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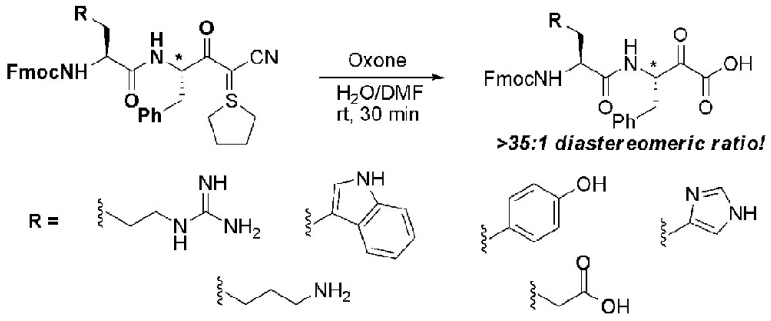

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Stereoretentive Synthesis and Chemoselective Amide-Forming Ligations of C-Terminal Peptide α-Ketoacids

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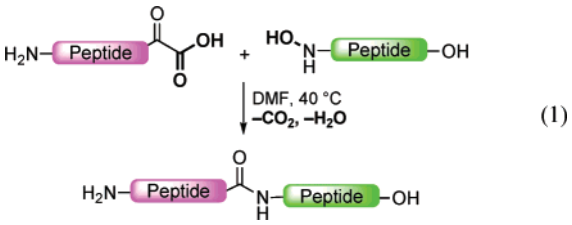
Chemoselective ligation reactions make possible the coupling of unprotected molecular fragments via selective bond forming reactions of unique functional groups. Particularly prized are ligation processes that lead to natural bond connections, such as the elegant and powerful native chemical ligation of C-terminal thioesters and N-terminal cysteines,[1] which allows access to large peptides and proteins with synthetic modifications.[2] In our own work, we have recently documented a novel amide-forming reaction[3] through the chemoselective coupling of α-ketoacids[4] and hydroxylamines under mild conditions, without reagents and without the need for side chain protecting groups (eq 1).[5] Although this reaction shows great



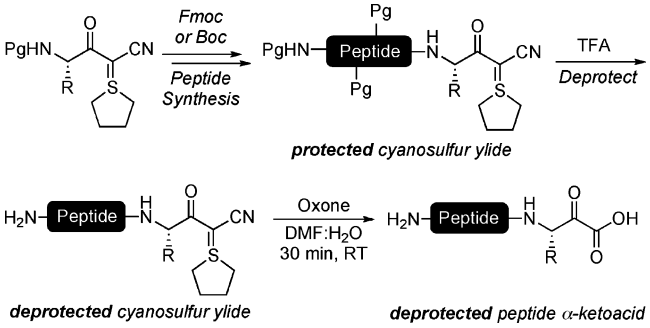
(1)

potential for general chemoselective ligation, a key obstacle to its practical utilization is the preparation of enantioenriched peptide-derived α-ketoacids.[6]

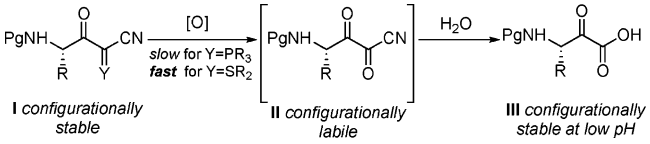
A viable method for α-ketoacid synthesis should address three key challenges: (1) it must provide the C-terminal α-ketoacids without epimerization; (2) it must be compatible with the preparation of side chain unprotected peptide α-ketoacids; and (3) it must interface with established methods and reagents used for iterative peptide synthesis. Herein, we document the first stereoretentive synthesis of unprotected C-terminal peptide α-ketoacids through an operationally friendly approach (Scheme 1).

At the outset of our studies there was, to the best of our knowledge, no prior report of the synthesis of enantiopure oligopeptide α-ketoacids. In contrast, considerable attention had been placed on the synthesis of peptide α-ketoesters and α-ketoamides.[7] We initially hoped to directly translate a leading method for α-ketoester formation, Wasserman's use of cyanophosphorus ylides, to the preparation of unprotected peptide-derived α-ketoacids.[8] The development of a solid-supported variant rendered this strategy additionally attractive.[9] Although we successfully employed phosphorus ylides for the synthesis of protected α-ketoacid monomers, attempts to extend this chemistry to the synthesis of longer and unprotected peptide-derived α-ketoacids were met with frustration. Of particular concern were the conditions required for oxidative conversion of the cyanophosphorus ylides into the α-ketoacids; typical protocols required exhaustive ozonolysis at low temperatures or highly reactive oxidants that were not compatible with unprotected functionality.[8b,c] We also observed epimerization under these conditions, which we attributed to slow turnover of acyl cyanide intermediate **II** (Scheme 2).

