α-Ketocarbonyl Peptides: A General Approach to Reactive Resin-Bound Intermediates in the Synthesis of Peptide Isosteres for Protease Inhibitor Screening on Solid Support

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Abstract: α-Ketocarbonyl peptides were generated from peptide precursors on solid support via a metal-ion-catalyzed transamination. The reaction proceeded to completion within 2 h with glyoxylate as electrophile and copper(II) ions as catalyst in an aqueous acetate buffer at pH 5.5−6.0. The variety of naturally occurring α-amino acid substrates gave rise to a diverse set of differentially functionalized ketones. The highly reactive terminal ketocarbonyls were prone to aldol-type dimerization and could be transferred into stable moieties by oxime formation, reduction to the alcohol, or reductive amination, respectively. The α-ketocarbonyl peptides were efficient in nucleophilic addition of C-nucleophiles such as phosphono-ylides and allylsilanes.

Introduction

There is continuing demand in combinatorial chemistry and drug discovery to expand the synthetic repertoire of solid-phase chemistry in order to extend its scope in the variety of compounds synthesized. Ideally, such concepts should employ easily available, structurally simple starting materials and yield products that are highly diverse in structure and functionality. Amino acids are among the simplest means to meet these demands. This notion has been pursued in the design of cysteine protease inhibitors.[1] Competitive and irreversible protease inhibitors can be obtained as analogues of natural peptide substrates incorporating a non-peptide insert acting as the inhibitory element. Peptide−isoster structures that irreversibly inactivate cysteine proteases are putative drug targets for diseases such as HIV, osteoporosis, cancer, and various parasitic diseases.

In previous work, *N*-terminal peptidyl aldehydes have been demonstrated as versatile intermediates for the synthesis of such peptide isosters.[2−4] This approach is now further explored with α-ketoamides. Although α-ketoamides exhibit high reactivity similar to that of glyoxaldehydes, the keto-alkyl substituent provides an additional dimension of diversity. Various keto-alkyl groups can be derived from the side chains of α-amino acids and hence exceed the potential of *N*-terminal glyoxaldehydes. The presence of this keto-alkyl group gives access to further synthetic routes.[5] Whereas *N*-terminal peptide glyoxaldehydes can react solely as electrophiles, ketones bearing α-protons are known to react as nucleophilic enols or enolates.

In connection to biological activity, ketocarbonyl peptides have been shown to be efficient and selective enzyme inhibitors. Several examples of electrophilic peptide isosters such as ketones,[6] aldehydes, epoxides,[7] and vinylsulfonamides[1] have been reported in the literature as cysteine and other protease inhibitors. Keto-amides have also been described as efficient inhibitors of cysteine[8] and aspartate proteases.[9] Furthermore, keto-amides can be employed as synthetic intermediates on the route to α-hydroxyamides, a class of compounds with high antitumor and antibacterial activities, such as the bestatin.[10] Bestatin was found to be a potent inhibitor of aminopeptidase B and metalloenzyme leukotriene A4 hydrolase; its diastereomer with (2*S*,3*S*)-configuration is an important structural component of several HIV-protease inhibitors.[11]

In Nature, α-ketocarbonyl compounds are found as metabolic intermediates in the biosynthesis of several amino acids and carbohydrates formed by a cofactor-mediated transaminase reaction. These enzymes have also been used in vitro for the enzymatic preparation of specific keto carbonic acids. Enzyme-free transamination was studied mostly with cofactors such as pyridoxalphosphate[12] or other electron-deficient aldehydes such as α-carbonylaldehydes, yielding an equilibrium between amino acid and ketocarbonyl product (Scheme 1). Usually, strong bases are employed to abstract the proton from the primary imine prior to the electron-rearrangement step, resulting in the secondary imine, which is finally hydrolyzed to the ketocarbonyl product. In a recent example, 4-(methylpyridinium)carboxyaldehyde was used as electrophile and 1,8-diazabicyclo[5.4.0]undec-7-ene

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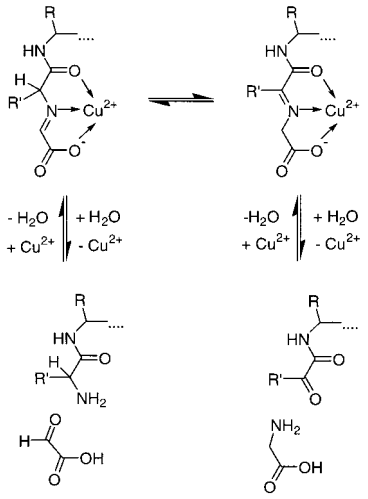
Scheme 1. Specific Modification of the *N*-Terminal α-Amino Group of a Peptide into an α-Ketocarbonyl by Transamination with Sodium Glyoxylate and Cu^{2+} as Catalyst



(DBU) as base;[13] however, the complete conversion of the starting material has not been achieved.

Furthermore, intermolecular side reactions such as aldol reactions render it difficult to isolate this class of compounds. The solid-phase transamination is superior to the reaction in solution for several reasons. Equilibrium reactions can be pushed to the products by replacing the soluble components during reactions. Anchoring of reactive intermediates to the solid support provides a pseudodilution effect which results in suppression of intermolecular side reactions. Therefore, reactions which are prone to side reactions in solution might be quite successful on solid phase. Furthermore, the immobilization of the product on the support allows the use of large excess of reagent which can drive the transamination reaction to proceed to completion.

