Site-selective Amide Functionalization by Catalytic Azoline Engrafting

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Abstract. Amide activation is a challenging transformation due to the stabilizing effect of the amide group. While enzymes can be considered as prototypical systems that have evolved to achieve high selectivity and specificity, small-molecule catalysts that functionalize the amide group may accommodate a much larger selection of substrates but currently remain scarce. Here, by combining the desired features from both catalytic regimes we designed an artificial cyclodehydratase, a catalytic system for site-selective modification of peptides and natural products by engrafting heterocyclic into their scaffolds. The catalytic system features molybdenum(VI) center that was decorated with a sterically congested tripod ligand. The optimized catalyst can introduce azolines into small molecules, natural products, and oligopeptides with high efficiency and minimal waste. We further demonstrate the utility of the new protocol in direct functionalization of a single amide group in the presence of up to seven other chemically similar positions, and direct conversion into amines and thioamides. This new mechanistic paradigm may address an unmet need for a general method for selective and sustainable functionalization of peptides and natural products.

Amides are an abundant functional group common in proteins, peptides, synthetic polymers, myriad natural products and pharmaceuticals.1 While methods to form this functionality are well-studied, chemical activation of amides represents a challenge due to harsh conditions required to break this resonance-stabilized bond.2, 3 Furthermore, differentiation between virtually identical positions in oligopeptides and proteins through synthetic means remains unprecedented, and recent advances in amide activation focus on compounds with one amide group and often utilize special substrates with reduced stability.4 However, direct conversions of amides into amines,5-8 thioamides,9 N-alkyl amides,10, 11 and various nitrogen-containing heterocycles in complex substrates12 are sought-after transformations due to their synthetic appeal and relevance of the products of these transformations in improving anti-bacterial activity,13 cell permeability,10 structural analysis,14-16 and as privileged ligands in asymmetric catalysis (Figure 1A).17 In addressing this challenge we were drawn to the logic of late-stage modification that allows complex organic molecules and biologics to undergo site- and chemoselective functionalization.18-20 Current site-selective strategies for peptide and protein modifications focus on reactions of functional groups located in the side-chains of amino acid but little effort has been devoted to direct transformations of the amide group itself.21, 22 In this respect, cyclodehydratases23 – a class of ATP-dependent bacterial enzymes installing azoline groups in ribosomally-synthesized peptides such as thiopeptides through serine, threonine, and cysteine cyclodehydration – can serve as a prototypical catalytic system that can directly modify either amide’s carbonyl group or the nitrogen atom (Figure 1B).24 Cyclodehydratases achieve their selectivity by binding to a leader peptide within the proximity of the substrate,25 and a similar logic could be applied to artificial systems where the (sulf)hydrol groups located in the side-chain of amino acid determine the site of amide modification through chelation to a catalyst. This approach would effectively mimic the biosynthetic logic of assembling post-translationally modified peptides and complement current synthetic strategies that feature linear coupling of pre-assembled building blocks (Figure 1B). Here, we describe the development of a site-selective functionalization method of amides promoted by bis-oxo Mo(VI) catalysts under exceptionally mild conditions producing water as the only by-product. This cyclodehydrative oxygen atom-transfer reaction is suitable for the preparation of five-membered heterocycles with high atom economy and minimal waste. In a broader context, our results demonstrate that the mimicry of enzymatic processes with rationally designed small-molecule catalysts which operate through mechanisms independent of the biosynthetic pathway may offer a promising strategy to streamline structure-activity relationship studies and discovery of small molecules with translational potential.

In our approach to selectively activate amides, oxazolines play a central role either as intermediates or as ultimate products due to electrophilic reactivity at C2/C5 (Figure 1C).26 Most established methods to introduce azolines use stoichiometric amounts of highly electrophilic reagents that are incompatible with
In the absence of catalyst, but a 93:7 mixture of regioselectivity in competing cyclodehydration towards modification of these solvents were not pursued further. In some cases, more polar co-solvents were found to be beneficial for substrate solubility, extend the reaction lifetime at elevated temperatures (60-140 °C), and introduce overall flexibility. All complexes 2a-2h are crystalline solids stable at ambient conditions and can be recovered (>85%) by chromatographic purification after completed cyclodehydration.

