

Synthesis of *N*-(Hydroxy)amide- and *N*-(Hydroxy)thioamide-Containing Peptides

Lu Wang and Otto Phanstiel IV*

Department of Chemistry, Center for the Discovery of Drugs and Diagnostics,
University of Central Florida, Orlando, Florida 32816-2366

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Methods developed with *N*-(benzoyloxy)amines and hydroxamic acids were used in the synthesis of *N*-(hydroxy)amide-containing pseudopeptides. Acylation of *N*-(benzoyloxy)phenethylamine with the acid chloride of *N*^t-Fmoc-L-leucine provided a *N*^t-Fmoc-*N*-(benzoyloxy)-L-leucinamide in 90% yield. Deprotection of the benzoyl group (using 10 vol % NH₄OH/MeOH) provided the *N*^t-Fmoc-*N*-(hydroxy)-L-leucinamide in 87% yield. In general, the appended Fmoc group allowed for further elaboration of the *N*-hydroxy-*N*-(alkyl)amides using classic peptide-coupling methods. A practical synthetic strategy was developed, and racemization issues were addressed using diastereomeric Val-Leu derivatives. In addition, *N*-(hydroxy)thioamides were generated from the corresponding *N*-(benzoyloxy)thioamides. *N*-(Benzoyloxy)thioamides were obtained in moderate yields (53–76%) from the reaction of the corresponding *N*-(benzoyloxy)amides with Lawesson's reagent (i.e., 2,4-bis(4-methoxyphenyl)-1,3-dithia-2,4-diphosphetane-2,4-disulfide). In summary, this new technology allows for the introduction of either *N*-hydroxyamide or *N*-(hydroxy)thioamide linkages into pseudopeptide chains without racemization.

Introduction

Structural modifications of biologically active peptides are often performed in order to improve the properties of these peptides for research in medicine and biochemistry. An important class of compounds in this field are the backbone-modified peptides or "pseudopeptides." These are peptides or peptide-like molecules that contain one or more non-peptide linkages between the α -amino acids or α -amino acid-like motifs. The hydroxamic acid functionality is a key structural constituent of a wide spectrum of bioactive agents including various antibacterial, antifungal, and anticancer agents.^{1,2} Hydroxamic acids (R-CONHOH) are very effective metal-ion chelators and have led to the rational design and discovery of potent inhibitors of metalloenzymes such as thermolysin,³ angiotensin-converting enzyme (ACE),⁴ and the matrix metalloprotease (MMP) family of enzymes.⁵ The terminal hydroxamic acids (RCONHOH) are readily accessible by a variety of methods and can be accessed from commercially available hydroxylamine hydrochloride.⁶ Conversely, the internal hydroxamic acid motifs (R₁CONR₂-OH) are often found in siderophores^{7,8} and apoptosis-inducing agents such as polyoxypeptin A.⁹ These internal architectures often require the synthesis of an N–O bond either by a direct amine or amide oxidation step. New

methods, which allow facile access to the internal hydroxamic acids, would be of clear value to medicinal chemists interested in using *N*-(hydroxy)amides as pseudopeptides.

The replacement of the amide bond by a thioamide bond in physiologically active peptides is another backbone modification introduced in the search for compounds that are more potent than their parent structures. The resistance afforded by the thioamide bond against enzymatic cleavage may enhance the potency of the pseudopeptide.¹⁰ During our development of the synthetic methods to access *N*-hydroxyamides,^{11,12} we recognized that the

(1) Miller, M. Hydroxamate Approach to The Synthesis of β -Lactam Antibiotics. *Acc. Chem. Res.* **1986**, *19*, 49–56. (b) Stearn, B.; Losee, K. A.; Bernstein, J. Hydroxyurea. A New Type of Potential Antitumor Agent. *J. Med. Chem.* **1963**, *6*, 201. (c) Young, C. W.; Schachetman, C. S.; Hodas, S.; Bolis, M. C. Inhibition of DNA Synthesis by Hydroxyurea: Structure–Activity Relationships. *Cancer Res.* **1967**, *27*, 535–540.

(2) Miller, M. J. *Chem. Rev.* **1989**, *89*, 1563–1579.

(3) Nishino, N.; Powers, J. C. Design of Potent Reversible Inhibitors for Thermolysin. Peptides Containing Zinc Coordinating Ligands and Their Use in Affinity Chromatography. *Biochemistry* **1979**, *18*, 4340–4347.

(4) Petrillo, E. W., Jr.; Ondetti, M. A. Angiotensin-converting Enzyme-Inhibitors Medicinal Chemistry and Biological Actions. *Med. Res. Rev.* **1982**, *2*, 1–41.

(5) (a) Gowravaram, M. R.; Tomczuk, B. E.; Johnson, J. S.; Delecki, D.; Cook, E. R.; Ghose, A. K.; Mathiowetz, A. M.; Spurlino, J. C.; Rubin, B.; Smith, D. L.; Pulvino, T.; Wahl, R. C. Inhibition of Matrix Metalloproteinases by Hydroxamates Containing Heteroatom-Based Modification of P-1' Group. *J. Med. Chem.* **1995**, *38*, 2570–2581. (b) Rockwell, A.; Melden, M.; Copeland, R. A.; Hardman, K.; Decicco, C. P.; Degrado, W. F. Complementarity of combinatorial chemistry and structure-based ligand design: Application to the discovery of novel inhibitors of matrix metalloproteinases. *J. Am. Chem. Soc.* **1996**, *118*, 10337–10338. (c) Hagmann, W. K.; Lark, M. W.; Becker, J. W. Inhibition of matrix metalloproteinases. Bristol, J. A., Ed. *Ann. Rep. Med. Chem.* **1996**, *31*, 231–240.

(6) Romine, J. L. bis-Protected Hydroxylamines as Reagents in Organic Synthesis. A Review. *Org. Prep. Proced. Int.* **1996**, *28*(3), 249–288 and references therein.