Scheme 1. Preparation of α-Ketoacid Peptides via Chemically and Configurationally Stable C-Terminal Sulfur Ylides



Scheme 2. Acyl Cyanides Are Configurationally Labile Intermediates in the Formation of α-Ketoacids by Oxidation of Cyanophosphorus or Cyanosulfur Ylides



In seeking to develop a strategy that would make possible the synthesis of enantiopure, side chain deprotected peptide α-ketoacids under mild, aqueous conditions, we turned to the use of sulfur ylides, which we had recently found to be useful in a different context.[10,11] Preliminary studies suggested that the C-terminal peptide ylides could be formed under a variety of convenient protocols without epimerization of the α-stereocenter (*vida infra*) and that both the acylation reactions and subsequent oxidations of the cyanosulfur ylide occurred more rapidly and cleanly than the phosphorus variant. The cyanosulfur ylides were not only rapidly oxidized by ozone and DMDO[12] but also could be transformed into α-ketoacids using mild and easy to handle Oxone[13] under aqueous conditions. Although Wasserman had previously shown that Oxone could oxidize certain highly activated phosphorus ylides, these reactions suffered poor chemoselectivity.[8f,14] Furthermore, our peptide-derived cyanophosphorous ylides were inert to aqueous Oxone.

Sulfur ylide **1** was chosen as a model substrate for the optimization of the Oxone oxidation conditions.[15] Due to the difficulty in isolating and analyzing the α-ketoacids, which exist as mixtures of the keto and hydrated forms and can also undergo decarboxylation upon concentration, we assayed the degree of epimerization by ligating the resulting α-ketoacid to HONH-Gly–Phe-O*t*Bu (**3**) under a set of standard but unoptimized conditions (Scheme 3). Oxidation conditions were screened with the goal of maximizing the yield of the α-ketoacid in preference to the formation of the corresponding carboxylic acid, which arises from oxidative decarboxylation of the initially formed α-ketoacid, probably via the generation of hydrogen peroxide.[16]

10.1021/ja800053t CCC: $40.75

Scheme 3. Bench Stable α-Peptide Sulfur Ylides Are Prepared via Acylations with Bromide Salt **5** with Either Boc or Fmoc Protection

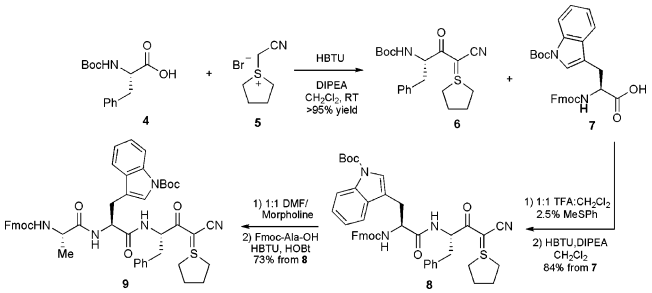
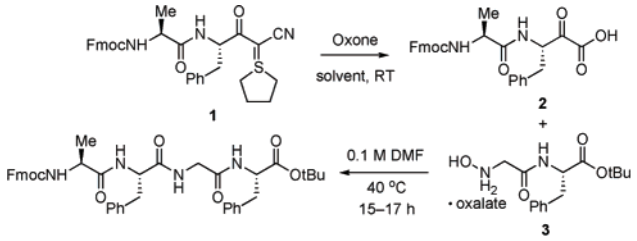


Table 1. Optimization of Oxone Oxidation of Sulfur Ylide **1** to α-Ketoacid **2**



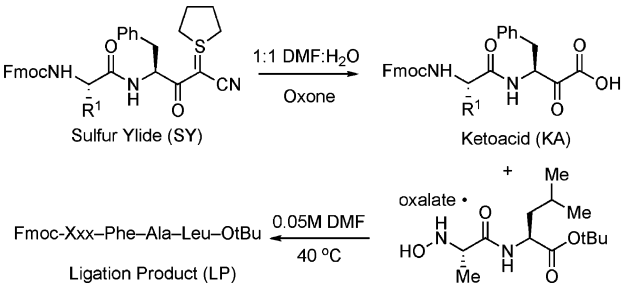
entry	conditions[a]	t / min	KA : SY : CA[b]	dr[c]
1	THF/H_2O (1:1)	30	95 : 5 : 0	21 : 1
2	Acetone/H_2O (1:1)	30	93 : trace : 6	21 : 1
3	MeCN/H_2O (1:1)	50	91 : trace : 9	15 : 1
4	DMF/H_2O (1:1)	30	94 : trace : 5	39 : 1
5	DMF/H_2O (1:1)	120	90 : 0 : 10	21 : 1
6	THF/H_2O (2:1)	30	89 : 5 : 1	50 : 1
7	DMF/H_2O (2:1)[d]	50	53 : 43 : 4	69 : 1
8	DMF/H_2O (1:3)	50	38 : 44 : 13	84 : 1
9	DMF/1M pH 1 HCl-KCl buffer (1:1)	50	21 : 7 : 69	19 : 1
10	DMF/1M pH 3 Gly-HCl buffer (1:1)	50	64 : 1 : 35	58 : 1
11	DMF/1M pH 4 acetate buffer (1:1)	50	90 : trace : 10	41 : 1
12	DMF/1M pH 10 carbonate buffer (1:1)	50	2 : 37 : 57	n.d.[e]