Figure 2A depicts examples of cyclizations with small molecules, natural products, and commercial drugs under the optimized conditions with catalyst 2h and azeotropic removal of water. Simple 5- and 6-membered heterocycles 5-9 were formed in excellent yields (68%-98%, Figure 2C). Similarly, substituted serine (10-12, 14), threonine (4, 13, 15) and cysteine (19) substrates were readily converted into their corresponding oxazoline and thiazoline heterocycles. Even azolines prone to facile epimerization such as conjugate (16) and di-phenyl (17) oxazolines could be formed with no loss of stereochemical integrity under the optimized conditions. The transformation of D-glucosamine hemiacetal represents a direct synthesis of a valuable glycosyl donor 18 that can be incorporated into chemoenzymatic synthesis of N-linked glycans. In some cases, more polar co-solvents such as 1,4-dioxane or PhCl were added to ensure full solubility of the substrates, but the inhibitory effects of potentially coordinating solvents such as N,N-dimethylformamide (DMF), dimethylsulfoxide (DMSO), N-methylpyrrolidone (NMP) on the reaction rates were significant, and these solvents were not pursued further. Taken together, the direct method offers an entry into a valuable class of heterocycles that can be converted into aromatic thia- or oxazoles, a structural motif that is relevant towards modification of bioactive natural products and commercial drugs.

Several complex substrates for site-selective cyclodehydration are noteworthy of a detailed analysis (Figure 2D). Commercial amides such as bactericidal antibiotic florfenicol (20) and a gap-junction modulator tonabersat (21) represent a subset of substrates with one available site for amide activation. In the case of a small-molecule inhibitor of MEK1/2, pirmaserftib, only the conjugated oxazoline 22 was formed. On the other hand, the reaction with chloramphenicol (21) represents a unique example demonstrating the control of regioselectivity in competing cyclodehydrations between alcohols in different steric arrangements resulting in a 2.3:1 ratio of 23 with the preference toward a more substituted oxazoline 23b derived from the secondary alcohol. We also note that compound 23b did not equilibrate under the reaction conditions in the absence of catalyst, but a 93:7 mixture of 23a:23b was formed upon heating 23a under the reaction.
conditions for 48 h. This suggests that isomerization is catalyzed by 2h and supports the notion that the cyclodehydration proceeds under the kinetic control. Other substrates with basic sites that may render the cyclization prohibitively slow such as aminonucleoside antibiotic puromycin45 (24) or HIV protease inhibitors indinavir43 (25) and lopinavir44 (26) underwent cyclodehydrations in consistently high yields. The reaction with paclitaxel45 (27), a potent anti-cancer agent, represents another extreme example of high regioselectivity and functional group compatibility in a substrate containing a number of potentially reactive functionalities prone to elimination such as esters, alcohols, and a strained oxetane ring that might react with Lewis acids. Indeed, attempted cyclodehydration of paclitaxel with a highly electrophilic phosphonium salt (the Hendrickson reagent)46 or Deoxo-Fluor (bis(2-methoxyethyl)aminosulfur trifluoride)47 afforded <5% of 27 (based on LC analysis) along with several unidentified products showing loss of water. Cyclization of a narrow-spectrum antibiotic lincomycin demonstrates also that the resultant oxazoline can undergo intramolecular opening with a neighboring alcohol to form an ether as shown in 28.

In addition to small organic molecules, we were interested in testing the efficiency of cyclization with peptide substrates (Figure 3). In the attempt to engraft a small azoline ring onto the macrocycle through direct cyclodehydration, the overall conformation of the macrocycle and arrangement of substituents around the amide of interest need to be considered. Furthermore, some amide bonds may be engaged in intramolecular H-bonding contributing to an even more challenging transformation.48 With these considerations in mind, we selected curacycline B (29),49 a cyclic peptide with immunomodulatory activities, as a model system. The individual serine group in 29 could be directly converted into β-chloroalanine 30, β-azidoalanine 31 and mutated into S-phenylcysteine 32 through a common azoline intermediate formed by Mo-catalyzed cyclodehydration and opened with HCl, TMSN₃, and PhSH, respectively. We note that in all transformations presented in Figure 3A no epimerization at any potentially susceptible positions was observed.

