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Chemoselective Protection of α-Ketoacids by Direct Annulations with Oximes

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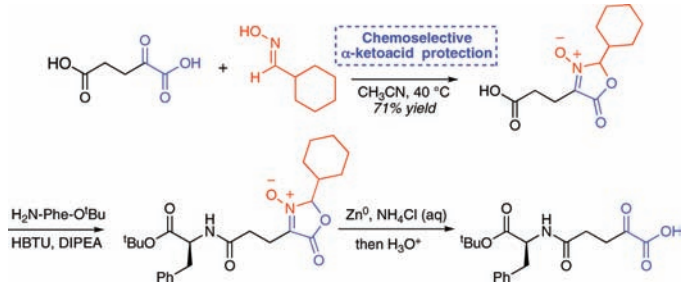
ABSTRACT



Oximes and α-ketoacids undergo an unexpectedly facile and chemoselective annulation to afford 2,5-dihydrooxazole 3-oxides. The resulting cyclic nitrones serve as chemically and configurationally stable masked α-ketoacids that can be easily elaborated and manipulated. Deprotection is achieved by mild reduction with zinc metal and hydrolysis. This methodology allows for the protection, elaboration, and deprotection of enantiopure peptide derived α-ketoacids, which are the key starting materials for the chemoselective ketoacid-hydroxylamine peptide ligation.

α-Ketoacids are uniquely reactive functional groups utilized in both biological processes and in synthetic organic chemistry.[1] While maintaining some characteristics of both ketones and carboxylic acids, they possess distinct reactivity that limits the application of the well-known transformations of the functional groups inherent to α-ketoacids. Unprotected α-ketoacids are also labile toward decarboxylation, restricting conditions for their synthesis, purification, and handling.[2]

Our recent discovery of the highly chemoselective amide-forming ligation reaction of α-ketoacids and hydroxylamines renews interest in synthetic methods for the preparation and manipulation of α-ketoacids.[3] Chemoselective amide ligations are in great demand for the preparation of peptides, proteins, and related structures but are currently limited to very specific amino acid residues.[4] Despite the potential power of the ketoacid-hydroxylamine ligation for complex molecule synthesis, limitations in the preparation and handling of the requisite α-ketoacids and hydroxylamines have slowed the adoption of this chemistry.[5,6] A critical inadequacy is the lack of suitable protecting groups for α-ketoacids. Common strategies such as protection as esters or amides promote epimerization or accentuate the electrophilic properties of the ketone.[7] In this report, we disclose an unexpectedly simple strategy for the chemoselective

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(6) We have recently disclosed a sulfur ylide-based linker for the solid phase synthesis of C-terminal α-ketoacid peptides. While this protocol works well for many complex, unprotected peptides it is not appropriate for simpler ketoacids or for peptides with cysteine or methionine residues: Ju, L.; Bode, J. W. *Org. Biomol. Chem.* **2009**, *7*, 2259.

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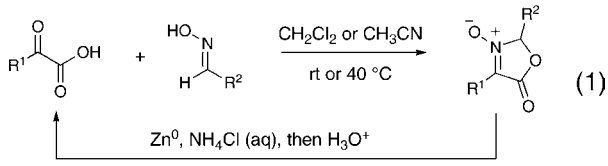
protection of α-ketoacids via direct annulations with oximes (eq 1). The resulting 2,5-dihydrooxazole 3-oxides are chemically and configurationally stable masked α-ketoacids that can be deprotected under mild reductive conditions.