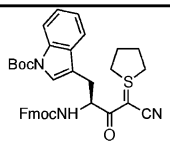
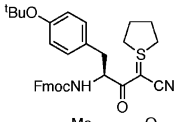
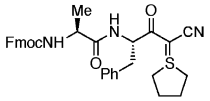
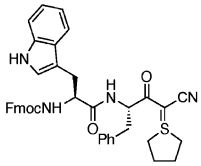
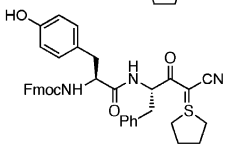
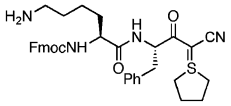
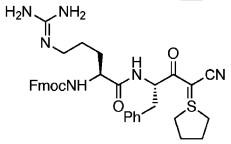
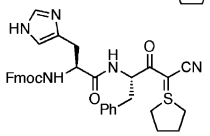
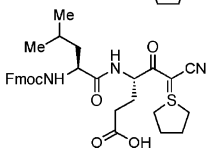
[a] Oxidations were conducted on a 0.05 mmol scale at 0.05 M. [b]KA = ketoacid, SY = sulfur ylide, CA = carboxylic acid (from oxidative decarboxylation), ratios determined by SFC analysis of the unpurified mixture at 300 nm. [c]Determined by SFC analysis of the unpurified ligation product. Ligations were performed using 1.2 equiv of hydroxylamine **3**. [d]1.1 equiv of Oxone was used in the oxidation. [e]Not determined.

Good yields of the α-ketoacid could be obtained in mixtures of water with a variety of organic cosolvents (Table 1, entries 1−4). We observed that the diastereomeric ratio of the ligation product was affected by the reaction time. When the oxidation was allowed to proceed for a longer time (entry 5), the yields of the carboxylic acid side product increased and the diastereomeric ratios of the ligation product decreased. This implies that epimerization originates in the oxidation process. Variation of the solvent ratios (entries 6−8) altered both the oxidation yield and the diastereomeric ratio of the coupling product as a combined result of changes in the solubility of the reactants and the nature of the solvent. Notably, 2:1 THF/H_2O (entry 6) resulted in a 50:1 ratio of diastereomers while still retaining high yields of the α-ketoacid. In general, lower levels of epimerization were observed at low conversion (entries 7−8).

The optimal pH range appears to be at pH 3−4 (entries 10−11), which gives good yields and high diastereomeric ratios. When the reaction was performed in a highly acidic environment (entry 9) or at a pH above 5 (entry 12), the α-ketoacid was rapidly overoxidized into the carboxylic acid. Performing the reaction at a lower temperature or adding the Oxone in portions simply slowed down the rate of the oxidation (not shown) but could not further suppress epimerization. In choosing our standard conditions we elected to balance the highest yield of the α-ketoacid and the lowest

Table 2. Side Chain Compatibility of Sulfur Ylide Oxidation and Ligation



entry	ylide	Oxidation[a] KA : SY : CA	Ligation[b] LP : SY : CA
1		80 : 15 : 5	75 : 12 : 7[d] (70%)[f]
2		89 : 0 : 10	49 : 0 : 8.8
3		86 : 3 : 7	63 : 3 : 10[g] (44%)[f]
4		58 : (31)[c]	43 : (37)[e] (41%)[f]
5		82 : 3 : 8	51 : 3 : 25
6		73 : (16)[c]	52 : (38)[e]
7		75 : (6)	42 : (11)[e] (34%)[f]
8		54 : (32)	32 : (39)[e]
9		77 : 9 : 1	48 : 9 : 1[h] (44%)[f]

[a] HPLC yields at 300 nm: α-ketoacid (KA):sulfur ylide (SY):carboxylic acid (CA). [b]HPLC yields at 300 nm: ligation product (LP):sulfur ylide (SY): carboxylic acid (CA) from oxidative decarboxylation. [c]Combined yield of sulfur ylide and carboxylic acid. [d]Ligation performed with HONH-Ala-O*t*Bu. [e]Ligation performed at 60 °C. [f]Isolated yield over two steps. [g]Ligation performed with HONH-Gly-Phe-O*t*Bu. [h]At 50 °C.

levels of epimerization and selected 2:1 THF/H_2O (entry 4) and 1:1 DMF/H_2O (entry 6), which could accommodate the solubility of most of our substrates and give excellent conversion and high diastereomeric ratios. Importantly, a 0.1 M solution of ketoacid **2** prepared in this manner was chemically and configurationally stable upon standing overnight at ambient temperature.