(7) Bergeron, R. J.; McManis, J. S.; Phanstiel, O., IV; Vinson, J. R. T. A versatile synthesis of Deferrrioxamine-B. *J. Org. Chem.* **1995**, *60*, 109–114.

(8) Bergeron, R. J.; Phanstiel, O., IV. The total synthesis of Nanchochelin-A, a novel cinnamoyl hydroxamate-containing siderophore. *J. Org. Chem.* **1992**, *57*, 7140–7143.

(9) Umezawa, K.; Nakazawa, K.; Ikeda, Y.; Naganawa, H.; Kondo, S. Polyoxypeptins A and B Produced by *Streptomyces*: Apoptosis-Inducing Cyclic Dipeptides Containing the Novel Amino Acid (2*S*,3*R*)-3-hydroxy-3-methylproline. *J. Org. Chem.* **1999**, *64*, 3034–3038.

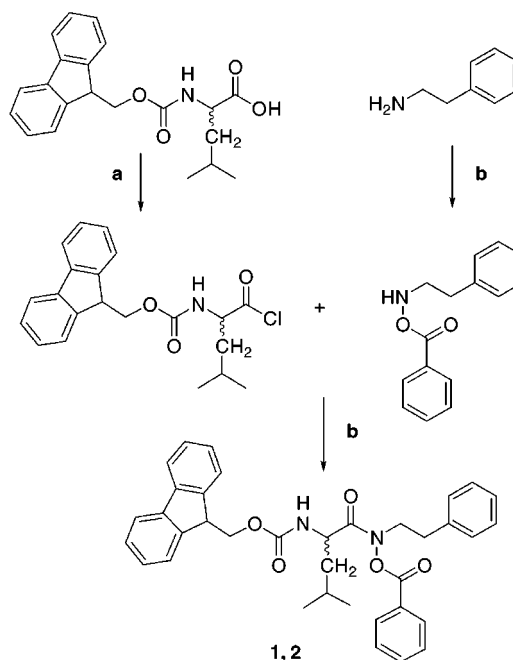
(10) Brian, C. T.; Hallett, A.; Ko, S. Y. Thioamide Synthesis: Thioacyl-*N*-phthalimides as Thioacylating Agents. *J. Org. Chem.* **1997**, *62*, 3808–3809.

(11) Wang, Q. X.; King, J.; Phanstiel, O., IV. An Improved Synthesis of *O*-Benzoyl Protected Hydroxamates. *J. Org. Chem.* **1997**, *62*, 8104–8108.

(12) Wang, Q. X.; Phanstiel, O., IV. Total Synthesis of Acinetoferrin. *J. Org. Chem.* **1998**, *63*, 1491–1495.

related *N*-hydroxythioamides ($R_2C=SNR_1OH$) could also be generated.¹³ These architectures are of interest due to the vast amount of literature illustrating the success of related hydroxamic acid containing MMP inhibitors.¹⁴ The sulfur derivatives would be of importance for comparison to their oxygen analogues and may actually be more potent. Of the three heteroatoms (nitrogen, oxygen, and sulfur) typically used to bind zinc (the metal cofactor in MMPs), replacement of oxygen by sulfur (in the carbonyl position) may be important for several reasons: (1) the observed stronger coordination of sulfur over oxygen for zinc¹⁵ may enhance the binding affinity of these substrates over their hydroxamic acid counterparts, (2) the fact that in a related zinc metalloprotease, i.e., angiotensin converting enzyme (ACE), many amine adducts (including guanidine derivatives) were not as effective as their sulfur analogues,¹⁶ (3) the fact that sulfur is naturally involved in the "cysteine switch" [i.e., proforms of certain MMPs are inhibited by internal coordination of the active site zinc atom by a cysteine side chain (e.g., R-SH) in the prosegment, which is cleaved upon activation].¹⁷

In 1997, we published an improved biphasic method, which allows access to hydroxamic acids in high yield and purity.¹¹ In particular, this method selectively oxidizes a primary amine to a *N*-(benzoyloxy)amine.¹⁸ This paper illustrates the subsequent acylation of a *N*-(benzoyloxy)amine with an *N*-Fmoc-amino acid chloride to introduce the hydroxamic acid functional group into peptides. This synthetic methodology provides α -amino hydroxamic acid containing peptides in high yield without racemization. The retention of chirality throughout this synthetic method was confirmed with a pair of Val-Leu diastereomers. This work also provided several α amino thiohydroxamic acid analogues by conversion of the *N*-(benzoyloxy)amide into the corresponding *N*-(benzoyloxy)thioamide using Lawesson's reagent.^{19,20} Future work will compare

Scheme 1^a

^a Reagents: (a) $SOCl_2$ in CH_2Cl_2 reflux, 2 h; (b) BPO (2 equiv)/ CH_2Cl_2 , pH =10.5 aqueous sodium carbonate buffer, rt.

the efficacy of the sulfur-containing ligands with their oxygen counterparts.

Results and Discussion

The mild conditions (room temperature and pH 10.5) associated with the previously described biphasic method make it ideally suited for converting a primary amine to the corresponding *N*-(benzoyloxy)amine.^{11,18} Initial efforts to apply this biphasic method to the direct oxidation of α -amino acids were unsuccessful. For example, attempts to generate *N*-(benzoyloxy)-L-phenylalanine ethyl ester via the oxidation of L-Phe ethyl ester gave a 0% yield of the desired product. An alternative strategy envisioned acylation of the *N*-benzoyloxyamine with an *N*-protected amino acid. Unfortunately, the use of the classic peptide coupling reagents such as DCC,²¹ or the BOP²¹ reagent, gave low yields of the *N*-(benzoyloxy)amides.²² Carpino has pointed out that the Fmoc amino-protecting group (e.g., the fluorenylmethoxycarbonyl group) is stable enough to survive conversion from the Fmoc-protected amino acid into an Fmoc-protected acid chloride form.²³ Under appropriate conditions, the amine coupling occurs without loss of chirality adjacent to the carboxylic acid site.²³ As we had previously demonstrated the facile acylation of *N*-benzoyloxyamines with acid chlorides,¹¹ Carpino's Fmoc-protected amino acid chlorides offered a promising alternative. As shown in Scheme 1, acylation of *N*-(benzoyloxy)phenethylamine¹⁸ with either the L- or D-Fmoc-protected leucine acid chloride²³ afforded the respective hydroxamate **1** (90%) or **2** (85%). This suc-

(13) Walter, W.; Schaumann, E. *The Chemistry of Thiohydroxamic Acids. Synthesis* **1971**, (3), 111-130.

