are relatively mobile. This observation can be interpreted as being the result of a stabilization of the position of the P<sub>3</sub>' and P<sub>4</sub>' residues due to the more tightly bound P<sub>2</sub>' Val. Compounds **3–5** have an alanine in P<sub>2</sub>', and the contribution of the P<sub>3</sub>'-P<sub>4</sub>' groups to binding is most probably minimal. Moreover, with the P<sub>3</sub>' AlaGlu-OH analogue **4**, the loss of the P<sub>4</sub>' Phe interactions is likely compensated by a favorable electrostatic interaction between the terminal carboxylate group and the guanidinium moiety of Arg128. The observed 60-fold decrease of the CathD activity in going from **3** to **4** indicates a stronger contribution of the P<sub>4</sub>' groups to CathD binding, which is not compensated by new interactions. Since Arg128 is substituted by a valine in Cath D,<sup>46</sup> the electrostatic interaction proposed hereabove for BACE cannot take place.

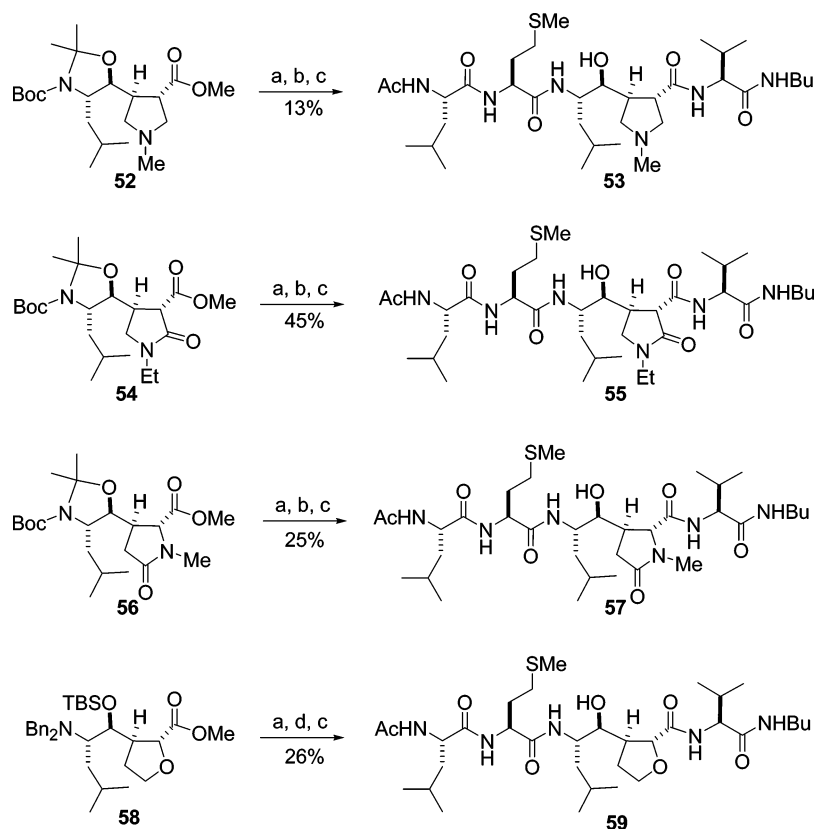
Scheme 2<sup>a</sup>

(a) i: NaOH, THF/H<sub>2</sub>O; ii: PyBOP, DIPEA, CH<sub>2</sub>Cl<sub>2</sub>, H-Ala-OCH<sub>2</sub>CCl<sub>3</sub>, 75% (**25**, two steps), 75% (**26**, two steps), 71% (**27**, two steps); (b) i: HCl, then satd NaHCO<sub>3</sub>; ii: PyBOP, DIPEA, CH<sub>2</sub>Cl<sub>2</sub>, Boc-Met-OH, 33% (**28**, two steps), 75% (**29**, two steps), 75% (**30**, two steps); (c) i: HCl, then satd NaHCO<sub>3</sub>; ii: PyBOP, DIPEA, CH<sub>2</sub>Cl<sub>2</sub>, Ac-Leu-OH, 44% (**31**, two steps), 76% (**32**, two steps), 52% (**33**, two steps); (d) Zn, KH<sub>2</sub>PO<sub>4</sub>, 71% (**34**), 77% (**35**), 91% (**36**); (e) i: KOH, THF/H<sub>2</sub>O; ii: 1 N HCl; iii: PyBOP, DIPEA, CH<sub>2</sub>Cl<sub>2</sub>, H-Val-OBn, 98% (two steps); (f) i: TFA, CH<sub>2</sub>Cl<sub>2</sub>, then satd NaHCO<sub>3</sub>; ii: PyBOP, DIPEA, CH<sub>2</sub>Cl<sub>2</sub>, Boc-Met-OH, 68% (two steps); (g) i: HCl, then satd NaHCO<sub>3</sub>; ii: EDC, HOBT, CH<sub>2</sub>Cl<sub>2</sub>/H<sub>2</sub>O, Ac-Leu-OH, 58% (two steps); (h) 10% HCO<sub>2</sub>H, MeOH, Pd Black, 74%.

Scheme 3<sup>a</sup>

(a) i: NaOH, THF/H<sub>2</sub>O; ii: PyBOP, DIPEA, CH<sub>2</sub>Cl<sub>2</sub>, H-Val-NHBu, 95%, (**41**, two steps), H-Ala-NHBu 75%, (**42**, two steps); (b) i: HCl, then satd NaHCO<sub>3</sub>; ii: PyBOP, DIPEA, CH<sub>2</sub>Cl<sub>2</sub>, Boc-Met-OH, 72% (**43**, two steps), 75% (**44**, two steps); (c) i: HCl, then satd NaHCO<sub>3</sub>; ii: EDC, HOBT, CH<sub>2</sub>Cl<sub>2</sub>/H<sub>2</sub>O, Ac-Leu-OH, 78% (**45**, two steps), 78% (**46**, two steps); (d) PyBOP, NMM, DMF, BuNH<sub>2</sub>, 20%; (e) i: NaOH, MeOH/H<sub>2</sub>O, then satd NH<sub>4</sub>Cl; ii: EDC, HOBT, DMAP, BuNH<sub>2</sub>, CH<sub>2</sub>Cl<sub>2</sub>, 89% (two steps); (f) i: TFA, CH<sub>2</sub>Cl<sub>2</sub>, then satd NaHCO<sub>3</sub>; ii: PyBOP, DIPEA, CH<sub>2</sub>Cl<sub>2</sub>, Boc-Met-OH, 74% (two steps); (g) i: HCl, dioxane, then satd NaHCO<sub>3</sub>; ii: EDC, HOBT, CH<sub>2</sub>Cl<sub>2</sub>/H<sub>2</sub>O, Ac-Leu-OH, 54% (**50**, two steps).



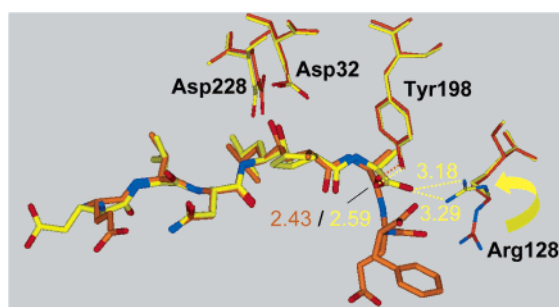
Scheme 4<sup>a</sup>

(a) i: NaOH, MeOH–H<sub>2</sub>O, 65 °C, and then 1 N HCl; ii: H-Val-NHBu, PyBOP, DIPEA, CH<sub>2</sub>Cl<sub>2</sub>; (b) i: TFA, DCM, and then NaHCO<sub>3</sub>; ii: Boc-Met-OH, PyBOP, DIPEA, CH<sub>2</sub>Cl<sub>2</sub>; (c) i: TMSI, CH<sub>2</sub>Cl<sub>2</sub>; and then Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub>, NaHCO<sub>3</sub>; ii: Ac-Leu-OH, EDC, HOBt, DCM/H<sub>2</sub>O; (d) i: Pd black, HCOOH/MeOH; ii: Boc<sub>2</sub>O, NaHCO<sub>3</sub>, MeOH; iii: TBAF, THF.

**Table 1.** Truncation of the P' Side



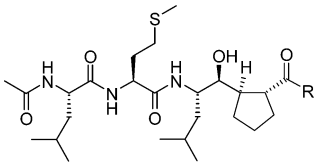
compd	R =	IC <sub>50</sub> BACE, μM	IC <sub>50</sub> CathD, μM	compd	R =	IC <sub>50</sub> BACE, μM	IC <sub>50</sub> CathD, μM
1	AlaGluPheOH	0.0006	0.006	3	AlaGluPheOH	0.016	<0.01 (90% @ 0.01 μM)
2	AlaOH	0.012	0.098	4	AlaGluOH	0.040	0.060
				5	AlaOH	0.039	6.0



**Figure 2.** Overlay of cocrystal structures of **5** (yellow) and **1**<sup>17</sup> (orange) with interactions discussed in text (distances in Å).

The P<sub>2</sub>' Ala-OH analogue **5** afforded suitable cocrystals with the enzyme that were amenable to an X-ray analysis at 2.25 Å resolution (Figure 2). The superposition of the cocrystal structures of **5** and **1** with the enzyme showed good agreement along the peptidic

backbone encompassed by the P<sub>2</sub>'–P<sub>2</sub> residues, confirming that the rigid CP moiety does not alter substantially the positioning of the P<sub>2</sub>' alanine and of the modified transition state mimic. Small positional shifts of 0.6–0.8 Å are observed for P<sub>1</sub>–P<sub>2</sub>' atoms of analogue **5** relative to the corresponding **1** atoms. The distance between the catalytic aspartic acid residues and the hydroxyethylene isostere are similar in the OM99-2 complex (2.4 Å to Asp32 Oδ1, 2.8 Å to Asp228 Oδ2) and in the complex with analogue **5** (2.6 Å to Asp32 Oδ1, 2.6 Å to Asp228 Oδ2). A remarkable feature of the X-ray of **5** are the two specific interactions of the Ala carboxylate with Tyr198 and Arg128. In the OM99-2 complex, the carbonyl oxygen atom of the P<sub>3</sub>'–P<sub>4</sub>' amide bond is hydrogen-bonded to the hydroxyl of Tyr198. In the complex with analogue **5**, one of the carboxylate oxygens is mimicking this interaction, while in addition, the second oxygen forms a tight interaction with Arg128. This is possible due to a more than 3 Å move of the Arg

**Table 2.** SAR in P<sub>2</sub>' : Further Truncation on P- and P'-Side


compd	R =	IC <sub>50</sub> BACE, μM	IC <sub>50</sub> CathD, μM
<b>36</b>	AlaOH	0.600	0.090
<b>40</b>	ValOH	0.190	0.025
<b>46</b>	AlaNHBu	0.185	<0.01 (95% @ 0.01 μM)
<b>47</b>	ValNHBu	0.025	<0.01 (100% @ 0.01 μM)
<b>50</b>	NHBu	1.82	0.060

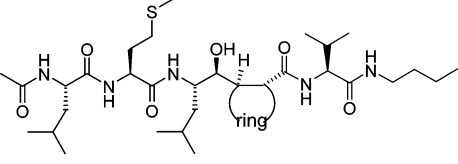
side-chain when compared to its position in the structure of **1** (Figure 2).







Thus, the X-ray data show that the loss of the P<sub>3</sub>' group in the Ala-OH analogue **5** can be compensated by new polar and electrostatic interactions between Tyr198, Arg128 and the free carboxylate of the inhibitor, explaining the relatively small effect of the truncation on the inhibitory potency. As already pointed out previously, the loss of activity of compound **5** against CathD can, at least in part, be ascribed to the Arg128 to Val substitution in CathD.<sup>46</sup>

**SAR in P<sub>2</sub>'.** On the basis of the subsite specificity published by Turner<sup>23</sup> and in accordance with published SAR data,<sup>26</sup> one can assume that it is beneficial to replace P<sub>3</sub> Val by Leu, and that the P<sub>2</sub> Asn can be replaced by the less hydrophilic Met without loss of activity. The mentioned published data also revealed that replacement of P<sub>4</sub> Glu in **1** by a hydrogen resulted in only about 20-fold loss of activity, suggesting that the P<sub>4</sub> residue can be truncated without too much penalty. Indeed, exchanging the P<sub>4</sub>-P<sub>2</sub> residues in **5** from GluValAsn to AcLeuMet as in **36**, led to only about 15-times lower inhibitory activity than **5**. However, as expected due to the more hydrophobic S<sub>2</sub> pocket in CathD,<sup>46</sup> the inhibitory activity for CathD was increased. Further studies were pursued using **36** as reference compound. Thus, in accordance with the results of Turner<sup>23</sup> replacement of the P<sub>2</sub>'-Ala by Val led to improved activity (compare **36** versus **40**, **46** versus **47**). A Val residue was shown to fill the S<sub>2</sub>' pocket better than Ala.<sup>18</sup> Capping the carboxylate as a butylamide further improved activity on BACE (compare **36** versus **46**, and **40** versus **47**). Deletion of P<sub>2</sub>' as in **50** resulted in only weak activity, while additional shortening of **50** on the N-terminal side going from Ac-Leu to 4-methylpentoyl (as a mimic of Leu) led to a compound without activity. Therefore, **47** was chosen as a suitable reference for an SAR study on the cyclopentyl peptidomimetic moiety.

#### SAR of the Cyclopentyl Peptidomimetic Moiety.

The water-filled S<sub>1</sub>' pocket<sup>17</sup> suggested an extension of the CP concept toward more polar analogues. An obvious example is the cyclopentanone **45** (Table 3). From a study of a cocrystal X-ray structure, the ring carbonyl of **45** forms a network of hydrogen bonds via water molecules to several residues lining the hydrophilic S<sub>1</sub>' pocket (see discussion below). This modified pattern of water-mediated interactions contributes to only a small improvement of the activity on BACE compared to **5**. On the other hand, the activity on CathD was decreased

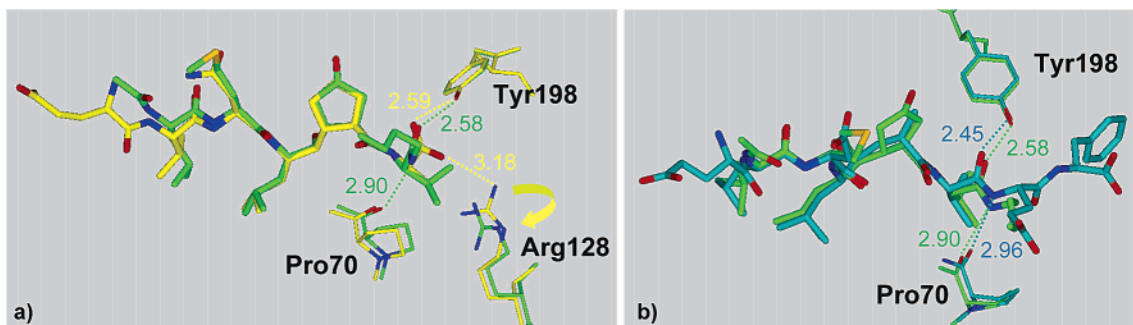
**Table 3.** SAR of Ring Element: Val-NHBu Derivatives


compd	ring	IC <sub>50</sub> BACE, μM	IC <sub>50</sub> CathD, μM
<b>47</b>		0.025	<0.01 (100% @ 0.01 μM)
<b>45</b>		0.010	0.056
<b>53</b>		3.44	1.23
<b>55</b>		3.35	0.13
<b>57</b>		<0.01 (79% @ 0.01 μM)	0.070
<b>59</b>		9.56	0.28

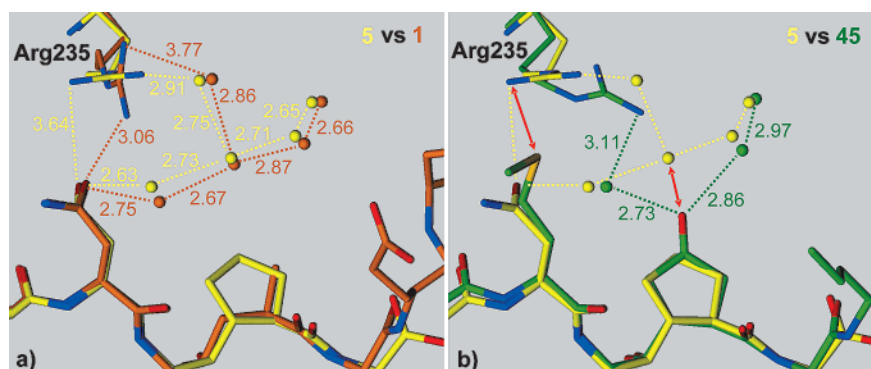
(Table 3), which might be explained by two facts. First, the S<sub>1</sub>' pocket in CathD is more lipophilic than in BACE (Arg235 in BACE corresponds to Val238 in CathD), hence less favorable to accommodate a polar carbonyl group. Second, the Val332 in BACE corresponds to Ile320 in CathD, resulting in a smaller pocket possibly leading to unfavorable steric interactions.

The cyclopentanone derivative **45** was cocrystallized in BACE and an X-ray structure was solved to 2.05 Å resolution. Overall, the binding conformation of **45** corresponded well with the binding conformation of **5** (Figure 3a). In particular, the positioning of the P<sub>1</sub> group and the modified transition state isostere in **45** is, within experimental error, identical in the two complexes. As mentioned above, the P<sub>2</sub>' residue has been shown to influence the binding of the P<sub>3</sub>' and P<sub>4</sub>' side chains. A structural comparison with the OM00-3<sup>18</sup> (P<sub>2</sub>' = Val) complex is therefore interesting (Figure 3b). It shows that the P<sub>2</sub>' Val binding is not affected by the rigid CP modification, and that the butylamide cap takes the place of the P<sub>3</sub>' Glu of OM00-3. The hydrogen-bonded interaction to Tyr198 is maintained in analogue **45**. Furthermore, the nitrogen atom of the butylamide is H-bonded to the carbonyl oxygen of Pro70 and its alkyl chain is in van der Waals contact to Pro70, Tyr71 and Thr72 of the enzyme flap, thereby contributing to the stabilization of the closed conformation of the enzyme.

An analysis of the solvent structure in the S<sub>1</sub>' pocket, as observed in the published complexes with OM99-2 and OM00-3<sup>18</sup> and in this study with compounds **5** and **45**, shows that it is affected by the nature of the groups in position P<sub>2</sub> and P<sub>1</sub>' of the inhibitors, and by Arg235,



**Figure 3.** (a) Overlay of cocrystal structures of **5** (yellow) and **45** (green). (b) Overlay of **45** (green) and **1a** (blue) (distances in Å).