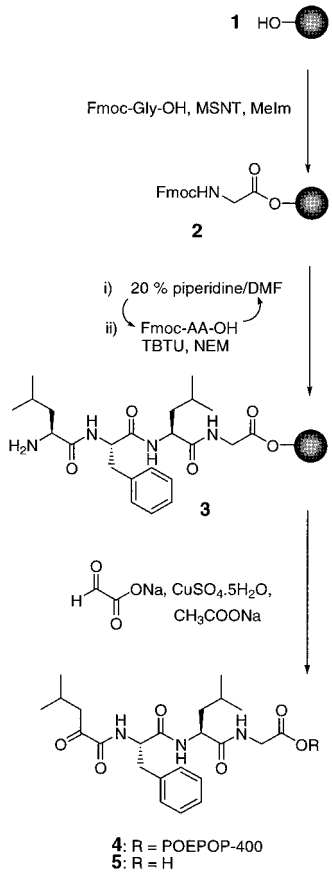
Described herein is the application of the transamination reaction for the formation of α-ketoamide peptides on solid support. The versatility of the modified peptides with regard to structural diversity and reactivity for use in combinatorial chemistry is evaluated.

Results and Discussion

Metal-Ion Catalyzed Transamination. The presented solid-phase reactions were performed on novel supports, recently developed for the purpose of solid-phase organic chemistry, namely POEPOP-400,[2] SPOCC-400, and SPOCC-1500 resins.[14] These resins have in common the fact that they are composed of long polyoxyethylene glycol (PEG) chains, cross-linked by anionic or cationic polymerization of oxirane- and oxetane-derivatized PEG, respectively. They are compatible with non-polar organic solvents and aqueous buffers and may be used in organic synthesis as well as in enzyme assays. They were developed to be used in on-bead assays for the identification of protease inhibitor structures.

The transamination reaction was investigated with resin-bound tetrapeptide **3** assembled on a POEPOP-400 resin **1**. Instead of using a linker, the carboxy terminus of **3** was attached directly to the hydroxy groups of the PEG resin (Scheme 2). Fmoc-glycine was esterified to the resin using the condensing agent

Scheme 2. Generation of α-Ketocarbonyl Peptide on POEPOP-400[a]



[a] POEPOP-400 **1** is esterified with Fmoc-Gly-OH to yield **2**. Tetrapeptide GLFL **3** is synthesized, and transamination yields the α-ketocarbonyl **4**.

1-(2-mesitylenesulfonyl)-3-nitro-1*H*-1,2,4-triazole (MSNT) in combination with *N*-methylimidazole as catalyst.[15] Following Fmoc deprotection, the glycine residue was elongated with TBTU-activated Fmoc-amino acids.[16] The deprotected tetrapeptide resin **3** was subjected to transamination conditions. Glyoxylate was used as an electron-deficient aldehyde in aqueous acetate buffer containing cuprate ions. Similar conditions were originally established as a tool in protein analysis;[17−19] however, the method has not been exploited for synthetic chemistry so far. Efficient and quantitative conversion of the α-amino group to the α-keto function of the *N*-terminal amino acid was achieved by treatment of resin **3** with a solution of glyoxylate in the presence of copper(II) sulfate and sodium acetate at pH 5.5. The reaction was monitored by HPLC at the wavelengths of 215 and 280 nm, as ketocarbonyl peptides show strong absorption at 280 nm in contrast to the starting peptides. The transamination product **4** appeared with an elongated retention time of 10 min (Figure 1) as compared to the starting material, and formation of the ketone was confirmed by ESI-MS determination. Three minor side products were formed and eluted with slightly prolonged retention times (1, 2, and 3 min, respectively), all displaying molecular ions (MH^+) in MS corresponding to dimeric structures of the ketone. It is assumed

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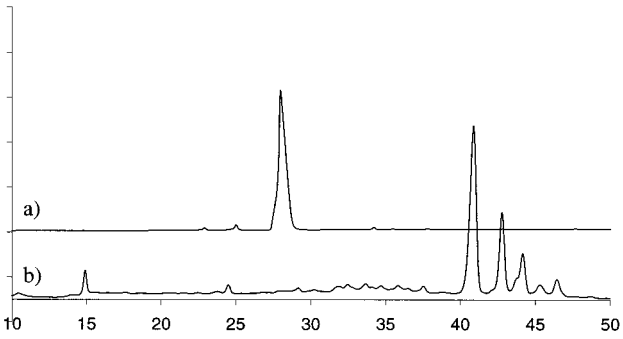

Figure 1. HPLC chromatogram of (a) tetrapeptide **3** and (b) the transamination product **4**. The minor peaks at prolonged retention times correspond to the dimeric structures of the ketone.

that the basic cleavage conditions instigate intermolecular aldol reactions of ketone **5** which result in the formation of these dimeric products. Furthermore, mass spectroscopic analysis of the isolated ketone **5** following storage for a few hours at room temperature gave evidence for the presence of dimers.

Rate of the Reaction. The reaction rate was investigated by analyzing the reaction after 10 min, 30 min, 1 h, 2 h, 4 h, and 8 h, removing all soluble reagents, cleaving the products off the resin with 0.1 M NaOH, and analyzing the neutralized product by HPLC. Copper(II) ions were required for the reaction, and complete conversion of the substrate was observed after 2 h. A comparative experiment without copper(II) ions did not result in any detectable conversion of the peptide substrate within 8 h. The transamination reaction was also investigated in a pyridine/acetic acid buffer since nonpolar substrates might require salt-free environments. Under these conditions, the reaction proceeded with essentially the same reaction rate.