(14) (a) Brown, F. K.; Brown, P. J.; Bickett, P. M.; Chambers, C. L.; Davies, H. G.; Deaton, D. N.; Drewry, D.; Foley, M.; McElroy, A. B.; Gregson, M.; McGeehan, G. M.; Myers, D. L.; Norton, D.; Salovich, J. M.; Schoenen, F. J.; Ward, P. Matrix Metalloproteinase inhibitors containing a (carboxyalkyl) amino zinc ligand modification of the P1 and P2' residues. *J. Med. Chem.* **1994**, *37*, 674-688 and references therein. (b) Otake, S.; Okayama, T.; Obata, M.; Morikawa, T.; Hattori, S.; Hori, H.; Nagai, Y. Vertebrate collagenase inhibitor. 1. Tripeptidyl hydroxamic acids. *Chem. Pharm. Bull.* **1990**, *38*, 1007-1011 and references therein. (c) Toba, S.; Damodaran, K. V.; Merz, K. M., Jr. Binding Preferences of Hydroxamate Inhibitors of the Matrix Metalloproteinase Human Fibroblast Collagenase. *J. Med. Chem.* **1999**, *42*, 1225-1234. (d) Yamamoto, M.; Tsujishita, H.; Hori, N.; Ohishi, Y.; Inoue, S.; Ikeda, S.; Okada, Y. Inhibition of Membrane-Type 1 Matrix Metalloproteinase by Hydroxamate Inhibitors: An Examination of the Subsite Pocket. *J. Med. Chem.* **1998**, *41*, 1209-1217.

(15) Cushman, D. W.; Ondetti, M. A. Inhibitors of Angiotensin-converting Enzyme. *Prog. Med. Chem.* **1980**, *42*-93.

(16) Redshaw, S. In *Medicinal Chemistry: The Role of Organic Chemistry in Drug Research*; Ganellin, C. R., Roberts, S. M., Eds.; Academic Press: London, 1993; pp 170-172.

(17) Springman, E. B.; Angleton, E. L.; Birkedalhansen, H.; Vanwart, H. E. Multiple-Modes of activation of latent human Fibroblast collagenase-Evidence for the role of a Cys-73 active-site zinc complex in latency and a cysteine switch mechanism for activation. *Proc. Natl. Acad. Sci. U.S.A.* **1990**, *87*, 356-368.

(18) This procedure was later improved in: Phanstiel, O., IV; Wang, Q. X.; Powell, D. H.; Ospina, M. P.; Leeson, B. A. Synthesis of Secondary Amines via *N*-(Benzoyloxy)amines and Organoboranes. *J. Org. Chem.* **1999**, *64*, 803-806.

(19) Jensen, D. E.; Lawesson, S. O. Studies on Amino Acids and Peptides VIII. *Tetrahedron* **1985**, *41*, 5595-5606.

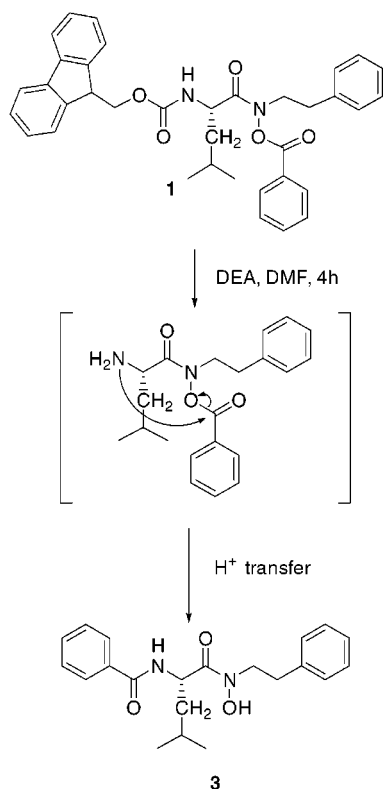
(20) Clausen, K.; Spatola, A. F.; Lenieux, C.; Schiller, P. W.; Lawesson, S. O. Evidence of a Peptide Backbone Contribution Toward Selective Receptor Recognition for Leucine Enkephalin Thioamide Analogue. *Biochem. Biophys. Res. Commun.* **1984**, *120*, 305-310.

(21) Bodanszky, M. *Principles of Peptide Synthesis* **1994**, 118, 124, 155.

(22) For the corresponding condensation of the related *N*-(methyl)-*O*-benzylhydroxylamine with *N*-Boc alanine and DCC, see ref 6, p 266.

(23) Carpino, L. A.; Cohen, B. J.; Stephens, K. E., Jr.; Sadat-Aalae, S. Y.; Tien, J.; Langridge, D. C. ((9-Fluorenylmethyl) oxy) carbonyl (Fmoc) Amino Acid Chlorides. Synthesis, Characterization, and Application to the Rapid Synthesis of Short Peptide Segments. *J. Org. Chem.* **1986**, *51*, 3732-3734.