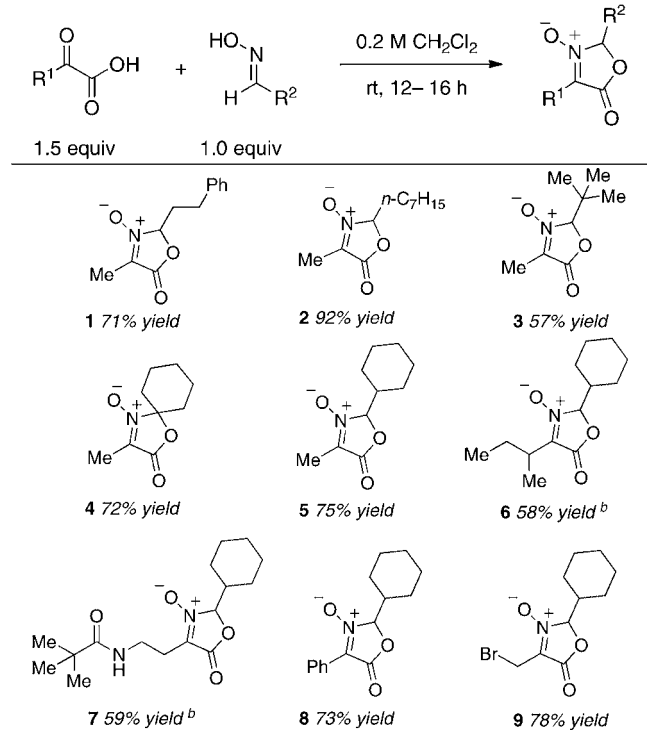


(1)

Our studies began by considering strategies to simultaneously protect both the acid and ketone functionalities of the α-ketoacid. Although certain cyclic imines have been employed as precursors to α-ketoacids, we found such compounds to be labile to most common purification methods and chemical transformations.[8] The corresponding *N*-oxides, that is, cyclic nitrones, were known to be stable compounds, but the reported methods for their preparation relied on cycloadditions of nitrosoketenes and ketones, a protocol that was limited to the preparation of glyoxylic acid derivatives.[9] Furthermore, we were primarily interested in identifying a method for the protection of an existing α-ketoacid rather than a de novo synthesis of this functionality.

In the course of our investigations of the properties and reactions of α-ketoacids, we were therefore surprised to find that α-ketoacids and aliphatic oximes undergo a spontaneous, chemoselective annulation to afford 2,5-dihydrooxazole 3-oxides in good yields under a variety of conditions.[10] The structure of the annulation product was confirmed by single crystal X-ray analysis of **8**. The reaction of oximes and α-ketocids under aqueous conditions has been previously studied as an effective method for oxime hydrolysis, but no reports of this annulation have appeared.[11,12] A screen of reaction parameters identified nonpolar solvents as preferred, with reaction temperatures ranging from room temperature to 40 °C. Oximes derived from either aliphatic aldehydes or aliphatic ketones were suitable for annulations with a variety of α-ketoacids (Scheme 1). The reaction usually failed for oximes adjacent to an sp^2 hybridized carbon, such as those derived from benzaldehyde or cinnamaldehyde (not shown). Bulky groups on the oxime did not significantly retard the reaction, while α-ketoacids containing β-branching coupled more slowly but were still viable substrates.[13]

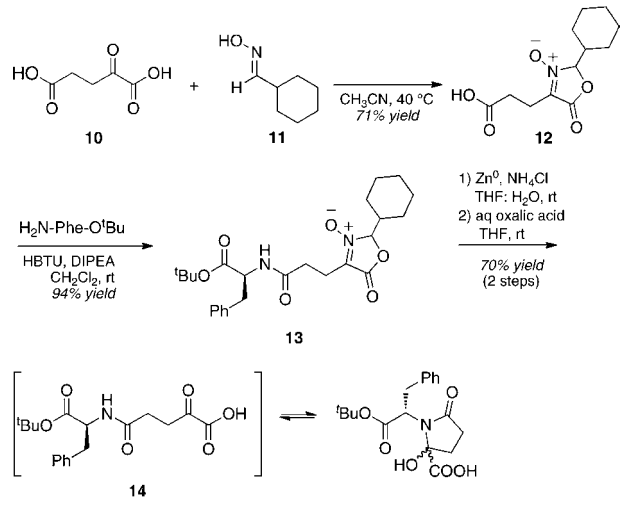
Scheme 1. Annulations of Oximes and α-Ketoacids[a]



[a] All reactions were preformed at 0.2 M CH_2Cl_2 for 12−16 h. Yields refer to mass yields of isolated, pure products [b] Reaction performed at 40 °C.

A key feature of the annulation of α-ketoacids and oximes is the chemoselectivity; selective protection of α-ketoacids in the presence of unprotected carboxylic acids is possible (Scheme 2). This enables the transient protection and

Scheme 2. Chemoselective Annulation, Elaboration, and Deprotection of α-Ketoacids



elaboration of bifunctional α-ketoacids, a finding that will extend the utility of the ketoacid-hydroxylamine amide-forming ligation.