Scope of the Transamination Reaction. The full potential of the transamination reaction, namely the facile generation of chemically diverse and highly reactive electrophiles on solid phase, can be exploited only if the reaction conditions are generally applicable with a variety of protected and unprotected side chains. Ten amino acids (Gly, Leu, Thr(tBu), Cys(Trt), Glu(tBu), Gln, Lys(Boc), Trp, Arg(Pmc), and His) were selected as being representative examples for the naturally occurring side chains. In a parallel synthesis block, resin **3** was elongated in 10 wells with each of these amino acids, respectively. A sample of each resin was cleaved and analyzed by RP-HPLC followed by MALDI-MS. The previously established transamination conditions were then applied to the 10 parallel reaction vessels for 6 h. The products were analyzed by a combination of HPLC and MALDI-MS. The formation of the α-ketocarbonyl compounds was confirmed in all wells with complete conversion of the starting material, except when histidine was the terminal amino acid. Beads containing peptides terminated with histidine changed their color to brown/black and gave no ketocarbonyl product. It is assumed that the copper ion binds to histidine via the *N*-terminal amino group and the *N*-3 of the imidazole side chain to form a six-membered chelate ring.[20] This participation of the histidine side chain results in a redox-active copper coordination sphere. In the presence of glyoxylate, the copper undergoes reduction to form a black precipitation. *π*-Trityl-protected histidine was employed in order to suppress the side-chain coordination and subjected to transamination. A minor amount of the ketocarbonyl product was identified by ES-MS analysis of the collected HPLC fractions. Finally, *τ*-Boc-Im protected histidine was subjected to the transamination conditions, and 40% conversion to the ketone adduct was achieved in 3 h.

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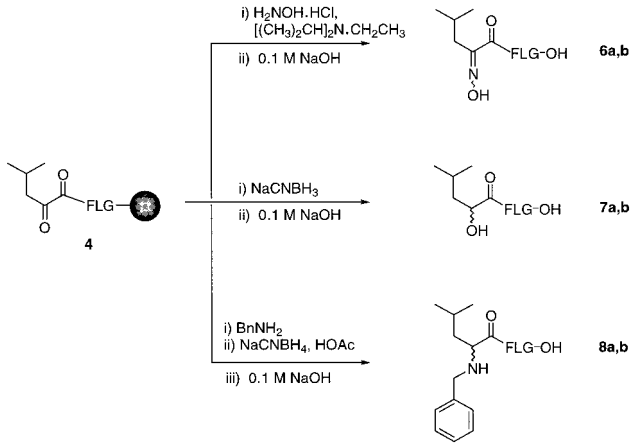
Scheme 3. Chemical Modifications of α-Ketocarbonyl Peptides for Investigation of Dimerization Tendency during Cleavage

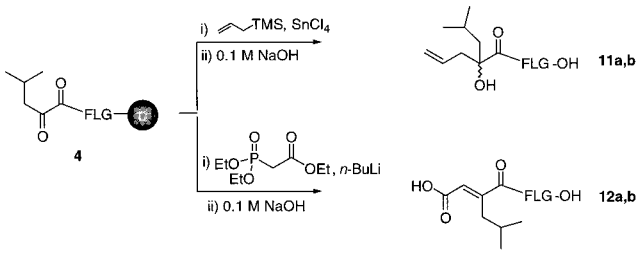


Chemical Transformations of Ketocarbonyl Peptides. The ketocarbonyl peptides were reacted with nucleophiles. In the proceeding experiments, ketocarbonyl peptides could only be isolated as mixtures of the monomeric and dimeric products. Their use as reactive intermediates requires sufficient stability toward dimerization on the resin; thus it was investigated whether dimerization occurred in the resin-bound state or during the cleavage reaction. For this purpose, resin **4** was reacted with hydroxylamine hydrochloride in the presence of diisopropylethylamine (Scheme 3). HPLC analysis of a cleaved sample of resin **6** revealed complete conversion of all the starting material into two products, both displaying the mass of the oxime cis/trans isomers **6a,b**. Reduction of resin **4** was feasible with sodium borohydride, yielding a 1:1 mixture of the two 2-hydroxy products **7a,b**. The reductive amination of **4** with benzylamine was also attempted. Initial experiments employing sodium cyanoborohydride as reducing agent left the starting material unchanged. However, reduction with sodium borohydride in the presence of acetic acid resulted in almost quantitative conversion (>95%) into two isomers of the *N*-benzylamino peptide **8a,b**. These results indicate that the electron-deficient enamine which is in equilibrium with the primarily formed imine requires a stronger reducing agent. Neither in oxime formation nor in reduction nor in reductive amination could any ketodimers be detected by HPLC. Consequently, it can be concluded that the ketocarbonyl moieties on the solid phase are sufficiently immobilized to avoid intermolecular aldol reactions and make the ketone a promising substrate for further transformations.

The α-ketocarbonyl peptide **4** was also employed as substrate for *C*-nucleophiles. Several reactions of *C*-nucleophiles with *N*-terminal peptide aldehydes were previously investigated, and the peptide substrates were compatible with the reaction conditions.[2] Sakurai reaction of **4** with allyltrimethylsilane and tin(IV) chloride as catalyst yielded a 3:2 mixture of two diastereomers **11a,b** (Scheme 4), which could be separated by HPLC. The conversion was 90% after 1 h. However, when resin **4** was treated under the same conditions with 1-(trimethylsiloxy)cyclohexene as *C*-nucleophile, no reaction was observed.

