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Metal-Free Selective Modification of Secondary Amides: Application in Late-Stage Diversification of Peptides

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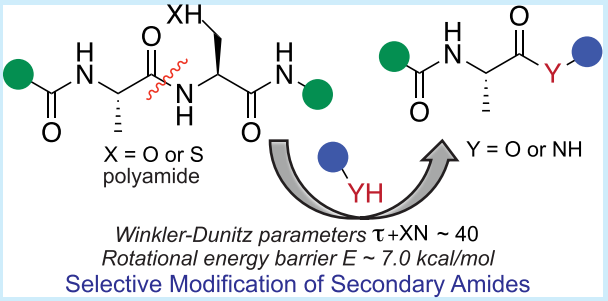
ABSTRACT: Here we solve a long-standing challenge of the site-selective modification of secondary amides and present a simple two-step, metal-free approach to selectively modify a particular secondary amide in molecules containing multiple primary and secondary amides. Density functional theory (DFT) provides insight into the activation of C−N bonds. This study encompasses distinct chemical advances for late-stage modification of peptides thus harnessing the amides for the incorporation of various functional groups into natural and synthetic molecules.



Amide bonds are fundamental building blocks for a broad range of compounds, from peptides and proteins to pharmaceutically active compounds and natural products.[1−4] The high abundance of amide bonds in a variety of molecules is due to their high stability by the resonance, resulting in the partial double bond character of the acyl C−N bond.[5,6] This stability impedes the direct cleavage of amide bonds to further introduce new functional groups by transamidation or esterification, making this synthetic feat highly challenging to achieve. In the last six years, several elegant studies have been reported for the transamidation and esterification of secondary amides including both metal-catalyzed[7−10] and metal-free approaches for applications in the synthesis of a variety of pharmaceutically active molecules and bioactive natural products.[11−13] In contrast to the substantial body of literature on secondary amide activation on small molecules by Szotask and Garg groups,[14−17] analogous methods for the selective activation of particular secondary amides in the presence of several other similar primary and secondary amides in polyamides such as in peptides or polymers, remain an unsolved challenge. Although enzymes selectively cleave amide bonds in proteins, transamidation has proven difficult to achieve both synthetically and enzymatically.[18,19]

Herein, we demonstrate the success of our two-step approach to achieve the selective transamidation and esterification of secondary amides in peptides. The methodology is metal-free and operationally simple, does not require water or air sensitive equipment, and proceeds at room temperature under mild reaction conditions (Figure 1a).

Based on our previous work on synthesis of C-terminal modified peptides[20,21] and acylation strategies for amide activation that are limited to C-terminal modification,[22−27] we hypothesized that the amide bond next to Ser/Thr or Cys could be weakened by the N-functionalization with the respective side chains of these residues through carbonyl or thiocarbonyl insertion (Figure 1a). The resulting oxazolidinone or thiazolidinone cyclic moiety will weaken the amide bond by introducing a twist that distorts the pi-pi overlap of the C−N bond.[20,21] Interception of these intermediates with primary/secondary amines and alcohols would furnish the desired transamidated and esterified products (Figure 1a). Physical organic parameters required for the activation and modification of acylated amides have never been reported before. Here we provide the guidelines/parameters to determine the reactivity of acylated amides toward varying reactions.

Selective Activation of Amides: Design

We initiated our studies with the DFT calculations on carbonyl and thiocarbonyl acylated derivatives selective for cysteine and serine amides (Figure 1b, Supplementary Figure 1).

Using B3LYP/6-311++G(d,p), we determined their amide bond distortion using Winkler−Dunitz parameters: twist angles (τ) and pyramidization at nitrogen (X_N) as well as N−C(O) and C=O bond lengths.[28] We found that the addition of carbonyl or thiocarbonyl group between the side chain of serine and backbone C−N amide bond generated oxazolidinone **1a** (τ = 21.30, X_N = 9.1) and oxazolidithione **1b** (τ = 24.0, X_N = 16.1) and resulted in the twisting of the amide bond relative to the unmodified analog **E** (twisted angle τ = 3.6, nitro X_N = 0.5) (Figure 1b). Similarly, the addition of