Scheme 2

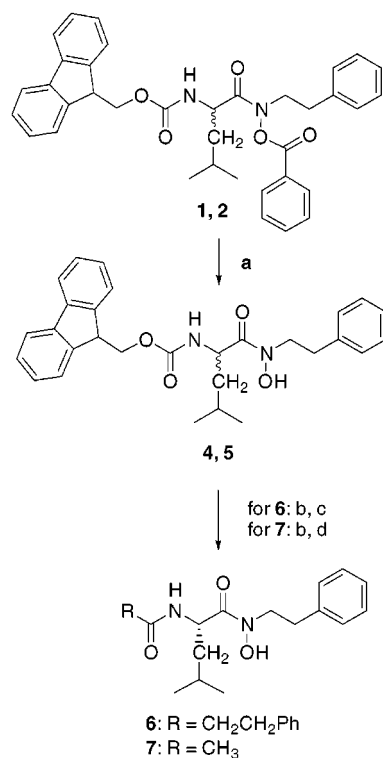


successful strategy was then used to generate several hydroxamic acids and *N*-hydroxythioamide-containing peptides. Significantly, this modular synthetic approach allows access to a diverse number of structures from a common *N*-(benzoyloxy)amine intermediate.

Having incorporated the protected *N*-hydroxy-*N*-alkyl-hydroxamic acid motif within an amino acid scaffold (see **1** in Scheme 2), we wanted to further elaborate the peptide chain via the α -amine. Upon addition of a 10% (by volume) solution of diethylamine (DEA) in DMF to compound **1**, the *N*^t-Fmoc group was removed. The newly generated amine rapidly deprotected the benzoyl group from the adjacent hydroxamate to give the corresponding benzamide hydroxamic acid **3** in 90% yield through a putative intramolecular transformation (Scheme 2).⁸

To generate the free amine without rearrangement, the benzoyl group was removed prior to the Fmoc deprotection step. The benzoyl groups of **1** and **2** were removed with a solution of 10% (by volume) concentrated NH₄-OH in MeOH at -23 °C to give the "free" hydroxamic acids **4** and **5** in 87% and 90% yield, respectively (Scheme 3). Subsequent deprotection of the Fmoc group with 10% DEA in DMF and acylation with either hydrocinnamic acid chloride or acetyl chloride gave the hydroxamic acids **6** and **7** (each in 75% yield).

This method was also extended to the synthesis of *N*-hydroxythioamide-containing peptides.²⁴ A recent paper by Ko et al.¹⁰ illustrated the use of thioacylating agents in synthesizing thiopeptides. Even though Ko's reagent (**A** in Figure 1) was shown to selectively convert amines to thioamides, it was not successful in thioacylating *N*-(benzoyloxy)phenethylamine. Lawesson's reagent

Scheme 3^a

^a Reagents: (a) 10% NH₄OH/MeOH; (b) 10% DEA, DMF; (c) hydrocinnamoyl chloride; (d) acetyl chloride.

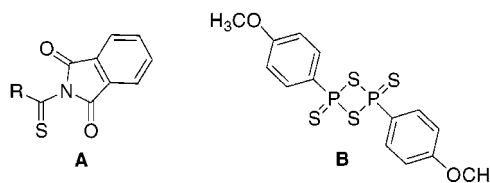
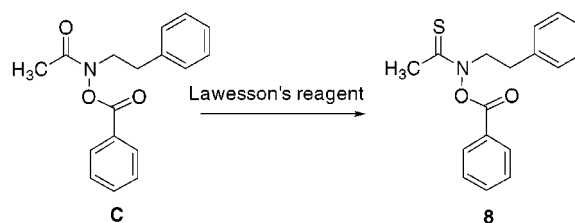


Figure 1. (A) Ko's reagent. (B) Lawesson's reagent.

Scheme 4



agent (**B** in Figure 1) has been shown to convert amides to thioamides and does not usually react with esters.^{19,20} Since the *N*-(benzoyloxy)amide system (e.g., RCONRO-COPh) contained both functional groups, it seemed reasonable that the reagent might selectively convert the amide portion to its thioamide derivative, while leaving the benzoyloxy group intact. Reaction of model compound **C**¹¹ with Lawesson's reagent gave the desired thioamide **8** (76%) (Scheme 4). ¹H NMR chemical shift and mass spectrum (see the Experimental Section) differences allowed for the confirmation of sulfur incorporation into **C** [δ CH₃: **8** (2.5 ppm) vs **C** (2.0 ppm)]. Indeed, reaction of **1** with Lawesson's reagent gave the *N*-(benzoyloxy)-thioamide derivative **9** in 53% yield (Scheme 5). The mass spectrum of **9** further corroborated the incorporation of sulfur into **1**.

(24) Shalaby, M. A.; Grote, C. W.; Rapoport, H. Thiopeptide Synthesis. Amino Thionoacid Derivatives of Nitrobenzotriazole as Thioacylating Agents. *J. Org. Chem.* **1996**, *61*, 9045–9048 and references therein.

Comparison of Peptides of 13 and 14 by ^{13}C NMR. The ^{13}C NMR comparison studies of **13** and **14** were performed at 75.4 MHz in CDCl_3 . The concentrations of **13** and **14** were 24.5 and 23.2 mg/mL, respectively. A spectrum was obtained on each sample, and then the samples were combined in an approximately 2:3 volume ratio and the "spiked" sample spectrum was obtained. Note: Although the chemical shifts are slightly off (~ 1 ppm) from those recorded at 50 MHz, the trends are identical. The ^1H NMR (300 MHz) and ^{13}C APT (attached proton test) experimental results with **13** and **14** are available as Supporting Information.

***N*-(2-Phenylethyl)-*N*-(benzoyloxy)-*N* $_{\alpha}$ -Fmoc-L-leucinamide (1).** A solution of benzoyl peroxide (2.35 g, 9.8 mmol) in CH_2Cl_2 was added to a vigorously stirred solution of phenethylamine (1.20 g, 9.9 mmol) in 25 mL of a carbonate buffer solution (pH = 10.5). The reaction was stirred under argon at room temperature overnight. A new spot was observed on TLC ($R_f = 0.47$, 20% EtOAc/hexane).