In comparison to the glyoxaldehydes, the present substrates have acidic α-protons, facilitating formation of enolic structures and hence complicating nucleophilic addition under basic

Scheme 4. Nucleophilic Reactions with the Solid-Phase-Bound α-Ketocarbonyl **4** Leading to Peptide Isosters



conditions. When ketocarbonyl **4** was reacted in toluene with an ylide of the Horner−Emmons type (5 equiv) with sodium hydride (4.5 equiv) as a base at room temperature for 2 h, 60% conversion to the products **12a,b** was observed. When this reaction was conducted in THF, a considerable portion of the substrate was cleaved off the resin, indicating the requirement for strictly anhydrous, nonpolar conditions.

Conclusions

A general method for the rapid, quantitative, and selective conversion of terminal α-amino acid amino groups of peptides into α-ketocarbonyl moieties on solid phase has been demonstrated. The reaction was carried out on PEG-based resins in an aqueous medium which also allows direct use of resin-bound products in on-bead screening for enzyme inhibition. Different nucleophilic reactions have been performed successfully to generate a range of new functional properties that enable the generation of peptide isoster libraries. The reaction is particularly valuable for solid-phase synthesis of new protease inhibitors.

Experimental Section

General Procedures. Organic solvents were purchased in HPLC grade, stored over molecular sieves, and used without further purification. Solid-phase peptide chemistry and solid-phase organic chemistry were performed in plastic syringes. Flat-bottom PE syringes were equipped with sintered Teflon filters (50-μm pores), Teflon tubing, and valves for applying suction to the syringes from below. Resin loadings were determined by Fmoc cleavage and optical density measurement at 290 nm and were calculated by employing a calibration curve. The loading of starting hydroxyl resins was determined after esterification with Fmoc-Gly using MSNT as condensing agent and MeIm as base.

Analysis of all solid-phase reactions was performed after product cleavage from a resin sample. A small portion of dry resin (1−2 mg) was weighed in an Eppendorf cup and treated with an aqueous 0.1 M solution of NaOH (50 μL, 2 h). The suspension was neutralized with 1 M hydrochloric acid (5 μL). A sample of this solution (20 μL) was examined on an analytical HPLC column (8 × 200 mm C-18 column, Millipore Delta Pak 15 μm) with detection at 215 and 280 nm using a photodiode array detector (Waters M 991). Eluents A (0.1% TFA in water) and B (0.1% TFA in acetonitrile/water, 9:1) were used in a linear gradient (0% B → 100% B in 50 min). Preparative HPLC was performed using a 25 × 200 mm semipreparative RP-18 column (Millipore Delta Pak 15 μm) employing a linear gradient starting with 85% A and 15% B, a slope of 0.5%/min, and a flow rate of 10 mL/min. Collected fractions were analyzed by ESI/MS (Fisons VG Quatro) or by MALDI-TOF-MS (Bruker) employing α-cyano-4-hydroxycinnamic acid as matrix. Solution-phase NMR spectroscopy was conducted on a Bruker DRX 250-MHz instrument or a Varian Unity Inova 500-MHz instrument. The calculated masses of all products are the average values. All HPLC retention times quoted refer to the analytical column. Chemical shifts were calibrated relative to the signal of tetramethylsilane (0 ppm) or internal solvent signals (3.30 ppm for MeOD-d_4 and 2.49 ppm for DMSO-d_6.

Transamination Reactions. A. General Conditions. Transaminations were conducted with a freshly prepared aqueous solution of 0.5 M sodium glyoxylate (57 mg/mL) and 50 mM copper(II) sulfate pentahydrate (15 mg/mL) in a buffer containing 3.3 M sodium acetate (165 mg/mL) and 0.5 M acetic acid (0.03 mL/mL solution). The pH of the resulting solution was 5.5−6.0, and 1 mL of the solution was employed for 100 mg of POEPOP-400 and SPOCC-400 resins. For 100 mg of SPOCC-1500 resin, 3 mL of the solution was used.

B. Salt-Free Conditions. The hydrate of glyoxalic acid (0.5 M, 46 mg/mL solution) and copper(II) sulfate pentahydrate were dissolved in water or in a mixture of dioxane and water, buffered with pyridine (2.5 M, 202 μL/mL of solution) and acetic acid (0.5 M, 30 μL/mL). The resultant pH in water was 6.0. The volume of solution per amount of resin employed was as described in section A (above).

Leu-Phe-Leu-Gly-POEPOP-400 (3). POEPOP-400 resin (0.5 g, 0.3 mmol) was treated with a solution of MSNT (268 mg, 3 equiv), Fmoc-Gly-OH (268 mg, 3 equiv), and MeIm (60 μL, 2.5 equiv) in CH_2Cl_2 (10 mL). After the resin was washed with dry CH_2Cl_2, the coupling procedure was repeated. Fmoc cleavage was effected with 20% piperidine in DMF (10 min), and the resin was washed again with DMF and CH_2Cl_2. The unprotected amine was acylated with 3 equiv of the Fmoc-protected amino acids of Leu, Phe, and Leu, using TBTU (280 mg, 2.9 equiv) as condensing agent and NEM (152 μL, 4 equiv) as base. Each acylation step was followed by a deprotection procedure as described. Finally the resin was washed with DMF, acetonitrile, and CH_2Cl_2 and dried in vacuo. Fmoc cleavage as described in the General Procedures section revealed a loading of 0.45 mmol/g. A sample of the resin was subjected to HPLC and MS analysis. HPLC: retention time, 28.0 min. ESI-MS: calcd (M = $C_{23}H_{36}N_4O_5$), 448.6 Da; found (MH^+), *m*/*z* 449.6.