Conveniently, and importantly, the peptide sulfur ylides could be easily prepared simply by direct coupling of an *N*-protected amino acid with sulfonium salt **5** under standard peptide coupling conditions (Scheme 3). Similar protocols with the corresponding phosphonium salts are possible, but we found these reactions to be capricious and highly sensitive to water and other factors.[9a] In contrast, couplings with the sulfonium salt require no special handling or precautions, save that the starting sulfonium bromide is hydroscopic and must be stored accordingly. The resulting α-keto-cyanosulfur ylides are configurationally and chemically stable and may be handled in air without concern. Standard deprotection reactions, such as acidic removal of Boc groups, does not protonate the highly stabilized ylide. Elongation of the peptide chain is readily achieved using standard peptide deprotection and coupling conditions (EDCI or HBTU).[17]

With standard protocols for both the synthesis of the C-terminal peptide cyanosulfur ylides and their conversion to the α-ketoacids established, we investigated the compatibility of these conditions with various amino acid side chains (Table 2). We prepared a series of dipeptides containing an unprotected side chain adjacent to a C-terminal phenylalanine. When placed away from the ligation site, unprotected tryptophan, tyrosine, lysine, arginine, histidine, and glutamic acid (entries 4−9) were all found to be compatible with sulfur ylide oxidations with aqueous Oxone (Table 2). Protected side chains, in most cases, were also tolerated. *tert*-butyl carbamates (Boc) and esters (^{t}Bu) were not affected, but we observed partial deprotection of trityl and Pbf protecting groups, probably due to the acidic nature of unbuffered Oxone. When placed at the ligation site, protected tryptophan and tyrosine residues underwent smooth oxidations (entries 1, 2). Unprotected C-terminal tryptophan or tyrosine sulfur ylides, however, were resistant to oxidation. It should be noted that the ligation step was unoptimized; higher yields can be obtained under other conditions or with excess hydroxylamine.

Examination of Table 2 reveals that in all cases the α-ketoacid is the major product. Together, the α-ketoacid product and the carboxylic acid byproduct resulting from the oxidative decarboxylation account for >85% of the material, indicating that the side chains do not form significant amounts of oxidized byproducts. Basic residues (entries 4, 6−8) appear to slow the rate of oxidation, resulting in lower conversion when using the reaction times and conditions optimized for Fmoc-Ala-Phe-SY (**2**). This is consistent with our findings that the pH of buffered solutions alters the rate of the oxidations.

In preliminary experiments with a methionine containing sulfur ylide, we isolated the sulfone-ligated product and HPLC and ESI-MS data of the crude oxidations evidenced a mixture of the sulfur ylide and α-ketoacid with the methionine sulfur at the sulfoxide and sulfone oxidation states. Addition of DMSO, tetrahydrothiophene, or thioanisole as oxidation competitors as well as varying Oxone equivalents, solvent, and temperature resulted in conditions providing a 29% yield of the sulfoxide α-ketoacid (see Supporting Information).

In summary, we have developed a robust, chemoselective method to produce C-terminal peptide α-ketoacids with minimal epimerization. Our studies also provide further testament to the impressive chemoselectivity of the α-ketoacid−hydroxylamine amide-forming ligation reaction. Current efforts are aimed at translating this work to solid-phase methods, which will allow the preparation of longer α-peptide derived α-ketoacids suitable for use in decarboxylative peptide ligation reactions.

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Supporting Information Available: Experimental procedures and characterization data for all compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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(14) Wasserman, H. H.; Baldino, C. M.; Coats, S. J. *J. Org. Chem.* **1995**, *60*, 8231−8235.
(15) We found the reproducibility of the oxidation was dependent on the source of the Oxone. The Oxone used in these studies was obtained from Alfa Aesar and generally resulted in more efficient oxidations than Oxone obtained from other sources.
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(17) Peptide couplings immediately adjacent to the cyanosulfur ylide peptide monomer (i.e., from **6** in Scheme 3) are somewhat sluggish. In solution phase, this is best overcome by using a small excess of the deprotected amino sulfur ylide during this coupling step. Subsequent peptide couplings proceed without unusual difficulties, save that their low solubility can lead to low isolated yields. This is not an issue with solid phase methods, and we have used this chemistry extensively for the synthesis of longer peptide α-ketoacids.

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