The following three transformations also showcase the power of site-selective direct amide functionalization: (A) A one-pot mutation of carbonyl group into thioamide 33 was achieved by exposing oxazoline to H₂S and produced 33 in 85% yield; (B) Regioselective reduction of 29 into amine 34 was accomplished with a borohydride; and (C) the azoline intermediate was oxidized directly into oxazole 35 albeit in a low yield, which represents a direct conversion of serine into a fully aromatic ring. We next applied the same protocol to site-selectively convert two cyclic peptides – dianthin G (36)50, 51 and pseudostellarin G (37)52 into thioamides in the presence of seven or five other amide groups that remained intact during the entire process (Figure 3B). This protocol adds to the palette of methods for carbonyl thionation but also offers high regioselectivity not available with other thiphosphorus reagents.53 The molybdenum catalyst 2h enabled the synthesis of a series of azoline-containing cyclic peptides through direct cyclodehydration of the parent macropeptide with concomitant removal of two (38) or even three (39, 40) molecules of water. The cyclization reactions are not restricted to oxazolines, and two thiazoline groups could be readily installed, as shown in the synthesis of 40.

High selectivities and exceptional substrate scope prompted us to undertake mechanistic investigations summarized in Figure 4. In the proposed mechanism, the spectator pyridine ligand in 41 opens a site for binding of amide 42 to molybdenum complex 41 (Figure 4A). The catalyst likely “samples” various amide positions and establishes bidentate configuration 43 with N⁻H bonding that can assist in rotation of the amide group into the required conformation for 5-exo-trig cyclization resulting in oxazoline/thiazolidine 45. The pyridine ring in this step plays an important role of a proton carrier from the N⁻H to the carbonyl's oxygen atom and reorganization into another H-bonded intermediate 46, which then releases oxazoline 48 and a molecule of water. This proposal is consistent with reactions of 18O O=C-labelled amides (95% 18O incorporation) catalyzed by 2h, which furnished the expected azolines together with recovered molybdenum catalyst 2h with <5% incorporation of 18O as confirmed by high-resolution mass-spectrometry analysis (for details, see SI). This data shows that the oxo ligands may play a role maintaining high Lewis-acidic character of Mo but remain intact throughout the entire catalytic cycle. Along similar lines, Hammett analysis of cyclodehydration of substituted benzamides 49 with first-order kinetics revealed accumulation of negative charge at the carbonyl group (Figure 4B). This result is supportive of with the hypothesis that breakage of the carbonyl group through addition of a nucleophile is the rate-determining step (conversion of 43 into 45).
The mechanism of Mo-catalyzed cyclodehydration was also probed computationally. We first evaluated four representative structures that correspond to complexes 2 based on the available crystallographic data (Figure 4C). Bis-oxo complexes 2 exist in two distinct and stable conformers schematically depicted as Z(N) (where two phenolic arms of the triad ligand face the pyridine group) and Z(O) (where both the phenoxide groups are located closer the oxo group). Regardless of the substitution of the phenolic group, the Z(N) structures are more stable (ΔG 9.3-11.7 kcal·mol⁻¹), but their relative stabilities vary depending on the size of the R¹ group located on the spectator pyridine ligand. The calculated barriers for interconversion of these structures are low (ΔG² 1.2-2.4 kcal·mol⁻¹), and at the reaction conditions, both conformers are readily available. This unique feature allows for accommodation of a broad selection of substrates as the catalyst can adopt optimal conformation in response to the geometry of the reactants. Next, we analyzed the reaction profile with model amides 51 and the Z(N) isomer of 52 (Figure 4D). The removal of water from amides is a thermodynamically unfavorable process that requires at least two challenging steps to take place: rotation to an amide with Z configuration and breakage of the carbonyl group. The DFT study supports the notion that the pyridine group can help to recruit the substrates to bind to the molybdenum center (53 and 54), but in the reactive conformer 55 that undergoes cyclization the assistance of pyridine moieties is not required. Furthermore, intermediate 55 shows favorable enthalpic binding (1.3-2 kcal·mol⁻¹) which is sufficient to overcome the barrier for amide rotation and supports the notion that two binding sites in the substrates are required for cyclization. It is also intriguing to note that the most stable structure 55 originates from hydrogen bonding between OH/SH groups and one of the phenolic ligands. Subsequent steps lead to two intermediates 56 and 57 that are stabilized by pyridine and the oxo ligand. From these two structures, the loss of water can proceed with the assistance of catalyst scaffolding (e.g., pyridine) but can also take place after dissociation of oxazolidine/thiazolidine from molybdenum.

