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Aspartic Protease Inhibitors: Expedient Synthesis of 2-Substituted Statines

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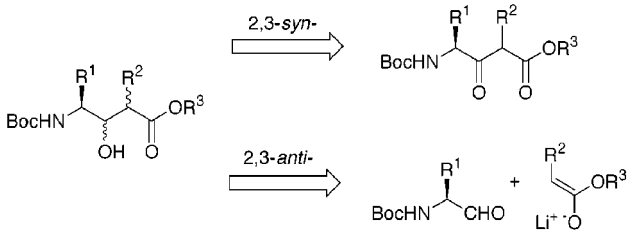
ABSTRACT



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 *β*-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 (plasmepsin), and Alzheimer's disease (*β*-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,[1] which contains two copies of the unnatural amino acid statine (Figure 1), a unit proposed to mimic the transition state for amide bond hydrolysis.[2] On the basis of this principle, a number of transition-state analogues have been developed and incorporated into peptides.[3]

The most common transition-state isosteres utilized in peptide-like aspartic protease inhibitors are the "hydroxyethylenes",[4] "hydroxyethylamines",[5] and statines[6] (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.[7] Since then, 2-substituted statines have not

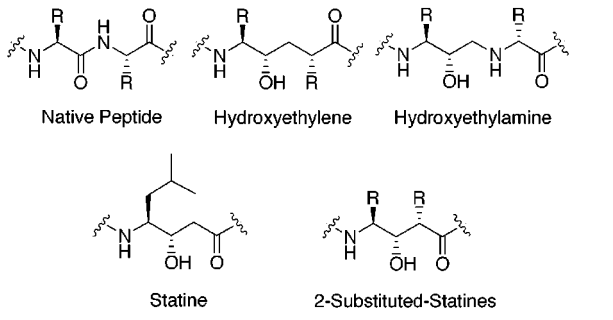


Figure 1. Transition-state isostere dipeptide mimetics.

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(1) Umezawa, H.; Aoyagi, T.; Morishima, H.; Matsazuki, 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) Marciniszyn, J., Jr.; Hartsuck, J. A.; Tang, J. *J. Biol. Chem.* **1976**, *251*, 7088.

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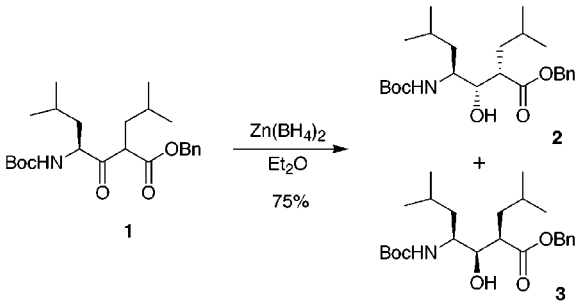
been investigated as aspartic protease inhibitors. However, since 2-substituted statines are components of bleomycin[8] and the dolastatins,[9] a number of syntheses specific to these natural products have been developed. The 2,3-*syn* stereoisomers have been accessed via a number of chiral auxiliary mediated reactions,[10] whereas the synthesis of the 2,3-*anti* stereoisomers has been less explored.[11] 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 β-ketoester **1** (Scheme 1),[12] obtained via

Scheme 1

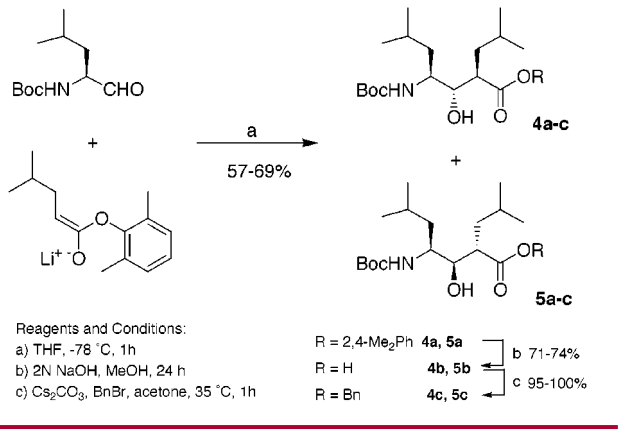


coupling of Boc-leucine acyl-imidazole with the enolate of benzylisocaproate. While this synthesis of β-ketoester **1** offers a quick entry to the β-ketoester, the product is difficult to purify from the side products.[13] Fortunately, reduction of crude **1** with ethereal zinc borohydride was chemoselective for the β-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 β-ketoester, with the 2,3-*syn* stereochemistry arising via intramolecular hydride delivery in the proposed zinc borohydride-β-ketoester six-membered chelate.[14] 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.[15]

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.[8] However, poor yields were obtained (≤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.[16] 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.[17] 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[18] 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.