**Figure 4.** Binding interactions in the water-filled S<sub>1</sub>' pocket (distances in Å). (a) comparison of **5** (yellow) and **1** (orange). (b) comparison of **5** (yellow) and **45** (green).

whose orientation varies in response to the bulkiness and chemical nature of the P<sub>2</sub> substituent. Nevertheless, two waters in the S<sub>1</sub>' pocket are conserved in all four complexes, and two additional waters are found in three complexes. As depicted in Figure 4a, the introduction of the cyclopentyl moiety in P<sub>1</sub>' (compound **5** compared to **1**) hardly effects the pattern of conserved water molecules. In contrast, introduction of the ring carbonyl group and replacement of P<sub>2</sub> Asn by Met (compound **45**) result in major changes in the interaction pattern (Figure 4b). The carbonyl oxygen forms hydrogen bonds with two conserved waters. The movement of Arg235 (now forming a hydrogen bond with one of the waters) together with the spatially more demanding carbonyl displaces two water molecules.

With the observed binding pattern of the carbonyl group in the cocrystal complex of **45** and BACE (Figure 4), we were not surprised that the lactam **57** which deployed its carbonyl group in the same direction exhibited almost equal inhibition compared to **45** and significantly better than the isomeric lactam **55**. The slight additional gain in activity for **57** might result from favorable conformational changes due to steric constraints imposed by the rigid lactam motif.

The substantial loss of activity of the oxa-analogue **59** and the isomeric lactam **55** might be explained by different conformational preferences due to the introduction of electronegative groups adjacent to the amide. In addition, the loss of activity for BACE of compound **55** is consistent with the negative effect of a hydrophobic group in the hydrophilic S<sub>1</sub>' environment. The inhibition of CathD, which comprises a hydrophobic S<sub>1</sub>' pocket, is much less affected by these changes. The loss of activity of the tertiary amine **53** is more difficult to explain. Most likely the positive charge is substantially influencing

**Table 4.** SAR of Ring Element: Ala-OH Derivatives

compd	ring	IC <sub>50</sub> BACE, μM	IC <sub>50</sub> CathD, μM
<b>36</b>		0.60	0.090
<b>35</b>		0.72	1.50
<b>34</b>		>10 (11% @ 10 μM)	0.43

the water-mediated interaction pattern in S<sub>1</sub>' resulting in less favorable interactions.

A comparable effect of the ring carbonyl was found in the P<sub>2</sub>'-Ala-OH series (Table 4): the keto derivative **35** showed similar activity on BACE as the unsubstituted compound **36**, while the activity for CathD was decreased. In agreement with the above-mentioned results for **55**, the lipophilic methylene derivative **34** exhibited an almost complete loss of activity for BACE, while it was still active on CathD.

## Conclusion

We have demonstrated the validity of introducing a carbocyclic constraint in the hydroxyethylene subunit of the original Tang-Ghosh BACE inhibitor **1**. A cocrys-



tal structure of the CP AlaOH analogue **5** with the enzyme showed a good match with **1**. Introduction of a keto group in the CP ring of P' truncated analogues as in **45** results in excellent BACE activity (IC<sub>50</sub> 10nM) as well as a 5-fold activity loss against CathD. A cocrystal structure of **45** with BACE shows excellent superposition with **1a**. Heterocyclic replacements of the CP unit resulted in the single digit nanomolar lactam inhibitor **57**. This compound exhibits an at least 7-fold selectivity toward BACE over CathD. Disappointingly, despite the high potency in the enzymatic assay (IC<sub>50</sub> < 10 nM), the compound exhibited an only weak activity (IC<sub>50</sub> 0.9 μM) in the cellular assay (Aβ-production in CHO cells transfected with APP<sub>wt</sub>). All other inhibitors showed even lower, mostly nonrelevant inhibition at 10 μM concentration.<sup>45</sup> The character of the compounds is still peptidic and is limiting penetration across cell membranes. Although much remains to be done, the results obtained so far demonstrate that the combination of truncation and functional manipulation in hydroxyethylene transition state mimetics can produce potent inhibitors of BACE with excellent potential for selectivity over CathD.

## Experimental Section

**Chemistry.** Solvents were distilled under positive pressure of dry argon before use and dried by standard methods; THF and ether, from Na/benzophenone; CH<sub>2</sub>Cl<sub>2</sub> and toluene, from CaH<sub>2</sub>. All commercially available reagents were used without further purification. All reactions were performed under argon atmosphere. NMR (<sup>1</sup>H, <sup>13</sup>C) spectra were recorded on Bruker AMX-300 and ARX-400 spectrometers in CDCl<sub>3</sub> or CD<sub>3</sub>OD or D<sub>2</sub>O with solvent resonance as the internal standard. Low- and high-resolution mass spectra were recorded on VG Micromass, AEIMS 902, or Kratos MS-50 spectrometers using fast atom bombardment (FAB). The purity of the target compounds was determined to be >95% by LC/MS obtained on a Finnigan Surveyor MSQ spectrometer. Purity of the compounds was determined by method [A] Alltech Prevail C18 column (250 × 4.6 mm) at 0.5 mL/min flow rate using a gradient of 20–90% acetonitrile-water (0.1% trifluoroacetic acid) and method [B] Alltech Prevail C18 column (250 × 4.6 mm) at 0.5 mL/min flow rate using 65% methanol-water (0.1% trifluoroacetic acid). Optical rotations were recorded on a Perkin-Elmer 241 polarimeter in a 1 dm cell at ambient temperature. Analytical thin-layer chromatography was performed on Merck 60F 254 precoated silica gel plates. Flash column chromatography was performed using (40–60 μm) silica gel at increased pressure. All melting points are uncorrected.

**(5S)-tert-Butoxycarbonylamino-(4S,R)-hydroxy-7-methyl-oct-2-ynoic Acid Methyl Ester (6).** To a solution of *i*-Pr<sub>2</sub>NEt (2.548 g, 25.2 mmol) in 20 mL of THF at –78 °C was added butyllithium (2.5 M in hexanes, 8.4 mL, 21 mmol). The solution was warmed to 0 °C for 0.5 h and then cooled to –78 °C. To the LDA solution was added methyl propiolate (2.548 g, 25.2 mmol) dropwise, and the reaction mixture was stirred at –78 °C for 0.5 h (solution A).

To a 250 mL flame-dried flask were added anhydrous CeCl<sub>3</sub> (5.16 g, 21 mmol) and THF (40 mL). The reaction mixture was vigorously stirred at room temperature for 2 h and then cooled to –78 °C. To this suspension was added solution A (–78 °C) in one portion. The reaction mixture was stirred at the same temperature for 1 h. A solution of Boc-leucinal (3.012 g, 14 mmol) in THF (12 mL) was added at –78 °C, and the resulting reaction mixture was stirred at –78 °C for 3 h, quenched by addition of a solution of acetic acid (4 mL) in THF (16 mL) at –78 °C, and allowed to warm to room temperature. The mixture was extracted with diethyl ether (100 mL) and washed with 10% citric acid (2 × 40 mL), and the separated organic

layer was washed with saturated NaHCO<sub>3</sub> (3 × 50 mL) and brine (50 mL), dried (Na<sub>2</sub>SO<sub>4</sub>), filtered, and concentrated in vacuo. The residue was purified by column chromatography (30% EtOAc in hexanes) to afford **6** as a 5:1 mixture of diastereomers (3.05 g, 72%) as a yellow oil; <sup>1</sup>H NMR (CDCl<sub>3</sub>) δ 4.74 (m, 1H), 4.50 (m, 1H), 3.84–3.70 (m, 1H), 3.76 (s, 3H), 1.67 (m, 1H), 1.58–1.20 (m, 2H), 1.43 (s, 9H), 0.93 (m, 6H); <sup>13</sup>C NMR (CDCl<sub>3</sub>) δ 156.6, 153.0, 86.2, 80.0, 65.1, 52.9, 52.7, 38.6, 28.1, 24.6, 23.2, 21.7, 21.5.

**(4S,5S)-Isobutyl-5-methoxycarbonylethynyl-2,2-dimethyl-oxazolidine-3-carboxylic Acid tert-Butyl Ester (7).** A solution of **6** (1.27 g, 3.74 mmol), 2,2-dimethoxypropane (15 mL, 0.123 mol), and dry acetone (70 mL) was heated to reflux for 5 min under argon atmosphere. Then TsOH was added until a permanent dark red solution was observed. The reaction mixture was refluxed for an additional 2 h, cooled to room temperature, and concentrated in vacuo. Saturated NaHCO<sub>3</sub> (20 mL) was added to the residue and extracted with ethyl acetate (3 × 60 mL). The combined organic layer was dried (Na<sub>2</sub>SO<sub>4</sub>), filtered, and concentrated to give a crude product as a yellow oil, which was purified by column chromatography (3–4% EtOAc in hexanes) to afford **7** as a single isomer (0.92 g, 64%) as a yellow oil; [α]<sub>D</sub><sup>20</sup> –25.6 (c 1.5, CHCl<sub>3</sub>); IR (neat) 2960, 2874, 2238, 1723, 1703, 1457, 1436, 1381, 1254, 1175, 1128, 1089, 1068, 1027, 851, 752 cm<sup>-1</sup>; <sup>1</sup>H NMR (CDCl<sub>3</sub>) δ 4.58 (s, 1H), 4.28–3.90 (m, 1H), 3.77 (s, 3H), 1.73 (s, 3H), 1.62–1.30 (m, 6H), 1.48 (s, 9H), 0.95 (t, *J* = 6.2 Hz, 6H); <sup>13</sup>C NMR (CDCl<sub>3</sub>) δ 153.9, 151.6, 118.1, 96.4, 91.0, 86.6, 68.8, 62.5, 53.1, 43.4, 42.5, 28.8, 28.0, 26.3, 24.0, 21.9; MS (FAB): *m/z* 340 [M + 1]<sup>+</sup>; HRMS calcd for C<sub>18</sub>H<sub>30</sub>NO<sub>5</sub> [M + 1]<sup>+</sup> 340.2124; found 340.2108.

**(4S,5S)-Isobutyl-5-(2-methoxycarbonyl-vinyl)-2,2-dimethyl-oxazolidine-3-carboxylic Acid tert-Butyl Ester (8).** Benzene (30 mL), Lindlar catalyst (60 mg, 5 wt %), and quinoline (0.24 mL) were placed in a 100 mL round-bottom flask and stirred for 20 min at room temperature. Compound **7** (1.2 g, 3.54 mmol) in benzene (20 mL) was added to the flask, and the mixture was stirred for an additional 20 min at room temperature. The flask was then partially charged with H<sub>2</sub> (not fully inflated balloon), and the suspension was stirred vigorously at room temperature overnight. The catalyst was removed by filtration through a pad of Celite and thoroughly washed with ethyl acetate. The filtrate was concentrated, and the residual yellow oil was purified by column chromatography (5% Et<sub>2</sub>O in CH<sub>2</sub>Cl<sub>2</sub>) to give **8** (1.09 g, 90%) as a yellow oil; [α]<sub>D</sub><sup>20</sup> +24.0 (c 1.2, CHCl<sub>3</sub>); IR (neat) 2959, 2873, 1743, 1700, 1388, 1367, 1256, 1175, 1092, 922, 870, 770 cm<sup>-1</sup>; <sup>1</sup>H NMR (CDCl<sub>3</sub>) δ 6.28 (m, 1H), 5.84 (m, 1H), 5.47 (m, 1H), 3.80–3.62 (m, 1H), 3.68 (s, 3H), 1.70–1.47 (m, 9H), 1.44 (s, 9H), 0.89 (d, *J* = 2.9 Hz, 3H), 0.85 (d, *J* = 6.2 Hz, 3H); <sup>13</sup>C NMR (CDCl<sub>3</sub>) δ 166.2, 152.1, 147.6, 121.5, 80.3, 75.6, 61.9, 53.3, 51.9, 43.6, 28.9, 28.0, 26.3, 25.4, 24.3, 21.9. MS (FAB): *m/z* 342 [M + 1]<sup>+</sup>; HRMS calcd for C<sub>18</sub>H<sub>32</sub>NO<sub>5</sub> [M + 1]<sup>+</sup> 342.2281; found 342.2274.

**(4S)-Isobutyl-(5S)-((2R)-methoxycarbonyl-4-methylene-cyclopentyl)-2,2-dimethyl-oxazolidine-3-carboxylic Acid tert-Butyl Ester (10).** To a solution of **8** (1.0 g, 2.93 mmol) in toluene (5 mL) was added 2-trimethylsilylanyl-methylallyl acetate **9** (0.63 g, 3.52 mmol) followed by triisopropyl phosphite (0.43 mL, 0.37 g, 1.76 mmol) and palladium acetate (46 mg, 0.2 mmol) at room temperature. The reaction mixture was heated to 100 °C under an argon atmosphere for 16 h and cooled, and toluene was evaporated under reduced pressure. The resulting residue was purified by column chromatography (5% EtOAc in hexanes) to afford an inseparable ~2:1 epimeric mixture as a colorless oil (1.04 g, 90%).

To a solution of the epimeric mixture (0.85 g, 2.15 mmol) in methanol (10 mL) was added a 0.5 M solution of NaOMe (8.6 mL, 4.3 mmol) in methanol at room temperature, and the reaction mixture was refluxed for 6 h. Methanol was removed under reduced pressure, H<sub>2</sub>O (5 mL) was added, and the aqueous layer was extracted with EtOAc (3 × 10 mL). The combined organic layers were dried (Na<sub>2</sub>SO<sub>4</sub>), filtered, and concentrated in vacuo. The residue was purified by column chromatography (5% EtOAc in hexanes) to afford exclusively

the trans isomer **10** as a colorless oil (0.85 g, 99%);  $[\alpha]_{20}^D$  -29.5 (*c* 2.9, CHCl<sub>3</sub>); <sup>1</sup>H NMR (CDCl<sub>3</sub>) δ 4.88 (d, *J* = 6.7 Hz, 2H), 3.71 (m, 1H), 3.70 (s, 3H), 2.60–2.30 (m, 6H), 1.60–1.45 (m, 10H), 1.47 (s, 9H), 0.93 (d, *J* = 4.8 Hz, 6H); <sup>13</sup>C NMR (CDCl<sub>3</sub>) δ 175.7, 152.0, 148.8, 106.9, 95.1, 80.8, 60.9, 59.2, 52.1, 46.6, 37.3, 34.2, 28.8, 27.7, 25.6, 24.6, 21.7, 14.5. MS (FAB): *m/z* 396 [M + 1]<sup>+</sup>; HRMS calcd for C<sub>22</sub>H<sub>37</sub>NO<sub>5</sub> [M]<sup>+</sup> 395.2672; found 395.2682.

**(4S)-Isobutyl-(5S)-((2R)-methoxycarbonyl-4-oxo-cyclopentyl)-2,2-dimethyl-oxazolidine-3-carboxylic Acid tert-Butyl Ester (11).** To a solution of **10** (700 mg, 1.77 mmol) in THF and H<sub>2</sub>O (27 mL, 2:1) was added NaIO<sub>4</sub> (1.52 g, 7.08 mmol), followed by addition of OsO<sub>4</sub> (44.3 mg, 0.177 mmol) at room temperature. The reaction mixture was stirred for 14 h at room temperature. A saturated solution of Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub> (10 mL) was added and stirred for 10 min. The partitioned aqueous layer was extracted with EtOAc (5 × 20 mL), and the combined organic layers were dried (Na<sub>2</sub>SO<sub>4</sub>), filtered, and concentrated in vacuo. The residue was purified by column chromatography (20% EtOAc in hexanes) to afford **11** (0.633 g, 90%) as a colorless oil;  $[\alpha]_{20}^D$  -38.8 (*c* 0.7 CHCl<sub>3</sub>); <sup>1</sup>H NMR (CDCl<sub>3</sub>) δ 3.95 (br, 1H), 3.75 (s, 3H), 3.61 (br, 1H), 3.03 (br, 1H), 2.72 (m, 1H), 2.56 (d, *J* = 33.5 Hz, *J* = 8.4 Hz, 1H), 2.44 (dd, *J* = 16.0 Hz, *J* = 8.2 Hz, 1H), 2.40 (m, 2H), 1.65–1.30 (m, 9H), 1.47 (s, 9H), 0.95 (d, *J* = 6.2 Hz, 3H), 0.93 (d, *J* = 5.7 Hz, 3H); <sup>13</sup>C NMR (CDCl<sub>3</sub>) δ 215.0, 174.8, 151.9, 94.8, 89.1, 82.0, 80.4, 59.2, 52.7, 43.7, 43.2, 42.0, 38.8, 28.9, 27.3, 25.6, 24.7, 21.8; MS (FAB): *m/z* 398 [M + 1]<sup>+</sup>; HRMS calcd for C<sub>21</sub>H<sub>36</sub>NO<sub>6</sub> [M + 1]<sup>+</sup> 398.2543; found 398.2550.

**(4S)-Isobutyl-(5S)-((2R)-methoxycarbonyl-cyclopentyl)-2,2-dimethyl-oxazolidine-3-carboxylic Acid tert-Butyl Ester (12).** To a solution of **11** (0.4 g, 1.01 mmol) in MeOH (20 mL) were added NH<sub>2</sub>NHTs (205 mg, 1.11 mmol) and Na<sub>2</sub>SO<sub>4</sub> (2 g). The reaction mixture was refluxed for 2 h, cooled to room temperature, and stirred overnight. Methanol was removed under reduced pressure, and the residue was purified by column chromatography (33% EtOAc in hexanes) to afford the hydrazone as a white solid (0.58 g, 99%).

To a solution of the hydrazone (0.58 g, 1.01 mmol) in CHCl<sub>3</sub> (6 mL) was added catecholborane (1 M in THF, 2.16 mL, 2.16 mmol) at 0 °C. The reaction mixture was stirred at 0 °C for 2 h and overnight at room temperature. NaOAc·3H<sub>2</sub>O (0.412 g) was added, and the reaction mixture was refluxed for 3 h, cooled to room temperature, diluted with CH<sub>2</sub>Cl<sub>2</sub> (30 mL), and washed with saturated Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub> (10 mL). The aqueous layer was extracted with CH<sub>2</sub>Cl<sub>2</sub> (3 × 10 mL), and the combined organic layers were dried (Na<sub>2</sub>SO<sub>4</sub>), filtered, and concentrated in vacuo. The residue was purified by column chromatography (10% EtOAc in hexanes) to afford **12** (283 mg, 68%) as colorless crystals; mp: 75–77 °C;  $[\alpha]_{20}^D$  -35.2 (*c* 0.8, CHCl<sub>3</sub>); <sup>1</sup>H NMR (CDCl<sub>3</sub>) δ 3.75–3.50 (m, 2H), 3.68 (s, 3H), 2.43 (m, 2H), 2.00–1.35 (m, 15H), 1.45 (s, 9H), 0.92 (d, *J* = 6.7 Hz, 6H); <sup>13</sup>C NMR (CDCl<sub>3</sub>) δ 176.9, 152.1, 0.0, 79.9, 59.6, 52.1, 47.2, 31.6, 28.9, 28.0, 27.7, 27.2, 25.8, 25.6, 25.1, 24.7, 21.6; MS (FAB): *m/z* 383 [M]<sup>+</sup>; HRMS calcd for C<sub>21</sub>H<sub>37</sub>NO<sub>5</sub> [M]<sup>+</sup> 383.2672; found 383.2658.

