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

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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^{α}-Fmoc-L-leucine provided a N^{α}-Fmoc-*N*-(benzoyloxy)-L-leucinamide in 90% yield. Deprotection of the benzoyl group (using 10 vol % NH_4OH/MeOH) provided the N^{α}-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 (R_1CONR_2-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

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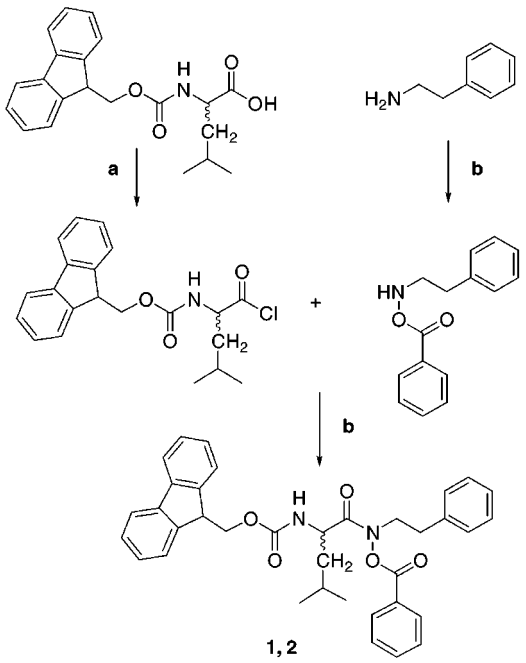
related *N*-hydroxythioamides ($R_2C{=}SNR_1OH$) could also be generated.[13] These architectures are of interest due to the vast amount of literature illustrating the success of related hydroxamic acid containing MMP inhibitors.[14] The sulfur derivatives would be of importance for comparison to their 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 zinc[15] 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 the efficacy of the sulfur-containing ligands with their oxygen counterparts.

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.

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 BOP[21] 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)phenethylamine[18] with either the L- or D-Fmoc-protected leucine acid chloride[23] afforded the respective hydroxamate **1** (90%) or **2** (85%). This suc-

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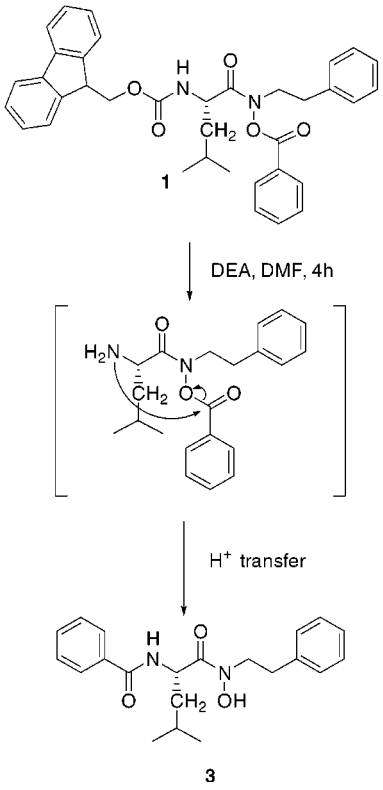
Scheme 2



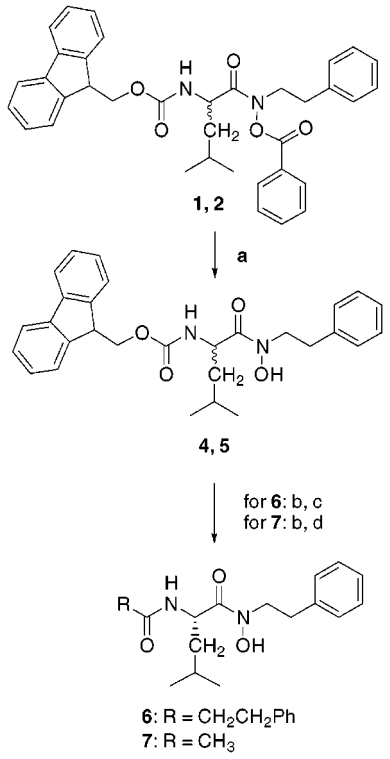
cessful strategy was then used to generate several hydroxamic acids and *N*-hydroxythioamide-containing peptides. Significantly, this modular synthetic approach allows access to a diverse number of structures from a common *N*-(benzoyloxy)amine intermediate.