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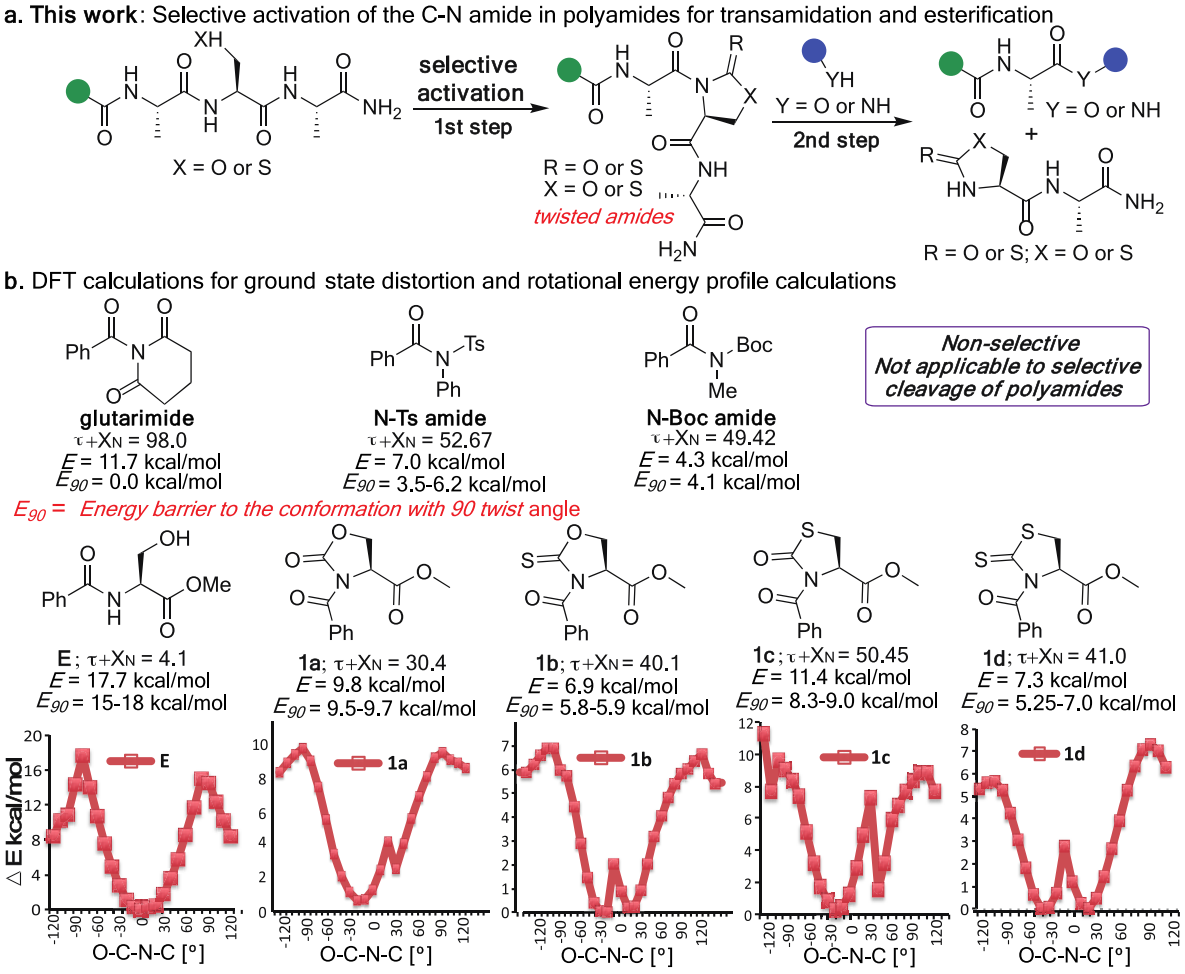

Figure 1. a) A two-step chemical approach to achieve selective amide bond activation of secondary amides at Ser or Cys followed by transamidation and esterification of the C−N amide bond under metal-free mild conditions. b) DFT calculations determined the ground state distortion and rotational profiles of amides **1a**−**1d** and **E** (Δ*E*, kcal/mol, vs O−C−N−C [deg]).