**Compound 13.** To a solution of KOH (28 mg, 0.5 mmol) in MeOH (4 mL) and H<sub>2</sub>O (2 mL) was added **12** (75 mg, 0.195 mmol). The reaction mixture was stirred at 65 °C for 3 h and at room-temperature overnight. 1 N HCl (10 mL) was added, and the aqueous phase was extracted with CH<sub>2</sub>Cl<sub>2</sub> (4 × 15 mL). The combined organic layers were dried (Na<sub>2</sub>SO<sub>4</sub>), filtered, and concentrated in vacuo. The residue was used without further purification. To a solution of Boc-Ala-Glu(OBn)-Phe-OBn (192 mg, 0.3 mmol) in CH<sub>2</sub>Cl<sub>2</sub> was added TFA (233 μL). The reaction mixture was stirred at room temperature for 3 h, quenched by addition of saturated NaHCO<sub>3</sub> (1.5 mL), and extracted with EtOAc (5 × 10 mL). The combined organic layers were dried (Na<sub>2</sub>SO<sub>4</sub>), filtered, and concentrated in vacuo. The residue was dissolved in CH<sub>2</sub>Cl<sub>2</sub> (3 mL) and followed by addition of NH<sub>2</sub>-Ala-Glu(OBn)-Phe-OBn (192 mg, 0.3 mmol), PyBOP (156 mg, 0.3 mmol), and *i*-Pr<sub>2</sub>NEt (134 μL, 0.6 mmol) at 0 °C. The reaction mixture was stirred for 4 h at 0 °C, then partitioned in EtOAc (20 mL) and 1 N HCl (10 mL). The organic layer

was separated and washed with saturated NaHCO<sub>3</sub> (10 mL) and brine (10 mL), dried (Na<sub>2</sub>SO<sub>4</sub>), filtered, and concentrated in vacuo. The residue was purified by column chromatography (50% EtOAc in hexanes) to afford **13** (100 mg, 57%) as a colorless oil;  $[\alpha]_{20}^D$  -40.9 (*c* 2.0, CHCl<sub>3</sub>); <sup>1</sup>H NMR (CDCl<sub>3</sub>) δ 7.50–7.00 (m, 15H), 5.11 (m, 6H), 4.86 (q, *J* = 6.2 Hz, 1H), 4.53 (q, *J* = 7.0 Hz, 2H), 3.75 (s, 1H), 3.60 (s, 2H), 3.11 (dd, *J* = 13.9 Hz, *J* = 6.0 Hz, 1H), 3.04 (dd, *J* = 13.9 Hz, *J* = 6.5 Hz, 1H), 2.49 (m, 1H), 2.41 (m, 3H), 2.06 (m, 1H), 1.96–1.34 (m, 17H), 1.46 (s, 9H), 1.29 (d, *J* = 5.2 Hz, 3H), 0.92 (m, 6H); <sup>13</sup>C NMR (CDCl<sub>3</sub>) δ 175.1, 173.0, 172.3, 170.8, 170.4, 151.6, 135.5, 134.9, 129.1, 128.4, 128.3, 128.2, 128.1, 126.9, 79.5, 67.1, 66.4, 58.8, 53.3, 52.1, 48.6, 48.3, 46.2, 37.6, 31.4, 30.0, 28.4, 27.6, 25.0, 24.2, 21.4, 18.3; MS (FAB): *m/z* 897.6 [M + 1]<sup>+</sup>; HRMS calcd for C<sub>51</sub>H<sub>69</sub>N<sub>4</sub>O<sub>10</sub> [M + 1]<sup>+</sup> 897.5014; found 897.5043.

**Compound 14.** Prepared from **12** according to the general procedure for preparation of **13** (124 mg, 76%);  $[\alpha]_{20}^D$  -35.1 (*c* 0.4, CHCl<sub>3</sub>); <sup>1</sup>H NMR (CDCl<sub>3</sub>) δ 7.40–7.22 (m, 10H), 7.20 (d, *J* = 7.6 Hz, 1H), 6.44 (d, *J* = 7.4 Hz, 1H), 5.13 (m, 2H), 5.05 (s, 2H), 4.63 (m, 2H), 3.72 (br, 2H), 2.56–2.12 (m, 5H), 1.98 (m, 1H), 1.96–1.10 (m, 23H), 1.45 (s, 9H), 1.33 (d, *J* = 6.9 Hz, 3H), 0.90 (d, *J* = 6.9 Hz, 6H); <sup>13</sup>C NMR (CDCl<sub>3</sub>) δ 175.6, 172.9, 172.8, 171.6, 152.1, 136.0, 135.6, 129.0, 128.9, 128.8, 128.7, 128.3, 94.1, 82.9, 81.6, 80.0, 78.4, 67.7, 66.9, 60.8, 59.4, 52.1, 49.0, 48.9, 46.9, 44.4, 43.0, 32.0, 30.5, 28.9, 27.5, 27.4, 25.6, 24.7, 22.0, 21.5, 18.9, 14.6; MS (FAB): *m/z* 750.3 [M + 1]<sup>+</sup>; HRMS calcd for C<sub>42</sub>H<sub>60</sub>N<sub>3</sub>O<sub>9</sub> [M + 1]<sup>+</sup> 750.4285; found 750.4324.

**Compound 15.** Prepared from **12** according to the general procedure for preparation of **13** (210 mg, 99%);  $[\alpha]_{20}^D$  -27.2 (*c* 0.5, CHCl<sub>3</sub>); <sup>1</sup>H NMR (CD<sub>3</sub>OD) δ 7.40–7.30 (m, 5H), 6.14 (d, *J* = 7.2 Hz, 1H), 5.19 (m, 2H), 4.66 (m, 1H), 3.75 (m, 1H), 3.64 (m, 1H), 2.52 (m, 1H), 2.37 (m, 1H), 1.93–1.39 (m, 15H), 1.45 (s, 9H), 1.42 (d, *J* = 5.2 Hz, 3H), 0.92 (d, *J* = 6.4 Hz, 6H); <sup>13</sup>C NMR (CDCl<sub>3</sub>) δ 175.2, 173.4, 152.1, 135.7, 129.0, 128.9, 128.6, 93.9, 82.9, 80.0, 67.6, 59.4, 48.8, 48.5, 46.8, 44.3, 32.6, 32.0, 30.0, 28.9, 27.9, 27.6, 25.6, 24.7, 21.9, 18.9; MS (FAB): *m/z* 531.2 [M + 1]<sup>+</sup>; HRMS calcd for C<sub>30</sub>H<sub>47</sub>N<sub>2</sub>O<sub>6</sub> [M + 1]<sup>+</sup> 531.3434; found 531.3459.

**Compound 16.** To a solution of **13** (80 mg, 0.089 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (0.5 mL) was added TFA (0.2 mL). The reaction mixture was stirred at room temperature for 1 h, then quenched by addition of saturated NaHCO<sub>3</sub> (5 mL) and extracted with EtOAc (4 × 10 mL). The combined organic layers were dried (Na<sub>2</sub>SO<sub>4</sub>), filtered, and concentrated in vacuo. The residue was dissolved in CH<sub>2</sub>Cl<sub>2</sub> (4 mL), followed by addition of Boc-Asn(NTr)-OH (63.7 mg, 0.134 mmol), PyBOP (70 mg, 0.134 mmol) and *i*-Pr<sub>2</sub>NEt (60 μL, 0.268 mmol) at 0 °C. The reaction mixture was stirred at room-temperature overnight, EtOAc (15 mL) was added, and the separated organic layer was washed with 1 N HCl (2 × 10 mL), saturated NaHCO<sub>3</sub> (2 × 10 mL) and brine (10 mL). The organic layer was dried (Na<sub>2</sub>SO<sub>4</sub>), filtered, and concentrated in vacuo. The residue was purified by column chromatography (66% EtOAc in hexanes) to afford **16** (80 mg, 74%) as a colorless oil;  $[\alpha]_{20}^D$  -30.8 (*c* 1.2, CHCl<sub>3</sub>); <sup>1</sup>H NMR (CDCl<sub>3</sub>) δ 7.50–7.00 (m, 30H), 6.92 (br, 1H), 6.31 (br, 1H), 5.08 (d, *J* = 2.6 Hz, 4H), 4.80 (m, 1H), 4.38 (m, 1H), 4.25 (m, 1H), 3.90 (m, 1H), 3.56 (br, 1H), 3.33 (m, 1H), 3.06 (m, 2H), 2.72 (m, 2H), 2.58 (m, 1H), 2.34 (m, 2H), 2.05 (m, 1H), 1.86 (m, 1H), 1.77–1.20 (m, 14H), 1.44 (s, 9H), 1.28 (d, *J* = 5.3 Hz, 3H), 0.90 (d, *J* = 4.9 Hz, 6H); <sup>13</sup>C NMR (CDCl<sub>3</sub>) δ 177.0, 174.7, 173.8, 173.6, 172.6, 171.8, 171.3, 170.6, 156.6, 144.7, 136.4, 136.1, 135.5, 129.6, 129.1, 129.0, 128.9, 128.8, 128.7, 128.6, 128.3, 127.4, 80.8, 74.8, 71.1, 67.6, 66.9, 60.8, 53.9, 53.0, 52.7, 50.9, 50.5, 48.2, 46.9, 41.6, 38.1, 31.0, 30.9, 28.7, 27.5, 27.0, 25.1, 24.6, 23.7, 22.3, 21.5, 19.6, 17.8, 14.6, 14.2; MS (FAB): *m/z* 1213.3 [M + 1]<sup>+</sup>.

**Compound 17.** Prepared from **14** according to the general procedure for preparation of **16** (110 mg, 66%);  $[\alpha]_{20}^D$  -44.8 (*c* 0.6, MeOH); <sup>1</sup>H NMR (CDCl<sub>3</sub>) δ 7.50–7.00 (m, 25H), 6.94 (d, *J* = 6.4 Hz, 1H), 6.86 (d, *J* = 4.8 Hz, 1H), 6.26 (s, 1H), 5.13 (d, *J* = 12.3 Hz, 1H), 5.08 (d, *J* = 12.3 Hz, 1H), 5.03 (s, 2H), 4.51 (q, *J* = 7.7 Hz, 1H), 4.30 (m, 2H), 3.85 (m, 1H), 3.38 (m, 1H), 2.80 (m, 1H), 2.66 (m, 1H), 2.51 (m, 1H), 2.41–2.06 (m, 5H), 2.00 (m, 1H), 1.90–1.01 (m, 13H), 1.40 (s, 9H), 0.86 (m, 6H);



$^{13}\text{C}$  NMR ( $\text{CDCl}_3$ )  $\delta$  176.3, 173.0, 172.6, 172.0, 171.3, 170.0, 144.1, 135.5, 135.0, 128.5, 128.4, 128.3, 128.1, 127.8, 126.9, 80.3, 74.4, 70.6, 67.2, 66.4, 60.3, 51.9, 51.5, 50.7, 49.5, 47.7, 46.4, 40.8, 37.5, 30.4, 30.0, 28.1, 26.8, 26.5, 24.5, 23.9, 23.1, 21.7, 20.9, 17.2, 14.0, 13.6; MS (FAB):  $m/z$  1067.0 [ $\text{M} + 1$ ] $^+$ .

**Compound 18.** Prepared from **15** according to the general procedure for preparation of **16** (120 mg, 67%);  $[\alpha]_{\text{D}}^{20} -51.0$  ( $c$  0.8,  $\text{CHCl}_3$ );  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  7.60–7.00 (m, 20H), 6.89 (d,  $J = 8.0$  Hz, 1H), 6.68 (d,  $J = 6.4$  Hz, 1H), 6.22 (d,  $J = 6.0$  Hz, 1H), 5.14 (q,  $J = 12.3$  Hz, 2H), 5.08 (q,  $J = 12.3$  Hz, 2H), 4.53 (m, 1H), 4.30 (m, 1H), 3.85 (m, 1H), 3.68 (d,  $J = 5.0$  Hz, 1H), 3.37 (m, 1H), 2.83 (m, 1H), 2.61 (m, 2H), 2.30 (m, 1H), 1.90–1.48 (m, 8H), 1.41 (s, 9H), 1.47–1.10 (m, 3H), 0.87 (m, 6H);  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ )  $\delta$  175.6, 173.2, 172.1, 170.0, 156.0, 144.2, 135.3, 129.1, 129.0, 128.7, 128.5, 128.4, 127.4, 80.7, 74.9, 71.1, 67.4, 60.8, 52.1, 51.9, 48.7, 48.2, 47.1, 41.2, 38.2, 31.0, 30.8, 28.7, 26.4, 25.8, 24.5, 24.0, 23.8, 22.2, 21.5, 19.5, 18.2, 14.6, 14.1; MS (FAB):  $m/z$  848.0 [ $\text{M} + 1$ ] $^+$ .

**Compound 19.** A solution of **16** (68 mg, 0.056 mmol) in HCl (4 M in dioxane, 1 mL, 4 mmol) was stirred at 0 °C for 2 h, then saturated  $\text{NaHCO}_3$  (10 mL) was added and the aqueous layer was extracted by EtOAc (4  $\times$  10 mL). The combined organic layers were dried ( $\text{Na}_2\text{SO}_4$ ), filtered, and concentrated in vacuo. To a solution of the residue in  $\text{CH}_2\text{Cl}_2$  (2 mL) were added Boc-Valine (36.4 mg, 0.168 mmol), PyBOP (88 mg, 0.168 mmol), and *i*-Pr<sub>2</sub>NEt (126  $\mu\text{L}$ , 0.56 mmol). The reaction mixture was stirred at room temperature for 24 h, EtOAc (15 mL) was added, and the separated organic layer was washed with 1 N HCl (2  $\times$  10 mL), saturated  $\text{NaHCO}_3$  (2  $\times$  10 mL), and brine (10 mL), dried ( $\text{Na}_2\text{SO}_4$ ), filtered, and concentrated in vacuo. The residue was purified by column chromatography (66% EtOAc in hexanes) to afford **19** (43 mg, 59%) as a white foam;  $[\alpha]_{\text{D}}^{20} -32.8$  ( $c$  2.0, MeOH);  $^1\text{H}$  NMR ( $\text{CD}_3\text{OD}$ )  $\delta$  7.41–7.05 (m, 30H), 5.08 (d,  $J = 3.4$  Hz, 4H), 4.67 (m, 2H), 4.38 (m, 1H), 4.26 (m, 1H), 4.05–3.70 (m, 2H), 3.08 (m, 2H), 2.80 (m, 2H), 2.55 (m, 1H), 2.36 (m, 2H), 2.06 (m, 2H), 1.86 (m, 1H), 1.78–1.18 (m, 25H), 1.28 (d,  $J = 7.2$  Hz, 3H), 0.91 (m, 12H);  $^{13}\text{C}$  NMR ( $\text{CD}_3\text{OD}$ )  $\delta$  178.5, 175.2, 174.3, 172.5, 171.8, 145.8, 137.8, 136.9, 130.3, 130.0, 129.5, 129.2, 128.7, 127.8, 75.2, 68.1, 67.4, 62.1, 61.3, 55.4, 53.5, 52.3, 52.0, 51.3, 50.8, 47.3, 38.7, 38.2, 32.4, 31.9, 31.1, 28.7, 28.1, 25.8, 25.7, 23.8, 22.5, 19.8, 19.7, 18.3, 18.2, 17.6; MS (FAB):  $m/z$  1315 [ $\text{M} + 2$ ] $^+$ .

**Compound 20.** Prepared from **17** according to the general procedure for preparation of **19** (87 mg, 85%);  $^1\text{H}$  NMR ( $\text{CD}_3\text{OD}$ )  $\delta$  7.29 (m, 25H), 5.14 (d,  $J = 7.1$  Hz, 2H), 5.09 (d,  $J = 3.5$  Hz, 2H), 4.63 (t,  $J = 7.0$  Hz, 1H), 4.51 (m, 1H), 4.30 (q,  $J = 7.0$  Hz, 1H), 3.95 (d,  $J = 5.6$  Hz, 1H), 3.87 (m, 1H), 2.79 (m, 2H), 2.55 (m, 1H), 2.43 (m, 2H), 2.31 (m, 1H), 2.18 (m, 1H), 1.99 (m, 3H), 1.82 (m, 1H), 1.64 (m, 7H), 1.42 (s, 9H), 1.45–1.20 (m, 6H), 1.27 (d,  $J = 7.1$  Hz, 3H), 0.94 (d,  $J = 6.6$  Hz, 6H), 0.88 (d,  $J = 6.8$  Hz, 6H);  $^{13}\text{C}$  NMR ( $\text{CD}_3\text{OD}$ )  $\delta$  177.3, 174.5, 173.1, 173.0, 171.1, 170.8, 167.5, 144.9, 136.5, 136.1, 129.1, 128.6, 128.5, 128.4, 128.2, 128.1, 127.7, 126.8, 79.7, 74.0, 70.8, 67.1, 66.4, 60.3, 51.9, 51.5, 51.1, 31.1, 30.0, 27.8, 26.7, 24.8, 24.7, 22.8, 21.5, 18.8, 17.3, 16.8; MS (FAB):  $m/z$  1168.8 [ $\text{M} + 1$ ] $^+$ .

**Compound 21.** Prepared from **18** according to the general procedure for preparation of **19** (91 mg, 78%);  $[\alpha]_{\text{D}}^{20} -23.9$  ( $c$  3.2,  $\text{CHCl}_3$ );  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  7.77 (d,  $J = 6.0$  Hz, 1H), 7.47 (s, 1H), 7.37–7.15 (m, 20H), 6.79 (d,  $J = 9.1$  Hz, 1H), 6.62 (br, 1H), 5.18–5.02 (m, 4H), 4.59–4.49 (m, 2H), 3.99–3.85 (m, 1H), 3.82 (t,  $J = 5.9$  Hz, 1H), 3.53 (br, 1H), 3.39 (br, 1H), 2.76–2.60 (m, 3H), 2.29 (m, 1H), 2.08–1.86 (m, 1H), 1.75 (m, 2H), 1.47 (m, 4H), 1.47–1.34 (m, 5H), 1.40 (s, 9H), 0.92 (m, 12H);  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ )  $\delta$  176.1, 174.6, 173.7, 172.8, 171.3, 170.6, 156.6, 156.3, 144.7, 135.9, 129.1, 129.0, 128.7, 128.5, 128.4, 127.4, 80.7, 80.3, 74.1, 71.1, 67.3, 60.7, 59.8, 51.7, 51.4, 48.5, 47.9, 47.1, 41.1, 38.2, 31.2, 31.0, 30.3, 28.7, 28.6, 26.1, 25.1, 24.5, 23.8, 23.2, 22.2, 19.8, 19.7, 18.3, 18.0; MS (FAB):  $m/z$  946.5 [ $\text{M} + 1$ ] $^+$ ; HRMS calcd for  $\text{C}_{55}\text{H}_{72}\text{N}_5\text{O}_9$  [ $\text{M} + 1$ ] $^+$  946.5330; found 946.5332.