4-Methyl-2-oxopentanoyl-Phe-Leu-Gly-POEPOP-400 (4). Resin **3** (0.2 g, 0.09 mmol) was subjected to transamination conditions as described in section A (above) for 10 min, 30 min, 1 h, 2 h, and 8 h, respectively. The resin was washed with water, acetonitrile, and CH_2Cl_2 and was dried in vacuo. The HPLC profile of the cleaved resin samples showed disappearance of the starting tetrapeptide after 2 h. HPLC: retention time, 37 min.

4-Methyl-2-oxopentanoyl-Phe-Leu-Gly (5). Resin **4** (100 mg, 0.045 mmol) was treated with an aqueous 0.1 M solution of NaOH (2 mL, 2 h). It was then washed with water and acetonitrile. The collected washing fractions were neutralized with 1 M HCl (0.2 mL) and lyophilized. Semipreparative RP-HPLC was conducted as described in the General Conditions section. Isolation of the largest peak yielded 7 mg (35%) of **5**, displaying the mass of the expected ketone product by ESI-MS. ^{1}H NMR analysis of this product revealed a nonpure sample of ketone **5**, displaying a reduced integration for the two protons of the β-CH_2 group of the terminal ketoamide.

HPLC: retention time, 37 (major product, *m*/*z* 448.5), 38, 39, 40 min (all with *m*/*z* 896.2). ^{1}H NMR (250 MHz, MeOD-d_4): δ = 0.75−0.95 (m, 12 H, CH_3), 1.60−1.75 (m, 3 H, Leu-β,γ), 2.05 (m, 1 H, C*H*), 2.50−2.80 (m, CH_2), 2.85, 3.03 (2 dd, 2 H, Phe-β-CH_2), 3.77 (2d, 2 H, Gly-α), 4.36 (t, 1 H, Leu-α), 4.53 (dd, 1 H, Phe-α), 7.10−7.30 (m, 5 H, aromatic protons). ESI-MS: calcd [M(ketone) = $C_{23}H_{33}N_3O_6$], 447.5 Da; found (MH^+, $2MH^+$), *m*/*z* 448.5, 896.2.

2-Oxime-4-methylpentanoyl-Phe-Leu-Gly (6a,b). Lyophilized resin **4** (50 mg, 0.023 mmol) was treated with a solution of hydroxylamine hydrochloride (5 equiv, 8 mg) and diisopropylethylamine (5 equiv, 20 μL) in DMF (0.5 mL) for 16 h at room temperature. The resin was washed with DMF, acetonitrile, and CH_2Cl_2 and dried in vacuo. HPLC analysis showed complete conversion of the starting material (>90%). Cleavage and semipreparative RP-HPLC were conducted as described for compound **5**, yielding 3.8 mg of **6a** and 2.4 mg of **6b** after lyophilization with a combined yield of 63%.

6a. RP-HPLC: retention time, 38 min. ^{1}H NMR (500 MHz, MeOD-d_4): δ = 0.86−0.98 (m, 12 H, CH_3), 1.60−1.75 (m, 3 H, Leu-β,γ), 1.95 (m, 1 H, ketoxime-γ-C*H*), 2.44 (d, 2 H, ketoxime-β-CH_2), 3.03, 3.21 (2 dd, 2 H, Phe-β-CH_2), 3.87 (m, 2 H, Gly-α), 4.49 (t, 1 H, Leu-α), 4.75 (t, 1 H, Phe-α), 7.20−7.30 (m, 5 H, aromatic Phe). MALDI-MS: calcd (M = $C_{23}H_{34}N_4O_6$), 462.54 Da; found (MNa^+), *m*/*z* 485.57.

6b. RP-HPLC: retention time, 39 min. ^{1}H NMR (500 MHz, MeOD-d_4): δ = 0.85−0.98 (m, 12 H, CH_3), 1.60−1.75 (m, 3 H, Leu-β,γ), 1.95 (m, 1 H, ketoxime-γ-C*H*), 2.44 (d, 2 H, ketoxime-β-CH_2), 3.03, 3.21 (2 dd, 2 H, Phe-β-CH_2), 3.87 (m, 2 H, Gly-α), 4.49 (t, 1 H,

Leu-α), 4.75 (t, 1 H, Phe-α), 7.20−7.30 (m, 5 H, aromatic Phe). MALDI-MS: calcd (M = $C_{23}H_{34}N_4O_6$), 462.54 Da; found (MNa^+), *m*/*z* 485.57.

2-Hydroxy-4-methylpentanoyl-Phe-Leu-Gly (7a,b). Lyophilized resin **4** (45 mg, 0.02 mmol) was reacted with sodium borohydride (10 equiv, 7.7 mg) dissolved in THF (0.5 mL). After 2 h, the resin was washed with THF, acetonitrile, and CH_2Cl_2 and was then dried in vacuo. Products were cleaved with 2 M NaOH (1 mL, 2 h), washed off the resin with water and acetonitrile, neutralized with 1 M HCl, and lyophilized. Analytical RP-HPLC indicated the formation of two diastereomeric products with >90% conversion. Semipreparative HPLC was conducted as described in the General Procedures section, and compounds **7a** (2.8 mg) and **7b** (2.2 mg) were isolated with a combined yield of 55%.