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(12) We have confirmed that hydrolysis of the oxime occurs upon treatment with an *a*-ketoacid under aqueous conditions ($^tBuOH/H_2O$, rt). We do not detect the 2,5-dihydrooxazole 3-oxide as an intermediate.

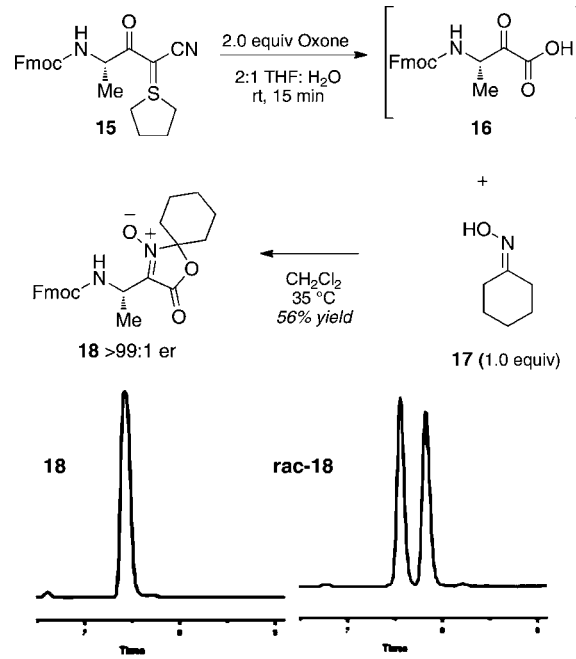
(13) The use of additives such as Lewis acids or dehydrating reagents did not significantly improve yield.

The cyclic nitrones derived from the annulation proved remarkably inert. Unlike other nitrones, they do not undergo cycloadditions even under forcing conditions or with highly reactive dipolarophiles.[14] They are to some extent hydrolytically unstable; prolonged exposure to aqueous acid or base results in release of the oxime-derived aldehyde along with formation of an α-oximino acid. They readily survive standard laboratory operations including aqueous workup, purification by column chromatography, reverse phase-HPLC, and common organic transformations including amide couplings, and deprotections of acids and amines. They resist reduction with reagents such as $NaCNBH_3$ but are readily deoxygenated at room temperature with zinc metal in aqueous NH_4Cl.[15,16] The resulting cyclic imine can be hydrolyzed to the unprotected α-ketoacid. In the case of nitrone **13**, deprotection results in an intermediate ketoacid that readily cyclizes and exhibits ring chain tautomerization.[17]

An outstanding need for the chemoselective protection and deprotection of α-ketoacids is in the application of the ketoacid-hydroxylamine ligation to the synthesis of α-oligopeptides. We have reported a stereoretentive method for the preparation of C-terminal peptide α-ketoacids by oxidation of cyanosulfur ylides.[18] This method provides a reliable route to enantiomerically pure α-ketoacids and longer peptides, but has a number of limitations including an incompatibility with sulfur containing side chains and the potential for overoxidation of the α-ketoacid if the reaction is not carefully monitored.