**Compound 22.** A solution of **19** (54 mg, 0.042 mmol) in HCl (4 M in dioxane, 3 mL, 12 mmol) was stirred at 0 °C for 1 h. Saturated  $\text{NaHCO}_3$  (5 mL) was added, and the separated

aqueous layer was extracted with EtOAc (4  $\times$  10 mL). The combined organic layers were dried ( $\text{Na}_2\text{SO}_4$ ), filtered, and concentrated in vacuo. The residue was dissolved in *N,N*-dimethylacetamide (1 mL), and followed by addition of *N*-Boc-Glu(OBn)-OH (42 mg, 0.125 mmol), PyBOP (66 mg, 0.125 mmol), and *i*-Pr<sub>2</sub>NEt (36  $\mu\text{L}$ , 0.21 mmol). The reaction mixture was stirred at room temperature for 24 h, EtOAc (15 mL) was added, and the separated organic layer was washed with 1 N HCl (2  $\times$  10 mL), saturated  $\text{NaHCO}_3$  (2  $\times$  10 mL), and brine (10 mL), dried ( $\text{Na}_2\text{SO}_4$ ), filtered, and concentrated in vacuo. The residue was purified by column chromatography (80% EtOAc in hexanes) to afford **22** (50 mg, 77%) as a white solid;  $[\alpha]_{\text{D}}^{20} -39.3$  ( $c$  0.1, MeOH);  $^1\text{H}$  NMR ( $\text{CD}_3\text{OD}$ )  $\delta$  7.39–7.02 (m, 35H), 5.08 (m, 6H), 4.65 (m, 2H), 4.36 (m, 1H), 4.23 (m, 2H), 3.95 (m, 1H), 3.04 (m, 2H), 2.80 (m, 2H), 2.60–2.22 (m, 7H), 2.07 (m, 4H), 1.89 (m, 4H), 1.63 (m, 5H), 1.46–1.10 (m, 14H), 1.40 (s, 9H), 0.90 (m, 12H);  $^{13}\text{C}$  NMR ( $\text{CD}_3\text{OD}$ )  $\delta$  177.5, 174.3, 173.6, 173.3, 172.2, 172.1, 172.0, 171.6, 170.9, 156.9, 144.9, 136.9, 136.5, 135.9, 129.3, 129.1, 128.6, 128.5, 128.4, 128.2, 127.8, 127.7, 126.9, 126.8, 126.5, 79.8, 74.3, 70.9, 67.1, 66.4, 60.6, 58.9, 54.4, 52.5, 49.8, 48.7, 48.5, 48.3, 48.0, 47.8, 47.6, 47.4, 37.3, 31.4, 30.5, 30.4, 30.1, 27.8, 27.7, 27.6, 24.9, 24.7, 22.9, 21.6, 17.4, 16.7, 13.5; MS (FAB):  $m/z$  1533.1 [ $\text{M} + 1$ ] $^+$ .

**Compound 23.** Prepared from **20** according to the general procedure for the preparation of **22** (57 mg, 56%);  $[\alpha]_{\text{D}}^{20} -35.4$  ( $c$  2.3, MeOH);  $^1\text{H}$  NMR ( $\text{CD}_3\text{OD}$ )  $\delta$  7.46–7.00 (m, 30H), 5.08 (m, 6H), 4.62 (m, 1H), 4.48 (m, 1H), 4.36–4.20 (m, 2H), 3.88 (m, 1H), 3.64 (m, 1H), 3.36 (m, 1H), 2.81 (m, 1H), 2.44 (m, 8H), 2.23–1.20 (m, 28H), 1.42 (s, 9H), 1.28 (d,  $J = 6.0$  Hz, 2H), 0.96 (m, 16H);  $^{13}\text{C}$  NMR ( $\text{CD}_3\text{OD}$ )  $\delta$  177.3, 174.9, 174.6, 173.3, 173.0, 172.1, 171.8, 156.9, 144.9, 136.6, 129.1, 128.6, 128.4, 128.2, 127.8, 126.8, 79.8, 74.1, 67.1, 66.4, 58.5, 54.3, 51.9, 51.5, 51.2, 49.5, 31.4, 31.1, 30.5, 30.1, 27.7, 27.1, 26.7, 24.8, 22.9, 21.6, 18.8, 17.3, 16.9; MS (FAB):  $m/z$  1384.9 [ $\text{M} + 1$ ] $^+$ .

**Compound 24.** Prepared from **21** according to the general procedure for the preparation of **22** (63 mg, 78%);  $[\alpha]_{\text{D}}^{20} -48.4$  ( $c$  1.0, MeOH);  $^1\text{H}$  NMR ( $\text{CD}_3\text{OD}$ )  $\delta$  7.33–7.16 (m, 25H), 5.10 (m, 4H), 4.37 (m, 1H), 4.20 (m, 1H), 4.08 (m, 1H), 3.90 (m, 1H), 3.70 (m, 1H), 3.36 (m, 1H), 2.86 (m, 1H), 2.70 (m, 1H), 2.58–2.26 (m, 4H), 2.06 (m, 3H), 1.95–1.52 (m, 8H), 1.51–1.22 (m, 8H), 1.42 (d,  $J = 3.3$  Hz, 9H), 0.92 (m, 12H);  $^{13}\text{C}$  NMR ( $\text{CD}_3\text{OD}$ )  $\delta$  178.5, 174.3, 173.1, 172.5, 171.8, 171.2, 157.9, 145.9, 137.5, 137.3, 130.0, 129.5, 129.2, 129.1, 128.7, 128.6, 127.8, 80.8, 75.2, 71.8, 71.7, 67.8, 67.4, 59.8, 55.2, 52.5, 52.0, 47.0, 42.1, 39.2, 33.1, 32.4, 31.5, 28.7, 28.4, 27.9, 26.5, 25.9, 25.7, 23.9, 23.4, 22.9, 22.5, 19.8, 18.3, 17.2; MS (FAB):  $m/z$  1165.4 [ $\text{M} + 1$ ] $^+$ .

**Compound 3.** A solution **22** (40 mg, 0.026 mmol) in TFA (1 mL) was stirred at room temperature for 1 h. MeOH (2 mL) was added to quench the reaction, and the solvents were removed under reduced pressure at room temperature. The residue was purified by column chromatography (16% MeOH in  $\text{CH}_2\text{Cl}_2$ ) to afford a white solid (13 mg, 42%);  $[\alpha]_{\text{D}}^{20} -47.5$  ( $c$  0.1, MeOH). The white solid was dissolved in MeOH (1 mL), 10% Pd on carbon (3 mg) was added, and the mixture was hydrogenated for 2 h at 1 atm. Filtration and evaporation afforded **3** as a white solid (7.1 mg, 71%);  $^1\text{H}$  NMR ( $\text{D}_2\text{O}$ )  $\delta$  7.35–7.05 (m, 5H), 5.00–4.78 (m, 4H), 4.33 (m, 1H), 4.10 (m, 3H), 3.90 (m, 1H), 3.86 (m, 1H), 3.10 (m, 1H), 2.87 (m, 1H), 2.72 (dd,  $J = 15.7$  Hz,  $J = 5.8$  Hz, 1H), 2.72 (dd,  $J = 15.7$  Hz,  $J = 5.8$  Hz, 1H), 2.61 (dd,  $J = 15.1$  Hz,  $J = 8.1$  Hz, 1H), 2.54 (m, 1H), 2.40–1.78 (m, 9H), 1.79–1.41 (m, 6H), 1.38–1.20 (m, 2H), 1.25 (d,  $J = 6.6$  Hz, 3H), 0.86 (d,  $J = 6.0$  Hz, 6H), 0.77 (m, 6H); MS (FAB):  $m/z$  919.2 [ $\text{M} + 1$ ] $^+$ ; HRMS calcd for  $\text{C}_{43}\text{H}_{67}\text{N}_8\text{O}_{14}$  [ $\text{M} + 1$ ] $^+$  919.4732; found 919.4772; LC/MS retention time: [A] 11.41 min; [B] 10.45 min.

**Compound 4.** Prepared from **23** according to the general procedure for the preparation of **3** (15.5 mg, 54%).  $[\alpha]_{\text{D}}^{20} -42.0$  ( $c$  0.6,  $\text{H}_2\text{O}$ );  $^1\text{H}$  NMR ( $\text{D}_2\text{O}$ )  $\delta$  4.30 (m, 1H), 4.20 (m, 1H), 4.05 (m, 1H), 3.90 (m, 1H), 2.65 (m, 1H), 2.44 (m, 1H), 2.40–2.10 (m, 8H), 2.06–1.95 (m, 9H), 1.86 (m, 2H), 1.67–1.38 (m, 5H), 1.28 (m, 4H), 0.90 (m, 22H);  $^{13}\text{C}$  NMR ( $\text{D}_2\text{O}$ )  $\delta$  179.7, 178.8, 176.0, 175.0, 172.7, 171.5, 170.1, 169.9, 74.2, 61.8, 60.1, 60.0, 52.9, 51.7, 50.2, 48.1, 46.1, 40.9, 37.1, 32.0, 31.6, 30.6, 27.8,

27.5, 27.0, 26.6, 25.5, 25.3, 24.5, 22.9, 21.6, 18.7, 18.1, 16.9; MS (FAB):  $m/z$  772.2 [M + 1]<sup>+</sup>; HRMS calcd for C<sub>34</sub>H<sub>58</sub>N<sub>7</sub>O<sub>13</sub> [M + 1]<sup>+</sup> 772.4048; found 772.4092; LC/MS retention time: [A] 9.32 min; [B] 8.54 min.

**Compound 5.** Prepared from **24** according to the general procedure for the preparation of **3** (10.9 mg, 60%) except that the hydrogenation was carried out in MeOH/H<sub>2</sub>O (1:1) instead of MeOH for 12 h. [ $\alpha$ ]<sub>D</sub><sup>20</sup> -37.8 (c 0.2, MeOH); <sup>1</sup>H NMR (D<sub>2</sub>O)  $\delta$  4.10 (m, 3H), 3.82 (m, 1H), 3.45 (m, 3H), 2.73 (m, 12H), 2.60–2.31 (m, 4H), 2.22 (m, 1H), 2.17–1.78 (m, 6H), 1.76–1.41 (m, 6H), 1.34 (m, 4H), 0.90 (m, 2H); <sup>13</sup>C NMR (D<sub>2</sub>O)  $\delta$  180.5, 180.0, 178.7, 175.2, 173.1, 172.9, 172.0, 170.3, 74.3, 74.1, 60.4, 52.4, 51.7, 51.0, 49.1, 48.1, 46.1, 40.9, 37.0, 31.4, 30.6, 29.3, 26.9, 26.3, 25.2, 24.5, 23.8, 22.8, 21.6, 18.7, 18.0, 16.4; MS (FAB):  $m/z$  645.5 [M + 1]<sup>+</sup>; HRMS calcd for C<sub>29</sub>H<sub>51</sub>N<sub>6</sub>O<sub>10</sub> [M + 1]<sup>+</sup> 643.3622; found 643.3662; LC/MS retention time: [A] 8.38 min; [B] 7.67 min.

**Compound 25.** Ac-Leu-OCH<sub>2</sub>CCl<sub>3</sub> (110 mg, 0.34 mmol) was stirred with HCl (4 M in dioxane, 1 mL) at room temperature for 1 h. The solvent was removed under reduced pressure, then the residue was dissolved in CH<sub>2</sub>Cl<sub>2</sub> (3 mL) and cooled to 0 °C. The acid from **10** (78 mg, 0.20 mmol) was added followed by PyBOP (120 mg, 0.20 mmol) and *i*-Pr<sub>2</sub>NEt (155  $\mu$ L, 0.80 mmol). The reaction mixture was allowed to warm to room temperature over a period of 3 h. The reaction mixture was diluted with EtOAc (15 mL) and washed with 1 N HCl (2 mL) and saturated NaHCO<sub>3</sub> (2 mL). The organic phase was dried over Na<sub>2</sub>SO<sub>4</sub> and concentrated in vacuo. The residue was purified by column chromatography (50% EtOAc in hexanes) to afford **25** (77 mg, 75%) as a colorless oil; [ $\alpha$ ]<sub>D</sub><sup>20</sup> -22.5 (c 1.5, CHCl<sub>3</sub>); <sup>1</sup>H NMR (CDCl<sub>3</sub>):  $\delta$  6.09 (d,  $J$  = 7.4 Hz, 1H), 4.93 (d,  $J$  = 11.9 Hz, 1H), 4.88 (m, 2H), 4.76 (m, 1H), 4.64 (d,  $J$  = 11.9 Hz, 1H), 3.81 (m, 1H), 3.64 (m, 1H), 2.62 (m, 3H), 2.52 (m, 2H), 2.42 (m, 1H), 1.75 (m, 1H), 1.58 (s, 3H), 1.50 (d,  $J$  = 7.3 Hz, 3H), 1.46 (s, 9H), 1.44 (s, 3H), 1.40 (m, 2H), 0.91 (d,  $J$  = 6.4 Hz, 6H); <sup>13</sup>C NMR (CDCl<sub>3</sub>):  $\delta$  174.1, 171.9, 152.1, 148.9, 107.2, 94.9, 80.2, 78.5, 77.7, 74.6, 58.9, 48.4, 48.0, 46.5, 37.8, 33.1, 28.9, 27.2, 25.5, 24.8, 21.9, 18.6; MS (FAB):  $m/z$  583, [M + 1]<sup>+</sup>; HRMS calcd for C<sub>26</sub>H<sub>42</sub>Cl<sub>3</sub>N<sub>2</sub>O<sub>6</sub> [M + 1]<sup>+</sup> 583.2108; found 583.2123.

**Compound 26.** Prepared from **11** according to the general procedure for the preparation of **25** (75%); [ $\alpha$ ]<sub>D</sub><sup>20</sup> -65.6 (c 0.4, CHCl<sub>3</sub>); <sup>1</sup>H NMR (CDCl<sub>3</sub>):  $\delta$  7.26 (m, 1H), 6.22 (d,  $J$  = 7.4 Hz, 1H), 4.94 (d,  $J$  = 11.9 Hz, 1H), 4.76 (m, 1H), 4.64 (d,  $J$  = 11.9 Hz, 1H), 3.57 (m, 1H), 2.93 (q,  $J$  = 9.1 Hz, 1H), 2.73 (dd,  $J$  = 8.2 Hz,  $J$  = 7.4 Hz, 1H), 2.56 (d,  $J$  = 9.4 Hz, 2H), 2.39 (m, 2H), 1.84 (m, 1H), 1.56 (s, 3H), 1.53 (d,  $J$  = 7.2 Hz, 3H), 1.45 (s, 12H), 1.38 (m, 2H), 0.91 (m, 6H); <sup>13</sup>C NMR (CDCl<sub>3</sub>):  $\delta$  214.8, 172.8, 171.6, 152.0, 94.8, 80.5, 74.7, 58.9, 48.6, 45.2, 43.6, 42.9, 38.4, 28.9, 27.0, 25.4, 24.7, 21.9, 18.6; MS (FAB):  $m/z$  585.1 [M + 1]<sup>+</sup>; HRMS calcd for C<sub>25</sub>H<sub>39</sub>Cl<sub>3</sub>N<sub>2</sub>O<sub>7</sub> [M + 1]<sup>+</sup> 585.1901; found 585.1889.

**Compound 27.** Prepared from **12** according to the general procedure for the preparation of **25** (71%); [ $\alpha$ ]<sub>D</sub><sup>20</sup> -27.8 (c 0.5, CHCl<sub>3</sub>); <sup>1</sup>H NMR (CDCl<sub>3</sub>):  $\delta$  6.01 (d,  $J$  = 7.3 Hz, 1H), 4.93 (d,  $J$  = 11.9 Hz, 1H), 4.73 (m, 1H), 4.62 (d,  $J$  = 11.9 Hz, 1H), 3.76 (m, 1H), 3.65 (m, 1H), 2.51 (m, 1H), 2.41 (m, 1H), 1.93 (m, 1H), 1.80 (m, 2H), 1.58 (s, 3H), 1.49 (d,  $J$  = 7.2 Hz, 3H), 1.45 (s, 12H), 1.80–1.40 (m, 6H), 0.91 (d,  $J$  = 6.2 Hz, 6H); <sup>13</sup>C NMR (CDCl<sub>3</sub>):  $\delta$  175.3, 172.0, 152.1, 94.9, 94.0, 80.1, 77.7, 74.6, 74.5, 59.3, 48.7, 48.4, 46.7, 32.0, 30.8, 28.9, 27.5, 26.2, 25.7, 25.6, 24.7, 21.9, 18.6; MS (FAB):  $m/z$  571 [M + 1]<sup>+</sup>; HRMS calcd for C<sub>26</sub>H<sub>42</sub>N<sub>2</sub>O<sub>6</sub>Cl<sub>3</sub> [M + 1]<sup>+</sup> 571.2108; found 571.2097.

**Compound 28.** A solution of (67.2 mg, 0.115 mmol) in a mixture of formic acid (98%, 2.0 mL), CH<sub>2</sub>Cl<sub>2</sub> (1.0 mL), and a drop of water was stirred at room temperature for 3 h. The solvents were removed at room temperature under reduced pressure, and the residue was dissolved in CH<sub>2</sub>Cl<sub>2</sub> (3 mL) and cooled to 0 °C. Boc-Met-OH (43.0 mg, 0.172 mmol) was added followed by PyBOP (58.0 mg, 0.115 mmol) and *i*-Pr<sub>2</sub>NEt (89  $\mu$ L, 0.46 mmol). The reaction mixture was allowed to warm to room temperature over a period of 3 h. The solution was diluted with EtOAc (10 mL) and washed with 1 N HCl (1 mL) and saturated NaHCO<sub>3</sub> (1 mL). The organic layer was dried

over Na<sub>2</sub>SO<sub>4</sub>, filtered, and concentrated in vacuo. The residue was purified by column chromatography (50% EtOAc in hexanes) to afford **28** (24 mg, 33%) as a white solid; [ $\alpha$ ]<sub>D</sub><sup>20</sup> -34.5 (c 0.4, CHCl<sub>3</sub>); <sup>1</sup>H NMR (CDCl<sub>3</sub>):  $\delta$  7.57 (m, 1H), 7.27 (m, 1H), 5.11 (d,  $J$  = 7.6 Hz, 1H), 5.03 (d,  $J$  = 12.1 Hz, 1H), 4.85 (d,  $J$  = 10.4 Hz, 1H), 4.62 (d,  $J$  = 7.8 Hz, 1H), 4.61 (m, 1H), 4.56 (m, 1H); 4.09 (m, 1H), 3.58 (m, 1H), 3.32 (m, 1H), 2.68 (m, 2H), 2.56 (m, 4H), 2.39 (m, 2H), 2.10 (s, 3H), 2.02 (m, 1H), 1.88 (m, 1H), 1.73 (m, 1H), 1.60 (m, 1H), 1.54 (d,  $J$  = 7.4 Hz, 3H), 1.42 (s, 9H), 1.37 (m, 1H), 0.91 (d,  $J$  = 6.5 Hz, 3H), 0.89 (d,  $J$  = 6.5 Hz, 3H); <sup>13</sup>C NMR (CDCl<sub>3</sub>):  $\delta$  175.6, 173.9, 173.7, 156.4, 148.6, 106.8, 95.0, 81.2, 76.8, 74.7, 54.7, 53.2, 49.7, 49.1, 46.9, 40.3, 38.5, 36.0, 30.9, 30.7, 28.7, 25.3, 23.7, 22.1, 17.4, 15.8; MS (FAB):  $m/z$  674.2 [M + 1]<sup>+</sup>; HRMS calcd for C<sub>28</sub>H<sub>47</sub>Cl<sub>3</sub>N<sub>3</sub>O<sub>7</sub>S [M + 1]<sup>+</sup> 674.2200; found 674.2218.

