

# Aspartic Protease Inhibitors: Expedient Synthesis of 2-Substituted Statines

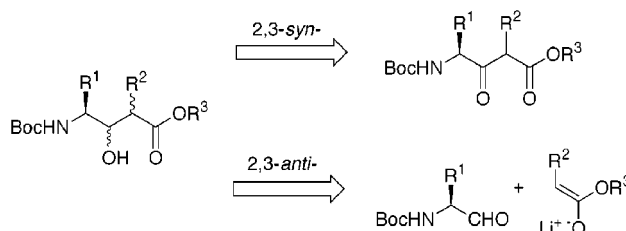
Jeremy M. Travins,<sup>†</sup> Matthew G. Bursavich,<sup>†</sup> Daniel F. Veber,<sup>‡</sup> and Daniel H. Rich<sup>\*,†</sup>

Department of Chemistry and School of Pharmacy, University of Wisconsin-Madison, Madison, Wisconsin 53706, and GlaxoSmithKline, 709 Sweedland Road, King of Prussia, Pennsylvania 19406

dhrich@facstaff.wisc.edu

Received June 22, 2001

## ABSTRACT



General stereocontrolled synthesis of all four (2,3)-stereoisomers of 2-substituted statines is described. The 2,3-*syn* and 2,3-*anti* isomers were synthesized via  $\beta$ -ketoester reduction and aldol reactions, respectively. Peptides containing 2-substituted statines inhibit porcine pepsin with nanomolar  $IC_{50}$  values.

Aspartic proteases play a crucial role in the onset or proliferation of many diseases including AIDS (HIV protease), hypertension (renin), malaria (plasmeprin), and Alzheimer's disease ( $\beta$ -secretase; BACE), and much work has been done to find both peptidomimetic and non-peptidomimetic inhibitors of these enzymes. A key structural element in many of these inhibitors is a hydroxyl or hydroxyl-like subunit that binds to the two catalytic aspartates in the enzyme active site. This structural feature was discovered in the peptide natural product pepstatin,<sup>1</sup> which contains two copies of the unnatural amino acid statine (Figure 1), a unit proposed to mimic the transition state for amide bond hydrolysis.<sup>2</sup> On the basis of this principle, a number of transition-state analogues have been developed and incorporated into peptides.<sup>3</sup>

The most common transition-state isosteres utilized in peptide-like aspartic protease inhibitors are the "hydroxyethylenes",<sup>4</sup> "hydroxyethylamines",<sup>5</sup> and statines<sup>6</sup> (Figure 1).

These molecules replace the dipeptide subunit that contains the scissile amide bond ( $P_1$ – $P_1'$  residues). Although statine peptides are good aspartic protease inhibitors, there are two obvious differences between a statine and a dipeptide: statine is one backbone atom shorter than a dipeptide and it lacks a  $P_1'$  side chain. The idea that functionalization of the statine C2 position would restore the  $P_1'$  side chain to yield improved or more selective aspartic protease inhibitors was explored by Veber et al.<sup>7</sup> Since then, 2-substituted statines have not

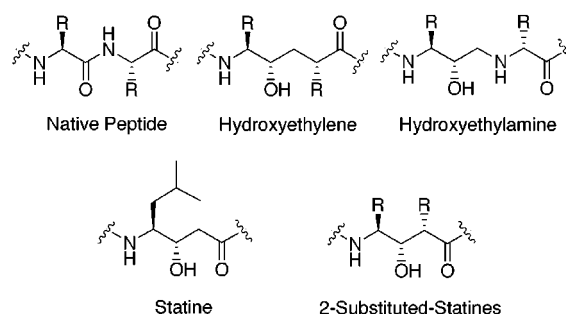


Figure 1. Transition-state isostere dipeptide mimetics.

<sup>†</sup> University of Wisconsin-Madison.

<sup>‡</sup> GlaxoSmithKline.