7a. RP-HPLC: retention time, 33.7 min. ^{1}H NMR (250 MHz, MeOD-d_4): δ = 0.84−0.95 (m, 12 H, C*H*$_3$), 1.38−1.46 (m, 2 H, 3-C*H*$_2$ of 2-hydroxypentanoyl), 1.61−1.82 (m, 3 H, Leu-β,γ), 2.98, 3.12 (2 dd, 2 H, Phe-β-C*H*$_2$), 3.84 (2d, 2 H, Gly-α), 3.96 (t, 1 H, 2-C*H* of 2-hydroxypentanoyl), 4.44 (dd, 1 H, Leu-α), 4.65 (t, 1 H, Phe-α), 6.90−7.30 (m, 5 H, aromatic protons). ESI-MS: calcd (M = $C_{23}H_{35}N_3O_6$), 449.5 Da; found (MNa^+), *m*/*z* 472.2.

7b. RP-HPLC: retention time, 34.6 min. ^{1}H NMR (250 MHz, MeOD-d_4): δ = 0.84−0.95 (m, 12 H, C*H*$_3$), 1.38−1.46 (m, 2 H, 3-C*H*$_2$ of 2-hydroxypentanoyl), 1.61−1.80 (m, 3 H, Leu-β,γ), 2.98, 3.12 (2 dd, 2 H, Phe-β-C*H*$_2$), 3.84 (2d, 2 H, Gly-α), 3.96 (t, 1 H, 2-C*H* of 2-hydroxypentanoyl), 4.44 (dd, 1 H, Leu-α), 4.66 (t, 1 H, Phe-α), 6.9−7.3 (m, 5 H, aromatic protons). ESI-MS: calcd (M = $C_{23}H_{35}N_3O_6$), 449.5 Da; found (MNa^+), *m*/*z* 472.2.

***N*-Benzyl-Leu-Phe-Leu-Gly (8).** Lyophilized resin **4** (80 mg, 0.036 mmol) was reacted with benzylamine (20 equiv, 79 μL) in CH_2Cl_2/DMF, 1:1 (1 mL), at room temperature for 2 h. Sodium borohydride (20 equiv, 27 mg) dissolved in DMF (0.3 mL) containing 1% acetic acid was added. After another 2 h the resin was washed with DMF, acetonitrile, and CH_2Cl_2 and dried in vacuo. RP-HPLC analysis indicated complete conversion (>90%) of the starting material. Products were cleaved and chromatographed by semipreparative RP-HPLC following lyophilization to give the isolated adducts **8a** (4.5 mg) and **8b** (6.8 mg) with a combined yield of 58%.

8a. RP-HPLC: retention time, 33.8 min. ^{1}H NMRm (250 MHz, MeOD-d_4): δ = 0.86−0.98 (m, 12 H, C*H*$_3$), 1.60−1.75 (m, 3 H, Leu-β,γ), 2.85, 3.03 (2 dd, 2 H, Phe-β-C*H*$_2$), 3.45 (2d, 2 H, PhC*H*$_2$), 3.66 (2d, 2 H, Gly-α), 4.35 (t, 1 H, D/L Leu-α), 4.55 (t, 1 H, L Leu-α), 4.75 (t, 1 H, Phe-α), 6.90−7.30 (m, 10 H, aromatic protons). MALDI-MS: calcd (M = $C_{30}H_{42}N_4O_5$), 538.3 Da; found (MH^+, MNa^+, MK^+), *m*/*z* 539.3, 561.3, 577.3.

8b. RP-HPLC: retention time, 35.5 min. ^{1}H NMR (250 MHz, MeOD-d_4): δ = 0.86−0.98 (m, 12 H, C*H*$_3$), 1.60−1.75 (m, 3 H, Leu-β,γ), 2.85, 3.03 (2 dd, 2 H, Phe-β-C*H*$_2$), 3.45 (2d, 2 H, PhC*H*$_2$), 3.66 (2d, 2 H, Gly-α), 4.35 (t, 1 H, D/L Leu-α), 4.55 (t, 1 H, L Leu-α), 4.75 (t, 1 H, Phe-α), 6.90−7.30 (m, 10 H, aromatic protons). MALDI-MS: calcd (M = $C_{30}H_{42}N_4O_5$), 538.3 Da; found (MH^+, MNa^+, MK^+), *m*/*z* 539.2, 561.3, 577.3.

X-Leu-Phe-Leu-Gly (9a−l). Twelve wells of a 20-well synthesis block were filled with dry resin **4** (10 mg, ca. 4.5 μmol each). Fmoc-protected pentafluorophenyl esters of amino acids (20 μmol per well) were dissolved in Eppendorf reaction vessels in DMF (0.5 mL). The solution was added to the resin and reacted for 4 h. To avoid the wells running dry, empty wells were filled with equal amounts of solvent. After the resin was washed with DMF, the Fmoc protective groups were cleaved with piperidine in DMF (20% v/v) for 10 min. The resin was filtered and washed with DMF, acetonitrile, and water. A resin sample of each well was analyzed by HPLC as described in the General Procedures section.

Gly-Leu-Phe-Leu-Gly (9a). HPLC: retention time, 27.6 min.

Leu-Leu-Phe-Leu-Gly (9b). HPLC: retention time, 30.6 min.