**Compound 29.** A solution of **26** (76 mg, 0.13 mmol) in HCl (4 M in dioxane, 2 mL) was stirred at room temperature for 1 h. The solvent was removed under reduced pressure, then the residue was dissolved in CH<sub>2</sub>Cl<sub>2</sub> (3 mL) and cooled to 0 °C. Boc-Met-OH (48.6 mg, 0.19 mmol) was added followed by PyBOP (74.3 mg, 0.14 mmol) and *i*-Pr<sub>2</sub>NEt (90  $\mu$ L, 0.52 mmol). The reaction mixture was allowed to warm to room temperature over a period of 3 h. The solution was diluted with EtOAc (15 mL), washed with 1 N HCl (1 mL) and saturated NaHCO<sub>3</sub> (1 mL). The organic phase was dried over Na<sub>2</sub>SO<sub>4</sub>, filtered, and concentrated in vacuo. The residue was purified by column chromatography (70% EtOAc in hexanes) to afford **29** (65 mg, 75%) as a white solid; [ $\alpha$ ]<sub>D</sub><sup>20</sup> -24.4 (c 0.5, CHCl<sub>3</sub>); <sup>1</sup>H NMR (CDCl<sub>3</sub>):  $\delta$  7.33 (d,  $J$  = 9.1 Hz, 1H), 7.26 (m, 1H), 5.21 (d,  $J$  = 8.6 Hz, 1H), 4.62 (m, 1H), 4.61 (d,  $J$  = 16.0 Hz, 1H), 4.13 (m, 1H), 3.97 (m, 1H), 3.38 (m, 1H), 3.02 (m, 1H), 2.54 (m, 4H), 2.40 (m, 3H), 2.10 (s, 3H), 2.04 (m, 1H), 1.93 (m, 1H), 1.62 (m, 2H), 1.54 (d,  $J$  = 9.8 Hz, 3H), 1.41 (s, 9H), 1.33 (m, 2H), 0.91 (d,  $J$  = 8.9 Hz, 3H), 0.89 (d,  $J$  = 8.9 Hz, 3H); <sup>13</sup>C NMR (CDCl<sub>3</sub>):  $\delta$  216.9, 174.0, 173.6, 172.4, 156.3, 95.0, 81.2, 74.8, 74.7, 54.9, 51.1, 48.9, 44.6, 43.9, 41.3, 41.0, 39.6, 31.1, 30.8, 28.6, 25.2, 23.7, 22.1, 17.7, 15.8; MS (FAB):  $m/z$  676.1 [M + 1]<sup>+</sup>; HRMS calcd for C<sub>27</sub>H<sub>45</sub>Cl<sub>3</sub>N<sub>3</sub>O<sub>6</sub>S [M + 1]<sup>+</sup> 676.1993; found 676.2004.

**Compound 30.** Prepared from **27** according to the general procedure for the preparation of **28** (75%); [ $\alpha$ ]<sub>D</sub><sup>20</sup> -40.4 (c = 1.9, CHCl<sub>3</sub>); <sup>1</sup>H NMR (CDCl<sub>3</sub>):  $\delta$  7.26 (br, 1H), 6.96 (br, 1H), 5.04 (d,  $J$  = 6.0 Hz, 1H), 5.00 (d,  $J$  = 12.1 Hz, 1H), 4.66 (m, 1H), 4.63 (d,  $J$  = 12.1 Hz, 1H), 4.11 (m, 1H), 3.58 (b, 1H), 3.32 (b, 1H), 2.55 (d,  $J$  = 11.9 Hz, 1H), 2.46 (m, 1H), 2.28 (m, 1H), 2.09 (s, 3H), 2.01 (m, 2H), 1.85 (m, 3H), 1.65 (m, 5H), 1.52 (t,  $J$  = 7.0 Hz, 2H), 1.43 (s, 9H), 0.90 (d,  $J$  = 6.6 Hz, 3H), 0.88 (d,  $J$  = 6.6 Hz, 3H); <sup>13</sup>C NMR (CDCl<sub>3</sub>):  $\delta$  176.5, 173.8, 156.4, 95.0, 81.0, 77.7, 74.7, 54.6, 53.5, 50.2, 49.0, 47.2, 40.4, 32.4, 31.0, 30.7, 28.8, 28.6, 25.4, 24.4, 23.6, 22.2, 17.4, 15.8; MS (FAB):  $m/z$  662.3 [M + 1]<sup>+</sup>; HRMS calcd for C<sub>27</sub>H<sub>47</sub>N<sub>3</sub>O<sub>6</sub>Cl<sub>3</sub> [M + 1]<sup>+</sup> 662.2200; found 662.2182.

**Compound 31.** A solution of **28** (20.0 mg, 0.030 mmol) in a mixture of formic acid (98%, 1 mL) and H<sub>2</sub>O (20  $\mu$ L) was stirred at room temperature for 2 h. The mixture was concentrated under reduced pressure, the residue was dissolved in EtOAc (5 mL) and washed with NaHCO<sub>3</sub> (1 N, 0.5 mL). The solvent was evaporated, and the residue was dissolved in a mixture of CH<sub>2</sub>Cl<sub>2</sub> (1.0 mL) and water (1.0 mL) and cooled to 0 °C. Ac-L-Leu-OH (10.4 mg, 0.060 mmol) was added followed by HOBt (8.1 mg, 0.060 mmol) and EDC (12.7 mg, 0.060 mmol). The mixture was stirred at 0 °C for 24 h, then diluted with EtOAc (15 mL) and washed with HCl (1 N, 0.2 mL) and NaHCO<sub>3</sub> (1 N, 0.2 mL). The organic layer was dried over Na<sub>2</sub>SO<sub>4</sub>, filtered, and concentrated in vacuo. The residue was purified by column chromatography (EtOAc then 10% MeOH in CH<sub>2</sub>Cl<sub>2</sub>) to afford **31** (9.5 mg, 44%) as a white solid; [ $\alpha$ ]<sub>D</sub><sup>20</sup> -50.3 (c 0.4, CHCl<sub>3</sub>); <sup>1</sup>H NMR (CDCl<sub>3</sub>):  $\delta$  7.39 (m, 1H), 7.26 (m, 2H), 6.59 (m, 1H), 5.00 (d,  $J$  = 12.0 Hz, 1H), 4.87 (m, 2H), 4.77 (t,  $J$  = 7.2 Hz, 1H), 4.62 (d,  $J$  = 12.0 Hz, 1H), 4.57 (m, 1H), 4.39 (m, 1H), 3.99 (m, 1H), 3.49 (m, 1H), 2.81 (m, 1H), 2.70 (m, 1H), 2.55 (m, 4H), 2.49 (m, 1H), 2.31 (m, 1H), 2.09 (m, 1H), 2.08 (s, 3H), 2.03 (s, 3H), 1.62 (m, 4H), 1.53 (d,  $J$  = 7.2 Hz, 3H), 1.49 (m, 2H), 1.34 (m, 2H), 0.90 (m, 12H); <sup>13</sup>C NMR (CDCl<sub>3</sub>):  $\delta$  175.0, 173.1, 173.0, 171.6, 171.4,



107.4, 95.0, 74.6, 73.9, 53.5, 52.9, 51.6, 48.4, 48.0, 41.8, 41.0, 36.3, 33.5, 30.6, 30.4, 30.1, 25.3, 23.7, 23.4, 23.1, 22.7, 22.3, 18.3, 15.7; MS (FAB):  $m/z$  729.2 [M + 1]<sup>+</sup>; HRMS calcd for C<sub>31</sub>H<sub>52</sub>Cl<sub>3</sub>N<sub>4</sub>O<sub>7</sub>S [M + 1]<sup>+</sup> 729.2622; found 729.2621.

**Compound 32.** A solution of **29** (42 mg, 0.066 mmol) in HCl (4 M in dioxane, 2.0 mL) was stirred at room temperature for 1 h. The solution was concentrated under reduced pressure at room temperature. The residue was dissolved in EtOAc (5 mL) and washed with NaHCO<sub>3</sub> (1 N, 0.5 mL). The organic layer was concentrated under reduced pressure, and the residue was dissolved in a mixture of CH<sub>2</sub>Cl<sub>2</sub> (1 mL) and water (1 mL). Ac-Leu-OH (11.4 mg, 0.066 mmol) was added at 0 °C followed by HOBt (8.9 mg, 0.066 mmol) and EDC (14.0 mg, 0.073 mmol). The reaction mixture was stirred at 0 °C for 24 h, diluted with EtOAc (15 mL), and then washed with HCl (1 N, 0.2 mL) and NaHCO<sub>3</sub> (1 N, 0.2 mL). The organic phase was dried over Na<sub>2</sub>SO<sub>4</sub>, filtered, and concentrated in vacuo. The residue was purified by column chromatography (EtOAc then 10% MeOH in CH<sub>2</sub>Cl<sub>2</sub>) to afford **32** (34.2 mg, 76%) as a white solid; [α]<sub>D</sub><sup>20</sup> -68.7 (c 0.6, CHCl<sub>3</sub>); <sup>1</sup>H NMR (CDCl<sub>3</sub>): δ 7.75 (d, *J* = 9.3 Hz, 1H), 7.58 (d, *J* = 9.8 Hz, 1H), 7.18 (d, *J* = 12.3 Hz, 1H), 7.11 (d, *J* = 7.6 Hz, 1H), 4.96 (d, *J* = 15.9 Hz, 1H), 4.75 (m, 1H), 4.60 (d, *J* = 15.9 Hz, 1H), 4.54 (m, 1H), 4.48 (m, 1H), 4.20 (m, 1H), 4.05 (m, 1H), 3.51 (m, 1H), 3.13 (m, 1H), 2.56 (m, 3H), 2.49 (m, 2H), 2.32 (m, 2H), 2.09 (m, 2H), 2.06 (s, 3H), 2.00 (s, 3H), 1.68 (m, 2H), 1.55 (d, *J* = 9.7 Hz, 3H), 1.31 (m, 2H), 0.89 (m, 13H); <sup>13</sup>C NMR (CDCl<sub>3</sub>): δ 217.5, 174.0, 173.6, 172.4, 172.3, 171.9, 94.9, 74.6, 73.4, 53.7, 53.6, 50.4, 48.4, 45.3, 44.0, 42.0, 40.92, 40.8, 38.6, 30.7, 30.0, 25.3, 23.7, 23.1, 22.3, 18.3, 15.7; MS (FAB):  $m/z$  731.1 [M + 1]<sup>+</sup>; HRMS calcd for C<sub>30</sub>H<sub>50</sub>Cl<sub>3</sub>N<sub>4</sub>O<sub>8</sub>S [M + 1]<sup>+</sup> 731.2415; found 731.2433.

**Compound 33.** Prepared from **30** according to the general procedure for the preparation of **32** (52%); [α]<sub>D</sub><sup>20</sup> -71.5 (c 1.2, CHCl<sub>3</sub>); <sup>1</sup>H NMR (CDCl<sub>3</sub>): δ 7.46 (d, *J* = 7.0 Hz, 1H), 7.20 (d, *J* = 8.9 Hz, 1H), 6.96 (d, *J* = 7.0 Hz, 1H), 6.60 (m, 1H), 4.97 (d, *J* = 11.9 Hz, 1H), 4.71 (m, 1H), 4.62 (d, *J* = 11.9 Hz, 1H), 4.54 (m, 1H), 4.44 (m, 1H), 4.06 (m, 1H), 3.92 (m, 1H), 3.45 (m, 1H), 2.62 (m, 1H), 2.53 (m, 1H), 2.26 (m, 1H), 2.08 (s, 3H), 2.04 (m, 2H), 2.02 (s, 3H), 1.71 (m, 3H), 1.61 (m, 6H), 1.51 (d, *J* = 7.3 Hz, 3H), 1.37 (m, 1H), 0.90 (m, 12H); <sup>13</sup>C NMR (CDCl<sub>3</sub>): δ 176.1, 173.0, 172.9, 171.8, 171.2, 95.0, 77.6, 74.6, 53.5, 52.7, 51.9, 48.6, 48.5, 48.3, 41.7, 41.4, 30.7, 30.6, 30.2, 26.5, 25.2, 24.6, 23.7, 23.4, 23.0, 22.8, 22.4, 18.2, 15.8; MS (FAB):  $m/z$  717.6 [M + 1]<sup>+</sup>; HRMS calcd for C<sub>30</sub>H<sub>52</sub>N<sub>4</sub>O<sub>7</sub>Cl<sub>3</sub> [M + 1]<sup>+</sup> 717.2622; found 717.2609.

**Compound 34.** To a solution of **31** in THF (0.4 mL) were added KH<sub>2</sub>PO<sub>4</sub> and zinc powder (100 mg) under Ar atmosphere. The reaction mixture was stirred for 1 h. After the disappearance of starting material on TLC, the Zn powder was filtered, and the filtrate was concentrated under reduced pressure. The residue was purified by column chromatography (20% MeOH in CH<sub>2</sub>Cl<sub>2</sub>) to afford **34** (5.0 mg, 71%) as a white solid; [α]<sub>D</sub><sup>20</sup> -51.9 (c 0.2, MeOH); <sup>1</sup>H NMR (CD<sub>3</sub>OD): δ 4.84 (m, 2H), 4.47 (m, 1H), 4.34 (m, 1H), 4.23 (m, 1H), 3.94 (m, 1H), 3.43 (t, *J* = 4.2 Hz, 1H), 2.76 (m, 1H), 2.61 (m, 1H), 2.53 (m, 2H), 2.40 (m, 2H), 2.29 (m, 1H), 2.09 (s, 3H), 2.00 (s, 3H), 1.68 (m, 1H), 1.59 (m, 4H), 1.45 (m, 1H), 1.38 (d, *J* = 7.1 Hz, 3H), 1.29 (m, 2H), 0.91 (m, 12H); <sup>13</sup>C NMR (CD<sub>3</sub>OD): δ 175.0, 174.5, 174.0, 172.5, 172.4, 150.3, 105.4, 72.1, 53.1, 52.5, 51.7, 50.8, 49.0, 48.8, 41.4, 40.8, 36.5, 32.4, 31.4, 30.2, 24.9, 24.8, 22.8, 22.3, 21.4, 21.3, 21.0, 18.3, 14.3; MS (FAB):  $m/z$  599.3 [M + 1]<sup>+</sup>; HRMS calcd for C<sub>29</sub>H<sub>51</sub>N<sub>4</sub>O<sub>7</sub>S [M + 1]<sup>+</sup> 599.3478; found 599.3471; LC/MS retention time: [A] 22.33 min; [B] 15.16 min.

**Compound 35.** Prepared from **32** according to the general procedure for the preparation of **34** (12 mg, 77%); [α]<sub>D</sub><sup>20</sup> -32.2 (c 0.6, MeOH); <sup>1</sup>H NMR (CD<sub>3</sub>OD): δ 4.58 (m, 1H), 4.48 (m, 1H), 4.34 (t, *J* = 4.7 Hz, 1H), 4.22 (m, 1H), 4.04 (m, 1H), 3.53 (m, 1H), 3.11 (m, 1H), 2.68 (m, 1H), 2.58 (m, 1H), 2.44 (m, 2H), 2.39 (m, 1H), 2.30 (m, 1H), 2.09 (s, 3H), 2.00 (s, 3H), 1.94 (m, 1H), 1.69 (m, 1H), 1.58 (m, 4H), 1.44 (m, 1H), 1.40 (d, *J* = 6.9 Hz, 3H), 1.29 (m, 1H), 0.93 (m, 12H); <sup>13</sup>C NMR (CD<sub>3</sub>OD): δ 218.4, 174.5, 174.4, 174.1, 172.7, 72.4, 53.2, 52.7, 51.0, 44.7, 44.6, 44.1, 41.2, 40.7, 38.4, 31.3, 30.3, 27.9, 24.9, 24.8, 24.9,

22.9, 22.4, 21.5, 21.2, 21.1, 17.9, 14.3; MS (FAB):  $m/z$  601.1 [M + 1]<sup>+</sup>; HRMS calcd for C<sub>28</sub>H<sub>49</sub>N<sub>4</sub>O<sub>8</sub>S [M + 1]<sup>+</sup> 601.3226; found 601.3267; LC/MS retention time: [A] 12.31 min; [B] 11.27 min.

**Compound 36.** Prepared from **33** according to the general procedure for the preparation of **34** (17.2 mg, 91%); [α]<sub>D</sub><sup>20</sup> -53.0 (c 0.4, MeOH); <sup>1</sup>H NMR (CD<sub>3</sub>OD): δ 4.44 (m, 1H), 4.35 (t, *J* = 6.8 Hz, 3H), 4.19 (q, *J* = 7.1 Hz, 1H), 3.89 (m, 1H), 3.41 (m, 1H), 2.57 (m, 3H), 2.24 (m, 1H), 2.09 (s, 3H), 2.02 (m, 1H), 2.00 (s, 3H), 1.86 (m, 1H), 1.70 (m, 5H), 1.59 (m, 3H), 1.48 (m, 1H), 1.37 (d, *J* = 7.1 Hz, 3H), 1.31 (m, 1H), 0.98 (d, *J* = 6.5 Hz, 3H), 0.93 (m, 9H); <sup>13</sup>C NMR (CD<sub>3</sub>OD): δ 180.6, 176.9, 174.3, 172.7, 172.5, 73.7, 53.4, 52.7, 51.4, 51.2, 41.7, 40.7, 31.2, 30.7, 30.3, 26.9, 24.9, 24.8, 24.7, 22.8, 22.4, 21.5, 21.4, 21.0, 17.5, 14.3; MS (FAB):  $m/z$  587.3 [M + 1]<sup>+</sup>; HRMS calcd for C<sub>28</sub>H<sub>51</sub>N<sub>4</sub>O<sub>7</sub>S [M + 1]<sup>+</sup> 587.3434; found 587.3474; LC/MS retention time: [A] 15.60 min; [B] 14.29 min

**Compound 37.** To a solution of KOH (280 mg, 5.0 mmol) in MeOH (20 mL) and H<sub>2</sub>O (10 mL) was added **12** (770 mg, 2 mmol). The reaction mixture was stirred at 65 °C for 3 h then at room-temperature overnight. HCl (1 N, 20 mL) was added, and the aqueous phase was extracted with CH<sub>2</sub>Cl<sub>2</sub> (4 × 20 mL). The combined organic layers were dried (Na<sub>2</sub>SO<sub>4</sub>), filtered, and concentrated in vacuo. The residue was dissolved in CH<sub>2</sub>Cl<sub>2</sub> (35 mL), followed by addition of H-Val-OBn-TsOH salt (1.14 g, 3 mmol), PyBOP (1.59 g, 3 mmol) and *i*-Pr<sub>2</sub>NEt (1.78 mL, 10.2 mmol) at 0 °C. The reaction mixture was stirred for 3.5 h at 0 °C, then was partitioned in EtOAc (80 mL) and 1 N HCl (20 mL). The separated organic layer was washed with saturated NaHCO<sub>3</sub> (20 mL) and brine (20 mL). The organic layer was dried (Na<sub>2</sub>SO<sub>4</sub>), filtered, and concentrated in vacuo. The residue was purified by column chromatography (20% EtOAc in hexanes) to afford **37** (1.1 g, 98%); as a colorless oil; [α]<sub>D</sub><sup>20</sup> -21.1 (c 1.0, CHCl<sub>3</sub>); <sup>1</sup>H NMR (CDCl<sub>3</sub>): δ 7.36-7.19 (m, 5H), 6.06 (d, *J* = 7.5 Hz, 1H), 5.10 (m, 2H), 4.60 (m, 1H), 3.77 (br, 1H), 3.60 (br, 1H), 2.42 (br, 2H), 2.14 (m, 1H), 1.96-1.20 (m, 15H), 1.40 (d, *J* = 5.5 Hz, 9H), 0.92 (m, 12H); <sup>13</sup>C NMR (CDCl<sub>3</sub>): δ 175.6, 172.3, 152.0, 135.6, 128.9, 128.8, 128.7, 94.1, 82.4, 79.9, 67.4, 60.7, 59.2, 57.2, 49.0, 47.1, 44.5, 43.0, 31.9, 31.7, 30.6, 28.8, 27.9, 27.3, 25.4, 24.7, 21.8, 21.4, 19.5, 19.3, 17.9, 14.6; MS (FAB):  $m/z$  559.4 [M + 1]<sup>+</sup>.

