Ligation Reactions

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Chemoselective Amide Ligations by Decarboxylative Condensations of *N*-Alkylhydroxylamines and α-Ketoacids**

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Chemoselective ligation reactions make possible covalent bond formation between two fragments containing unprotected functional groups. An ideal ligation process proceeds

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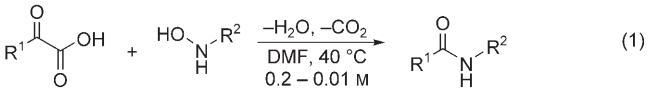
under mild, often aqueous, conditions at low molar concentrations, does not require reagents or catalysts, and produces no chemical by-products.[1] The few known reactions that approach these criteria, including oxime formation,[2] thioester-α-bromocarbonyl alkylations,[3] and the copper-promoted alkyne–azide cycloaddition[4] have found diverse and significant applications in drug discovery,[5] functionalized polymer synthesis,[6] and the fabrication of novel nanostructures.[7] Their utility in biomolecule synthesis, however, is limited by the fact that they produce unnatural, and often relatively labile, covalent bonds. The development of chemical ligation reactions that create amide bonds have been a long standing goal.[8] The identification of the native chemical ligation of C-terminal peptide thioesters and N-terminal cysteines has revolutionized the field of synthetic protein chemistry by making possible the coupling of large, unprotected-peptide fragments.[9,10] Despite the utility of this process, the requirement of ligation at relatively rare cysteine residues has encouraged investigations into alternative amide bond-forming reactions.[11,12]

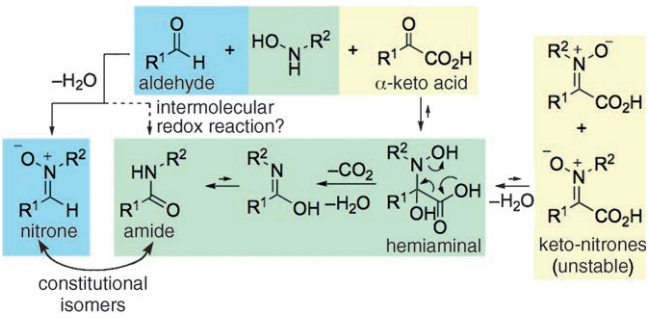
A significant obstacle to the development of new amide ligations is the paucity of reaction types for amide synthesis; nearly all known intermolecular-amide syntheses proceed by addition–elimination reactions of activated carboxylates. Herein we document our discovery of a novel approach to amide synthesis by the decarboxylative condensation of *N*-alkylhydroxylamines and α-keto carboxylic acids [Eq. (1); DMF = *N*,*N*-dimethylformamide]. This process proceeds in polar protic and aprotic solvents, requires no reagents or catalysts, produces only water and carbon dioxide as by-products, and readily tolerates unprotected functional groups. We provide preliminary evaluations of its application to the chemoselective ligation of unprotected-peptide fragments. Investigations into the mechanism and substrate scope of the reaction reveal a rich chemistry that will lead to new solutions for the synthesis of complex molecules and functionalized materials.



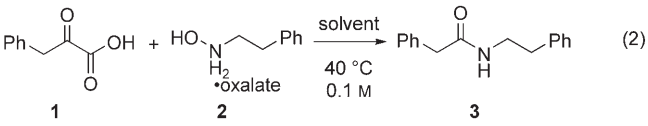
(1)

The discovery of this reaction stemmed from our efforts to develop new approaches to amide and ester synthesis by intramolecular redox reactions of aldehydes.[13] We reasoned that intermolecular redox reactions, for example, between an aldehyde and a hydroxylamine, could also lead to amide formation under appropriate conditions (Scheme 1). These studies, however, were complicated by the propensity of aldehydes and hydroxylamines to rapidly form nitrones. In contrast, ketones rarely condense with *N*-alkylhydroxylamines under mild conditions. Instead, metastable hemiaminals are formed,[14] and we hypothesized that *N*-alkylhydroxylamines would react with α-ketoacids to produce a hemiaminal poised for oxidative decarboxylation to give amide products.[15]

Our hypothesis was tested by mixing two readily available substrates, phenylpyruvic acid (**1**) and *N*-phenethylhydroxylamine (**2**), under a variety of reaction conditions [Eq. (2), Table 1]. Although no amide products were formed in nonpolar solvents, we were pleased to find that simply warming a solution of these two reactants in DMF produced the desired amide product in >70% yield (Table 1, entry 1). A concern in these initial studies was the preparation and handling of the hydroxylamine in its unstable free-base form. Conveniently, we found that salts of the hydroxylamine are equally, and possibly more, efficient in the amidation reaction and as such, we selected the stable, highly crystalline hydroxylamine oxalate salts for further studies (Table 1, entry 2). Likewise, either the protonated ketoacids or their carboxylate salts were suitable reactants (Table 1, entry 3). Amide bond formation occurred in dimethyl sulfoxide (DMSO; Table 1, entry 4), MeOH (Table 1, entry 5), aqueous DMF (Table 1, entry 6), or as suspensions of the reactants in aqueous buffers (Table 1, entries 7–8). Reactions were typically performed at 0.1M by using ketoacid (1.0 equiv) and hydroxylamine oxalate (1.2 equiv) at 40°C, but lower concentrations (0.01M, 0.005M) and other stoichiometric ratios were also viable.