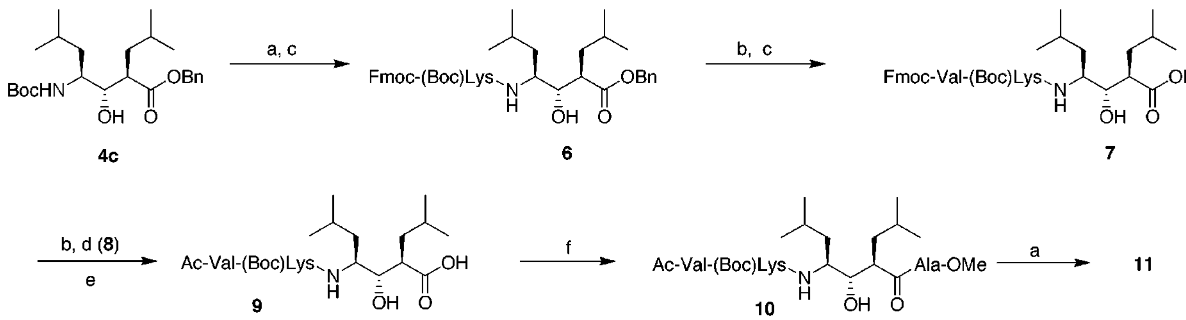
(15) Removal of the Boc-group followed by cyclization to the γ-lactam and ^{1}H NMR analysis provided absolute stereochemical assignment for each diastereomer based on the L-leucine starting material. See Supporting Information.

(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 ^{1}H NMR analysis of the corresponding lactams. See Supporting Information.

Scheme 3



11a = (2*R*, 3*S*, 4*S*)-2-^{i}BuSta-
11b = (2*S*, 3*S*, 4*S*)-2-^{i}BuSta-
11c = (2*S*, 3*R*, 4*S*)-2-^{i}BuSta-
11d = (2*R*, 3*R*, 4*S*)-2-^{i}BuSta-
12 = (2*R*, 3*S*, 4*S*)-2-^{i}BuSta-Val-OMe

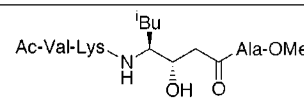
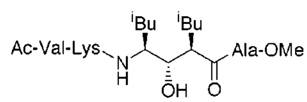
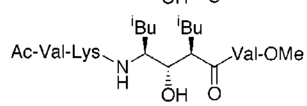
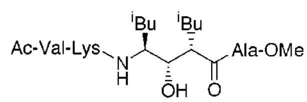
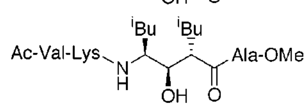
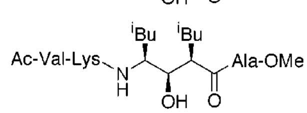
Reagents and conditions: a) 50% CF_3COOH in CH_2Cl_2, 0 °C b) 20% piperidine in DMF c) Fmoc-Xaa, EDCI, HOBt, DIEA, DMF, 0°C d) Ac_2O, DIEA, DMF, 0°C e) H_2, Pd-C, MeOH f) HCl•Ala-OMe or HCl•Val-OMe, HATU, HOAt, DIEA, DMF, 0 °C

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

Each of the four Boc-2-^{i}Bu-Sta-OBn diastereomers, **2**, **3**, **4c**, and **5c**, was incorporated into a peptide of the sequence Ac-Val-Lys-(2-^{i}BuSta)-Ala-OMe (Scheme 3). Initially we chose this amino acid sequence to target β-amyloid converting enzyme (BACE; β-secretase).[19] The sequence is a hybrid of the normal Alzheimer precursor protein (APP) cleavage site (VK*M*−*D*AE) and the APP Swedish mutant (VN*L*−*D*AE)[20] and an unexpectedly effective inhibitor of BACE (EVN-*StaV*-AEF).[21] The peptide synthesis began with the removal of the 2-^{i}Bu-statine N-Boc group, followed by coupling with Fmoc-Lys(N_ϵ-Boc)-OH to give pseudotripeptide **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-^{i}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_2 position of the peptide diminished binding to porcine pepsin by about 2 orders of magnitude.[22] 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 μM IC_{50} 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 fluorometric assay,[23] Ac-V-K-Sta-Ala-OMe gave an IC_{50} of 2 μM, in good agreement with prediction. The diastereomeric

Table 1. Activities of statine and 2-^{i}Bu-Statine peptides against porcine pepsin

cmpd #	structure	IC_{50} (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.; Suomensaari, S. M.; Wang, S.; Walker, D.; Zhao, J.; McConlogue, L.; John, V. *Nature* **1999**, *402*, 537.

(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 μM range. However, none of these peptides showed inhibition of BACE at concentrations of 10 μM.

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,[6] 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 $RuCl_3/NaIO_4$ 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.[24] On the basis of the inhibitory activity of the 2-^{i}Bu-Sta-containing peptides against aspartic proteases, these molecules may offer new templates for the design of selective aspartic protease inhibitors.

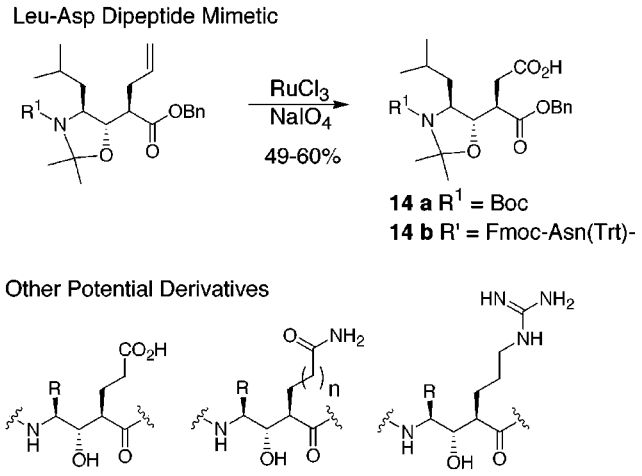


Figure 2. Functionalization of the C2 side chain.

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

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(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.