Having incorporated the protected *N*-hydroxy-*N*-alkylhydroxamic 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^{α}-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 NH_4-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 (**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., RCONROCOPh) 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). ^{1}H NMR chemical shift and mass spectrum (see the Experimental Section) differences allowed for the confirmation of sulfur incorporation into **C** [δ CH_3: **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**.

Scheme 3[a]



[a] Reagents: (a) 10% NH_4OH/MeOH; (b) 10% DEA, DMF; (c) hydrocinnamoyl chloride; (d) acetyl chloride.

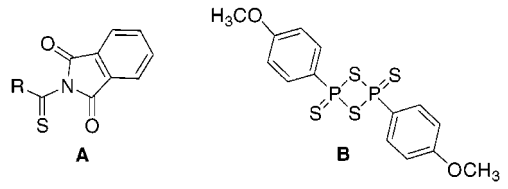
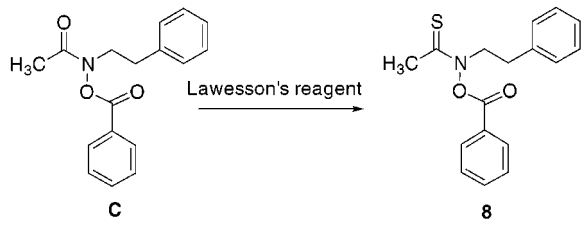


Figure 1. (**A**) Ko's reagent. (**B**) Lawesson's reagent.

Scheme 4



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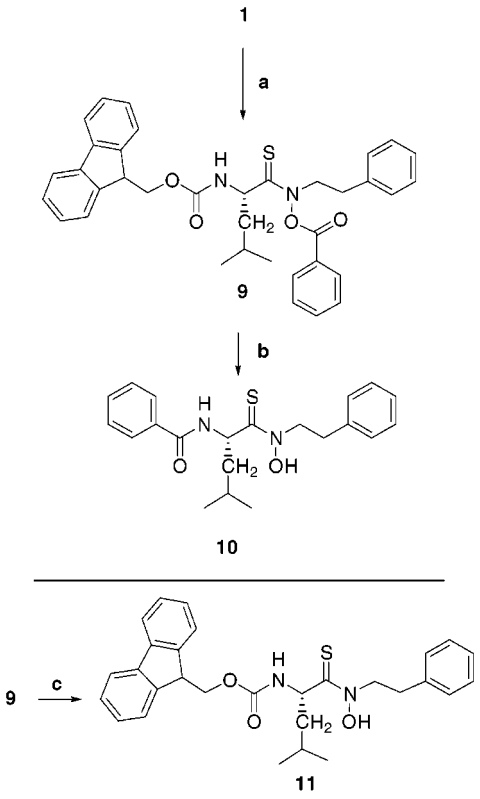
Scheme 5[a]