Scheme 1. Reactions of *N*-alkylhydroxylamines with aldehydes and ketones.



(2)

Table 1: Reaction conditions for amide formation from hydroxylamine **1** and α-ketoacid **2**.

Entry	Conditions[a]	*t* [h]	Yield[b] [%]
1	DMF, hydroxylamine free base	15	70
2	DMF	15	79 (88)[c]
3	DMF, ketoacid sodium salt	15	75
4	MeOH	24	72
5	DMSO	15	80
6	DMF/H_2O (5:1)	15	72 (77)[c]
7	acetate buffer (pH 4)	24	(70)[c]
8	6N NH_4Cl, 60°C	15	68 (70)[c]

[a] All reaction performed on a 0.2 mmol scale; [b] Yields following chromatography; [c] HPLC yields of unpurified reaction mixtures given in parentheses.

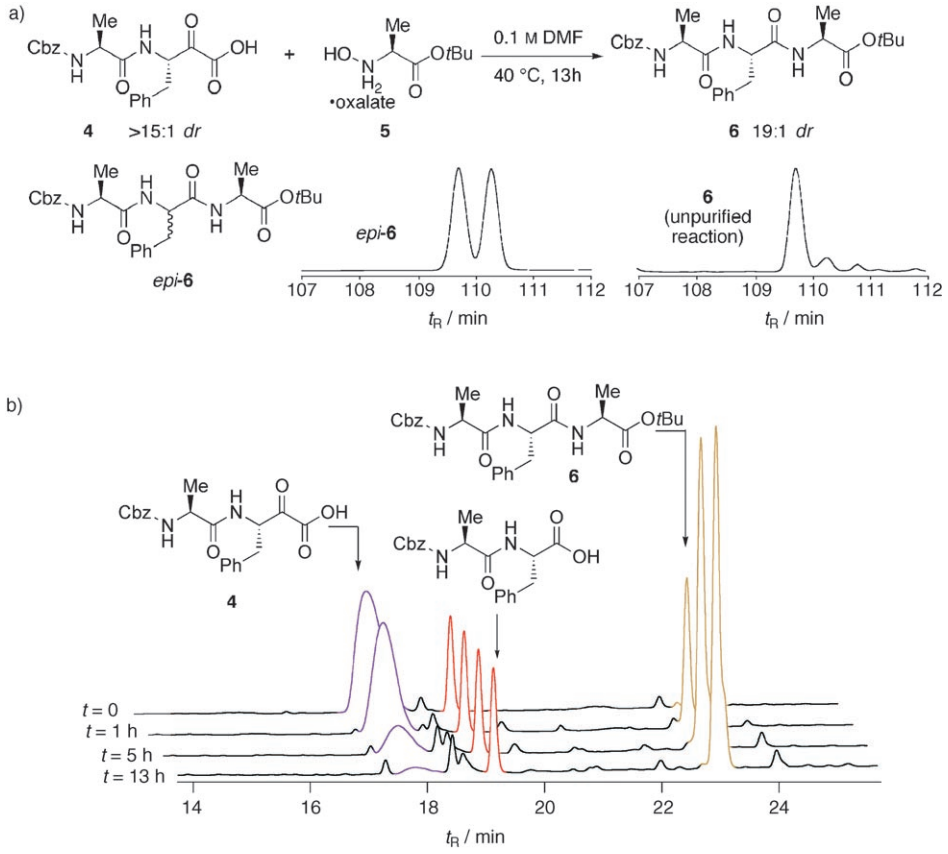
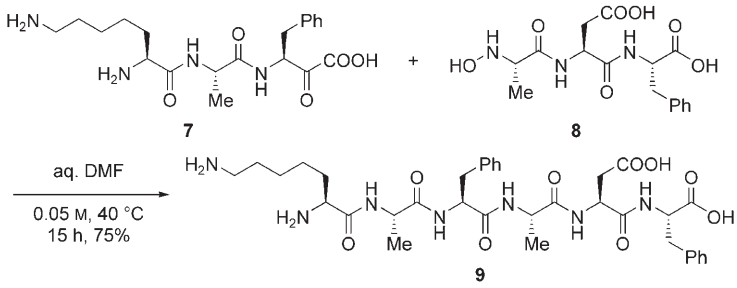


Figure 1. Ketoacid–hydroxylamine ligations of peptide substrates **4** and **5**. a) Demonstration of preservation of stereochemistry during the reaction; b) Monitoring of the reaction by HPLC (samples taken directly from the reaction mixture without purification or workup). A small amount of the carboxylic acid is formed during the synthesis of ketoacid **4**. Cbz = carbobenzyloxycarbonyl.

