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Facile Synthesis of Internal and C-Terminal Peptide α -Ketoamides with Fmoc-Solid Phase Peptide Synthesis

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Dedication ((optional))

We report a general and operationally simple method for the solid phase synthesis of α -ketoamide peptides using standard Fmoc solid phase peptide synthesis. The method delivers deprotected peptide α -ketoamides directly upon resin cleavage without any additional steps, and tolerates all peptide functional groups. A small collection of C-terminal and internal α -ketoamide peptides – including two reported protease inhibitors (HCV and SUB1) – were prepared in good yields. In addition, we demonstrate that our method serves as versatile platform for the convenient preparation of cyclic α -ketoamide peptides, photocaged peptide α -ketoamides, and fluorescently labeled peptides.

Keywords: α -ketoamides • peptides • amides • protease inhibitors • solid phase synthesis

Introduction

Peptide-derived α -ketoamides are present in natural products and serve as valuable pharmacophores for drug development, particularly in the construction of protease inhibitors where they serve as key elements for reversible covalent enzyme inhibition.^{i, ii, iii} Despite the widespread use and interest in α -ketoamide peptides, there are only a limited number of strategies for their synthesis and most require several steps to install this reactive functional group.^{iv} One of the most common approaches is the oxidation of α -hydroxy- β -amino acid-containing peptides to the corresponding α -ketoamides.^v An elegant method developed by Wassermann allows the synthesis of certain C-terminal α -ketoamides from cyanophosphorous ylides, which are oxidized with O₃ or dimethyloxirane (DMDO) to give an α -keto acylcyanide that can be intercepted with an amine.^{vi} Rademann has implemented this chemistry into a solid phase approach to peptide α -ketoamides using a solid supported C-terminal cyanophosphorous ylide to form α -ketoamides in solution following resin cleavage.^{vii} Meldal employed dithiolane-masked α -ketoamide dipeptides to synthesize internal α -ketoamide peptides, which are cleaved after peptide assembly with *N*-bromosuccinimide (NBS).^{viii} A recent report on enzymatic formation of internal peptide α -ketoamides from alpha-peptides is an exciting, and unexpected, development in this field.^{ix} Although all these approaches offer valuable access to α -ketoamides, each have considerable limitations in substrate scope and throughput.

To the best of our knowledge there is no method that allows for the solid phase synthesis of α -ketoamide peptides without deviation from standard Fmoc solid phase peptide synthesis (SPPS) protocols (coupling reagents under basic conditions, deprotection with piperidine or similar amines, and TFA cleavage). Based on our recent development of direct approaches to the formation of C-terminal peptide α -ketoacids,^x we now report an approach to the fully automated solid phase synthesis of both internal and C-terminal α -ketoamide peptides directly upon resin cleavage and without the need for additional deprotection or oxidation steps. This approach relies on acid-labile, protected α -ketoacid building blocks and standard Fmoc-amino acids with acid-labile side chain protecting groups including *tert*-butyl, trityl and Boc.^{xi} Importantly – and in contrast to other methods – our approach allows the formation of enantiopure peptide α -ketoamides without detectable epimerization during their synthesis or purification by reverse phase HPLC.^{xii} This chemistry can be applied to the preparation of both linear and cyclic peptide α -ketoamides.

Results and Discussion

The basis for our approach is the synthesis of enantiopure protected α -ketoacids, which can be prepared on a multi-gram scale in four steps from commercially available Fmoc α -amino acids.^{xi} More than 10 different protected α -ketoacid starting materials have been prepared by this convenient, unified route.^{xiii} The overall synthetic route and selected examples, including two novel examples prepared for this study, are shown in Scheme 1.

Scheme 1. Synthesis of protected peptide α -ketoacid monomers for SPPS. For additional information, including the synthesis of many other protected amino acids, please see ref. xi.

In prior work from our group, these monomers were first coupled to a linker by alkylation of a benzylic halide; the linker served as a handle for attachment to a solid support.^{xi} Given the steric hindrance of the protecting group, we were unsure if direct coupling onto a Rink Amide PS (polystyrene) resin would be successful. After some optimization, we were pleased to find that the leucine-derived monomer **1a** could be cleanly coupled to the resin using HATU to give **2**, which was used for the preparation of model α -ketoamide peptide **4** by standard Fmoc SPPS. Cleavage from the resin using 95:2.5:2.5 TFA/H₂O/DODT (2,2'-(ethylenedioxy)diethanethiol) and purification by preparative HPLC directly afforded the pure α -ketoamide peptide **4**; high resolution MALDI mass spectrometry confirmed the identity of the product (Scheme 2). To further confirm the structure and to check for possible epimerization we fully characterized **4** by NMR (¹H, ¹³C, COSY, HSQC, HMBC) and were pleased to find that the product was obtained as a single diastereomer.