carbonyl and thiocarbonyl to cysteine generated twisted thiazolidinone **1c** (τ = 28.65, X_N = 21.8) and thiazolidithione **1d** (τ = 27.3, X_N = 13.7). Based on the Winkler−Dunitz parameters, **1c** with maximum value ($\tau + X_N$ = 50.45) should be the most reactive among these twisted amides but another factor that influences the reactivity of twisted amides is their rotational energy profile.[15,29] We determined rotational energy profile and barrier (E) of twisted molecules **1a**−**1d** and the unmodified analog E (Figure 1b). The rotational energy profile calculations revealed that all the twisted amides **1a**−**1d** have the lowest energy at dihedral angles (21.3−28.6) and thus prefer to remain in a twisted conformation as compared to unmodified analog E with lowest energy at dihedral angle at 3.6 that thus prefers to remain in a planar form. These calculations showed that compound **1c** has a maximum rotational energy barrier (E = 11.4 kcal/mol and rotational energy barrier to most reactive conformation (twist angle ∼90) (E_{90} ∼ 8.5 to 9.5 kcal/mol) as compared to other twisted amides **1a** (E = 9.8 kcal/mol and E_{90} ∼ 9.5−9.7 kcal/mol), **1b** (E = 6.9 kcal/mol and E_{90} ∼ 5.8−5.9 kcal/mol), and **1d** (E = 7.3 kcal/mol and E_{90} ∼ 5.2−7.0 kcal/mol) (Figure 1b). We compared the twist angle and rotational energy barrier of the twisted amides **1a**−**1d** with well-known twisted amides N-Boc (τ = 32.06, X_N = 17.36, E = 4.26 kcal/mol and E_{90} ∼ 4.1 kcal/mol), N-Ts amides (τ = 30.39, X_N = 22.28, E = 7.00 kcal/mol and E_{90} ∼ 3.5−6.2 kcal/mol) and N-acyl-glutarimides (τ = 91.4, X_N = 6.6, E = 11.7 kcal/mol and E_{90} = 0 kcal/mol) (Figure 1b).[29] Based on these calculations and results from previous twisted amides, we hypothesized that twisted amides **1b** and **1d** with lower E_{90} ∼ 6 kcal/mol will be more reactive as compared to **1a** and **1c** with higher E_{90} (∼9.5 kcal/mol).

Key Experiments and Reaction Discovery for Transamidation

To correlate twisted amide reactivity with the rotational energy profile, we carried out transamidation reactions on small twisted molecules **1a**−**1d** generated by incorporation of carbonyl or thiocarbonyl groups into the serine and cysteine methyl esters by using carbonyl donor (*N*,*N*′-disuccinimidyl carbonate DSC (1.3 equiv) or thiocarbonyl donor (1,1′-thiocarbonyldiimidazole (thio-CDI) (1.2 equiv) and Et_3N (1.1 equiv) in THF for 16h at room temperature, Supplementary Figure 2).[20,21] Optimization studies toward transamidation were carried out with benzylamine on thiazolidithione **1d** (lowest barrier E_{90} ∼ 5.2 kcal/mol) using varying bases and solvents (Supplementary Figure 3). We found that under mild reaction conditions (**1d** (1 equiv), benzylamine (1.5 equiv), triethylamine (1.5 equiv) in DCM at room temperature for 2 h) the transamidation product **2a** was obtained in 96% yield (entry 4, Figure 2). **1b** also generated transamidated product in high yield (94%, entry 2, Figure 2). As predicted, twisted

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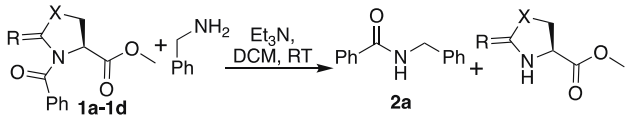

Probe	X	R	Time (h)	Yield (%)
1a	O	O	16	71
1b	O	S	2	94
1c	S	O	12	69
1d	S	S	2	96

Figure 2. Metal-free transamidation reaction on **1a−1d** (0.07 mm), amine (1.5 equiv), triethylamine (1.5 equiv), and DCM (5 mL) at RT.

amides **1a** and **1c** with a higher energy barrier generated transamidation products with moderate yields (69−71%, Figure 2, Supplementary Figure 3). As expected, the attempted transamidation of unmodified analog E with benzylamine failed, thus highlighting the unique ability of this approach to activate secondary amide bonds (Supplementary Figure 4).

Scope of the Transamidation

Examination of the scope of twisted amides **1b** and **1d** revealed that remarkably broad ranges of primary amines such as propargyl amine, dimethoxyethylamine, including sterically hindered isopropylamine and linear octylamine are suitable for this transamidation protocol and generated corresponding products **2b−2e** with high yields in 2 h under optimized reaction conditions (70−89%, Figure 3a, Supplementary Figure 5). Transamidation of **1b** and **1d** proceeded smoothly for the synthesis of tertiary amides **2f−2i** by the use of secondary amines such as N-Boc protected piperazine, piperidine, morpholine, pyrrolidine with yields 60−79% at room temperature in 2 h (Figure 3a, Supplementary Figure 5). The reaction generated transamidation product **2j** with 2-bromoethylamine in moderate yields (50%) without any functionalization of the bromo group (Figure 3a, Supplementary Figure 5). Finally, to test the scalability of this method, **2a** was synthesized by the reaction of **1d** with benzylamine on a gram scale reaction with 90% yield under the optimized reaction conditions (Supplementary Figure 5). Further, we performed the transamidation of **1b** and **1d** using a variety of chiral amino esters such as proline and phenylalanine methyl ester generated secondary amides **2k−2l** in high yields (60−70%, Figure 3a, Supplementary Figure 5).