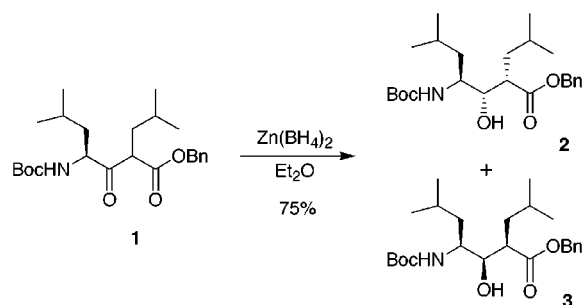
(1) Umezawa, H.; Aoyagi, T.; Morishima, H.; Matsuzaki, M.; Hamada, M.; Takeguchi, T. *J. Antibiot.* **1972**, 25, 251.

(2) (a) Marshall, G. R. *Fed. Proc., Fed. Am. Soc. Exp. Biol.* **1976**, 35, 2494. (b) Marciniuszyn, J., Jr.; Hartsuck, J. A.; Tang, J. *J. Biol. Chem.* **1976**, 251, 7088.

been investigated as aspartic protease inhibitors. However, since 2-substituted statines are components of bleomycin<sup>8</sup> and the dolastatins,<sup>9</sup> a number of syntheses specific to these natural products have been developed. The 2,3-*syn* stereoisomers have been accessed via a number of chiral auxiliary mediated reactions,<sup>10</sup> whereas the synthesis of the 2,3-*anti* stereoisomers has been less explored.<sup>11</sup> Herein, we describe a general and expedient synthesis of four C2,C3 diastereomers of 2-substituted statines, their incorporation into peptides, and porcine pepsin inhibition data.

The synthesis of the (2,3)-*syn*-2-*i*Bu-statines began with the synthesis of  $\beta$ -ketoester **1** (Scheme 1),<sup>12</sup> obtained via

**Scheme 1**



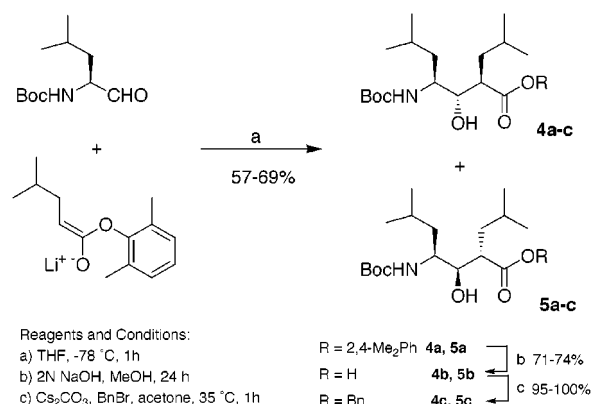
coupling of Boc-leucine acyl-imidazole with the enolate of benzylisocaproate. While this synthesis of  $\beta$ -ketoester **1** offers a quick entry to the  $\beta$ -ketoester, the product is difficult to purify from the side products.<sup>13</sup> Fortunately, reduction of crude **1** with ethereal zinc borohydride was chemoselective for the  $\beta$ -ketoester, yielding the desired (2*S*,3*S*,4*S*)- and (2*R*,3*R*,4*S*)-alcohols **2** and **3** in a ratio of 1:1.4. The

diastereoselectivity corresponds to the diastereomeric ratio of the starting  $\beta$ -ketoester, with the 2,3-*syn* stereochemistry arising via intramolecular hydride delivery in the proposed zinc borohydride- $\beta$ -ketoester six-membered chelate.<sup>14</sup> The individual diastereomers were easily purified using standard silica gel flash chromatography. Removal of the Boc group or the benzyl ester allows for peptide coupling the N- or C-terminus, respectively.<sup>15</sup>

The (2,3)-*anti* stereoisomers were more difficult to obtain. Initial attempts involved alkylation of *N,N*-dibenzyl statine ethyl ester, using methodology similar to that used for the synthesis of dolastatins.<sup>8</sup> However, poor yields were obtained ( $\leq 25\%$ ) even when reactive alkylating agents such as allyl bromide were used. The low yields, limited availability of reactive alkylating agents, and number of steps compelled us to develop a new route.