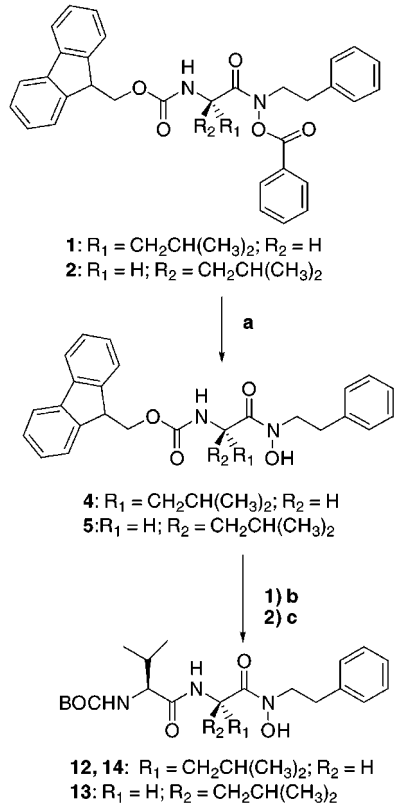
[a] Reagents: (a) Lawesson's reagent, THF, rt; (b) 10% DEA, DMF, 4 h; (c) 10% NH_4OH/MeOH.

Treatment of **9** with 10% NH_4OH/CH_3OH afforded the rearranged benzamide adduct **10**, albeit in 39% yield. Therefore, both the sulfur analogue **9** and the oxygen analogue **1** were observed to undergo the intramolecular rearrangement to the benzamide adducts **10** and **3**, respectively. The benzoyl group was removed from **9** using the same procedure described previously with **1** to give the Fmoc-protected *N*-(hydroxy)thioamide **11** in 54% yield (Scheme 5). Although the reactions that involve sulfur afforded moderate yields, the *N*-(hydroxy)thioamide-containing peptides were still accessible.

Since most biologically active compounds are chiral, racemization is a very important issue in peptide or "pseudopeptide" synthesis. To monitor the chiral integrity throughout the synthesis, a pair of diastereomers (L−L and L−D of Val-Leu-hydroxamic acid derivatives) were synthesized (Scheme 6). The respective Fmoc groups in compounds **4** and **5** were removed in the presence of 10% DEA in DMF. Addition of BOC-L-valine and dicyclohexylcarbodiimide (DCC) to either the free amine of **4** or **5** gave the respective diastereomer **12** (L−L) or **13** (L−D) in 45% and 63% overall yield, respectively. The free amine of **4** was also coupled to Boc-L-Valine using the BOP[21] reagent to provide **14** (L−L) isomer in 70% yield. It should be noted that BOP method gave higher yields and less byproduct formation by TLC.

After isolation the optical rotation of each compound (**4**, **5**, **12**, **13**, and **14**) was measured.[25] The overall racemization at the leucine α carbon center was addressed by ^{13}C NMR experiments in $CDCl_3$. The L−L and L−D Val-Leu diastereomers gave distinct CH_3 signals at δ 17.2 and 16.6 ppm, respectively.[26] As a control, a "spiked" sample containing both diastereomers also gave the two signals at 17.2 and 16.6 ppm. Inspection of the ^{13}C NMR spectrum of **14** (L−L, δ 17.2 ppm) gave no detectable signal for the other diastereomer (L−D) at δ 16.6 ppm. These results suggest that little (<3%) if any racemization occurred during the coupling step involving either the leucine amine or acid functional groups.[23]

Scheme 6[a]



[a] Reagents: (a) 10% NH_4OH/MeOH; (b) 10% DEA, DMF, 4 h; (c) BOC-L-valine.

In summary, enantiomeric amino acids containing the *N*-hydroxyamide motif can be introduced into peptides by coupling *N*-(benzoyloxy)amines with Fmoc-protected amino acid derivatives. This technology provides promising reagents to be used in the generation of natural products such as polyoxypeptin A[9] or combinatorial libraries which contain internal *N*-hydroxyamide subunits. These pseudopeptides, which contain metal binding ligands within their architecture, may have unique biological properties. Inhibition studies evaluating these ligands (**4**, **5**, **13**, and **14**) with a variety of metalloproteins are in progress.

Experimental Section

General Methods. Nuclei were observed at 200 and 50 MHz for ^1H NMR and ^{13}C NMR, respectively. The only exceptions were the ^1H and ^{13}C comparison experiments (see below).