Scheme 2: Preparation of C-terminal α -ketoamide peptide **4** using protected α -ketoacid building block **1a** and standard conditions for Fmoc SPPS.

Encouraged by this result we explored the scope of the method by synthesizing a small library of C-terminal α -ketoamide peptides (Table 1). All peptides were isolated in good yields and without any deviation from standard protocols. We observed, however, that heating analytical and preparative HPLC columns to 60 °C improved the peak shape significantly as the α -ketoamide peptides in general tended to elute as broad peaks, probably due to facile formation of hydrates.

Table 1: Synthesis of C-terminal α -ketoamides.

entry	sequence ^a	yield ^b	
		[mg]	[%]
1	Tyr-Phe-Lys-Lys-Leu-Pro-Ser-Gly	36.6	34
2	Gly-Leu-Gly-Lys-Pro-Gly-Arg	14.2	17
3	Pro-Glu-Asn-Ile-Ser	6.7	10
4	Gly-Val-Ile-Arg-Gly	12.9	20
5	Phe-Gly-Val-Leu-Ile	17.5	25

^aSynthesized by automated Fmoc SPPS (2 x 45 min couplings; 4.00 equiv Fmoc-aminoacid; 4.00 equiv HCTU; 8.00 equiv ^tPr₂NEt; 100 μ mol scale; cleavage with 95:2.5:2.5 TFA/DODT/H₂O for 1.5 h) on leucine protected α -ketoamide resin **2**. ^bIsolated yield of pure peptide based on initial resin loading.

Since many biologically active peptides possess an internal α -ketoamide moiety, we were curious if we could use our building blocks for the assembly of internal α -ketoamide peptides. We were initially concerned that the acetal adjacent to the carboxylic acid might reduce the reactivity of our building blocks and render them unsuited for the coupling into a peptide sequence. We were pleased to find that coupling was easily achieved using only 1.5 equiv (based on initial resin loading) of the protected α -ketoacid using HATU for 1.5 h. We speculated that the increased sterics of the protected α -ketoacids was offset by increased electrophilicity due to the electron-withdrawing acetal. Using these optimized coupling conditions, we prepared a collection of internal α -ketoamide peptides in good yields (Table 2).

Table 2: Synthesis of internal α -ketoamides.

entry	sequence ^a		yield ^b	
	Sequence 1	Sequence 2	[mg]	[%]
1	Ala-Asn-Ala	Phe-Ile-Ser-Thr-Ile-Asn-Gly	44.8	39
2	Gly-Ile-Asn-Ala-Trp-Ile	Asp	22.9	25
3	Tyr-Phe-Lys-Lys	Ser-Gly-Leu	41.4	42
4	Thr-Tyr-Arg	Gly-Ile-Asn	43.1	50
5	Tyr-Cys-Lys	Pro-Met-Asn	42.3	47
6	Lys-Glu	Val-Ile-Asn	21.9	30

^aSynthesized by automated Fmoc SPPS (2 x 45 min couplings; 4.00 equiv Fmoc-protected amino acid; 4.00 equiv HCTU; 8.00 equiv ^tPr₂NEt; protected α -ketoacid **1** was coupled using 1.50 equiv **1**, 1.50 equiv HATU and 3.00 equiv ^tPr₂NEt for 1.5 h; 100 μ mol scale; cleavage with 95:2.5:2.5

TFA/DODT/H₂O for 1.5 h) on Rink Amide resin. ^bIsolated yield of pure peptide based on initial resin loading.