The (2,3)-*anti* stereoisomers were synthesized (Scheme 2)

**Scheme 2**



as separable diastereomers by employing aldol methodology developed by Heathcock and co-workers.<sup>16</sup> Deprotonation of 2,6-dimethylphenyl isocaproate yielded the lithium *E*-enolate, which reacted with Boc-leucinal to give the pair of 2,3-*anti* products **4a** and **5a** via the Zimmerman–Traxler transition state.<sup>17</sup> Limiting the amount of LDA in the reaction proved to be critical as an excess caused epimerization of the C2 carbon. Separation of the diastereomers<sup>18</sup> from each other was facile; however, each product was contaminated with recovered Boc-leucinal. Saponification of the aryl ester took place via the methyl ester by employing 2 N NaOH in methanol, yielding the Boc-2-*i*Bu-statines **4b** and **5b** ready for peptide coupling. Alkylation of the acid with cesium

(3) (a) Rich, D. H. *Peptidase Inhibitors*. In *Comprehensive Medicinal Chemistry. The Rational Design, Mechanistic Study and Therapeutic Application of Chemical Compounds*; Hansch, C., Sammes, P. G., Taylor, J. B., Eds.; Pergamon Press: New York; 1990; Vol. 2, pp 391–441. (b) Rich, D. H. *Med. Res. Rev.* **1993**, *13*, 327.

(4) (a) Szelke, M.; Jones, D. M.; Hallett, A. European Patent Application EP 45665, 1982; *Chem. Abstr.* **1982**, *97*, 39405p. (b) Szelke, M.; Jones, D. M.; Atrash, B.; Hallett, A.; Leckie, B. J. *Proc. Am. Pept. Symp. 8th* **1983**, 579. (c) Holladay, M. W.; Rich, D. H. *Tetrahedron Lett.* **1983**, *24*, 4401. (d) Greenlee, W. J. *Med. Res. Rev.* **1990**, *10*, 173.

(5) (a) Gordon, E. M.; Godfrey, J. D.; Pluscec, J.; Von Langen, D.; Natarajan, S. *Biochem. Biophys. Res. Commun.* **1985**, *126*, 419. (b) Rich, D. H.; Green, J.; Toth, M. V.; Marshall, G. R.; Kent, S. B. H. *J. Med. Chem.* **1990**, *33*, 1285.

(6) Rich, D. H. *J. Med. Chem.* **1985**, *28*, 263.

(7) Veber, D. F.; Bock, M. G.; Brady, S. F.; Ulm, E. H.; Cochran, D. W.; Smith, G. H.; LaMont, B. I.; DiPardo, R. M.; Poe, Freidinger, R. M.; Evans, B. E.; Boger, J. S. *Trans. Biochem. Soc.* **1984**, *12*, 956.

(8) (a) Narita, M.; Otsuka, M.; Kobayashi, S.; Ohno, M. *Tetrahedron Lett.* **1982**, *23*, 525. (b) DiPardo, R. M.; Bock, M. G. *Tetrahedron Lett.* **1983**, *24*, 4805.

(9) (a) Tomioka, K.; Kanai, M.; Koga, K. *Tetrahedron Lett.* **1991**, *32*, 2395. (b) Shioiri, T.; Hayashi, K.; Hamada, Y. *Tetrahedron* **1993**, *49*, 1913.

(10) (a) Bock, M. G.; DiPardo, R. M.; Evans, B. E.; Rittle, K. E.; Boger, J. S.; Freidinger, R. M.; Veber, D. F. *Chem. Commun.* **1985**, 109. (b) Rivero, R. A.; Greenlee, W. J. *Tetrahedron Lett.* **1991**, *32*, 2453.

(11) Hayashi, K.; Hamada, Y.; Shioiri, T. *Tetrahedron Lett.* **1991**, *32*, 7287.