**Compound 38.** To a solution of **37** (559 mg, 1 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (16 mL) was added TFA (4 mL). The reaction mixture was stirred at room temperature for 1 h, then quenched by addition of saturated NaHCO<sub>3</sub> (15 mL) and extracted with EtOAc (4 × 20 mL). The combined organic layers were dried (Na<sub>2</sub>SO<sub>4</sub>), filtered, and concentrated in vacuo. The residue was dissolved in CH<sub>2</sub>Cl<sub>2</sub> (15 mL), followed by addition of Boc-Met-OH (499 mg, 2 mmol), PyBOP (1.059 g, 2 mmol), and *i*-Pr<sub>2</sub>NEt (696 μL, 4 mmol) at 0 °C. The reaction mixture was stirred at 0 °C for 3 h. EtOAc (80 mL) was added, and the separated organic layer was washed by 1 N HCl (2 × 20 mL), saturated NaHCO<sub>3</sub> (2 × 20 mL), and brine (20 mL). The organic layer was dried (Na<sub>2</sub>SO<sub>4</sub>), filtered, and concentrated in vacuo. The residue was purified by column chromatography (66% EtOAc in hexanes) to afford **38** (440 mg, 68%) as a colorless oil; [α]<sub>D</sub><sup>20</sup> -101.2 (c 0.1, MeOH); <sup>1</sup>H NMR (CDCl<sub>3</sub>): δ 7.42-7.19 (m, 5H), 6.91 (d, *J* = 6.4 Hz, 1H), 5.84 (d, *J* = 8.3 Hz, 1H), 5.63 (d, *J* = 7.6 Hz, 1H), 5.12 (m, 2H), 4.67 (br, 1H), 4.51 (m, 1H), 4.41 (m, 1H), 4.16 (m, 1H), 3.56 (m, 1H), 3.39 (br, 1H), 2.50 (m, 3H), 2.29 (m, 1H), 2.13 (m, 1H), 2.01 (s, 3H), 1.87 (m, 2H), 1.74 (m, 2H), 1.61 (m, 5H), 1.40-1.21 (m, 1H), 1.36 (d, *J* = 8.6 Hz, 9H), 0.84 (m, 12H); <sup>13</sup>C NMR (CDCl<sub>3</sub>): δ 176.7, 174.0, 173.7, 156.4, 135.7, 129.0, 128.8, 128.7, 127.8, 80.7, 76.6, 67.5, 58.7, 54.5, 53.6, 50.3, 47.4, 40.5, 32.1, 31.2, 30.9, 30.6, 28.7, 27.8, 25.3, 24.4, 23.7, 22.1, 19.7, 19.1, 15.7; MS (FAB):  $m/z$  650.4 [M + 1]<sup>+</sup>; HRMS calcd for C<sub>34</sub>H<sub>56</sub>N<sub>3</sub>O<sub>7</sub>S [M + 1]<sup>+</sup> 650.3839; found 650.3811.

**Compound 39.** A solution of **38** (325 mg, 0.5 mmol) in HCl (4 M in dioxane, 10 mL, 40 mmol) was stirred at room temperature for 1 h, then the solvent was removed under reduced pressure. The residue was dissolved in a saturated solution of NaHCO<sub>3</sub> (10 mL) and extracted with EtOAc (4 × 15 mL). The combined organic layers were dried (Na<sub>2</sub>SO<sub>4</sub>),

filtered, and concentrated in vacuo. The residue was dissolved in  $\text{CH}_2\text{Cl}_2$  (5 mL), followed by addition of water (5 mL), Ac-Leu-OH (86.5 mg, 0.5 mmol), and HOBt (67.7 mg, 0.5 mmol), then cooled in an ice bath to 0 °C, and EDC (105 mg, 0.5 mmol) was added, then stirred at 0 °C for 36 h. Aqueous HCl (1 N, 5 mL) was added, the layers were partitioned, and the organic layer was washed sequentially with aqueous HCl (0.5 N, 10 mL), brine (10 mL),  $\text{NaHCO}_3$  (1 N, 2 × 10 mL), and brine (10 mL). The organic layer was dried ( $\text{Na}_2\text{SO}_4$ ), filtered, and concentrated in vacuo. The residue was purified by column chromatography (EtOAc) to afford **39** (204 mg, 58%) as a white foam;  $[\alpha]_D^{20} -23.9$  (c 3.2, MeOH);  $^1\text{H NMR}$  ( $\text{CD}_3\text{OD}$ )  $\delta$  7.40–7.22 (m, 5H), 5.21 (d,  $J = 12.3$  Hz, 1H), 5.11 (d,  $J = 12.3$  Hz, 1H), 4.43 (q,  $J = 5.0$  Hz, 1H), 4.32 (m, 2H), 3.90 (m, 1H), 3.43 (t,  $J = 4.6$  Hz, 1H), 2.66 (m, 1H), 2.53 (m, 2H), 2.31 (m, 1H), 2.20–1.80 (m, 6H), 2.08 (s, 3H), 1.98 (s, 3H), 1.68 (m, 7H), 1.56 (t,  $J = 7.4$  Hz, 3H), 1.45 (m, 1H), 1.27 (m, 1H), 0.94 (m, 18H);  $^{13}\text{C NMR}$  ( $\text{CD}_3\text{OD}$ )  $\delta$  178.5, 175.0, 173.3, 173.1, 172.9, 137.5, 129.6, 129.5, 129.4, 74.4, 67.7, 59.7, 54.7, 53.6, 52.7, 42.4, 41.9, 32.0, 31.8, 31.7, 31.5, 26.9, 25.9, 25.8, 25.7, 23.8, 23.2, 22.2, 22.0, 19.2, 18.8, 15.2; MS (FAB):  $m/z$  705.8  $[\text{M} + 1]^+$ .

**Compound 40.** To a solution of **39** (84 mg, 0.119 mmol) in 10% formic acid in MeOH was added Pd black (90 mg) under Ar atmosphere. The mixture was stirred at room temperature for 30 min. The reaction mixture was filtered through a pad of Celite and washed with MeOH. The collected filtrate was evaporated under reduced pressure, and the residue was purified by column chromatography (20% MeOH in  $\text{CH}_2\text{Cl}_2$ ) to afford **40** (54 mg, 74%) as a white solid;  $[\alpha]_D^{20} -104.5$  (c 0.5, MeOH);  $^1\text{H NMR}$  ( $\text{CD}_3\text{OD}$ )  $\delta$  4.45 (q,  $J = 5.0$  Hz, 1H), 4.32 (m, 2H), 3.91 (m, 1H), 3.48 (t,  $J = 4.3$  Hz, 1H), 2.71 (m, 1H), 2.52 (m, 2H), 2.26 (m, 1H), 2.20–1.80 (m, 5H), 2.09 (s, 3H), 1.98 (s, 3H), 1.68 (m, 7H), 1.56 (t,  $J = 7.4$  Hz, 3H), 1.43 (m, 1H), 1.28 (m, 1H), 0.95 (m, 18H);  $^{13}\text{C NMR}$  ( $\text{CD}_3\text{OD}$ )  $\delta$  177.5, 174.4, 174.1, 172.5, 172.3, 73.2, 58.6, 53.1, 52.6, 51.8, 41.3, 40.8, 31.1, 30.7, 30.5, 30.1, 25.0, 24.9, 24.8, 23.0, 22.4, 21.4, 21.3, 21.1, 18.8, 17.8, 14.3; MS (FAB):  $m/z$  637.3  $[\text{M} + 23]^+$ ; HRMS calcd for  $\text{C}_{30}\text{H}_{55}\text{N}_4\text{O}_7\text{S}$   $[\text{M} + 1]^+$  615.3747; found 615.3789; LC/MS retention time: [A] 17.89 min; [B] 16.38 min.

**Compound 41.** Boc-Val-NHBu (43 mg, 0.158 mmol) was stirred with HCl (4 M in dioxane, 1 mL) at room temperature for 1 h. The solvent was removed under reduced pressure, and the compound was dissolved in  $\text{CH}_2\text{Cl}_2$  (2 mL) and cooled to 0 °C. The acid from **11** (48 mg, 0.125 mmol) was added followed by PyBOP (65 mg, 0.125 mmol) and *i*-Pr<sub>2</sub>NEt (65  $\mu\text{L}$ , 0.375 mmol). The reaction mixture was allowed to warm to room temperature over a period of 3 h, diluted with EtOAc (10 mL), and washed with HCl (1 N, 1 mL) and saturated  $\text{NaHCO}_3$  (1 mL). The organic phase was dried over  $\text{Na}_2\text{SO}_4$ , filtered, and concentrated in vacuo. The residue was purified by column chromatography (50% EtOAc in hexanes) to give **41** (64 mg, 95%) as a white solid;  $[\alpha]_D^{20} -66.6$  (c 0.9,  $\text{CHCl}_3$ );  $^1\text{H NMR}$  ( $\text{CDCl}_3$ ):  $\delta$  6.59 (br, 1H), 6.08 (br, 1H), 4.27 (t,  $J = 7.0$  Hz, 1H), 4.02 (m, 1H), 3.56 (br, 2H), 3.30 (m, 1H), 3.20 (m, 1H), 4.27 (q,  $J = 8.8$  Hz, 1H), 2.62 (m, 1H), 2.56 (m, 1H), 2.49 (m, 1H), 2.38 (m, 2H), 2.08 (m, 1H), 1.88 (br, 2H), 1.47 (s, 3H), 1.40 (m, 5H), 1.46 (s, 9H), 1.34 (m, 3H), 0.95 (m, 15H);  $^{13}\text{C NMR}$  ( $\text{CDCl}_3$ ):  $\delta$  214.6, 172.6, 170.5, 156.0, 79.9, 58.5, 58.1, 45.2, 42.2, 39.2, 37.4, 31.4, 28.3, 26.30, 24.8, 24.2, 21.2, 19.8, 19.1, 18.0, 13.5; MS (FAB):  $m/z$  538.2  $[\text{M} + 1]^+$ ; HRMS calcd for  $\text{C}_{29}\text{H}_{52}\text{N}_3\text{O}_6$   $[\text{M} + 1]^+$  538.3856; found 538.3834.

**Compound 42.** Prepared from **10** according to the general procedure for the preparation of **41** (26.8 mg, 75%);  $[\alpha]_D^{20} -51.1$  (c 1.3,  $\text{CHCl}_3$ );  $^1\text{H NMR}$  ( $\text{CDCl}_3$ ):  $\delta$  6.63 (br, 1H); 6.40 (t,  $J = 6.8$  Hz, 1H), 4.52 (t,  $J = 7.0$  Hz, 1H), 3.74 (m, 1H), 3.63 (m, 1H), 3.24 (q,  $J = 6.7$  Hz, 2H), 2.47 (m, 1H), 2.37 (m, 1H), 1.88 (m, 1H), 1.72 (m, 1H), 1.67 (m, 2H), 1.64 (m, 4H), 1.58 (s, 3H), 1.48 (m, 2H), 1.45 (s, 12H), 1.36 (d,  $J = 6.9$  Hz, 3H), 1.33 (m, 3H); 0.92 (m, 9H);  $^{13}\text{C NMR}$  ( $\text{CDCl}_3$ ):  $\delta$  175.6, 172.6, 152.1, 80.0, 77.6, 59.4, 49.2, 48.0, 46.9, 39.6, 32.0, 28.9, 27.5, 25.6, 24.7, 21.9, 20.4, 19.2, 14.1; MS (FAB):  $m/z$  496.2  $[\text{M} + 1]^+$ ; HRMS calcd for  $\text{C}_{27}\text{H}_{50}\text{N}_3\text{O}_5$   $[\text{M} + 1]^+$  496.3750; found 496.3768.

**Compound 43.** A solution of **41** (49 mg, 0.091 mmol) in HCl (4 M in dioxane, 1 mL) was stirred at room temperature

for 1 h. The solvent was removed under reduced pressure, and the residue was dissolved in  $\text{CH}_2\text{Cl}_2$  (2.0 mL) and cooled to 0 °C. Boc-Met-OH (25 mg, 0.101 mmol) was added followed by PyBOP (52.6 mg, 0.101 mmol) and *i*-Pr<sub>2</sub>NEt (48  $\mu\text{L}$ , 2.73 mmol). The mixture was allowed to warm to room temperature over a period of 2 h, diluted with EtOAc (10 mL), and washed with HCl (1 N, 1 mL) and saturated  $\text{NaHCO}_3$  (1 mL). The organic phase was dried over  $\text{Na}_2\text{SO}_4$ , filtered, and concentrated in vacuo. The residue was purified by column chromatography (EtOAc) to afford **43** (41 mg, 72%) as a white solid;  $[\alpha]_D^{20} -41.7$  (c 0.6, MeOH);  $^1\text{H NMR}$  ( $\text{CDCl}_3$ ):  $\delta$  8.19 (br, 1H), 7.56 (br, 1H), 6.29 (br, 1H), 5.22 (br, 1H), 4.28 (m, 1H), 4.28 (m, 1H), 3.87 (m, 1H), 3.61 (m, 2H), 3.31 (m, 1H), 3.20 (m, 1H), 2.86 (m, 1H), 2.56 (m, 4H), 2.36 (m, 1H), 2.11 (s, 3H), 2.09 (m, 1H), 1.92 (m, 1H), 1.50 (m, 2H), 1.46 (m, 2H), 1.41 (s, 9H), 1.33 (m, 3H), 0.85–0.98 (m, 15H);  $^{13}\text{C NMR}$  ( $\text{CDCl}_3$ ):  $\delta$  215.0, 173.8, 173.4, 172.3, 156.1, 80.6, 75.4, 54.2, 52.8, 47.1, 43.6, 43.2, 39.8, 39.2, 31.2, 30.4, 30.1, 29.9, 28.1, 24.7, 23.7, 23.1, 21.5, 19.9, 19.3, 15.5, 15.2, 15.1; MS (FAB):  $m/z$  651.4  $[\text{M} + 23]^+$ ; HRMS calcd for  $\text{C}_{31}\text{H}_{57}\text{N}_4\text{O}_7\text{S}$   $[\text{M} + 1]^+$  629.3947; found 629.3931.

**Compound 44.** Prepared from **42** according to the general procedure for the preparation of **43** (26.8 mg, 75%);  $[\alpha]_D^{20} -45.5$  (c 1.0,  $\text{CHCl}_3$ );  $^1\text{H NMR}$  ( $\text{CDCl}_3$ ):  $\delta$  8.28 (br, 1H), 7.27 (br, 1H), 6.58 (br, 1H), 6.9 (m, 1H), 5.26 (d,  $J = 6.9$  Hz, 1H), 5.00 (br, 1H), 4.31 (t,  $J = 6.4$  Hz, 1H), 4.22 (q,  $J = 7.0$  Hz, 1H), 3.40 (m, 1H), 3.28 (m, 1H), 3.21 (m, 2H), 2.57 (m, 2H), 2.38 (m, 1H), 2.36 (m, 1H), 2.10 (s, 3H), 1.91 (m, 2H), 1.88 (m, 2H), 1.74 (m, 2H), 1.63 (m, 4H), 1.47 (m, 2H), 1.43 (s, 9H), 1.37 (d,  $J = 7.2$  Hz, 3H), 1.34 (m, 3H), 0.91 (m, 9H);  $^{13}\text{C NMR}$  ( $\text{CDCl}_3$ ):  $\delta$  176.8, 174.3, 173.8, 156.4, 80.8, 54.8, 53.6, 50.9, 50.4, 47.3, 40.6, 39.7, 32.9, 31.9, 31.4, 30.7, 29.1, 28.6, 25.4, 24.2, 23.6, 22.2, 20.4, 18.2, 15.7, 14.2; MS (FAB):  $m/z$  587.1  $[\text{M} + 1]^+$ ; 609.2  $[\text{M} + 23]^+$ ; HRMS calcd for  $\text{C}_{29}\text{H}_{55}\text{N}_4\text{O}_6\text{S}$   $[\text{M} + 1]^+$  587.3842; found 587.3854.