Fmoc-L-leucine (3.9 g, 11 mmol) was dissolved in CH_2Cl_2 (80 mL) followed by addition of SOCl_2 (13.06 g, 110 mmol). The reaction mixture was refluxed for 2 h under argon. The solvent and excess of SOCl_2 were removed under vacuum. The remaining residue was added to the biphasic reaction mixture above at room temperature under argon. The reaction was monitored by TLC ($R_f = 0.47$, 20% EtOAc/hexane) for the disappearance of starting material. After the coupling was completed, the organic phase was separated, dried over anhydrous Na_2SO_4 , filtered, and concentrated. The crude residue was subjected to flash chromatography and eluted with 20% EtOAc/hexane to furnish the desired *O*-benzoyl hydroxamate **1** (5.08 g, 90%). **1**: $R_f = 0.30$ in 20% EtOAc/hexane; ^1H NMR (CDCl_3) δ 8.00 (d, 2H), 7.30 (broad m, 16H), 5.40 (d, 1H), 4.60 (m, 1H), 4.30 (d, 1H), 4.15 (m, 2H), 3.00 (t, 2H), 1.50 (broad m, 3H), 0.65 (broad m, 6H); ^{13}C NMR δ 173.0, 164.0, 156.0, 144.0, 142.0, 141.0, 138.0, 134.2, 130.0, 128.4, 128.2, 127.5, 127.2, 127.0, 125.0, 120.0, 67.0, 50.0, 47.0, 42.0, 33.0, 24.0, 23.0, 21.0; $[\alpha]^{25}_{\text{D}} + 23.2$ ($c = 1$, CHCl_3). Anal. Calcd for $\text{C}_{36}\text{H}_{36}\text{N}_2\text{O}_5$: C, 74.98; H, 6.29; N, 4.86. Found: C, 74.70; H, 6.31; N, 4.84.

***N*-(2-Phenylethyl)-*N*-(benzoyloxy)-*N* $_{\alpha}$ -Fmoc-D-leucinamide (2).** Benzoyl peroxide (0.6 g, 2.5 mmol), phenethylamine (0.3 g, 2.5 mmol), Fmoc-D-leucine (1.0 g, 2.8 mmol), and SOCl_2 (3.32 g, 28 mmol) in CH_2Cl_2 were reacted using the same procedure described for **1**. Column chromatography (20% EtOAc/hexane) furnished 1.2 g (85%) of **2**. **2**: $R_f = 0.30$ in 20% EtOAc/hexane; ^1H NMR (CDCl_3) δ 8.00 (d, 2H), 7.30 (broad m, 16H), 5.40 (d, 1H), 4.60 (m, 1H), 4.30 (d, 1H), 4.15 (m, 2H), 3.00 (t, 2H), 1.50 (broad m, 3H), 0.65 (broad m, 6H); ^{13}C NMR δ 173.0, 164.0, 156.0, 144.0, 142.0, 141.0, 138.0, 134.2, 130.0, 128.4, 128.2, 127.5, 127.2, 127.0, 125.0, 120.0, 67.0, 50.0, 47.0, 42.0, 33.0, 24.0, 23.0, 21.0; $[\alpha]^{25}_{\text{D}} - 22.9$ ($c = 1$, CHCl_3).

***N*-(2-Phenylethyl)-*N*-(hydroxy)-*N* $_{\alpha}$ -benzoyl-L-leucinamide (3).** Compound **1** (0.21 g, 0.36 mmol) was dissolved in DMF (3.30 mL) followed by the addition of DEA (0.38 mL). The reaction mixture was stirred for 4 h at room temperature under an inert atmosphere. The reaction was monitored by TLC ($R_f = 0.30$, 20% EtOAc/hexane). The mixture was concentrated under vacuum and purified by flash chromatography eluting with 40% EtOAc/hexane to give benzamide **3** (0.13 g, 90%). **3**: $R_f = 0.30$, in 40% EtOAc/hexane; ^1H NMR (CDCl_3) δ 10.10 (s, 1H), 7.70 (d, 2H), 7.25 (m, 8H), 5.30 (m, 1H), 4.00 (m, 1H), 3.65 (m, 1H), 2.80 (t, 2H), 1.70 (broad m, 3H), 0.95 (m, 6H); ^{13}C NMR δ 170.5, 168.5, 138.2, 133.0, 132.0, 129.0, 128.5, 128.0, 127.0, 126.0, 49.0, 48.0, 40.0, 32.5, 25.0, 23.0, 22.2. Anal. Calcd for $\text{C}_{21}\text{H}_{26}\text{N}_2\text{O}_3 \cdot 0.5 \text{H}_2\text{O}$: C, 69.40; H, 7.49; N, 7.71. Found: C, 69.37; H, 7.32; N, 7.48.

***N*-(2-Phenylethyl)-*N*-(hydroxy)-*N* $_{\alpha}$ -Fmoc-L-leucinamide (4).** Compound **1** (1.50 g, 2.6 mmol) was dissolved in 10% $\text{NH}_4\text{OH}/\text{MeOH}$ (3 mL) and stirred for 30 min under argon. The disappearance of starting material was monitored by TLC ($R_f = 0.30$, 20% EtOAc/hexane). After the reaction was completed, the solvent was removed under vacuum. The crude was purified by flash chromatography eluting with 20% EtOAc/hexane to give hydroxamic acid **4** (1.30 g, 87%). **4**: $R_f = 0.19$, 20% EtOAc/hexane; ^1H NMR (CDCl_3) δ 8.90 (s, 1H), 7.70 (d, 2H), 7.50 (d, 2H), 7.20 (m, 9H), 5.65 (d, 1H), 4.90 (q, 1H), 4.10

(broad m, 5H), 2.90 (t, 2H), 1.95 (s, 1H), 1.60 (broad m, 2H), 0.90 (m, 6H); ^{13}C NMR δ 171.6, 157.5, 143.6, 141.6, 138.2, 129.0, 128.8, 128.5, 127.8, 127.0, 126.2, 125.0, 120.0, 67.8, 49.0, 48.5, 47.0, 40.0, 32.5, 24.5, 23.0, 22.0; $[\alpha]^{25}_{\text{D}} - 14.9$ ($c = 1$, CHCl_3). Anal. Calcd for $\text{C}_{29}\text{H}_{32}\text{N}_2\text{O}_4$: C, 73.70; H, 6.82; N, 5.93. Found: C, 72.50; H, 6.72; N, 5.83.