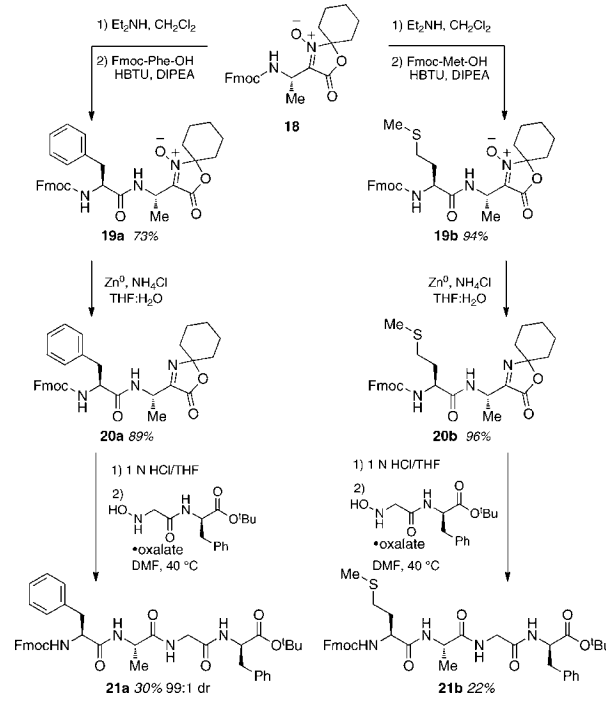
Anticipating the utility of the 2,5-dihydrooxazole 3-oxides as protecting groups for the preparation and elaboration of α-peptide ketoacids, we performed the following studies. Fmoc-Alanine α-ketoacid was prepared using the sulfur ylide method and annulated with the oxime derived from cyclohexanone. We elected to employ an oxime that would not give diastereomeric annulation products so that we could rigorously establish the stereochemical integrity of the amino acid during the protection, deprotection, and elaboration steps. Analysis of annulation product **18** by SFC on chiral columns demonstrated that the annulation proceeded without epimerization. Subsequent Fmoc deprotection and peptide coupling with either phenylalanine or methionine were achieved without difficulty giving dipeptide nitrone **19a** and **19b**, respectively. Reduction with zinc metal afforded the oxazolone in good yield; acid hydrolysis revealed the α-ketoacid poised for amide bond formation with a dipeptide *N*-alkylhydroxylamine. The ligation product **21a** was found to be 99:1 dr at the relevant stereocenter, confirming that epimerization does not occur during the protection, manipulation, deprotection, or ligation steps. In the case of methionine-containing peptide **19b**, no interference from the thioether was detected at any stage.

Scheme 3. Stereoretentive Protection of α-Peptide Ketoacids



A probable reaction pathway would involve either nucleophilic attack of the oxime nitrogen onto the α-ketoacid ketone (path a) or by the carboxylate of the α-ketoacid onto the oxime carbon (path b). Either process would be followed

Scheme 4. Stereoretentive Protection of α-Peptide Ketoacids



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(17) Despite the ring-chain tautomerization, this α-ketoacid may be used for decarboxylative condensation with an *N*-alkylhydroxylamine. See Supporting Information.

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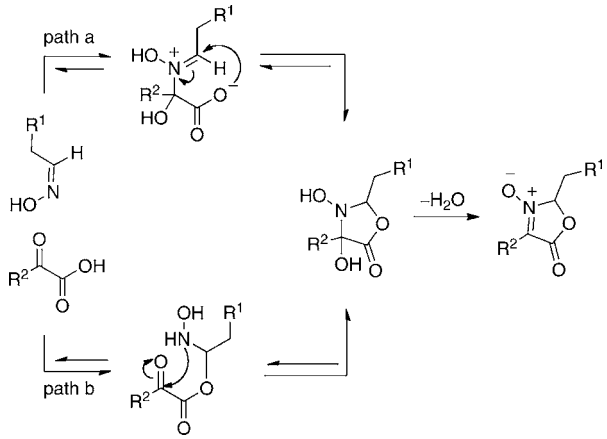
Scheme 5. Postulated Reaction Pathways



by ring closure and elimination of water to give the final 2,5-dihydrooxazole 3-oxide. (Scheme 5). Our initial efforts to distinguish these two pathways have proven inconclusive; however we currently favor path a based on several observations. Oximes derived from benzaldehyde, cinnamaldehyde, or any other aldehyde with an adjacent sp^2 hydridized carbon fail to give the annulation product, probably due to the reduced nucleophilicity of the oxime nitrogen in the conjugated system. Likewise, oximes are known to be resistant to nucleophilic attack and are considerably less basic than other amine derivatives, also making path b unlikely.[19]

In summary, we have disclosed a novel, chemoselective annulation of oximes and α-ketoacids that proceeds without reagents or catalysts and affords synthetically valuable 2,5-dihydrooxazole 3-oxides. We have exploited this finding for the development of a selective method for the protection and deprotection of α-ketoacids, which will find utility in the preparation of substrates for the ketoacid-hydroxylamine amide-forming ligation. Efforts to extend these findings to the solid-phase synthesis of peptide-ketoacids without the need for our previously reported late stage oxidation are currently underway.

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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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