***O*-*tert*-Butyl-Thr-Leu-Phe-Leu-Gly (9c).** HPLC: retention time, 31.9 min.

***S*-Trityl-Cys-Leu-Phe-Leu-Gly (9d).** HPLC: retention time, 41.6 min.

***O*-*tert*-Butyl-Gln-Leu-Phe-Leu-Gly (9e).** HPLC: retention time, 30.0 min.

Gln-Leu-Phe-Leu-Gly (9f). HPLC: retention time, 30.0 min.

***N*-*tert*-Butyloxycarbonyl-Lys-Leu-Phe-Leu-Gly (9g).** HPLC: retention time, 33.1 min.

Trp-Leu-Phe-Leu-Gly (9h). HPLC: retention time, 32.9 min.

***N*-Pentamethylchromylsulfonate-Arg-Leu-Phe-Leu-Gly (9i).** HPLC: retention time, 38.7 min.

His-Leu-Phe-Leu-Gly (9j). HPLC: retention time, 26.4 min.

***N*-Trityl-His-Leu-Phe-Leu-Gly (9k).** HPLC: retention time, 39.0 min.

***N*-*tert*-Butyloxycarbonyl-His-Leu-Phe-Leu-Gly (9l).** HPLC: retention time, 27.6 min.

2-Oxoacyl-Leu-Phe-Leu-Gly (10a−l). Resin samples **9a−l** (ca. 4 μmol) were treated with the transamination solution (0.5 mL) described under section A (above) for 16 h. After being washed with water, the resin samples were analyzed as described in the General Procedures section. The conversion of the starting material was >90% in all cases, except when histidine was the terminal amino acid.

***N*-Oxalyl-Leu-Phe-Leu-Gly (10a).** HPLC: retention time, 30.9 min. ESI-MS: calcd (M = $C_{25}H_{36}N_4O_7$), 504.3 Da; found (MH_2OH^+), *m*/*z* 523.3.

4-Methyl-2-oxopentanoyl-Leu-Phe-Leu-Gly (10b). HPLC: retention time, 37.4 min. ESI-MS: calcd (M = $C_{29}H_{44}N_4O_7$), 560.3 Da; found (MNa^+, MK^+), *m*/*z* 583.3, 599.3.

3-*tert*-Butyloxy-2-oxopentanoyl-Leu-Phe-Leu-Gly (10c). HPLC: retention time, 37.4 min. ESI-MS: calcd (M = $C_{31}H_{48}N_4O_8$), 604.4 Da; found (MNa^+, MK^+), *m*/*z* 627.4, 643.3.

3-Mercapto-2-oxopropanoyl-Leu-Phe-Leu-Gly (10d). HPLC: retention time, 31.5 min. ESI-MS: calcd (M = $C_{26}H_{38}N_4O_7S_1$), 550.3 Da; found (MH^+, MNa^+), *m*/*z* 551.6, 573.3.

5-Carboxylate-2-oxopentanoyl-Leu-Phe-Leu-Gly (10e). HPLC: retention time, 32.6 min, 34.4 min (tBu ester). ESI-MS: calcd (M = $C_{28}H_{40}N_4O_9$), 576.3 Da; found (MNa^+, MK^+), *m*/*z* 599.3, 615.3.

5-Carboxyamide-2-oxopentanoyl-Leu-Phe-Leu-Gly (10f). HPLC: retention time, 29.5 min. ESI-MS: calcd (M = $C_{28}H_{41}N_5O_8$), 575.3 Da; found (MNa^+, MK^+), *m*/*z* 598.3, 614.2.

(*N*-*tert*-Butyloxycarbonyl-5-amino)-2-oxopentanoyl-Leu-Phe-Leu-Gly (10g). HPLC: retention time, 39.8 min. ESI-MS: calcd (M = $C_{34}H_{53}N_5O_9$), 675.4 Da; found (MNa^+, MK^+), *m*/*z* 698.2, 714.2.

3-(Ind-2-yl)-2-oxopropanoyl-Leu-Phe-Leu-Gly (10h). HPLC: retention time, 35.8 min. ESI-MS: calcd (M = ($C_{34}H_{43}N_5O_7$), 633.3 Da; found (MNa^+, MK^+), *m*/*z* 656.1, 672.1.

5-(Pentamethylchromylsulfonyl-guanidino)-2-oxopentanoyl-Leu-Phe-Leu-Gly (10i). HPLC: retention time, 43.1 min. ESI-MS: calcd (M = $C_{43}H_{63}N_7O_{10}S_1$), 869.4 Da; found (MH^+, MNa^+, MK^+), *m*/*z* 870.4, 892.4, 908.4.

3-(Imidazo-4-yl)-2-oxopropanoyl-Leu-Phe-Leu-Gly (10k). After 6 h of treatment under the transamination conditions, 40% conversion of the Boc-protected starting material was observed. HPLC: retention time, 28.8 min. ESI-MS: calcd (M = $C_{29}H_{40}N_6O_7$), 584.7 Da; found (MH^+, MH_2OH^+), *m*/*z* 585.6. 603.6.