(12) Paris, M.; Fehrentz, J. J.; Heitz, A.; Martinez, J. *Tetrahedron Lett.* **1998**, *39*, 1569.

(13) Boc-Leu-OBn was a major identified impurity, along with unidentified products of benzyl ester self-condensation.

(14) (a) Nakata, T.; Oishi, T. *Tetrahedron Lett.* **1980**, *21*, 1641. (b) Oishi, T.; Nakata, T. *J. Synth. Org. Jpn.* **1981**, *39*, 633.

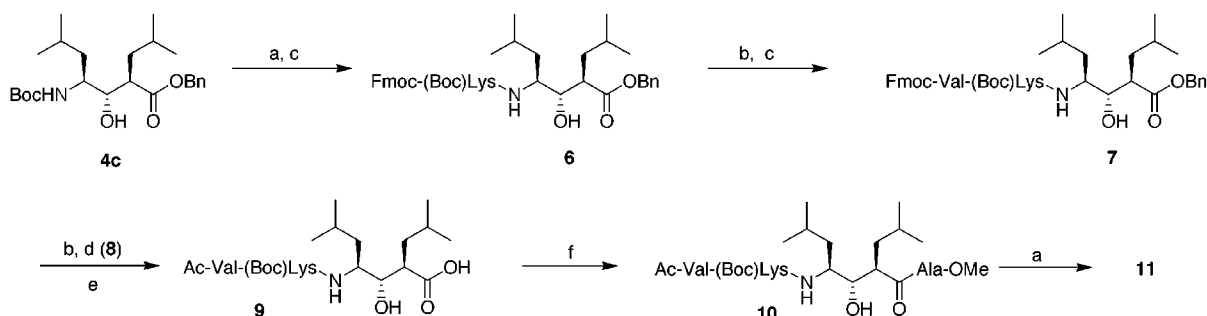
(15) Removal of the Boc-group followed by cyclization to the  $\gamma$ -lactam and <sup>1</sup>H NMR analysis provided absolute stereochemical assignment for each diastereomer based on the L-leucine starting material. See Supporting Information.

(16) Heathcock, C. H.; Pirrung, M. C.; Montgomery, S. H.; Lampe, J. *Tetrahedron* **1981**, *37*, 4081.

(17) Zimmerman, H. E.; Traxler, M. D. *J. Am. Chem. Soc.* **1957**, *79*, 1920.

(18) Absolute stereochemistry was again assigned via <sup>1</sup>H NMR analysis of the corresponding lactams. See Supporting Information.

### Scheme 3



Reagents and conditions: a) 50% CF<sub>3</sub>COOH in CH<sub>2</sub>Cl<sub>2</sub>, 0 °C b) 20% piperidine in DMF c) Fmoc-Xaa, EDCI, HOBt, DIEA, DMF, 0 °C d) Ac<sub>2</sub>O, DIEA, DMF, 0 °C e) H<sub>2</sub>, Pd-C, MeOH f) HCl-Ala-OMe or HCl-Val-OMe, HATU, HOAt, DIEA, DMF, 0 °C

11a = (2*R*, 3*S*, 4*S*)-2-<sup>i</sup>BuSta-  
11b = (2*S*, 3*S*, 4*S*)-2-<sup>i</sup>BuSta-  
11c = (2*S*, 3*R*, 4*S*)-2-<sup>i</sup>BuSta-  
11d = (2*R*, 3*R*, 4*S*)-2-<sup>i</sup>BuSta-  
12 = (2*R*, 3*S*, 4*S*)-2-<sup>i</sup>BuSta-Val-OMe

carbonate and benzyl bromide yielded the benzyl esters **4c** and **5c**.