Ketoacid–hydroxylamine amide ligations occur for a wide range of simple and complex substrates. We have focused our initial investigations on the exploration of the suitability of this process for peptide ligations. Peptide ketoacid **4** was prepared as a >15:1 mixture of diastereomers (with **4** being the major diastereomer) by a variant of Wasserman's ylide method[16,17] and reacted with (*S*)-alanine hydroxylamine oxalate salt **5** (1.2 equiv).[18] The ligation reaction occurred cleanly over the course of 12 h (Figure 1) to give peptide **6** as a 19:1 mixture of diastereomers (peptide **6** is the major form), which demonstrates that epimerization of the ketoacid does not occur during the ligation reaction. Encouraged by these results, we prepared a range of protected- and unprotected-peptide substrates and examined their ligations (Table 2). To further validate the tolerance of this reaction to fully unprotected peptides, ketoacid **7** and hydroxylamine **8** were prepared and shown to undergo efficient ligation in aqueous DMF [Eq. (3)]. Notably, a variety of ligation sites including Phe-Ala, Ala-Phe, Pro-Ala, Val-Gly, and Ala-Ala were feasible.

Several possible reaction pathways can give rise to amide formation, and we have initiated efforts to elucidate the reaction mechanism. Interestingly, although peptide substrates such as **4** and **5** do not appear to condense to form nitrones under the reaction conditions (Figure 1b), less-hindered substrates, including our model reaction [Eq. (2)], rapidly form the isomeric nitrones *cis*-**11** and *trans*-**11** (Figure 2). These nitrone intermediates were detected by reverse-phase HPLC (Figure 2b), liquid chromatography–MS, and in situ ^{1}H and ^{13}C NMR spectroscopy studies of the reaction mixtures. They could be isolated as their methyl esters by being trapped with diazomethane. Although it is clear that these nitrones can form under the reaction



(3)

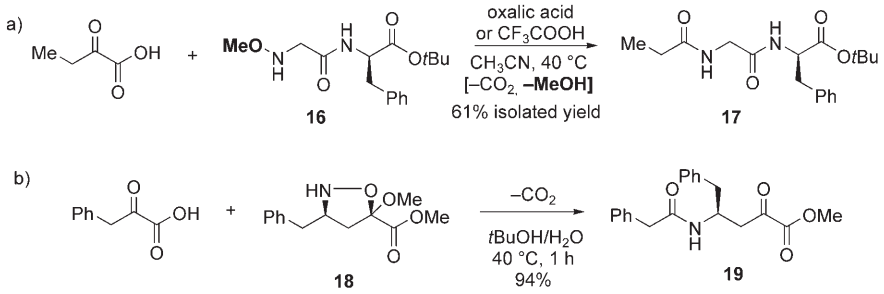
Table 2: Ketoacid–hydroxylamine peptide ligations of selected protected- and unprotected-peptide substrates.

Entry	Ketoacid	Hydroxylamine	Product[a]	Yield[b] [%]
1	FmocAlaPro	AlaO*t*Bu	Fmoc-AlaProAla-O*t*Bu	72
2	FmocAlaVal	GlyOEt	Fmoc-AlaValGly-OEt	58
3	FmocLys(Boc)-Glu(*t*Bu)PheAla	AlaO*t*Bu	Fmoc-Lys(Boc)Glu(*t*Bu)Phe-AlaAla-O*t*Bu[c]	80
4	H_2N-LysAlaPhe	AlaAsp(*t*Bu)PheO*t*Bu	H_2N-LysAlaPhe-AlaAsp(*t*Bu)Phe-O*t*Bu	74
5	FmocAspAlaPhe	AlaAsp(*t*Bu)PheO*t*Bu	Fmoc-AspAlaPhe-AlaAsp(*t*Bu)PheO*t*Bu	74

[a] All reaction performed at 0.02–0.1 M in DMF or DMSO containing ca. 5% H_2O at 40°C for 10–24 h using 1 equiv ketoacid and 1.2–2 equiv hydroxylamine oxalates; [b] Yields of pure products following preparative TLC or RP-HPLC. The reported yields include the preparation of the ketoacids by oxidation of the appropriate cyanoylide followed by coupling with the hydroxylamine; [c] 0.01 M, 48 h.