Esterification of Amides

Next, we extended our strategy of the selective "twist activation" of secondary amides for esterification (Figure 3b). Non-nucleophilic phenol with pK_a of 10.0 was used for the optimization of esterification on twisted amides **1a−1d** (Supplementary Figure 6). K_3PO_4 (5 equiv) in the presence of twisted amide **1b** or **1d**, and excess of phenol (1.5 equiv) was found to be the optimized reaction condition for the generation of the ester product **3a** (Figure 3b). Electronically diverse phenols with both electron-rich (*p*-bromo, *p*-methoxy), and electron-poor groups (*m*-formyl, *p*-acetyl, *p*-trifluoromethyl, naphthyl) including sterically hindered bis-*o*-tolyl alcohol and benzylic alcohols such as 2-nitrobenzyl alcohol and benzyl alcohol were found to be excellent substrates and generated corresponding esters **3b−3j** in good to excellent yields with twisted amides **1b** and **1d** (58−99%, Figure 3b, Supplementary Figure 7). The reaction with aliphatic alcohols such as methanol require high amounts of alcohol for the efficient

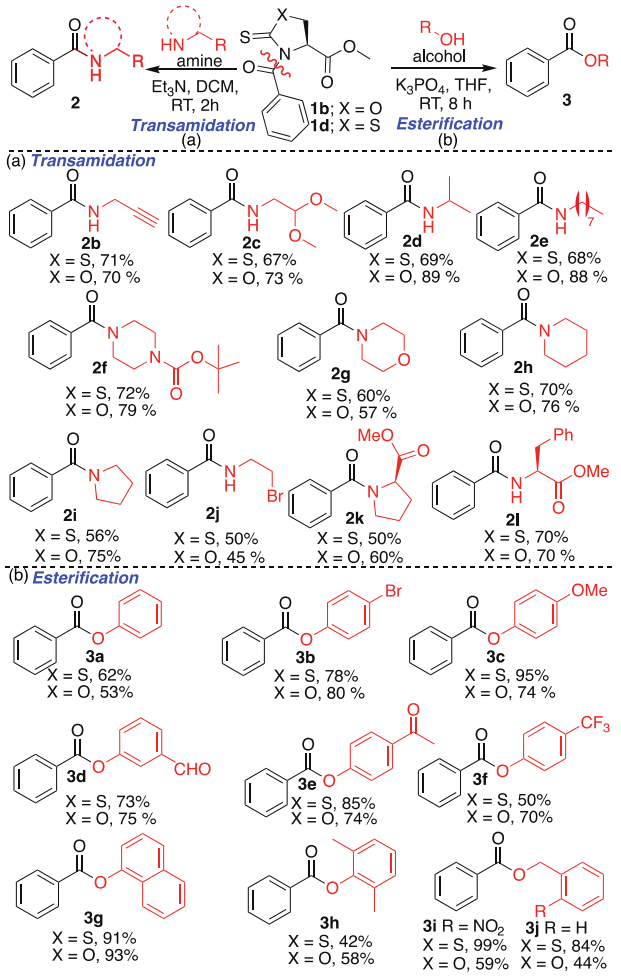

Figure 3. Scope of a) amine and b) alcohol nucleophiles for transamidation and esterification with amide substrates **1b** and **1d**.

modification.[20] Notably, the reaction tolerates substrates that are incompatible with metal-catalyzed and high-temperature protocols, including halides, aldehydes, ketones, highly electron-deficient arenes (e.g., *p*-trifluoromethyl and naphthyl); thus this protocol would be widely applicable in the synthesis of biomolecules and pharmaceutically active compounds.