Each of the four Boc-2-<sup>i</sup>Bu-Sta-OBn diastereomers, **2**, **3**, **4c**, and **5c**, was incorporated into a peptide of the sequence Ac-Val-Lys-(2-<sup>i</sup>BuSta)-Ala-OMe (Scheme 3). Initially we chose this amino acid sequence to target  $\beta$ -amyloid converting enzyme (BACE;  $\beta$ -secretase).<sup>19</sup> The sequence is a hybrid of the normal Alzheimer precursor protein (APP) cleavage site (VKM-DAE) and the APP Swedish mutant (VNL-DAE)<sup>20</sup> and an unexpectedly effective inhibitor of BACE (EVN-*Sta*V-AEF).<sup>21</sup> The peptide synthesis began with the removal of the 2-<sup>i</sup>Bu-statine N-Boc group, followed by coupling with Fmoc-Lys(N<sub>ε</sub>-Boc)-OH to give pseudotriptide **6**. The N-Fmoc group was cleaved, and the resulting amine was coupled with Fmoc-Val-OH to afford **7**. Removal of the N-Fmoc group, acetylation of the resulting amine, and subsequent hydrogenolysis of the benzyl ester yielded peptide acid **9**. Coupling of **9** with an amino acid ester, followed by treatment of the resultant peptide with TFA, yielded the functional 2-<sup>i</sup>Bu-statine-containing peptides **11a–d** and **12** in 40–50% overall yields.

To estimate the contribution to binding provided by the additional 2-isobutyl C2-substituent, the analogous statine-containing peptide, Ac-V-K-Sta-Ala-OMe (**13**), was synthesized. A series of lysine-substituted, statine-containing peptides previously synthesized here demonstrated that lysine in the P<sub>2</sub> position of the peptide diminished binding to

porcine pepsin by about 2 orders of magnitude.<sup>22</sup> Furthermore, replacement of the N-Iva unit with N-acetyl also weakens binding. Consequently, we predicted that control peptide Ac-V-K-Sta-Ala-OMe would inhibit porcine pepsin with a low  $\mu$ M IC<sub>50</sub> value. This unoptimized peptide sequence was chosen so that deleterious or improved binding contributions would be readily observable. When tested against porcine pepsin (Table 1) using a previously described

**Table 1.** Activities of statine and 2-<sup>i</sup>Bu-Statine peptides against porcine pepsin

compd #	structure	IC <sub>50</sub> (μM)
13		2
11a		0.1
12		0.1
11b		0.5
11c		3
11d		10

(19) Vassar, R.; Bennett, B. D.; Babu-Khan, S.; Kahn, S.; Mendiaz, E. A.; Denis, P.; Teplow, D. B.; Ross, S.; Amarante, P.; Leoloff, 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. *Science* **1999**, 286, 735.

(20) Citron, M.; Oltersdorf, T.; Haass, C.; McConlogue, L.; Hung, A. Y.; Seubert, P.; Vigo-Pelfrey, C.; Lieberburg, I.; Selkoe, D. J. *Nature* **1992**, 360, 672.

(21) Sinha, S.; Anderson, J. P.; Barbour, R.; Basi, G. S.; Caccavello, R.; Davis, D.; Doan, M.; Dovey, H. V.; Frigon, N.; Hong, J.; Jacobsen-Croak, K.; Jewett, N.; Keim, P.; Knops, J.; Lieberburg, I.; Power, M.; Tan, H.; Tatsuno, G.; Tung, J.; Schenk, D.; Seubert, P.; Suomensaar, S. M.; Wang, S.; Walker, D.; Zhao, J.; McConlogue, L.; John, V. *Nature* **1999**, 402, 537.

fluorometric assay,<sup>23</sup> Ac-V-K-Sta-Ala-OMe gave an IC<sub>50</sub> of 2 μM, in good agreement with prediction. The diastereomeric

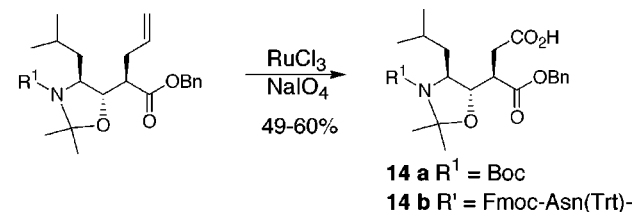
(22) Kuzmic, P.; Sun, C. Q.; Zhao, Z. C.; Rich, D. H. *Tetrahedron* **1991**, 47, 2519.