Prior approaches to peptide α -ketoamides by late-state oxidation of hydroxyl groups did not tolerate substrates containing unprotected serine or threonine residues. Using our approach, these peptides can be conveniently prepared in excellent yield without deviation from the standard protocol (Table 2 entries 1 and 4). The compatibility with other oxidation-prone residues such as tryptophan, methionine and cysteine (Table 2 entries 2 and 5) – which do not survive oxidation of cyanophosphorous ylides – is another advantage of our method.^{xv} As a benchmark, we synthesized α -ketoamide based *Plasmodium* SUB1 inhibitor **8**. Using protected alanine α -ketoacid for automated Fmoc solid phase synthesis, we isolated the product in 61% yield with a single purification step by preparative RP-HPLC. In comparison, the originally reported solution phase route afforded **8** as a mixture of two diastereomers in an overall yield of less than 2%.^{xv} As another example we prepared HCV protease inhibitor **7** in 22% yield using protected norvaline α -ketoacid.^{xvi}

Having established a convenient process for the fully automated synthesis of both C-terminal and internal α -ketoamide peptides, we sought to apply this approach to the synthesis of cyclic α -ketoamide peptides – a naturally occurring class of biologically active peptides for which there are few synthetic approaches.^{xvii} We were pleased to find that the acetal protecting group was unaffected by the conditions (1% TFA in CH₂Cl₂) used to cleave side-chain protected peptides from a 2-Cl-trityl resin. This allowed us to utilize standard, well-established workflows for the cyclization of fully protected peptides in solution. In brief, the fully protected peptide – containing an internal protected α -ketoamide – was cleaved from the resin, cyclized in solution (PyAOP, ^tPr₂NEt, DMF, 6.5 mM, 5 h), deprotected (95:2.5:2.5 TFA/H₂O/DODT; 2 h) and purified by preparative HPLC. Using this protocol, cyclic α -ketoamide peptide **9** was prepared in 20% overall yield (Scheme 3). Cyclic peptides could also be prepared by construction of a protected α -ketoacid by Fmoc-SPPS on 2-Cl-trityl resin and resin cleavage with HFIP to give the side chain and α -ketoacid protected linear peptide, followed by cyclization and global deprotection (see Supporting Information). This strategy may prove useful for cyclic peptide synthesis as it avoids the notorious problem of epimerization during cyclization.^{xviii}

Scheme 3. Applications of protected α -ketoacid building blocks for the synthesis of protease inhibitors and cyclic peptides bearing internal α -ketoamides. The α -ketoamide products are readily conjugated by hydrazone formation. The use of a photoprotected variant leads to photocaged α -ketoamides.

As the α -ketoamide moiety offers a unique, orthogonal electrophilic handle for post-assembly functionalization, we demonstrated the potential use of our method for the convenient synthesis of labeled peptides. Fluorescently labeled peptide **5** was prepared by hydrazone formation in 55% yield using the corresponding hydrazine-dye in 4:2:1 DMSO/H₂O/CH₃CO₂H for 10 h.

We have recently introduced α -ketoacids orthogonally protected with a photolabile protecting group.^{xi} By using these same starting materials, we successfully prepared a peptide bearing an internal photocaged α -ketoamide. The synthesis of the photoprotected peptide **6** proceeded in good yield under standard conditions for Fmoc SPPS, including purification by RP-HPLC. Upon irradiation of photocaged **6**, complete unmasking of the α -ketoacid occurred within 15 minutes. The identity of the uncaged α -ketoamide was confirmed by comparison to an authentic standard, prepared using the acid-labile α -ketoamide protecting group. This approach may prove useful for photocaged enzyme inhibitors^{xx} or in the emerging field of photopharmacology.^{xxi}

Conclusions

In summary, we have developed a convenient and general method for the fully automated solid phase synthesis of C-terminal and internal α -ketoamide peptides. The method is compatible with all functional groups present in canonical peptides and is to

the best of our knowledge the first method that allows the direct synthesis of α -ketoamide peptides upon resin-cleavage without any deviation from standard SPPS protocols. We have demonstrated the application of this method for the synthesis of protease inhibitors, cyclic α -ketoamide peptides, and fluorescently labeled peptides. We believe a method for the preparation of linear and cyclic peptide α -ketoamides without the need for post-assembly manipulations will facilitate further exploration of this important class of bioactive molecules.

Experimental Section

Please see the online Supplementary Materials.

Supplementary Material

Full experimental procedures for the preparation of protected α -ketoacids **1c** and **1d**, the synthesis of internal and C-terminal peptide α -ketoamides, the formation of cyclic peptides, and HPLC and NMR data for compounds described in the manuscript can be found under <http://dx.doi.org/10.1002/MS-number>.

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Author Contribution Statement

F.R. and A.Z. designed the experiments and conducted the research. F.R. and J.W.B wrote the manuscript. All authors contributed to the interpretation of the results and preparing data for publication.

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