Published in final edited form as:

Org Biomol Chem. 2009 June 7; 7(11): 2259–2264. doi:10.1039/b901198f.

A general strategy for the preparation of C-terminal peptide α -ketoacids by solid phase peptide synthesis†

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Abstract

A new cyanosulfur-ylide based linker makes possible the synthesis of C-terminal peptide α -ketoacids by solid phase synthesis. The preparation of the requisite linker and its application to a variety of C-terminal peptide α -ketoacids with unprotected side chains is reported.

As part of the intense interest in the development of new methods for native amide bond formation *via* chemoselective ligation reactions,^{1,2} our group has identified a novel and unexpectedly simple amide-forming ligation reaction that enables the coupling of the peptide fragments by decarboxylative condensation between α -ketoacids and *N*-alkyl hydroxylamines.³ This reaction offers great promise as a general method for the chemoselective ligation of two unprotected peptide fragments, but is currently limited by the lack of practical methods for the preparation of ligation partners bearing the requisite C-terminal α -ketoacids⁴ and N-terminal hydroxylamines.⁵

In addressing this, we have recently reported a robust, chemos-elective method to produce peptide α -ketoacids with minimal epimerization *via* oxidation of cyanosulfur ylides.⁶ This method is operationally friendly, high yielding and provides peptide α -ketoacids in high enantiopurity. It is also compatible with all unprotected amino acid side chains save cysteine and methionine. To extend this strategy further in preparing larger peptide derived ketoacids, we now report the synthesis and utility of a solid-supported cyanosulfur ylide that makes possible the preparation of C-terminal α -ketoacids using standard Fmoc-based solid phase peptide synthesis (Scheme 1).⁷

Inspired by Rademann *et al.*'s work,⁸ which performed Wasserman *et al.*'s elegant phosphorus ylide⁹ chemistry on a solid support, we hoped to enable the acylation of a cyanosulfur ylide moiety and elongation of the peptide chain on a standard solid support. We were encouraged by our previous results that the cyanosulfur ylides could tolerate the standard procedures employed for Fmoc-based N-terminal deprotection and extension of the peptide chain in the C to N direction.⁶ This finding anticipated an alkyl sulfide-containing linker as a precursor to peptide cyanosulfur ylides for synthesis on a suitable solid support.

Tetrahydrothiophene-derived linker **5** was prepared in five steps from commercially available tetrahydrothiophen-3-one (Scheme 2). It contains a free acid for loading onto an amine or alcohol derivatized solid support, and a four atom-spacer from the acid to the tetrahydrothiophene core. Horner–Wadsworth–Emmons reaction¹⁰ provided α,β -unsaturated

†Electronic supplementary information (ESI) available: Experimental procedures and characterization data. See DOI: 10.1039/b901198f

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ester **1**, which was subjected to 1,4-conjugate reduction to afford **2**. All attempts at metal catalyzed hydrogenation (Pd, Pt, Rh) failed due to catalyst poisoning by the sulfur.¹¹ We found that the conjugated ester could be reduced by *in situ* generated nickel boride,¹² accompanied with a small amount of desulfurization as a side reaction. Subsequent reduction of the ester with LiAlH₄ provided alcohol **3**.¹³ Elaboration of **3** presented the challenge of chemoselective *O*-vs. *S*-alkylation with *tert*-butyl bromoacetate, which was achieved under biphasic conditions to provide ester **4**.¹⁴ A small amount of recovered starting material **3** was resubjected to the alkylation. Finally, removal of the *tert*-butyl protecting group under acidic conditions provided the completed linker **5**.

Anchoring of the linker onto Rink amide MBHA resin (0.54–0.72 mmol/g) was accomplished with a standard HBTU protocol (Scheme 3).¹⁵ Following alkylation of the sulfide, the sulfonium salt could be converted to its ylide *in situ* and coupled to N-terminal Fmoc-protected amino acids in the presence of excess base. In solution phase studies, the sulfonium bromide could be generated by treating tetrahydrothiophene with neat bromoacetonitrile. Although this reaction proceeded in almost quantitative yield,⁶ the bromide salt was extremely hygroscopic and we anticipated that an alternative counterion would be necessary for a stable, storable resin-bound sulfur-ylide precursor.