(25) Under the same conditions (c = 1, $CHCl_3$), the BOP-generated peptide **14** gave a higher absolute value for its optical rotation (α^{25}_D = −34°) than the corresponding DCC derived **12** (α^{25}_D = −22°). This observation is consistent with partial racemization during the DCC-mediated coupling step.

(26) Other ^{13}C spectral differences were clearly apparent such as the BOC carbonyl resonances at δ 154.35 and 154.40 for **13** and **14**, respectively. The ^1H NMR ($CDCl_3$) for **14** gave a deceptively simple doublet centered at 0.82 ppm, whereas derivative **13** gave a more complex pattern in this range (see the Supporting Information).

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_α-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 phenethyamine (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; $[\alpha]^{25}_D$ +23.2 (c = 1, $CHCl_3$). Anal. Calcd for $C_{36}H_{36}N_2O_5$: 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_α-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; $[\alpha]^{25}_D$ −22.9 (c = 1, $CHCl_3$).

***N*-(2-Phenylethyl)-*N*-(hydroxy)-N_α-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 $C_{21}H_{26}N_2O_3 \cdot 0.5\ H_2O$: 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_α-Fmoc-L-leucinamide (4).** Compound **1** (1.50 g, 2.6 mmol) was dissolved in 10% NH_4OH/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; $[\alpha]^{25}_D$ −14.9 (c = 1, $CHCl_3$). Anal. Calcd for $C_{29}H_{32}N_2O_4$: 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_α-Fmoc-D-leucinamide (5). 2** (1.2 g, 2.1 mmol) was dissolved in 10% NH_4OH/MeOH (2.4 mL). The hydroxamic acid **5** (1.08 g, 90%) was isolated using the same procedure used with **4**. Anal. Calcd for $C_{29}H_{32}N_2O_4 \cdot 0.3H_2O$: 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; $[\alpha]^{25}_D$ +12.2 (c = 1, $CHCl_3$).

***N*-(2-Phenylethyl)-*N*-(hydroxy)-N_α-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 $C_{23}H_{30}N_2O_3$: 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_α-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 remperature 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 $C_{16}H_{24}N_2O_3 \cdot 0.4\ H_2O$: 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 **C**[11] (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 ($C_{17}H_{17}N_1O_2S$) M + 1 = 300.1058, found 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; ^{1}H NMR ($CDCl_3$) 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); ^{13}C 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 $C_{36}H_{36}N_2O_3S\cdot H_2O$: 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; ^{1}H NMR ($CDCl_3$) 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 ($C_{21}H_{26}N_2O_2S$) 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% NH_4-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**: ^{1}H NMR ($CDCl_3$) 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); ^{13}C 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 ($C_{29}H_{32}N_2O_2S$) M + 1 = 473.2263, found M + 1 = 473.2248. Anal. Calcd for $C_{29}H_{32}N_2O_3S\cdot 0.3H_2O$: 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 up 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 CH_2Cl_2 (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 Na_2SO_4, 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; ^{1}H NMR ($CDCl_3$) 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); ^{13}C 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; $[\alpha]^{25}_D$ −22.4 ($c = 1$, $CHCl_3$). Anal. Calcd for $C_{24}H_{40}N_3O_5\cdot 0.5\ H_2O$: 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 CH_2Cl_2 (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 Na_2SO_4, 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; ^{1}H NMR ($CDCl_3$) 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); ^{13}C 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; $[\alpha]^{25}_D$ −0.6 ($c = 1$, $CHCl_3$). Anal. Calcd for $C_{24}H_{40}N_3O_5\cdot 0.5H_2O$: 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 Na_2SO_4, 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; ^{1}H NMR ($CDCl_3$) 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); ^{13}C 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; $[\alpha]^{25}_D$ −33.6 ($c = 1$, $CHCl_3$). Anal. Calcd for $C_{24}H_{40}N_3O_5$: 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: ^{1}H and ^{13}C NMR and ^{13}C 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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