conditions, we do not currently believe that they are involved in product formation; all attempts to trap the postulated nitrilium ion **12** by the addition of nucleophiles (MeOH as the reaction solvent, thiophenol, cysteine, glycine) afforded only the usual amide product. Thus, although the rapid formation of nitrone **11** may contribute to the efficiency of the reaction, conversion to the product is likely to proceed through decarboxylation of the tetrahedral intermediate **10**. The question of whether the decarboxylation proceeds through a stepwise or concerted pathway remains to be addressed, although the isolation of small amounts of nitrone **15** suggests that a stepwise reaction is at least a possibility. We note, however, that decarboxylated nitrones are never isolated when peptide-derived ketoacids and hydroxylamines are employed. Although the details remain to be elucidated, the available evidence points to a distinct mechanistic manifold that may have further implications including a role in the prebiotic origin of higher peptides.

This chemoselective amide formation is not limited to *O*-unsubstituted hydroxylamines. For example, *N*-methoxy peptide **16** was coupled with 2-ketobutyric acid in acetonitrile in 61% yield (Scheme 2a).[19] Unexpectedly, and in contrast to the *O*-unsubstituted hydroxylamines, these substrates required added acid and showed increased efficiency in acetronitrile, but were slower in the presence of DMF, DMSO, or water. Cyclic hydroxylamines are exceptional substrates (Scheme 2b); isoxazolidine **18** reacted cleanly with α-ketoacids in either nonpolar (CH_2Cl_2, toluene) or aqueous (0.2 M 1:1 *t*BuOH/H_2O) conditions. The stability of the resultant ketoester product under the reaction conditions is noteworthy.[20]



Scheme 2. Amide-forming ketoacid ligations with *O*-alkyl hydroxylamines.

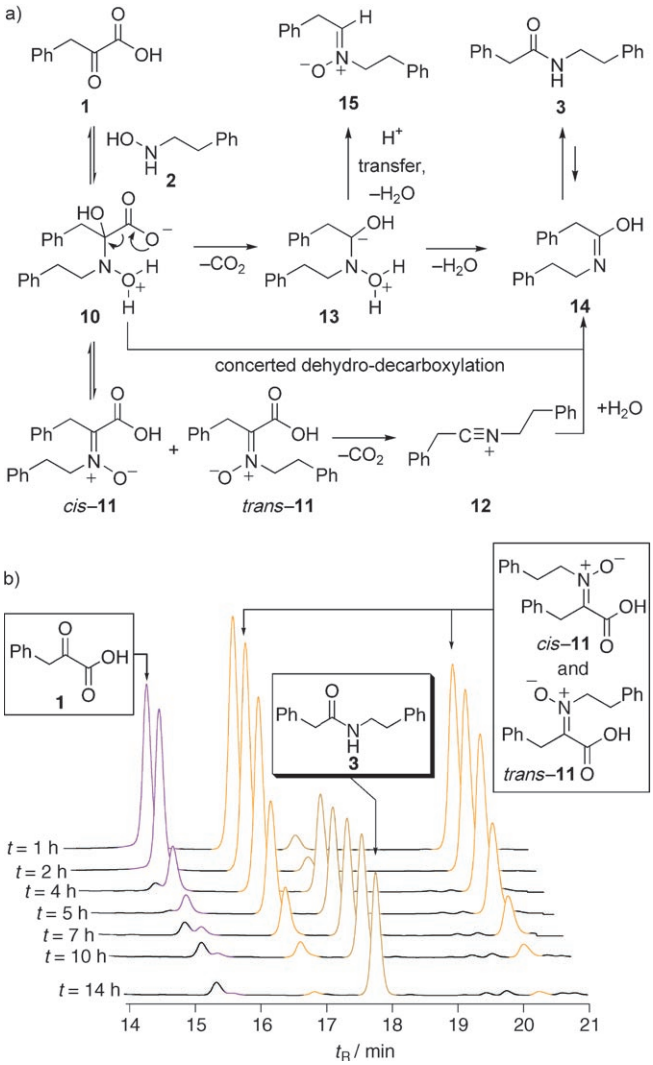


Figure 2. a) Possible reaction pathways for amide formation; b) detection of nitrone intermediates by HPLC.

The coupling of α-ketoacids and hydroxylamines is a powerful, chemoselective amide bond formation that proceeds in the presence of reactive functional groups, requires no reagents or catalysts, and produces only water and CO_2 as by-products. This reaction should be useful for diverse applications that require the coupling of unprotected molecules.

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