**Compound 45.** A solution of **43** (24.4 mg, 0.038 mmol) in HCl (4 M in dioxane, 1 mL) was stirred at room temperature for 1 h. After removal of the solvent under reduced pressure, the residue was dissolved in EtOAc and washed with  $\text{NaHCO}_3$  (1 N, 0.5 mL) and water. The solvent was removed and the residue dissolved in a mixture of  $\text{CH}_2\text{Cl}_2$  (0.75 mL) and water (0.75 mL) and cooled to 0 °C. Ac-Leucine (13.1 mg, 0.076 mmol) was added followed by EDC (16.1 mg, 0.076 mmol) and HOBt (10.2 mg, 0.076 mmol). The reaction mixture was stirred at 0 °C for 24 h, then diluted with EtOAc (5 mL), washed with HCl (1 N, 0.2 mL) and  $\text{NaHCO}_3$  (1 N, 0.2 mL). The organic phase was dried over  $\text{Na}_2\text{SO}_4$ , filtered, and concentrated in vacuo. The residue was purified by column chromatography (10% MeOH in  $\text{CH}_2\text{Cl}_2$ ) to give the **45** (20.8 mg, 78%) as a white solid;  $[\alpha]_D^{20} -60.0$  (c 0.2,  $\text{CHCl}_3$ );  $^1\text{H NMR}$  ( $\text{CD}_3\text{OD}$ ):  $\delta$  4.46 (m, 1H), 4.32 (t,  $J = 7.0$  Hz, 1H), 4.12 (d,  $J = 7.8$  Hz, 1H), 3.98 (m, 1H), 3.52 (m, 1H), 3.24 (m, 1H), 3.16 (m, 2H), 2.60 (m, 1H), 2.51 (m, 1H), 2.41 (m, 2H), 2.28 (m, 1H), 2.23 (s, 3H), 2.05 (m, 2H), 1.98 (s, 3H), 1.69 (m, 1H), 1.58 (m, 4H), 1.50 (m, 4H), 1.37 (m, 2H), 1.28 (m, 1H), 0.88–0.99 (m, 21H);  $^{13}\text{C NMR}$  ( $\text{CD}_3\text{OD}$ ):  $\delta$  218.0, 174.9, 174.2, 172.6, 172.5, 172.4, 72.3, 59.7, 53.1, 52.7, 51.3, 44.7, 44.1, 41.2, 41.0, 40.8, 39.1, 37.9, 31.5, 31.2, 31.1, 30.3, 24.9, 24.8, 22.9, 22.3, 21.4, 20.1, 18.8, 18.3, 14.3, 13.0; MS (FAB):  $m/z$  684.6  $[\text{M} + 1]^+$ ; HRMS calcd for  $\text{C}_{34}\text{H}_{62}\text{N}_5\text{O}_7\text{S}$   $[\text{M} + 1]^+$  684.4325; found 684.4363; LC/MS retention time: [A] 20.33 min; [B] 18.28 min.

**Compound 46.** Prepared from **44** according to the general procedure for the preparation of **45** (17 mg, 78%);  $[\alpha]_D^{20} -55.1$  (c 0.2,  $\text{CHCl}_3$  and MeOH (1:1));  $^1\text{H NMR}$  ( $\text{CD}_3\text{OD}$ ):  $\delta$  4.64 (m, 1H), 4.42 (t,  $J = 6.4$  Hz, 1H), 4.31 (q,  $J = 7.0$  Hz, 1H), 3.92 (m, 1H), 3.36 (m, 1H), 3.19 (m, 2H), 2.59 (m, 2H), 2.50 (m, 1H), 2.27 (m, 1H), 2.16 (s, 3H), 2.09 (m, 3H), 1.98 (s, 3H), 1.90 (m, 2H), 1.70 (m, 6H), 1.56 (m, 3H), 1.46 (m, 3H), 1.35 (d,  $J = 7.3$  Hz, 3H), 1.30 (m, 2H), 0.96 (m, 15H);  $^{13}\text{C NMR}$  ( $\text{CD}_3\text{OD}$ ):  $\delta$  177.1, 174.5, 174.3, 172.5, 172.3, 73.8, 53.1, 52.5, 51.2, 49.7, 47.1, 41.5, 40.8, 39.1, 31.5, 31.1, 30.7, 30.2, 26.7, 24.9, 24.8, 24.7, 22.9, 22.3, 21.4, 21.3, 21.1, 20.0, 17.4, 14.3, 13.1; MS (FAB):  $m/z$  642.3  $[\text{M} + 1]^+$ ; 664.4  $[\text{M} + 23]^+$ ; HRMS calcd for



$C_{32}H_{60}N_5O_6$  S [M + 1]<sup>+</sup> 642.4264; found 642.4242; LC/MS retention time: [A] 20.21 min; [B] 18.51 min.

**Compound 47.** To a solution of **40** (30 mg, 0.049 mmol) in *N,N*-dimethylacetamide (0.5 mL) were sequentially added PyBOP (27.2 mg, 0.051 mmol), *N*-methylmorpholine (5.6  $\mu$ L, 0.051 mmol), and BuNH<sub>2</sub> (5.8  $\mu$ L, 0.058 mmol). The reaction mixture was stirred at 0 °C for 3 h. The reaction mixture was diluted with EtOAc (40 mL) and washed with 1 N HCl (2  $\times$  8 mL), NaHCO<sub>3</sub> (2  $\times$  8 mL) and brine (8 mL). The organic layer was dried (Na<sub>2</sub>SO<sub>4</sub>), filtered, and concentrated in vacuo. The residue was purified by column chromatography (4% of MeOH in CH<sub>2</sub>Cl<sub>2</sub>) to afford **47** (6.5 mg, 20%) as a white solid; [ $\alpha$ ]<sub>D</sub><sup>20</sup> -78.4 (c 0.3, MeOH); <sup>1</sup>H NMR (CD<sub>3</sub>OD)  $\delta$  4.45 (q, *J* = 5.0 Hz, 1H), 4.35 (m, 1H), 4.09 (m, 1H), 3.90 (m, 1H), 3.46 (m, 2H), 3.19 (m, 2H), 2.68 (m, 1H), 2.60 (m, 1H), 2.52 (m, 1H), 2.28 (m, 1H), 2.09 (s, 3H), 1.99 (s, 3H), 1.87 (m, 3H), 1.80–1.08 (m, 22H), 0.95 (m, 18H); <sup>13</sup>C NMR (CD<sub>3</sub>OD)  $\delta$  177.3, 174.1, 172.7, 172.5, 172.4, 73.2, 59.7, 53.8, 52.6, 40.8, 31.5, 30.9, 30.3, 30.2, 24.9, 24.8, 22.4, 21.4, 21.3, 21.1, 20.1, 18.8, 18.3; MS (FAB): *m/z* 670.5 [M + 1]<sup>+</sup>; HRMS calcd for C<sub>34</sub>H<sub>64</sub>N<sub>5</sub>O<sub>6</sub>S [M + 1]<sup>+</sup> 670.4533; found 670.4571; LC/MS retention time: [A] 26.98 min; [B] 24.71 min.

**(5S)-((2R)-Butylcarbamoyl-cyclopentyl)-(4S)-isobutyl-2,2-dimethyl-oxazolidine-3-carboxylic Acid tert-Butyl Ester (48).** To a solution of NaOH (40 mg, 1.0 mmol) in MeOH (4.5 mL) and H<sub>2</sub>O (2.3 mL) was added **12** (192.2 mg, 0.5 mmol), then stirred at 65 °C for 3 h and at room-temperature overnight. 1 N HCl (10 mL) was added, the aqueous phase was extracted with CH<sub>2</sub>Cl<sub>2</sub> (4  $\times$  10 mL), and the combined organic layers were dried (Na<sub>2</sub>SO<sub>4</sub>), filtered, and concentrated in vacuo. The residue was used without further purification.

To a solution of the above acid and BuNH<sub>2</sub> (50  $\mu$ L, 0.525 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (11.5 mL) were added EDC (101 mg, 0.525 mmol) and HOBt (71 mg, 0.525 mmol), followed by DMAP (64.3 mg, 0.525 mmol) at 0 °C. The reaction mixture was stirred for 24 h at room temperature and then partitioned between CH<sub>2</sub>Cl<sub>2</sub> (20 mL) and 1 N HCl (10 mL). The organic layer was separated and washed by brine (10 mL), dried (Na<sub>2</sub>SO<sub>4</sub>), filtered, and concentrated in vacuo. The residue was purified by column chromatography (20% EtOAc in hexanes) to afford **48** (190 mg, 89%) as a white solid; [ $\alpha$ ]<sub>D</sub><sup>20</sup> -23.1 (c 0.4, CHCl<sub>3</sub>); <sup>1</sup>H NMR (CDCl<sub>3</sub>)  $\delta$  5.69 (s, 1H), 3.68 (br, 1H), 3.59 (br, 1H), 3.19 (m, 2H), 2.41 (m, 1H), 2.24 (br, 1H), 1.92–1.10 (m, 19H), 1.39 (s, 9H), 0.83 (m, 9H); <sup>13</sup>C NMR (CDCl<sub>3</sub>)  $\delta$  175.3, 152.1, 93.9, 82.9, 81.6, 59.2, 49.2, 46.8, 44.5, 43.0, 39.6, 32.2, 31.0, 28.8, 27.4, 26.2, 25.5, 25.3, 24.7, 21.8, 20.4, 14.1; MS (FAB): *m/z* 425.4 [M + 1]<sup>+</sup>; HRMS calcd for C<sub>24</sub>H<sub>45</sub>N<sub>2</sub>O<sub>4</sub> [M + 1]<sup>+</sup> 425.3379; found 425.3374.

**Compound 49.** To a solution of **48** (170 mg, 0.4 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (6 mL) was added TFA (1.5 mL). The reaction mixture was stirred at room temperature for 1 h. The reaction was quenched by addition of saturated NaHCO<sub>3</sub> (10 mL) and extracted by EtOAc (4  $\times$  10 mL). The combined organic layers were dried (Na<sub>2</sub>SO<sub>4</sub>), filtered, and concentrated in vacuo. The residue was dissolved in CH<sub>2</sub>Cl<sub>2</sub> (7 mL) followed by addition of *N*-Boc-Met-OH (299 mg, 1.2 mmol), PyBOP (636 mg, 1.2 mmol) and *i*-Pr<sub>2</sub>NEt (418  $\mu$ L, 2.4 mmol) at 0 °C. The reaction mixture was stirred at 0 °C for 3 h, EtOAc (80 mL) was added, and the separated organic layer was washed with 1 N HCl (2  $\times$  10 mL), saturated NaHCO<sub>3</sub> (2  $\times$  10 mL), and brine (10 mL). The organic layer was dried (Na<sub>2</sub>SO<sub>4</sub>), filtered, and concentrated in vacuo. The residue was purified by column chromatography (66% EtOAc in hexanes) to afford **49** (152 mg, 74%) as a colorless oil; [ $\alpha$ ]<sub>D</sub><sup>20</sup> -58.6 (c 0.5, CHCl<sub>3</sub>); <sup>1</sup>H NMR (CDCl<sub>3</sub>)  $\delta$  6.63 (d, *J* = 8.4 Hz, 1H); 6.30 (br, 1H); 5.40 (br, 1H); 4.19 (m, 1H); 3.94 (m, 2H), 3.40 (s, 1H), 3.19 (m, 2H), 2.53 (m, 3H), 2.17 (m, 1H), 2.00 (m, 1H), 2.06 (d, *J* = 3.2 Hz, 3H), 1.86 (m, 2H), 1.79–1.16 (m, 13H), 1.40 (d, *J* = 2.8 Hz, 9H), 0.86 (m, 9H); <sup>13</sup>C NMR (CDCl<sub>3</sub>)  $\delta$  175.5, 172.1, 155.6, 80.1, 73.8, 53.8, 50.7, 47.7, 47.6, 41.4, 39.2, 31.6, 31.0, 30.1, 29.0, 28.1, 25.5, 24.5, 23.9, 23.2, 21.7, 20.0, 15.2, 13.6; MS (FAB): *m/z* 516.3 [M + 1]<sup>+</sup>; HRMS calcd for C<sub>26</sub>H<sub>50</sub>N<sub>3</sub>O<sub>5</sub>S [M + 1]<sup>+</sup> 516.3471; found 516.3466.

**Compound 50.** A solution of **49** (103.2 mg, 0.2 mmol) in HCl (4 M in dioxanes, 5 mL, 20 mmol) was stirred at room temperature for 1 h. The solvent was removed under reduced pressure, and the residue was dissolved in saturated NaHCO<sub>3</sub> (10 mL) and extracted with EtOAc (4  $\times$  10 mL). The combined organic layers were dried (Na<sub>2</sub>SO<sub>4</sub>), filtered, and concentrated in vacuo. The residue was dissolved in CH<sub>2</sub>Cl<sub>2</sub> (2 mL) followed by addition of water (2 mL), Boc-Leucine (38.1 mg, 0.22 mmol), and HOBt (26.9 mg, 0.22 mmol). The reaction mixture was cooled in an ice bath to 0 °C, and EDC (42 mg, 0.22 mmol) was added. The resulting reaction mixture was stirred at 0 °C for 36 h. Aqueous HCl (1 N, 5 mL) was added, and the layers were partitioned. The organic layer was washed sequentially with aqueous HCl (0.5 N, 10 mL), brine (10 mL), NaHCO<sub>3</sub> (1 N, 2  $\times$  10 mL), and water (10 mL). The organic layer was dried (Na<sub>2</sub>SO<sub>4</sub>), filtered, and concentrated in vacuo. The residue was purified by column chromatography (10% MeOH in EtOAc) to afford **50** (62.0 mg, 54%) as a white foam; [ $\alpha$ ]<sub>D</sub><sup>20</sup> -94.1 (c 1.0, MeOD); <sup>1</sup>H NMR (CD<sub>3</sub>OD)  $\delta$  4.46 (q, *J* = 5.1 Hz, 1H), 4.35 (t, *J* = 7.6 Hz, 1H), 3.95 (m, 1H), 3.43 (t, *J* = 4.6 Hz, 1H), 3.20 (m, 2H), 2.54 (m, 3H), 2.28 (m, 1H), 2.12 (m, 1H), 2.00 (s, 3H), 1.99 (m, 1H), 1.84 (m, 1H), 1.70 (m, 6H), 1.60–1.23 (m, 10H), 0.95 (m, 15H); <sup>13</sup>C NMR (CD<sub>3</sub>OD)  $\delta$  177.2, 174.0, 172.5, 172.2, 73.4, 59.7, 53.8, 53.1, 52.6, 51.6, 48.8, 41.5, 40.8, 39.3, 31.7, 31.2, 30.9, 30.2, 25.5, 24.9, 24.8, 22.3, 21.4, 21.1, 20.2, 14.3, 13.1; MS (FAB): *m/z* 571.39 [M + 1]<sup>+</sup>; HRMS calcd for C<sub>29</sub>H<sub>55</sub>N<sub>4</sub>O<sub>5</sub>S [M + 1]<sup>+</sup> 571.3893; found 571.3900; LC/MS retention time: [A] 21.72 min; [B] 19.89 min.

**Compound 53.** Prepared from intermediate **52**<sup>45</sup> according to the general procedure for the preparation of **46**; [ $\alpha$ ]<sub>D</sub><sup>20</sup> -19.56 (c 0.23, MeOH); <sup>1</sup>H NMR (CD<sub>3</sub>OD)  $\delta$  8.28 (br, 1H); 7.27 (br, 1H); 6.58 (b, 1H); 5.26 (d, *J* = 6.9 Hz, 1H); 5.0 (br, 1H); 4.31 (t, *J* = 6.4 Hz, 1H); 4.22 (q, *J* = 7.0 Hz, 1H); 3.40 (m, 1H); 3.28 (m, 1H); 3.21 (m, 2H); 2.57 (m, 2H); 2.38 (m, 1H); 2.36 (m, 1H); 2.10 (s, 3H); 1.91 (m, 2H); 1.88 (m, 2H); 1.74 (m, 2H); 1.63 (m, 4H); 1.47 (m, 2H); 1.43 (s, 9H); 1.37 (d, *J* = 7.2 Hz, 3H); 1.34 (m, 3H); 0.91 (m, 9H); <sup>13</sup>C NMR (CD<sub>3</sub>OD)  $\delta$  174.7, 174.2, 172.6, 172.3, 172.2, 73.2, 59.8, 59.3, 56.6, 52.7, 48.6, 48.4, 46.3, 40.8, 39.1, 31.5, 30.2, 24.9, 24.8, 22.8, 22.4, 21.4, 21.1, 20.1, 18.8, 18.2, 13.08; MS (FAB): *m/z* [M + 1]<sup>+</sup> 685.4; HRMS calcd for C<sub>34</sub>H<sub>65</sub>N<sub>6</sub>O<sub>6</sub> [M + 1]<sup>+</sup> 685.5686; found 685.4688; LC/MS retention time: [A] 19.40 min; [B] 17.77 min.

**Compound 55.** Prepared from intermediate **54**<sup>45</sup> according to the general procedure for the preparation of **46**; [ $\alpha$ ]<sub>D</sub><sup>20</sup> -49.1 (c 0.64, MeOH). <sup>1</sup>H NMR (CD<sub>3</sub>OD):  $\delta$  0.91 (m, 9H), 1.34 (m, 3H), 1.37 (d, *J* = 7.2 Hz, 3H), 1.43 (s, 9H), 1.47 (m, 2H), 1.63 (m, 4H), 1.74 (m, 2H), 1.88 (m, 2H), 1.91 (m, 2H), 2.10 (s, 3H), 2.36 (m, 1H), 2.38 (m, 1H), 2.57 (m, 2H), 3.21 (m, 2H), 3.28 (m, 1H), 3.40 (m, 1H), 4.22 (q, *J* = 7.0 Hz, 1H), 4.31 (t, *J* = 6.4 Hz, 1H), 5.0 (1H, b), 5.26 (d, *J* = 6.9 Hz, 1H), 6.58 (b, 1H), 7.27 (b, 1H), 8.28 (b, 1H); <sup>13</sup>C NMR (CD<sub>3</sub>OD):  $\delta$  175.26, 175.04, 173.41, 173.37, 173.11, 81.05, 72.73, 70.03, 59.47, 53.92, 53.45, 52.50, 46.89, 42.37, 41.75, 40.03, 32.50, 32.39, 32.20, 31.17, 27.00, 26.50, 25.87, 25.70, 24.94, 23.89, 23.31, 22.63, 22.32, 22.20, 22.06, 21.05, 19.64, 18.69, 15.22, 14.03; IR (film) 3288, 3081, 2958, 2872, 1644, 1538; MS (FAB): *m/z* 713.5 [M + 1]<sup>+</sup>; LC/MS retention time: [A] 18.71 min; [B] 17.14 min.

**Compound 57.** Prepared from intermediate **56**<sup>45</sup> according to the general procedure for the preparation of **46**; [ $\alpha$ ]<sub>D</sub><sup>20</sup> -75.0 (c 1.0, MeOH); <sup>1</sup>H NMR (MeOD)  $\delta$  8.18 (t, *J* = 5.2 Hz, 1H), 7.54 (d, *J* = 9.3 Hz, 1H), 4.49 (dd, *J* = 9.6 Hz, *J* = 4.6 Hz, 1H), 4.36 (t, *J* = 7.5 Hz, 1H), 4.18 (d, *J* = 3.3 Hz, 1H), 4.14 (d, *J* = 11.7 Hz, 2H), 3.47 (dd, *J* = 6.3 Hz, *J* = 2.6 Hz, 1H), 3.31 (m, 1H), 3.24 (m, 1H), 2.75 (s, 3H), 2.73–2.50 (m, 4H), 2.38 (m, 1H), 2.26–1.92 (m, 3H), 2.10 (s, 3H), 2.01 (s, 3H), 1.75 (m, 1H), 1.65 (m, 4H), 1.60 (m, 2H), 1.42–1.24 (m, 3H), 0.96 (m, 21H); <sup>13</sup>C NMR (MeOD)  $\delta$  176.6, 174.3, 172.6, 172.2, 172.1, 171.5, 72.5, 65.9, 60.0, 52.7, 31.5, 31.0, 30.4, 28.0, 24.9, 24.8, 22.9, 22.4, 21.5, 21.2, 21.1, 20.1, 18.7, 18.5, 14.3, 13.1; MS (FAB): *m/z* 699.3 [M + 1]<sup>+</sup>; HRMS calcd for C<sub>34</sub>H<sub>63</sub>N<sub>6</sub>O<sub>7</sub>S [M + 1]<sup>+</sup> 699.4434; found 699.4474; LC/MS retention time: [A] 18.10 min; [B] 16.58 min.