2-*i*Bu-Sta-containing peptides also inhibited porcine pepsin in the 0.1–10  $\mu$ M range. However, none of these peptides showed inhibition of BACE at concentrations of 10  $\mu$ M.

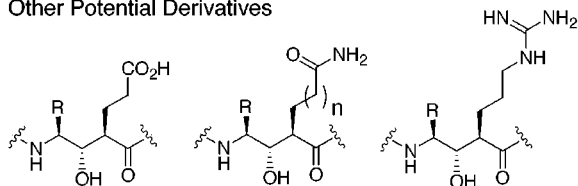
The inhibition data demonstrate that the *S*-hydroxyl 2-*i*-Bu-Sta peptides are potent inhibitors of porcine pepsin, with activities in the 100 nM range (Table 1). As expected for peptides of this length, the 2-*i*Bu-Sta peptides containing the *R*-hydroxyl group are weaker inhibitors, having low micromolar potencies. Similar to the observed inhibition of renin by 2-substituted statine peptides,<sup>6</sup> the (2*R*,3*S*,4*S*)-2-*i*Bu-Sta peptides are 3–4 times more active against porcine pepsin than the (2*S*,3*S*,4*S*)-diastereomer. The preference for the 2*R* stereochemistry can be rationalized on the basis of the positions of the statine-C2 pro-*R* and pro-*S* protons in the X-ray structures of statine peptides bound to porcine pepsin. Most importantly, the (3*S*)-2-*i*Bu-Sta peptides **11a** and **12** are at least 10-fold more active than the statine peptide **13** for inhibition of porcine pepsin. Further optimization is expected to provide improved inhibitors against this and other aspartic peptidases.

These strategies are being applied to synthesize other 2-substituted statines, as illustrated by 2-allylstatine (Figure 2). The allyl side chain permits entry to a variety of derivatives designed to mimic Asn, Gln, Glu, and Arg side chains. Oxidative cleavage of the allyl side chain using RuCl<sub>3</sub>/NaIO<sub>4</sub> gave the Leu-Asp dipeptide mimics **14a** and **14b** in good yields. Alternatively, hydroboration–oxidation of the alkene can provide the terminal alcohol, which can be converted into a number of functional groups. Elaboration of the allyl side chain into the Arg side chain was recently accomplished in the synthesis of a Phe-Arg hydroxyethylene isostere, utilizing a similar substrate.<sup>24</sup> On the basis of the inhibitory activity of the 2-*i*Bu-Sta-containing peptides

#### Leu-Asp Dipeptide Mimetic



#### Other Potential Derivatives



**Figure 2.** Functionalization of the C2 side chain.

against aspartic proteases, these molecules may offer new templates for the design of selective aspartic protease inhibitors.

**Acknowledgment.** This work was supported in part by grants from GlaxoSmithKline and from the National Institutes of Health (GM50113). We thank Dr. Thomas Meek for his support and encouragement, Lindsay J. Howett (GSK) for providing BACE assay data, and Dr. Martha Vestling for obtaining MS data.

**Supporting Information Available:** Detailed experimental procedures for synthesis and characterization of representative compounds and absolute stereochemical assignments via <sup>1</sup>H NMR analysis. This material is available free of charge via the Internet at <http://pubs.acs.org>.

OL016331D

(23) (a) Flentke, G. R.; Glinski, J.; Satyshur, K.; Rich, D. H. *Protein Expression Purif.* **1999**, *16*, 213. (b) Peranteau, A. G.; Kuzmic, P.; Angell, Y.; Rich, D. H. *Anal. Biochem.* **1995**, *227*, 242.

(24) Brewer, M.; Rich, D. H. *Org. Lett.* **2001**, *3*, 945.