***N*-(2-Phenylethyl)-*N*-(hydroxy)-*N* $_{\alpha}$ -Fmoc-D-leucinamide (5).** **2** (1.2 g, 2.1 mmol) was dissolved in 10% $\text{NH}_4\text{OH}/\text{MeOH}$ (2.4 mL). The hydroxamic acid **5** (1.08 g, 90%) was isolated using the same procedure used with **4**. Anal. Calcd for $\text{C}_{29}\text{H}_{32}\text{N}_2\text{O}_4 \cdot 0.3\text{H}_2\text{O}$: C, 72.87; H, 6.87; N, 5.87. Found: C, 72.62; H, 6.87; N, 6.27. **29**: $R_f = 0.19$, 20% EtOAc/hexane; ^1H NMR (CDCl_3) δ 8.90 (s, 1H), 7.70 (d, 2H), 7.50 (d, 2H), 7.20 (m, 9H), 5.65 (d, 1H), 4.90 (q, 1H), 4.10 (broad m, 5H), 2.90 (t, 2H), 1.95 (s, 1H), 1.60 (broad m, 2H), 0.90 (m, 6H); ^{13}C NMR δ 171.6, 157.5, 143.6, 141.6, 138.2, 129.0, 128.8, 128.5, 127.8, 127.0, 126.2, 125.0, 120.0, 67.8, 49.0, 48.5, 47.0, 40.0, 32.5, 24.5, 23.0, 22.0; $[\alpha]^{25}_{\text{D}} + 12.2$ ($c = 1$, CHCl_3).

***N*-(2-Phenylethyl)-*N*-(hydroxy)-*N* $_{\alpha}$ -hydrocinnamoyl-L-leucinamide (6).** **4** (0.20 g, 0.35 mmol) was dissolved in DMF (7 mL) and DEA (0.78 mL) and stirred at room temperature until all of the starting material ($R_f = 0.19$, 20% EtOAc/hexane) was consumed. The solvent was removed under vacuum, and the residue was redissolved in CH_2Cl_2 (10 mL) followed by the addition of an aqueous Na_2CO_3 solution (10 mL).

In a separate step, hydrocinnamic acid (0.066 g, 0.44 mmol) and SOCl_2 (1.00 mL) were mixed and refluxed for 2 h under an inert atmosphere. The excess SOCl_2 was removed under vacuum. The residue was added to the biphasic solution mentioned above and stirred overnight under argon at room temperature. The organic phase was separated, dried over anhydrous Na_2SO_4 , filtered, concentrated, and purified by flash chromatography eluting with 40% EtOAc/hexane to give amide **6** (0.10 g, 75%). **6**: $R_f = 0.30$ in 40% EtOAc/hexane; ^1H NMR (CDCl_3) δ 10.10 (broad s, 1H), 7.15 (broad m, 10H), 5.05 (m, 1H), 4.00 (m, 1H), 3.70 (m, 1H), 2.80 (m, 4H), 2.45 (m, 2H), 1.60 (m, 1H), 1.40 (m, 2H), 0.85 (m, 6H); ^{13}C NMR δ 174.0, 171.5, 140.6, 138.8, 129.2, 129.0, 128.9, 128.8, 126.5, 49.8, 48.0, 40.0, 38.0, 33.5, 32.0, 25.5, 23.5, 22.5. Anal. Calcd for $\text{C}_{23}\text{H}_{30}\text{N}_2\text{O}_3$: C, 72.22; H, 7.90; N, 7.32. Found: C, 72.30; H, 7.91; N, 7.42.

***N*-(2-Phenylethyl)-*N*-(hydroxy)-*N* $_{\alpha}$ -acetyl-L-leucinamide (7).** **4** (0.15 g, 0.26 mmol) was dissolved in DMF (5 mL) and DEA (0.58 mL) and stirred at room temperature until the starting material ($R_f = 0.19$, 20% EtOAc/hexane) was consumed. The solvent was removed under vacuum, and the residue was redissolved in CH_2Cl_2 (10 mL) followed by the addition of an aqueous Na_2CO_3 solution (10 mL) and acetyl chloride (0.045 mL). The reaction mixture was stirred overnight under argon at room temperature. The organic phase was separated, dried over anhydrous Na_2SO_4 , filtered, concentrated, and purified by flash chromatography eluting with 70% EtOAc/hexane to give the acetamide **7** (0.10 g, 75%). **7**: $R_f = 0.20$ in 70% EtOAc/hexane; ^1H NMR (CDCl_3) δ 9.90 (s, 1H), 7.20 (m, 5H), 6.35 (d, 1H), 4.95 (q, 1H), 3.90 (m, 2H), 2.95 (t, 2H), 2.00 (s, 3H), 1.50 (broad m, 3H), 0.90 (m, 6H); ^{13}C NMR δ 172.0, 170.0, 138.5, 129.0, 128.5, 126.2, 48.5, 47.5, 39.5, 33.0, 25.0, 23.0, 22.5. Anal. Calcd for $\text{C}_{16}\text{H}_{24}\text{N}_2\text{O}_3 \cdot 0.4 \text{H}_2\text{O}$: C, 64.15; H, 8.34; N, 9.35. Found: C, 64.54; H, 8.30; N, 8.95.

***N*-(2-Phenylethyl)-*N*-(benzoyloxy)thioacetamide (8).** Compound **C¹¹** (0.10 g, 0.35 mmol) was dissolved in anhydrous THF (1 mL) followed by the addition of 2,4-bis(4-methoxyphenyl)-1,3-dithia-2,4-diphosphetane-2,4-disulfide (Lawesson's reagent, 0.072 g, 0.18 mmol) portionwise. The reaction mixture was covered with aluminum foil and stirred for 4 h at room temperature until the starting material ($R_f = 0.26$, 20% EtOAc/hexane) was consumed. The solvent was removed under vacuum, and the residue was purified by flash chromatography eluting with 20% EtOAc/hexane to give the desired thioacetamide **8** (0.08 g, 76%). **8**: $R_f = 0.4$ in 20% EtOAc/hexane; ^1H NMR (CDCl_3) 7.92 (dd, 2H), 7.70 (t, 1H), 7.50 (t, 2H), 7.25 (m, 5H), 4.60 (t, 2H), 3.20 (t, 2H), 2.50 (s, 3H); high-resolution mass spectrum (FAB) theory for $(\text{C}_{17}\text{H}_{17}\text{N}_1\text{O}_2\text{S}) \text{M} + 1 = 300.1058$, found $\text{M} + 1 = 300.1058$.

