Reaction Mechanisms

DOI: 10.1002/ange.201107198

The Mechanism of the α-Ketoacid–Hydroxylamine Amide-Forming Ligation**

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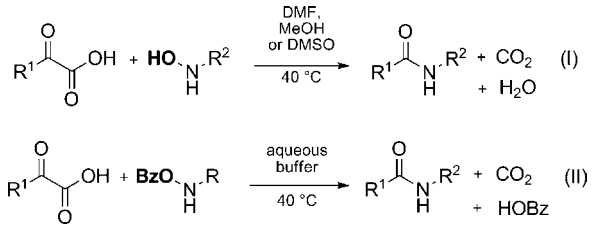
Chemoselective amide-forming ligations are among the most important and sought-after reactions in organic chemistry, as they can provide synthetic access to large peptides and proteins by the union of unprotected peptide fragments.[1] The most successful example, the native chemical ligation of peptide thioesters with peptides containing an N-terminal cysteine residue, has revolutionized the field of synthetic protein chemistry.[2] Our own efforts have identified the combination of C-terminal α-ketoacids and N-terminal hydroxylamine peptides as a remarkably selective and facile ligation reaction for the formation of amide bonds.[3] It proceeds in the presence of unprotected side chains, does not require any reagents or catalysts, and produces only CO_2 and water as by-products. Recently, we demonstrated that the α-ketoacid–hydroxylamine amide-forming ligation reaction (KAHA ligation) can be used for the chemoselective synthesis of therapeutic peptides (30 residues) without interference from unprotected side chain functional groups.[4]

We have identified two prototypical variants of this amide formation: type I, the reaction of α-ketoacids with *N*-alkyl hydroxylamines, in which the hydroxy group is unmodified; and type II, the reaction of α-ketoacids with O-substituted hydroxylamines, such as *O*-benzoyl (Bz) hydroxylamines[5] and isoxazolidines (Scheme 1).[6] At least two other examples of type II ligations have been documented: Phanstiel and co-workers have reported that *O*-Bz hydroxylamines ligate with acyl phosphonates to give amides,[7] and Fang et al. have reported the ligation of *N*-iodo amines and α-ketoacids.[8]



Scheme 1. Prototypical KAHA ligations with O-unsubstituted (type I) and O-substituted (type II) hydroxylamines. Bz = benzyl.

Although both type I and type II KAHA ligations give identical amide products, their reaction conditions are quite different: O-substituted hydroxylamines (type II) react faster under aqueous conditions, whereas O-unsubstituted species (type I) perform better in solvents such as DMF, DMSO, or MeOH; water is tolerated, but detrimental to the reaction rate. On the other hand, O-unsubstituted hydroxylamines undergo ligation at lower concentration (10 mM), whereas many of the substituted variants require higher concentrations (50–100 mM). To explain these discrepancies and to identify ligation partners ideally suited for the synthesis of larger peptides and proteins, we have elucidated the mechanism of the type I ligation. Herein we present the results of these studies, showing that the KAHA ligation of O-unsubstituted hydroxylamines follows a remarkably complex and unexpected reaction pathway.

At the outset of our studies, we considered six possible mechanistic pathways for amide formation from an α-ketoacid and an O-unsubstituted hydroxylamine (Scheme 2). Path **A** involves nitrone **II**, an intermediate we have occasionally detected, followed by decarboxylation to give a nitrilium ion (akin to a Ritter amidation).[9] This mechanism has also been postulated by Sucheck et al. in related investigations.[10] Path **B**, which we proposed in our original report,[3] would proceed via oxidative decarboxylation of hemiaminal **I**, in analogy to the known reaction of α-ketoacids with hydrogen peroxide.[11] Paths **C** and **C′** would proceed via oxazetidinone **III**, an intermediate recently detected by Yamamoto and Payette in an oxidative decarboxylation of α-hydroximino esters.[12] Paths **D** and **E** would proceed via an oxaziridine **IV**, although the details of both the decarboxylation and the formation of the oxaziridine were unclear.