(4-Methyl-2-hydroxy-2-prop-2'-en)-pentanoyl-Phe-Leu-Gly (11). Lyophilized resin **4** (37 mg, 0.016 mmol) was treated with a solution of allyltrimethylsilane (10 equiv, 28 μL) in CH_2Cl_2 (0.5 mL). Tin tetrachloride (2 equiv, 33 μL of a 1 M solution of $SnCl_4$ in CH_2Cl_2) was added and allowed to react for 4 h. The resin was washed with CH_2Cl_2, acetonitrile, and again with CH_2Cl_2 and dried in vacuo. Cleavage was conducted with 0.1 M NaOH (0.8 mL, 2 h). The products were washed off the resin with water and acetonitrile and lyophilized. HPLC analysis determined conversion of >90% of the starting products. Semipreparative RP-HPLC afforded **11a** (2.6 mg) and **11b** (1.8 mg), with a combined yield of 56%.

11a. HPLC: retention time, 36.9 min. ^{1}H NMR (250 MHz, MeOD-d_4): δ = 0.75−0.95 (m, 12 H, C*H*$_3$), 1.60−1.75 (m, 3 H, Leu-β,γ), 2.12, 2.28 (2 dd, 2 H, C*H*$_2$), 2.85, 3.03 (2 dd, 2 H, Phe-β-C*H*$_2$), 3.66 (2d, 2 H, Gly-α), 4.43 (t, 1 H, Leu-α), 4.75 (t, 1 H, Phe-α), 4.80−5.00 (m, 2 H, olefinic C*H*$_2$), 5.50 (1 H, m, olefinic C*H*), 7.10−7.30 (m, 5 H, aromatic protons). ESI-MS: calcd (M = $C_{26}H_{39}N_3O_6$), 489.6 Da; found (MH^+, MNa^+), *m*/*z* 490.6, 512.5.

11b. HPLC: retention time, 37.7 min. ^{1}H NMR (250 MHz, MeOD-d_4): δ = 0.75−0.95 (m, 12 H, C*H*$_3$), 1.60−1.75 (m, 3 H, Leu-β,γ), 2.12, 2.28 (2 dd, 2 H, C*H*$_2$), 2.85, 3.03 (2 dd, 2 H, Phe-β-C*H*$_2$), 3.66

(2d, 2 H, Gly-α), 4.43 (t, 1 H, Leu-α), 4.75 (t, 1 H, Phe-α), 4.85−4−97 (m, 2 H, olefinic C*H*$_2$), 5.50 (1 H, m, olefinic C*H*), 7.10−7.30 (m, 5 H, aromatic protons). ESI-MS: calcd (M = $C_{26}H_{39}N_3O_6$), 489.6 Da; found (MH^+, MNa^+), *m*/*z* 490.5, 512.6.

2-(2'-Methylbutyl)-4-carboxylate-but-2-enoyl-Phe-Leu-Gly (12a,b). Triethylphosphono acetate (5 equiv, 16.0 μL) in toluene (0.5 mL) was treated with sodium hydride (4.5 equiv, 0.065 mmol) at room temperature under N_2. The obtained solution was added to lyophilized resin **4** (35 mg, 0.016 mmol) and reacted for 2 h. The resin was washed with DMF, THF, and CH_2Cl_2. Analysis of the reaction by RP-HPLC showed 60% conversion of the starting material. Semipreparative RP-HPLC afforded **12a** (2.9 mg) and **12b** (1.3 mg), with a combined yield of 52%.

12a (*E* Isomer). HPLC: retention time, 33.4 min. ^{1}H NMR (500 MHz, MeCN-d_3/H_2O): δ = 0.67 (d, 6 H, methyl butyl C*H*$_3$), 0.82, 0.86 (2d, 6 H, Leu-δ), 1.23 (m, 1 H, methyl butyl C*H*), 1.55 (m, 3 H, Leu-β,γ), 2.40 (d, 2 H, methyl butyl C*H*$_2$), 2.90, 3.14 (2 dd, 2 H, Phe-β-C*H*$_2$), 3.54, 3.68 (2 dd, 2 H, Gly-α), 4.32 (m, 1 H, Leu-α), 4.65 (m, 1 H, Phe-α), 6.13 (s, 1 H, olefinic C*H*), 7.16 (dd, 1 H, Gly-NH), 7.22−7.28 (m, 5 H, aromatic protons), 7.73 (d, 1 H, Leu-NH), 7.75 (d, 1 H, Phe-NH). ESI-MS: calcd (M = $C_{25}H_{35}N_3O_7$), 489.3 Da; found (MH^+, MNa^+), *m*/*z* 490.3, 512.5.

12b (*Z* Isomer). HPLC: retention time, 35.5 min. ^{1}H NMR (500 MHz, MeCN-d_3/H_2O): δ = 0.81, 0.85 (2d, 6 H, Leu-δ), 0.85 (d, 6 H, methyl butyl C*H*$_3$), 1.52 (m, 3 H, Leu-β,γ), 1.74 (m, 1 H, methyl butyl C*H*), 2.11, 2.15 (2 dd, 2 H, methyl butyl C*H*$_2$), 2.91, 3.12 (2 dd, 2 H, Phe-β-C*H*$_2$), 3.72, 3.78 (2 dd, 2 H, Gly-α), 4.29 (m, 1 H, Leu-α), 4.57 (m, 1 H, Phe-α), 5.93 (s, 1 H, olefinic C*H*), 7.18−7.28 (m, 5 H, aromatic protons), 7.35 (dd, 1 H, Gly-NH), 7.61 (d, 1 H, Leu-NH), 7.95 (d, 1 H, Phe-NH). ESI-MS: calcd (M = $C_{25}H_{35}N_3O_7$), 489.3 Da; found (MH^+, MNa^+), *m*/*z* 490.4, 512.4.

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