**Compound 59.** Prepared from intermediate **58**<sup>45</sup> according to the general procedure for the preparation of **46**; [ $\alpha$ ]<sub>D</sub><sup>20</sup> -49.1

(c 0.6, MeOH);  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  8.28 (b, 1H), 7.27 (b, 1H), 7.27 (b, 1H), 6.58 (b, 1H), 5.26 (d,  $J = 6.9$  Hz, 1H), 5.0(b, 1H), 4.31 (t,  $J = 6.4$  Hz, 1H), 4.22 (q,  $J = 7.0$  Hz, 1H), 3.40(m, 1H), 3.28(m, 1H), 3.21 (m, 2H), 2.57(m, 2H), 2.38(m, 1H), 2.36(m, 1H), 2.10(s, 3H), 1.91(m, 2H), 1.88 (m, 2H), 1.74 (m, 2H), 1.63 (m, 4H), 1.47 (m, 2H), 1.43 (s, 9H), 1.37 (d,  $J = 7.2$  Hz, 3H), 1.34 (m, 3H), 0.91 (m, 9H);  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ ):  $\delta$  175.26, 175.04, 173.41, 173.41, 173.37, 173.11, 81.05, 72.73, 70.03, 59.47, 53.92, 53.45, 52.50, 46.89, 42.37, 41.75, 40.03, 32.50, 32.39, 32.20, 31.17, 27.00, 26.50, 25.87, 25.70, 24.94, 23.89, 23.31, 22.63, 22.32, 22.20, 22.06, 21.05, 19.64, 18.69, 15.22, 14.03; IR (film) 3288, 3081, 2958, 2872, 1644, 1538; MS (FAB)  $m/z$  672.4  $[\text{M} + 1]^+$ ; HRMS calcd for  $\text{C}_{33}\text{H}_{62}\text{N}_5\text{O}_7\text{S}$   $[\text{M} + 1]^+$  672.4370; found 672.4371; LC/MS retention time: [A] 22.28 min; [B] 20.79 min.

**Modeling of Compounds in BACE.** A  $10 \text{ \AA}$  shell around the inhibitor in the BACE OM99-2 cocrystal structure (PDB ref 1FKN) was used for calculations. In this binding site model the Monte Carlo docking/energy minimization protocol of the MCDOCK routine in the QXP program<sup>47</sup> (within the Flow96 package) was applied. Depending on the size and flexibility of the ligands 1000 or 2000 search and energy minimization cycles were performed in order to ensure an in depth conformational search and the exploration of different possible binding modes.

**Enzyme Inhibition Measurements.** Materials: Recombinant human BACE (SwissProt accession number P56817, amino acids 1–453 + a thrombin-cleavable C-kappa tag) was produced in-house using Sf9 insect cells. The proenzyme was activated autocatalytically at pH 4.5. Human proCathepsin D (SwissProt accession number P07339) was expressed in Sf9 cells, purified, and autoactivated by incubation at pH 3.7. Peptide substrates were obtained from Bachem, Bubendorf, Switzerland, and all other biochemicals were obtained from Fluka, Buchs, Switzerland.

Enzyme assays: Human BACE activity was assayed in 100 mM acetate buffer, pH 4.5, containing 0.1% CHAPS, using Mca-SEVNLDAEFK(DNP)-OH ( $3 \mu\text{M}$ ), and Cathepsin D activity was measured in 100 mM formate buffer, pH 3.1, using Mca-GKPILFFRLK(DNP)-D-R-NH<sub>2</sub> ( $2 \mu\text{M}$ ). Test substances were serially diluted from  $10 \mu\text{M}$  to 10 nM, inhibition assays were done in black 96-wellplates (Costar) by adding the respective enzyme in assay buffer. Plates were incubated at room temperature for 1 h, and residual enzymatic activity was measured after addition of substrate in a Spectramax Gemini fluorescence plate reader (Molecular Devices, Sunnyvale, CA). IC<sub>50</sub> values were calculated using the Microsoft Excel extension XL-fit.

**Acknowledgment.** We thank NSERC (Canada) for financial assistance, Dr. Michel Simard for X-ray analysis, and Dr. Dalbir Sekon (Université de Montréal) for LC/MS analyses. S.H. thanks Novartis Pharma AG, Basel/Canada, for generous financial support. We also thank René Hemmig, Sylvie Lehmann, and Sebastien Rieffel for technical support. X-ray data collection for the enzyme complexes was performed at the Swiss Light Source, Paul Scherrer Institut, Villigen, Switzerland. We are grateful to the machine and beamline groups whose outstanding efforts have made these experiments possible.

**Supporting Information Available:** Additional experimental procedures, copies of selected NMR spectra, LC/MS data, experimental procedures for crystallization and crystal structure data, and X-ray crystallographic data for compound 12. This material is available free of charge via the Internet at <http://pubs.acs.org>.

## References

- Hardy, J.; Selkoe, D. J. The amyloid hypothesis of Alzheimer's disease: progress and problems on the road to therapeutics. *Science* **2002**, *297*, 353–356.

- Citron, M. Strategies for disease modification in Alzheimer's disease. *Nature Rev. Neurosci.* **2004**, *5*, 677–685.
- Golde, T. E. Alzheimer disease therapy: Can the amyloid cascade be halted? *J. Clin. Invest.* **2003**, *111*, 11–18.
- Selkoe, D. J.; Schenk, D. Alzheimer's Disease: Molecular Understanding Predicts Amyloid-Based Therapeutics. *Annu Rev. Pharmacol. Toxicol.* **2003**, *43*, 545–584.
- Conway, K. A.; Baxter, E. W.; Felsenstein, K. M.; Reitz, A. B. Emerging  $\beta$ -Amyloid Therapies for the Treatment of Alzheimer's Disease. *Curr. Pharm. Des.* **2003**, *9*, 427–447.
- Wolfe, S. M. Therapeutic Strategies for Alzheimer's Disease. *Nature Rev. Drug Discovery* **2002**, *1*, 859–866.
- 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*, 735–741.
- 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*, 419–427.
- Yan, R.; Bienkowski, M. J.; Shuck, M. E.; Miao, H.; Tory, M. C.; Pauley, A. M.; Brashler, J. R.; Stratman, N. C.; Mathews, W. R.; Buhl, A. E.; Carter, D. B.; Tomasselli, A. G.; Parodi, L. A.; Henrikson, R. L.; Gurney, M. E. Membrane-anchored aspartyl protease with Alzheimer's disease  $\beta$ -secretase activity. *Nature* **1999**, *402*, 533–537.
- Lin, X.; Koelsch, G.; Wu, S.; Downs, D.; Dashti, A.; Tang, J. Human aspartic protease memapsin 2 cleaves the  $\beta$ -secretase site of  $\beta$ -amyloid precursor protein. *Proc. Natl. Acad. Sci. U.S.A.* **2000**, *97*, 1456–1460.
- Review: Vassar, R. BACE1, The  $\beta$ -Secretase Enzyme in Alzheimer's Disease. *J. Mol. Neurosci.* **2004**, *23*, 105–113.
- Wilquet, V.; De Strooper, B. Amyloid-beta precursor protein processing in neurodegeneration. *Curr. Opin. Neurobiol.* **2004**, *14*, 1–7.
- Citron, M.  $\beta$ -Secretase inhibition for the treatment of Alzheimer's disease – promise and challenge. *Trends Pharmacol. Sci.* **2004**, *25*, 92–97.
- Hussain, I. The potential for BACE1 inhibitors in the treatment of Alzheimer's disease. *Investig. Drugs* **2004**, *7*, 653–658.
- Tang, J.; Ghosh, A. K.; Hong, L.; Koelsch, G.; Turner, R. T., III; Chang, W. Study of memapsin 2 ( $\beta$ -Secretase) and Strategy of Inhibitor Design. *J. Mol. Neurosci.* **2003**, *20*, 299–304.
- John, V.; Beck, J. P.; Bienkowski, M. J.; Sinha, S.; Henrikson, R. L. Human  $\beta$ -secretase (BACE) and BACE inhibitors. *J. Med. Chem.* **2003**, *46*, 4626–4630.
- Hong, L.; Koelsch, G.; Lin, X.; Wu, W. S.; Terzyan, S.; Ghosh, A. K.; Zhang, X. C.; Tang, J. Structure of the protease domain of memapsin 2 ( $\beta$ -secretase) complexed with inhibitor. *Science* **2000**, *290*, 150–153.
- Hong, L.; Turner, R. T.; Koelsch, G.; Shin, D.; Ghosh, A. K.; Tang, J. Crystal Structure of Memapsin 2 ( $\beta$ -secretase) in Complex with an Inhibitor OM00-3. *Biochemistry* **2002**, *41*, 10963–10967.
- Patel, S.; Vuillard, L.; Cleasby, A.; Murray, C. W.; Yon, J. Apo and Inhibitor Complex Structures of BACE ( $\beta$ -secretase). *J. Mol. Biol.* **2004**, *343*, 407–416.
- Coburn, C. A.; Stachel, S. J.; Li, Y.-M.; Rush, D. M.; Steele, T. G.; Chen-Dodson, E.; Holloway, M. K.; Xu, M.; Huang, Q.; Lai, M.-T.; DiMuzio, J.; Crouthamel, M.-C.; Shi, X.-P.; Sardana, V.; Chen, Z.; Munshi, S.; Kuo, L.; Makara, G. M.; Annis, D. A.; Tadikonda, P. K.; Nash, H. M.; Vacca, J. P. Identification of a Small Molecule Nonpeptide Active Site  $\beta$ -Secretase Inhibitor That Displays a Nontraditional Binding Mode for Aspartyl Proteases. *J. Med. Chem.* **2004**, *47*, 6117–6119.
- Ghosh, A. K.; Devasamudram, T.; Hong, L.; DeZutter, C.; Xu, X.; Weerasena, V.; Koelsch, G.; Bilcer, G.; Tang, J. Structure-based design of cycloamide-urethane-derived novel inhibitors of human brain memapsin 2 ( $\beta$ -secretase). *Bioorg. Med. Chem. Lett.* **2005**, *15*, 15–20.
- Turner, R. T., III; Hong, L.; Koelsch, G.; Ghosh, A. K.; Tang, J. Structural Locations and Functional Roles of New Subsites S<sub>5</sub>, S<sub>6</sub>, and S<sub>7</sub> in Memapsin 2 ( $\beta$ -Secretase). *Biochemistry* **2005**, *44*, 105–112.
- Turner, R. T.; Koelsch, G.; Hong, L.; Castanheira, P.; Ghosh, A. K.; Tang, J. Subsite specificity of memapsin 2 ( $\beta$ -secretase); implications in inhibitor design. *Biochemistry* **2001**, *40*, 10001–10006.
- Gruninger-Leitch, F.; Schlatter, D.; Kung, E.; Nelbock, P.; Dobeli, H. Substrate and Inhibitor Profile of BACE ( $\beta$ -secretase) and Comparison with Other Mammalian Aspartic Proteases. *J. Biol. Chem.* **2002**, *277*, 4687–4693.
- Ghosh, A. K.; Shin, D.; Downs, D.; Koelsch, G.; Lin, X.; Ermolieff, J.; Tang, J. Design of potent inhibitors of human brain memapsin 2 ( $\beta$ -secretase). *J. Am. Chem. Soc.* **2000**, *122*, 3522–3523.



- (26) Ghosh, A. K.; Bilcer, G.; Harewood, C.; Kawahama, R.; Shin, D.; Hussain, K. A.; Hong, L.; Loy, J. A.; Nguyen, C.; Koelsch, G.; Ermolieff, J.; Tang, J. Structure-based design: potent inhibitors of human brain memapsin 2 ( $\beta$ -secretase). *J. Med. Chem.* **2001**, *44*, 2865–2868.
- (27) Cumming, J. N.; Iserloh, U.; Kennedy, M. E. Design and development of BACE-1 inhibitors. *Curr. Opin. Drug Discov. Dev.* **2004**, *7*, 536–556.
- (28) Hom, R. K.; Gailunas, A. F.; Mamo, S.; Fang, L. Y.; Tung, J. S.; Walker, D. E.; Davis, D.; Thorsett, E. D.; Jewett, N.; Moon, J. B.; John, V. Design and synthesis of hydroxyethylene-based peptidomimetic inhibitors of human  $\beta$ -secretase. *J. Med. Chem.* **2004**, *47*, 158–164.
- (29) Lamar, J.; Hu, J.; Bueno, A. B.; Yang, H.-C.; Guo, D.; Copp, J. D.; McGee, J. E.; Gitter, B.; Timm, D.; May, P. C.; McCarthy, J. R.; Chen, S.-H. Phe-Ala-based pentapeptide mimetics are BACE inhibitors: P2 and P3 SAR. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 239–243.
- (30) Chen, S.-H.; Lamar, J.; Guo, D.; Kohn, T.; Yang, H.-C.; McGee, J. E.; Timm, D.; Erickson, J. A.; Yip, Y.; May, P.; McCarthy, J. R. P3 Cap modified Phe-Ala series BACE inhibitors. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 245–250.
- (31) Brady, S. F.; Singh, S.; Crouthamel, M.-C.; Holloway, M. K.; Coburn, C. A.; Garsky, V. M.; Bogusky, M.; Pennington, M. W.; Vacca, J. P.; Hazuda, D.; Lai, M.-T. Rational design and synthesis of selective BACE-1 inhibitors. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 601–604.
- (32) Tung, T. S.; Davis, D. L.; Anderson, J. P.; Walker, D. E.; Mamo, S.; Jewett, N.; Hom, R. K.; Sinha, S.; Thorsett, E. D.; John, V. Design of Substrate-Based Inhibitors of Human  $\beta$ -Secretase. *J. Med. Chem.* **2002**, *45*, 259–262.
- (33) Hom, R. K.; Fang, L. Y.; Mamo, S.; Tung, J. S.; Guinn, A. C.; Walker, D. E.; Davis, D. L.; Gailunas, A. F.; Thorsett, E. D.; Sinha, S.; Knops, J. E.; Jewett, N. E.; Anderson, J. P.; John, V. Design and Synthesis of Statine-Based Cell-Permeable Peptidomimetic Inhibitors of Human  $\beta$ -Secretase. *J. Med. Chem.* **2003**, *46*, 1799–1802.
- (34) Hu, J.; Cwi, C. L.; Smiley, D.; Timm, D.; Erickson, J. A.; McGee, J. E.; Yang, H.-C.; Mendel, D.; May, P. C.; Shapiro, M.; McCarthy, J. R. Design and Synthesis of Statine-Containing BACE Inhibitor. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 4335–4339.
- (35) Hu, B.; Fan, K. Y.; Bridges, K.; Chopra, R.; Lovering, F.; Cole, D.; Zhou, P.; Ellingboe, J.; Jin, G.; Cowling, R.; Bard, J. Synthesis and SAR of bis-statine based peptides as BACE 1 inhibitors. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 3457–3460.
- (36) Kimura, T.; Shuto, D.; Kasai, S.; Liu, P.; Hidaka, K.; Hamada, T.; Hayashi, Y.; Hattori, C.; Asai, M.; Kitazume, S.; Saido, T. C.; Ishiura, S.; Kiso, Y. KMI-358 and KMI-370, highly potent and small-sized BACE1 inhibitors containing phenylnorstatine. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 1527–1531.
- (37) Kimura, T.; Shuto, D.; Hamada, Y.; Igawa, N.; Kasai, S.; Liu, P.; Hidaka, K.; Hamada, T.; Hayashi, Y.; Kiso, Y. Design and synthesis of highly active Alzheimer's  $\beta$ -secretase (BACE1) inhibitors, KMI-420 and KMI-429, with enhanced chemical stability. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 211–215.
- (38) Shuto, D.; Kasai, S.; Kimura, T.; Liu, P.; Hidaka, K.; Hamada, T.; Shibakawa, S.; Hayashi, Y.; Hattori, C.; Szabo, B.; Ishiura, S.; Kiso, Y. KMI-008, a novel  $\beta$ -secretase inhibitor containing a hydroxymethylcarbonyl isostere as a transition-state mimic: Design and synthesis of substrate-based octapeptides. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 4273–4276.
- (39) Tamamura, H.; Kato, T.; Otaka, A.; Fujii, N. Synthesis of potent  $\beta$ -secretase inhibitors containing a hydroxyethylamine dipeptide isostere and their structure–activity relationship studies. *Org. Biomol. Chem.* **2003**, *1*, 2468–2473.
- (40) Stachel, S. J.; Coburn, C. A.; Steele, T. G.; Jones, K. G.; Loutzenhiser, E. F.; Gregro, A. R.; Rajapakse, H. A.; Lai, M. T.; Crouthamel, M. C.; Xu, M.; Tugusheva, K.; Lineberger, J. E.; Pietrak, B. L.; Espeseth, A. S.; Shi, X. P.; Chen-dodson, E.; Holloway, M. K.; Munshi, S.; Simon, A. J.; Kuo, L.; Vacca, J. P. Structure-Based Design of Potent and Selective Cell-Permeable Inhibitors of Human  $\beta$ -Secretase (BACE-1). *J. Med. Chem.* **2004**, *47*, 6447–6450.
- (41) Fray, A. H.; Kaye, R. L.; Kleinmann, E. F. A Short, Stereo-selective Synthesis of the Lactone Precursor to 2R, 4S, 5S Hydroxyethylene Dipeptide Isosteres. *J. Org. Chem.* **1986**, *51*, 4828–4833.
- (42) Takeda, K.; Yano, S.; Yoshii, E. Synthesis of 28, 29-bisnor ( $\pm$ ) Kijanolid, *Tetrahedron Lett.* **1988**, *29*, 6951–6954.
- (43) Trost, B. M.; Chan, D. M. T. Palladium-mediated cycloaddition approach to cyclopentanoids. Introduction and initial studies. *J. Am. Chem. Soc.* **1983**, *105*, 2315–2325.
- (44) Kabalka, G. W.; Yang, D. T. C.; Baker, J. D. Deoxygenation of  $\alpha,\beta$ -unsaturated aldehydes and ketones via the catecholborane reduction of the corresponding tosylhydrazones. *J. Org. Chem.* **1976**, *41*, 574–575.
- (45) See Supporting Information.
- (46) Baldwin, E. T.; Bhat, T. N.; Gulnik, S.; Hosur, M. V.; Sowder, R. C., II.; Cachau, R. E.; Collins, J.; Silva, A. M.; Erickson, J. W. Crystal structures of native and inhibited forms of human cathepsin D: Implications for lysosomal targeting and drug design. *Proc. Natl. Acad. Sci. U.S.A.* **1993**, *90*, 6796–6800.
- (47) McMartin, C.; Bohacek, R. S. QXP: Powerful, rapid computer algorithms for structure-based drug design. *J. Comput.-Aided Mol. Des.* **1997**, *11*, 333–344.

JM050142+