We recognized that many of these pathways could be excluded by isotopic labeling studies. We therefore prepared the necessary ^{18}O-labeled substrates to perform the experiments shown in Table 1. Much to our surprise, the oxygen atom of the amide product originated from the hydroxylamine (entry 3) rather than from the ketone of the α-ketoacids (entry 2) or $H_2{}^{18}O$ (entry 1). An experiment with phenyl ester **1** further disfavored the oxazetidinone pathway (entry 4).[13] Taken together, these results excluded all of the pathways except Path **E** for type I ligations and implicated oxaziridine **IV** as a key intermediate in amide formation. In contrast, an ^{18}O label in the ketoacid was retained when *O*-Bz hydroxylamines were used (entry 5), implicating pathway **B** or **D** for type II ligations.

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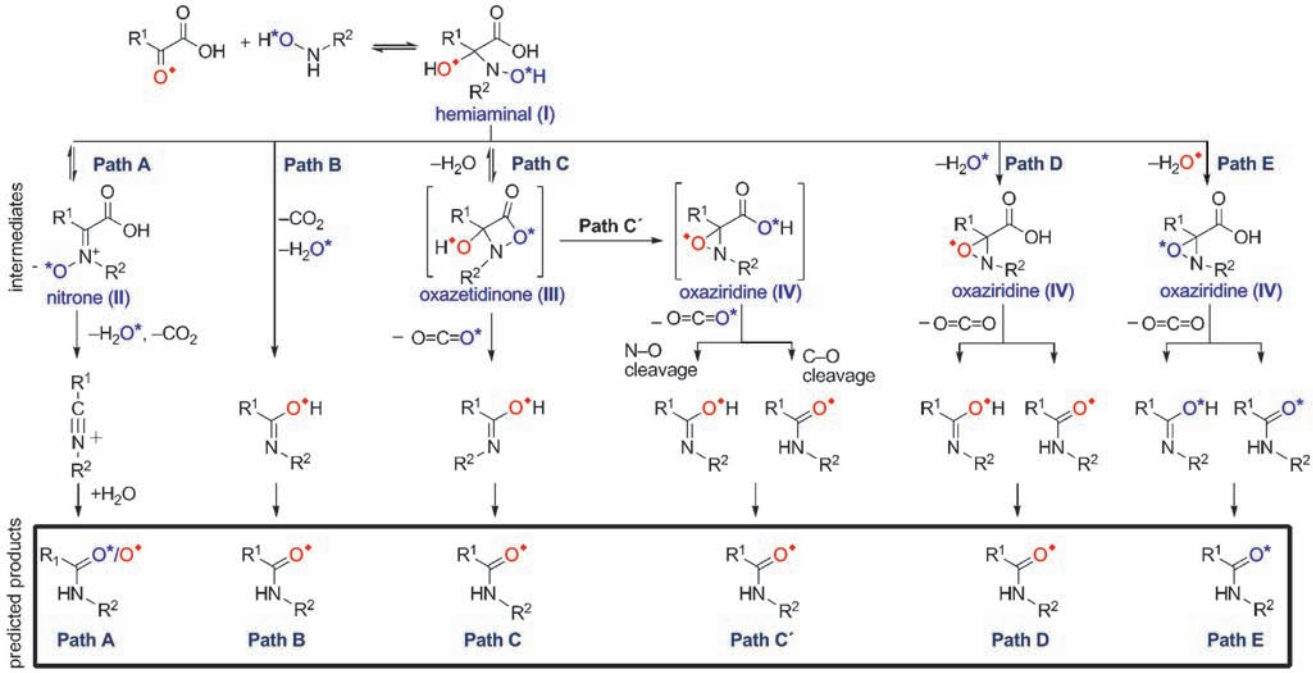
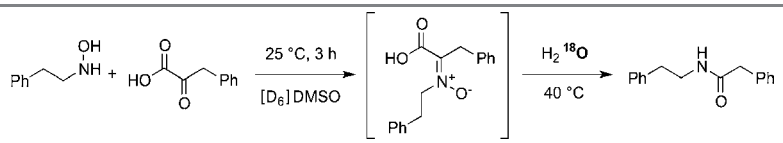
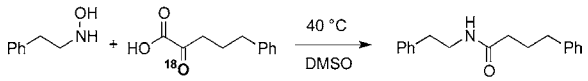
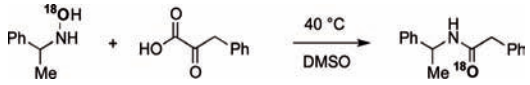
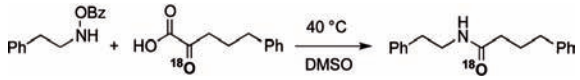
[**] This work was supported by the Swiss National Science Foundation (200021_131957).