Site-Selective Transamidation and Esterification of Peptides

As a defining feature of this chemistry, we performed the site-selective modification of particular amides in peptides containing similar secondary and primary amides (Figure 4). Since **1b** and **1d** exhibited low rotational energy barrier based on the DFT calculations, we attempted selective insertions of thiocarbonyl at cysteine amide in tripeptides Boc-FCF-OMe and AcO-RFC-solid support by using various thiocarbonylating agents (Supplementary Figure 8). These reactions failed to generate thiocarbonyl-modified peptides. This might be due to the low reactivity of an intermediate obtained by the reaction of the side chain of serine/cysteine with thiocarbonylating reagent resulting in its rapid decomposition/hydrolysis before the nucleophilic attack from the backbone amide bond. Therefore, we site-selectively incorporated carbonyl on a tripeptide AcO-FSF-NH_2 by reaction with a carbonyl donor (DSC) using DMAP as a base to generate corresponding twisted oxazolidinone AcO-FOxaF-NH_2 **4** (Supplementary

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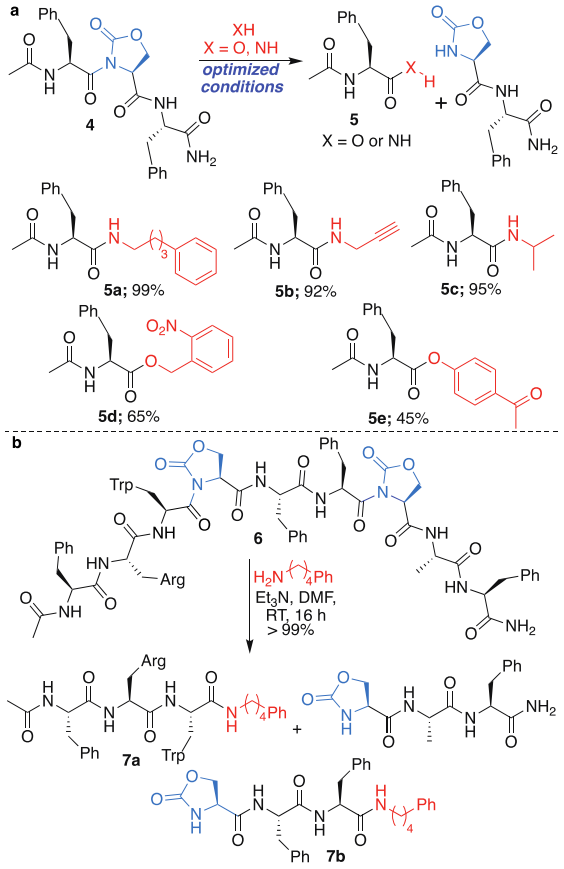

Figure 4. Selective modification of secondary amides in peptides a) **4** and b) **6** by transamidation and esterification.

Figure 9). Exposure of twisted tripeptide **4** to optimized transamidation and esterification conditions with 4-phenylbutylamine, propargyl amine, and sterically hindered isopropylamine and both nucleophilic (2- nitrobenzyl alcohol) and non-nucleophilic alcohols (*p*-acetyl phenol) gave the corresponding secondary amides **5a**−**5c** (92−99%) and ester functionalized peptides **5d**−**5e** (45−65%) Figure 4a, Supplementary Figure 10). We did not observe any epimerization of chiral center on selective amidation on twisted tetrapeptides AcO-FA$_L$SF and AcO-FA$_D$SF under the reaction conditions (Supplementary Figure 11).

In case of a nonapeptide AcO-FRWSFFSAF with two serines, double amide bond activation was observed at N-side of both serine residues to generate double twisted nonapeptide AcO-FRW**Oxa**FF**Oxa**AF **6** (Figure 4b). The treatment with 4-phenyl butylamine under optimized conditions generated two amidation products **7a** and **7b** in full conversions (Figure 4b, Supplementary Figure 12). These studies showed the potential of our method in introducing new functional groups in various amide-containing biomolecules, synthetic molecules and polymers.

In summary, we have developed a mild, two-step, transition-metal-free and operationally simple approach for selective transamidation and esterification of secondary amides. The methodology circumvents the classic problem of selective activation of particular secondary amides in the presence of other similar amides by using activation of Ser-, Cys- and Thr to enable selective modification with varying amines and alcohols. The experimental outputs of the transamidation and esterification correlate with our hypothesis based on DFT calculations that installation of C = X bond reduces π bond character in the amide C−N bond and the twisted molecule with lowest energy barrier E_{90} to reach the most reactive twist conformation exhibits highest reactivity. The reaction showed broad scope with amines, phenolic and benzylic alcohols, and applicable for late-stage modification of peptides. Given the importance of multiple amide bonds in biomolecules, polymers and materials, we anticipate that selective amidation and esterification approaches will be of a great significance in the field of chemical biology, biotechnology and material science. Future efforts focused on construction of C−heteroatom or C−C bonds with these activated acylated amides on peptides is currently underway in our laboratories.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.orglett.1c01622.

Supporting figures (energy profiles), Cartesian coordinates, experimental procedures, analytical data, and spectra for new compounds (PDF)

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Notes

The authors declare no competing financial interest.

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