After evaluating a variety of sulfonium salts (BF₄, TFA, OTf, PF₆, SbF₆), we selected the hexafluorophosphate counterion for further studies aimed at developing a reliable alkylation protocol for the preparation of an easily handled solid-supported sulfur ylide precursor. Cyanomethylation of the solid-supported linker initially proved more challenging than anticipated, due in part to the need to employ a co-solvent for the synthesis of the PF₆, rather than the bromide, salt. Optimization of the cyanomethylation conditions was carried out by assaying the amount of sulfide **7** and Fmoc-Phe-sulfur ylide **11** generated after the first acylation, as determined by ¹H NMR after cleavage from Rink amide MBHA resin (Table 1). Cyanomethylation of sulfide **7** proceeded cleanly in 1:2 CH₃CN:PhCH₃ upon heating with hexafluorophosphate salt **8**, which was generated by filtering the silver iodide precipitate from the combination of AgPF₆ and iodoacetonitrile. This procedure was faster and cleaner than using bromoacetonitrile alone (Table 1, entries 1–2) and left only a trace amount of sulfide unreacted. We found it essential to carefully filter the silver iodide, as contamination with silver caused aggregation of the resin and darkened its colour. The alkylated sulfide gave a bright orange colour to the beads. The polymer-bound sulfonium salt **9** was bench stable, and its reactivity remained the same after storing for months. In contrast, the corresponding resin-bound sulfonium bromide salt was hygroscopic and prone to decomposition.

While related solid-supported phosphorus ylides are highly water sensitive and require strong activation for the acylation step, the resin-supported sulfonium ylides showed improved reactivity and scope. For example, standard coupling conditions including DIC/HOBt, TBTU, and PyBOP protocols failed for the phosphorous ylide,⁸ while these conditions were readily applied to the synthesis of the C-terminal peptide cyanosulfur ylides. Acylation of the resin-bound sulfur ylide could be easily accomplished using HBTU/HOBt, HATU/HOBt,¹⁶ or carbodiimide reagents¹⁷ (DIC and EDC). The coupling efficiency of the first amino acid to the resin was quantified by spectrophotometric Fmoc-UV determination (0.37–0.42 mmol/g from 0.54 mmol/g Rink amide MBHA resin) upon Fmoc deprotection with piperidine, and by TFA cleavage and analysis of Fmoc-Phe-derived ylide **11**. The expected peptide sulfur ylide was obtained in 80% isolated yield after purification. Following introduction of the first amino acid, Fmoc-deprotection and subsequent elongation of the peptide could be carried out under standard conditions. Kaiser tests after the coupling of the first amino acid were not reliable due to trace amounts of residual silver salts from the cyanomethylation step, but could be used in subsequent steps. Following resin

cleavage, PTLC purification provided the desired dipeptide **13** with 65–74% yield over five synthetic steps. Wang resin also gave similar results for a carboxylic acid terminated peptide sulfur ylide.

We have previously documented that oxidative conversion of peptide sulfur ylides could generate the corresponding α -ketoacids in high enantiopurity.⁵ To confirm that the solid phase synthesis protocol preserved the stereochemistry, our previously examined model dipeptide derived sulfur ylide **13** was prepared and subjected to standard aqueous Oxone¹⁸ oxidation (Scheme 4) to give α -ketoacid **14** (92% yield by SFC analysis at 300 nm). Ligation of the crude ketoacid to HONH-Gly-Phe-O^tBu¹⁹ **15** gave comparable yield and diastereomeric ratio of the ligation product **16** (38% yield, d.r. =33:1) as obtained using a sulfur ylide prepared in solution phase. This confirms the practical extension of our peptide sulfur ylide methodology to a polymer support as a means of preparing C-terminal peptide α -ketoacids for chemoselective ligations.

To establish the utility of this strategy for the synthesis of larger peptide α -ketoacids with unprotected amino acid residues, a variety of different amino acids were loaded onto the polymer-supported sulfur ylide. The peptide chain could be easily elongated using standard Fmoc-based peptide coupling protocols. Scheme 5 shows detailed, standard conditions for the synthesis of C-terminal peptide sulfur ylides, using the preparation of **21** as a representative example. The loading of the first amino acid, which becomes the α -ketoacid following ylide oxidation, was performed twice to ensure complete loading due to the unreliability of Kaiser tests at this stage. After the coupling of the second amino acid, the couplings could be monitored by Kaiser tests and standard Fmoc-based peptide synthesis protocols were applied. Cleavage from the resin provided unprotected peptide sulfur ylide **21** in good yield (78% mass yield, >70% HPLC yield), and could be readily purified by preparative HPLC.

The potential of the α -ketoacid–hydroxylamine peptide ligation lies in its application to fragment couplings at a variety of C-terminal and N-terminal amino acid residues. To establish that resin **9** and the optimized coupling procedures detailed in Scheme 5 were applicable to a variety of different peptide sequences, we prepared four additional sulfur ylide sequences with distinct C-terminal residues (Ala, Ile, Glu, Pro) and a full complement of unprotected side chains. In all cases, the crude yields and purities were within the expected range for tetrapeptides prepared by Fmoc-based chemistry on other linkers (Fig. 1). These sulfur ylides could be further purified by preparative HPLC without difficulty.