***N*-(2-Phenylethyl)-*N*-(benzoyloxy)-*N*_α-Fmoc-L-leucin(thio)amide (9).** **1** (4.52 g, 7.8 mmol) was dissolved in anhydrous THF (20 mL) followed by the addition of 2,4-bis-(4-methoxyphenyl)-1,3-dithia-2,4-diphosphetane-2,4-disulfide (Lawesson's reagent, 1.59 g, 3.9 mmol) portionwise. The reaction mixture was covered with aluminum foil and stirred for 72 h at room temperature. The solvent was removed under vacuum, and the residue was purified by flash chromatography eluting with 15% EtOAc/hexane to give the desired *N*-benzoyloxythioamide **9** (0.9 g, 53% taking into account recovered starting material). **9**: *R*_f = 0.22 in 15% EtOAc/hexane; ¹H NMR (CDCl₃) δ 7.95 (d, 2H), 7.75 (d, 2H), 7.60 (t, 2H), 7.30 (broad m, 12H), 5.75 (d, 1H), 4.90 (broad m, 1H), 4.60 (m, 1H), 4.30 (m, 4H), 3.15 (m, 2H), 1.60 (broad m, 1H), 0.75 (dd, 6H); ¹³C NMR δ 202.0, 163.0, 156.0, 144.0, 143.8, 141.2, 137.8, 135.0, 130.0, 128.0, 127.9, 127.8, 127.5, 127.0, 126.8, 125.2, 125.0, 120.0, 67.0, 56.0, 54.0, 47.0, 45.5, 32.0, 24.5, 23.5, 21.5. Anal. Calcd for C₃₆H₃₆N₂O₃S·H₂O: C, 72.69; H, 6.44; N, 4.71. Found: C, 72.57; H, 6.30; N, 4.69.

***N*-(2-Phenylethyl)-*N*-(hydroxy)-*N*_α-benzoyl-L-leucin(thio)amide (10).** **9** (0.13 g, 0.22 mmol) was dissolved in DEA (0.23 mL) and DMF (2.0 mL) and stirred for 4 h at room temperature. The reaction vessel was covered with aluminum foil. The disappearance of the starting material was monitored by TLC (*R*_f = 0.22 in 15% EtOAc/hexane). Then the solvent was removed under vacuum at room temperature, and the residue was purified by flash chromatography eluting with 15% EtOAc/hexane to give the benzamide **10** (0.032 g, 39%). **10**: *R*_f = 0.44 in 30% EtOAc/hexane; ¹H NMR (CDCl₃) δ 8.01 (broad s, 1H), 7.80 (m, 2H), 7.35 (m, 8H), 4.70 (q, 1H), 4.00 (m, 2H), 3.00 (m, 2H), 1.60 (m, 3H), 0.90 (m, 6H); high-resolution mass spectrum (FAB) theory for (C₂₁H₂₆N₂O₂S) M + 1 = 371.1793, found M + 1 = 371.1783.

***N*-(2-Phenylethyl)-*N*-(hydroxyl)-*N*_α-Fmoc-L-leucin(thio)amide (11).** **9** (0.40 g, 0.67 mmol) was dissolved in 10% NH₄-OH/MeOH (1 mL) and stirred for 30 min under argon. The reaction vessel was covered with aluminum foil. The disappearance of starting material was monitored by TLC (*R*_f = 0.22, 15% EtOAc/hexane). After the reaction was complete, the solvent was removed under vacuum. The residue was purified by flash chromatography eluting with 20% EtOAc/hexane to give the unstable *N*-hydroxythioamide **11** (0.18 g, 55%). **11**: *R*_f = 0.22 and 0.31, 20% EtOAc/hexane. The mixture **11**: ¹H NMR (CDCl₃) δ 10.75 (s, 1H), 7.78 (d, 2H), 7.58 (d, 2H), 7.15 (m, 9H), 5.38 (d, 1H), 4.70 (m, 1H), 4.40 (m, 2H), 4.20 (m, 2H), 3.20 (m, 2H), 1.50 (m, 3H), 0.90 (m, 6H); ¹³C NMR δ 204.5, 176.0, 144.0, 140.5, 138.0, 128.5, 127.8, 127.0, 126.8, 125.0, 120.0, 67.0, 60.0, 47.0, 46.5, 45.0, 34.0, 25.0, 23.0, 22.5; high-resolution mass spectrum (FAB) theory for (C₂₉H₃₂N₂O₂S) M + 1 = 473.2263, found M + 1 = 473.2248. Anal. Calcd for C₂₉H₃₂N₂O₃S·0.3H₂O: C, 70.50; H, 6.65; N, 5.67. Found: C, 70.65; H, 6.74; N, 5.61. Note: the TLC spot with *R*_f = 0.31 rapidly equilibrated to form a mixture of two spots (*R*_f = 0.22 and 0.31). When the spot with *R*_f = 0.31 was isolated from the TLC plate and re-eluted on a new plate two spots were again observed (*R*_f = 0.22 and 0.31, respectively).