Supporting information for this article is available on the WWW under http://dx.doi.org/10.1002/anie.201107198.

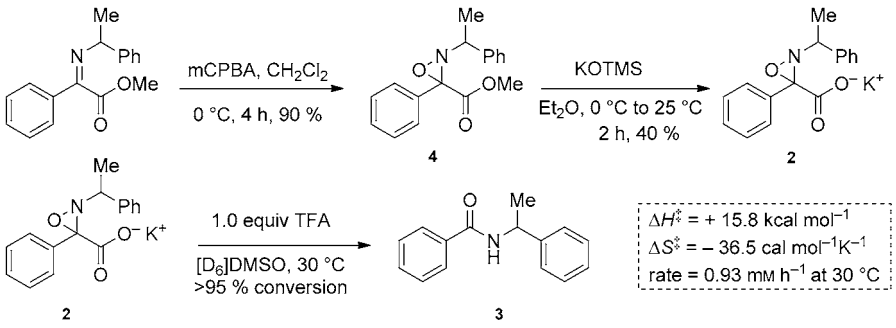


Scheme 2. Possible mechanistic pathways for type I KAHA ligations.

Table 1: Exclusion of pathways and mechanistic probes.

Entry		Label transfer	Path excluded
1		0%	A
2		0%	B, C, C′, D
3		>80%	A, B, C, C′, D
4		–	C
5		>80%	A, C, C′, E

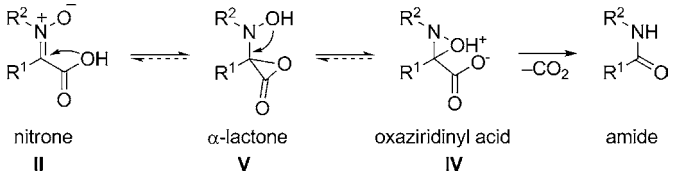
A survey of the literature revealed that at least one α-oxaziridinyl acid has been described,[14] and we sought to prepare and isolate such an intermediate to probe its ability to undergo an amide-forming oxidative decarboxylation. Potassium carboxylate **2** was prepared as shown in Scheme 3 and proved to be a stable, easily handled solid. Upon treatment of this salt with 1.0 equivalent of trifluoacetic acid (TFA), clean decarboxylation occurred to give amide **3**.



Scheme 3. Synthesis and rearrangement of α-oxaziridinyl acid **2**. mCPBA = *m*-chloroperoxybenzoic acid, TMS = trimethylsilyl, TFA = trifluoroacetic acid.

Eyring analysis (see Supporting Information) revealed a highly ordered transition state with an activation entropy ($\Delta S^{\ddagger}$) of -36.5 cal mol^{-1} K^{-1}).

The finding that α-oxaziridinyl acids are the probable intermediates in the ligation leaves the question of how they are formed under the reaction conditions. Direct cyclization of hemiaminal **I**, as shown in Path **E**, seems unfeasible. Furthermore, we have noted that amide formation also proceeds cleanly from nitrone **II** and that the rate of amide formation from the nitrone does not change, even in the presence of an additional 20 equivalents of H_2O. Also, both *E*- and *Z*-nitrones undergo the rearrangement, albeit at slightly different rates. These results imply that oxaziridine **IV** arises through the formation and rearrangement of nitrone **II**. Although such rearrangements are known under photochemical conditions,[15] this ligation proceeds cleanly in the dark. We therefore postulated a mechanism for the nitrone–oxaziridine rearrangement unique to the α-nitrone acids: attack of the carboxylate to give an α-lactone[16] followed by opening of this intermediate by the hydroxylamine to give oxaziridine **IV**. Decarboxylation of this intermediate gives the observed amide product (Scheme 4).