With purified, unprotected sulfur ylides **21–25**, we confirmed that oxidation to the α -ketoacid proceeds smoothly under our previously reported, operationally simple conditions. Exposure of a dilute (0.02–0.05 M in 2:1 DMF:H₂O) solution of the peptide sulfur ylide to Oxone (2 equiv.) effected rapid formation of the desired α -ketoacid, as determined by LCMS. The oxidations were generally completed within 10 to 30 minutes, and were monitored by HPLC; extended reaction times led to decomposition of the α -ketoacid to the corresponding carboxylic acid. For the purposes of this study, the reactions were quenched by the addition of dimethylsulfide and taken directly to the ligation reaction with simple peptide hydroxylamines (Scheme 6).²¹ The conversion of these diverse, unprotected peptide ylides to α -ketoacids and their subsequent ligation confirms the remarkable tolerance of these two reactions to unprotected side chains including amines, carboxylic acids, alcohols, and electron-rich aromatics. As noted in our prior studies, they are not, however, compatible with unprotected cysteine or methionine residues.

While the ligation reactions performed for this study were not optimized, the results provide qualitative data on the viability of ligation pairs that will inform the application of this

process to larger fragment coupling reactions. When amino acids with bulkier side chains were placed adjacent to the sulfur ylide (Ile, Glu), the ligation proceeded more slowly with sterically hindered hydroxylamines. A notable amount of ketoacid still persisted after stirring for 48 hours in DMF at 60 °C when the peptide α -ketoacids were subjected to ligation with phenylalanine derived hydroxylamines. Ligation with glycine derived hydroxylamines, in contrast, proceeded more smoothly under similar conditions.

In conclusion, we have documented the preparation and utility of sulfonium resin **9** for the Fmoc-based, solid phase synthesis of enantiopure C-terminal peptide sulfur ylides. These products serve as immediate precursors to C-terminal peptide α -ketoacids *via* chemoselective oxidation under simple conditions. The ready availability of C-terminal peptide α -ketoacids using this method will enable further research and application of the α -ketoacid–hydroxylamine ligation to complex peptide synthesis.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

This work was supported by the NIH NIGMS [R01-GM076320]. Unrestricted support from Bristol Myers Squibb, Eli Lilly, and Roche is greatly appreciated. J.W.B. is a fellow of the Beckman Foundation, the Packard Foundation, and the Sloan Foundation.

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20. Fmoc-His-Ser-Phe-Pro sulfur ylide **25** was prepared by loading dipeptide Fmoc-Phe-Pro-OH onto sulfonium resin **9** followed by Fmoc-deprotection and elongation. Stepwise coupling with Fmoc-Pro-OH also provided the desired tetrapeptide sulfur ylide with somewhat lower yield.
21. Peptide targets **26–30** are fragments chosen from either a Fuzeon or human prostate-specific antigen protein with the sole purpose of demonstrating disconnection at ligation site with different amino acid residues.

Fig. 1. Peptide sulfur ylides prepared from resin **9**. The crude purities of sulfur ylides (**22–25**) were assayed by HPLC; yields refer to mass yields of crude peptides after washing with cold ether; isolated yields obtained after purification by HPLC are given in parentheses.²⁰

Scheme 1.

Preparation of α -ketoacid peptides *via* side-chain unprotected C-terminal sulfur ylides.

Scheme 2.
Preparation of linker **5** from tetrahydrothiophen-3-one.

Scheme 3.
Generation of dipeptide derived sulfur ylide from sulfide-containing linker on a solid support.

Scheme 4.

Oxone oxidation of solid phase generated dipeptide sulfur ylide and ligation with a hydroxylamine.

Scheme 5.

Representative conditions for Fmoc-based synthesis of C-terminal peptide sulfur ylides on solid support.

Scheme 6.

Chemoselective oxidation of sulfur ylides to α -ketoacids and subsequent ligations with peptide hydroxyamines.

Table 1

Optimization of sulfide cyanomethylation

Entry	Reagents ^{a,b}	Solvent	Time/h	Temp/°C	Sulfide 7: Ylide 11 ^{c,d}
1	Br-CH ₂ -CN	PhCH ₃	8	60	14:1
2	Br-CH ₂ -CN	Neat	17	rt	1.3:1
3	PF ₆ -CH ₂ -CN	MeCN:PhCH ₃ 1:2	32	rt	1:1.5
4	PF ₆ -CH ₂ -CN	MeCN:PhCH ₃ 1:2	18	60	1:20
5	PF ₆ -CH ₂ -CN	MeCN:PhCH ₃ 1:2	8	60	1:4
6	PF ₆ -CH ₂ -CN	MeCN:PhCH ₃ 1:4	14	60	1:1.6
7	PF ₆ -CH ₂ -CN	MeCN:PhCH ₃ 1:5	13	60	1:1.3

^aThe reactions were agitated in a glass vial filled under a N₂ atmosphere in an oven shaker.

^bThe reactions were carried out with 5 equiv. alkylating reagent (entries 1, 3–7).

^cThe ratio of sulfide 7: ylide 11 was determined by ¹H NMR.

^dThe subsequent acylations were performed using a standard procedure with Fmoc-Phe-OH (5 equiv.), HATU (5 equiv.), HOBT (5 equiv.), DIPEA (20 equiv.) in DMF for 4 h.