***N*_α-Amido[*N*_α-BOC-L-valinyl]-*N*-(2-phenylethyl)-*N*-(hydroxy)-L-leucinamide (12).** **4** (0.26 g, 0.55 mmol) was dissolved in a solution containing DMF (5.4 mL) and diethylamine (DEA, 0.6 mL) and stirred at room temperature until the starting material (*R*_f = 0.19, 20% EtOAc/hexane) was consumed. The solvent was removed under vacuum, and the residue was redissolved in CH₂Cl₂ (5 mL) followed by the addition of dicyclohexylcarbodiimide (DCC, 0.11 g, 0.53 mmol) and BOC-L-valine (0.12 g, 0.55 mmol). The reaction mixture was stirred overnight under argon at room temperature. After filtration, the filtrate was extracted with brine, and the organic layer was separated, dried over anhydrous Na₂SO₄, filtered, and concentrated. The residue was purified by flash chromatography eluting with 30% EtOAc/hexane to give the L,L

derivative **12** (0.11 g, 45%). **12**: *R*_f = 0.30 in 30% EtOAc/hexane; ¹H NMR (CDCl₃) δ 9.80 (s, 1H), 7.25 (m, 5H), 5.40 (d, 1H), 5.00 (q, 2H), 3.90 (m, 3H), 2.90 (m, 2H), 2.00 (m, 1H), 1.60 (m, 3H), 1.40 (s, 9H), 0.90 (m, 12H); ¹³C NMR δ 173.8, 170.0, 156.0, 138.2, 129.1, 129.0, 128.5, 126.5, 80.0, 59.6, 49.2, 47.8, 39.1, 33.9, 32.8, 31.1, 24.9, 24.8, 22.9, 22.7, 22.1, 19.1, 18.3; [α]_D²⁵ -22.4 (*c* = 1, CHCl₃). Anal. Calcd for C₂₄H₄₀N₃O₅·0.5 H₂O: C, 62.72; H, 8.99; N, 9.14. Found: C, 62.88; H, 8.88; N, 9.06.

***N*_α-Amido[*N*_α-BOC-L-valinyl]-*N*-(2-phenylethyl)-*N*-(hydroxy)-D-leucinamide (13).** Fmoc-protected D-hydroxamic acid **5** (0.50 g, 1.0 mmol) was dissolved in a solution containing DMF (14.4 mL) and DEA (1.6 mL) and stirred until the starting material (*R*_f = 0.19, 20% EtOAc/hexane) was consumed. The solvent was removed under vacuum, and the residue was redissolved in CH₂Cl₂ (10 mL) followed by the addition of DCC (0.26 g, 1.30 mmol) and BOC-L-valine (0.28 g, 1.30 mmol). The reaction mixture was stirred overnight under argon at room temperature. After filtration, the filtrate was extracted and separated, dried over anhydrous Na₂SO₄, filtered, and concentrated. The residue was purified by flash chromatography eluting with 30% EtOAc/hexane to give the L,D derivative **13** (0.30 g, 63%). **13**: *R*_f = 0.30 in 30% EtOAc/hexane; ¹H NMR (CDCl₃) δ 9.80 (s, 1H), 7.25 (m, 5H), 5.40 (d, 1H), 5.00 (q, 2H), 3.90 (m, 3H), 2.90 (m, 2H), 2.00 (m, 1H), 1.60 (m, 3H), 1.40 (s, 9H), 0.90 (m, 12H); ¹³C NMR (50 MHz) δ 173.5, 170.6, 155.9, 138.6, 129.3, 129.0, 128.9, 128.7, 128.6, 126.5, 80.3, 60.0, 49.4, 49.3, 47.7, 41.0, 40.8, 39.5, 35.8, 34.0, 32.9, 31.2, 25.1, 25.0, 24.9, 23.2, 22.9, 22.2, 19.4, 17.8; [α]_D²⁵ -0.6 (*c* = 1, CHCl₃). Anal. Calcd for C₂₄H₄₀N₃O₅·0.5H₂O: C, 62.72; H, 8.99; N, 9.14. Found: C, 62.88; H, 8.88; N, 9.06.

***N*_α-Amido[*N*_α-BOC-L-valinyl]-*N*-(2-phenylethyl)-*N*-(hydroxy)-L-leucinamide (14).** **4** (0.17 g, 0.36 mmol) was dissolved in a solution containing EtOAc (3.6 mL) and DEA (0.4 mL) and stirred until the starting material (*R*_f = 0.19, 20% EtOAc/hexane) was consumed. The solvent was removed under vacuum, and the residue was redissolved in acetonitrile (5.4 mL). BOC-L-valine (0.09 g, 0.41 mmol) and benzotriazol-1-yloxytris(dimethylamino)phosphonium hexafluorophosphate (BOP reagent, 0.16 g, 0.36 mmol) were added, followed by the addition of TEA (0.05 mL). Stirring was continued under argon at room temperature overnight. A saturated sodium chloride solution (brine) was added, and the product was extracted with ethyl acetate. The organic phase was separated, dried over anhydrous Na₂SO₄, filtered, and concentrated. The residue was purified by flash chromatography eluting with 30% EtOAc/hexane to give the L,L derivative **14** (0.11 g, 70%). **14**: *R*_f = 0.30 in 30% EtOAc/hexane; ¹H NMR (CDCl₃) δ 9.80 (s, 1H), 7.25 (m, 5H), 5.25 (d, 1H), 5.00 (q, 2H), 3.90 (m, 3H), 2.90 (m, 2H), 2.00 (m, 1H), 1.60 (m, 3H), 1.40 (s, 9H), 0.90 (m, 2H); ¹³C NMR δ 173.8, 170.0, 156.0, 138.2, 129.1, 129.0, 128.5, 126.5, 80.0, 60.0, 49.1, 47.5, 39.2, 32.8, 31.0, 28.3, 24.8, 22.6, 22.2, 19.1, 18.0; [α]_D²⁵ -33.6 (*c* = 1, CHCl₃). Anal. Calcd for C₂₄H₄₀N₃O₅: C, 63.97; H, 8.95; N, 9.33. Found: C, 64.27; H, 8.93; N, 9.30.

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Supporting Information Available: ¹H and ¹³C NMR and ¹³C NMR APT spectra for compounds **13** and **14** and a 2:3 mixture of **13** and **14**. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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