In sum, we described a catalytic cyclodehydration process suitable for direct site-selective editing of small molecules, drugs, and peptides. This reaction is characterized by exceptional mildness and generality, and enables introduction of various amide modifications.

Accession codes. CCDC 2108077-2108078 contain the supplementary crystallographic data for this paper. These data can be obtained free of charge via www.ccdc.cam.ac.uk/data_request/cif.

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Author contributions. W.P. designed the experiments, collected and analyzed the data, wrote the manuscript and supplementary files. G. E. collected and analyzed the data. M. W. conceived and oversaw the project, secured external funding, analyzed the data, and wrote the manuscript and supplementary files. All authors approved the final version.

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A. Amide Modifications and Their Role in Representative Examples

Figure 1. Overview of the conceptual framework for site-selective amide functionalization.

B. Strategies in the Synthesis of Post-translationally Modified Peptides

C. Oxazolines as Activated Amides
Figure 2. Catalyst identification and scope of Mo(VI)-catalyzed cyclodehydration. General conditions: substrate (10 mM), 2h (1 mM), PhMe, reflux. Reaction run in PhCl. Reaction run with 5 mM of the substrate. Reaction run in PhCl and 20 mol% of 2h. PhMe:1,4-dioxane (9:1) used as a solvent. Reaction run with 15 mol% of 2h. Abbreviations: Ac=acetyl; Ad=1-adamantyl; Bn=benzyl; Boc = tert-butoxycarbonyl; t-Bu=tert-butyl; Bz=benzoyl; Me=methyl; Ph=phenyl; Piv=pivaloyl; Pr=1-propyl; i-Pr=2-propyl; TBDPS=tert-butyldiphenylsilyl.
A. Diversity of Direct Amide Functionalization in Curacycline B

![Chemical structures and functionalization reactions](image)

B. Scope of Site-selective Modification of Cyclic Peptides

![Chemical structures and site-selective modifications](image)

Figure 3. Site-selective amide functionalization of cyclic peptides. A. Reaction conditions: curacycline B (1 mM), 2h (1 mM), PhCl, reflux, 3 d then (a) TMSCl, MeOH, 1,4-dioxane (1:9), 60 °C, 24 h; (b) TMSN₃, BF₃·Et₂O, MeOH-1,4-dioxane (1:9), 70 °C, 15 h; (c) PhSH, MeOH, reflux, 36 h; (d) H₂S, Et₃N-MeOH (1:2); (e) NaBH₃CN, AcOH, 40 °C; (f) MnO₂, 1,4-dioxane, 110 °C. B. Reaction conditions: cyclic peptide (1 mM), 2h (1 mM), PhCl, reflux. Reaction was run in PhMe followed by H₂S, Et₃N-MeOH (1:2) after cyclization. bReaction was treated with H₂S, Et₃N-MeOH (1:2) after cyclization.
A. Proposed Mechanism of Cyclodehydration

B. Hammett Analysis

C. Thermodynamic Data for Z(N) and Z(O) Conformers

D. Computed Reaction Profile

Figure 4. Studies on the mechanism of amide cyclodehydration. Computational analysis of all molybdenum complexes and reaction pathways calculated at B3LYP/GD3/sVTZpp(SDM toluene)//B3LYP/GD3/SDD-6-31G*(SDM toluene) level of theory. Gibbs free energy and standard enthalpy (in brackets) calculated at 298 K and given in kcal·mol⁻¹.