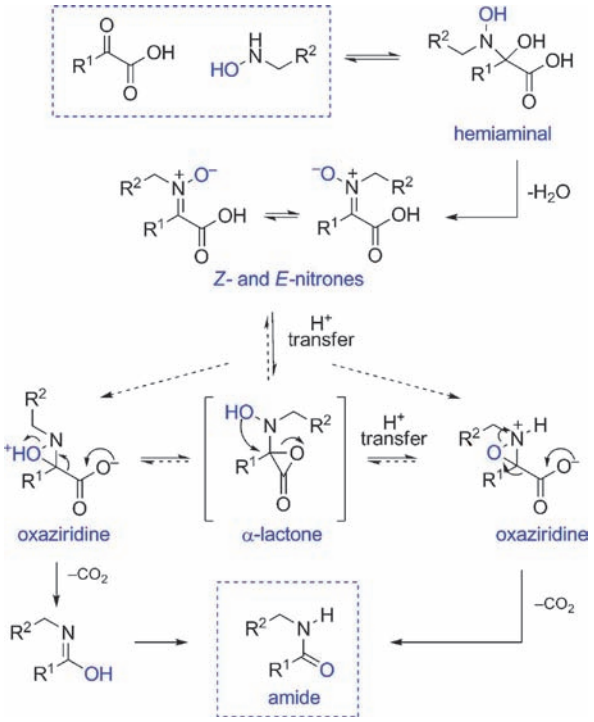


Scheme 4. Proposed pathway from nitrone to oxaziridine via an α-lactone intermediate.

Although not common intermediates, α-lactones have been implicated in related α-carboxyl displacements, including the facile hydrolysis of α,α-dichloro acids.[17] Extensive mechanistic studies carried out by Williams and co-workers have shown that α-lactones are intermediates in the addition of aqueous bromide to disodium dimethylmaleate and dimethylfumarate.[18] Importantly, both the formation of α-lactone **V** (3-*exo*-trig) and its opening to give oxazidirine **IV** (3-*exo*-tet) are favored by Baldwin's rules; the direct formation from the nitrone (3-*endo*-tet) is not.[19]

These investigations support the overall mechanism for amide formation from α-ketoacids and unsubstituted hydroxylamines shown in Scheme 5. This mechanism requires the formation of a nitrone—a process that we had previously considered to be a non-productive pathway and sought to avoid—and is likely the reason why water is often detrimental to this particular ligation. The precise mechanism of the ligation with O-substituted hydroxylamines remains open. Either direct oxidative decarboxylation (Path **B**) or formation of the oxaziridine by nucleophilic displacement at nitrogen to give the same oxaziridine intermediate **IV** (Path **D**) are possibilities. Further studies to determine the exact mechanistic pathway are underway.

The chemoselectivity of the KAHA ligation is remarkable in light of the complexity of the reaction mechanism. Its success lies in the selective formation of the key intermediates; nitrones and oxaziridines can arise only from α-ketoacids and hydroxylamines. However, this mechanism also points to a fundamental limitation of the type I ligation. It is unlikely that it will be fully compatible with the aqueous conditions usually required for protein synthesis and semisynthesis. These studies therefore suggest that type II ligations with O-substituted hydroxylamines, which may follow a different mechanism and do not proceed via a nitrone intermediate, are likely to be better suited for this purpose. We have adjusted our investigations accordingly and will report a new class of ligation partners that better tolerate aqueous conditions and operate at low reaction concentrations in due course.



Scheme 5. Overall mechanism for type I KAHA ligation.

Received: October 11, 2011
Published online: November 29, 2011

Keywords: amides · isotopic labeling · ligation reactions · peptides · reaction mechanisms

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