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Iterative Design of a Biomimetic Catalyst for Amino Acid Thioester Condensation

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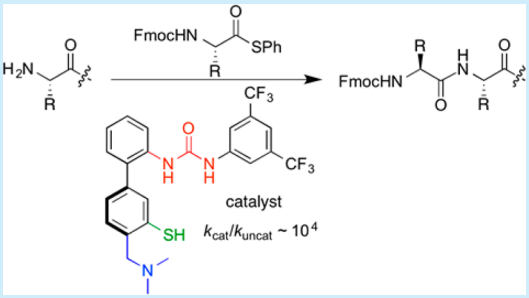
Supporting Information

ABSTRACT: Herein, the design of a catalyst that combines lessons learned from peptide biosynthesis, enzymes, and organocatalysts is described. The catalyst features a urea scaffold for carbonyl recognition and elements of nucleophilic catalysis. In the presence of 10 mol % of the organocatalyst, the rate of peptide bond formation is accelerated by 10000-fold over the uncatalyzed reaction between Fmoc-amino acid thioesters and amino acid methyl esters.



The amide bond is ubiquitous in peptides, polymers, and small molecules. Efficient and mild reagents that activate the carboxylic acid functionality for subsequent reaction with the desired amine have enabled the routine synthesis of this bond, such that synthesis of long polyamides can now be automated. However, contemporary amide bond synthesis is also wasteful.[1] We sought to evaluate if amino acid thioesters offer a practical approach to access peptides. Thioesters are nature's carboxylate activating groups: thioester condensation is a critical reaction in biosynthesis of natural products and non-ribosomal peptides.[2] Inspired by biosynthetic precedents, we envisioned a two-step process to convert amino acids to amides. The first step would involve efficient conversion of carboxylic acids to the more electrophilic thioesters. Examples of catalytic thioesterification have been demonstrated, albeit involving nonamino acid examples.[3] We are currently investigating several strategies to efficiently prepare amino acid thioesters. Alternatively, simple thioesters are readily accessible from condensation of carboxylic acids with triphenylphosphine and appropriate disulfides.[4] In the second step, a biomimetic catalyst would couple amino acid thioesters to a growing peptide chain with high efficiency. Results of our preliminary studies aimed at organocatalyst design for thioester−amine coupling are described herein. We focused on organocatalysis because metal catalysts often suffer from nonspecific coordination with amide bonds.

Our catalyst design combines elements of protease active sites and lessons learned from peptide and protein ligation methodologies. The oxyanion hole represents a critical component of protease active sites; ureas and other hydrogen bonding scaffolds have been invoked as mimics of the oxyanion hole for anion recognition.[5] Only a few examples of hydrogen bonding catalysts aiding acylation or deacylation chemistries are known.[6] We began by evaluating the ability of diphenylurea **1a** (Figure 1A) to enhance the rate of amide bond formation between model substrates *p*-bromobenzoic acid thiophenylester **A** (10 mM) and benzylamine **B** (20 mM) in acetonitrile at room temperature. Under these conditions, the uncatalyzed reaction requires more than 130 h for completion. We found the reaction rate to be only slightly faster with urea **1a** (Figure 2). These model studies quickly revealed that simple urea scaffolds are not sufficient to coalesce and activate the reactants for amide bond formation. We hypothesized that a catalyst that forms a transient covalent intermediate with the substrates, as observed in serine hydrolases, may enhance the reaction rate (Figure 1B).

We prepared urea **1b**, which features a thiol group on one aromatic ring, with the premise that a thioester exchange will transiently bond the starting thioester with the catalyst, turning a three-component reaction (thioester, catalyst, and amine) to a more manageable two-component system (Figure 1C). Incorporation of the thiol group builds on lessons from nucleophilic catalysis in enzymes as well as native chemical ligation (NCL).[7] Facile thioester exchange is a critical aspect responsible for the high reactivity and chemoselectivity observed with NCL. As part of this modification, we also elaborated one of the aromatic rings with trifluoromethane groups at the *meta* positions. Trifluoromethane groups have been shown to enhance the hydrogen-bonding potential of the urea group.[8] Urea **1b** was significantly more active than **1a**. After 2 h, roughly 55% of thioester **A** converted to amide **C** in the presence of **1b** as compared to 13% with urea **1a** and 23% with (bis)trifluoromethaneurea **1c**, which serves as a control to delineate the contribution of the trifluoromethane groups (Figure 2).

The rate enhancement observed with urea **1b** supports the hypothesis that transient capture of one reactant provides a significant boost. To be sufficiently competitive with carboxylic acids activated with conventional coupling agents, the catalyst will need to provide high yields of the amide product in minutes. We argued that the rate of the reaction may be further enhanced

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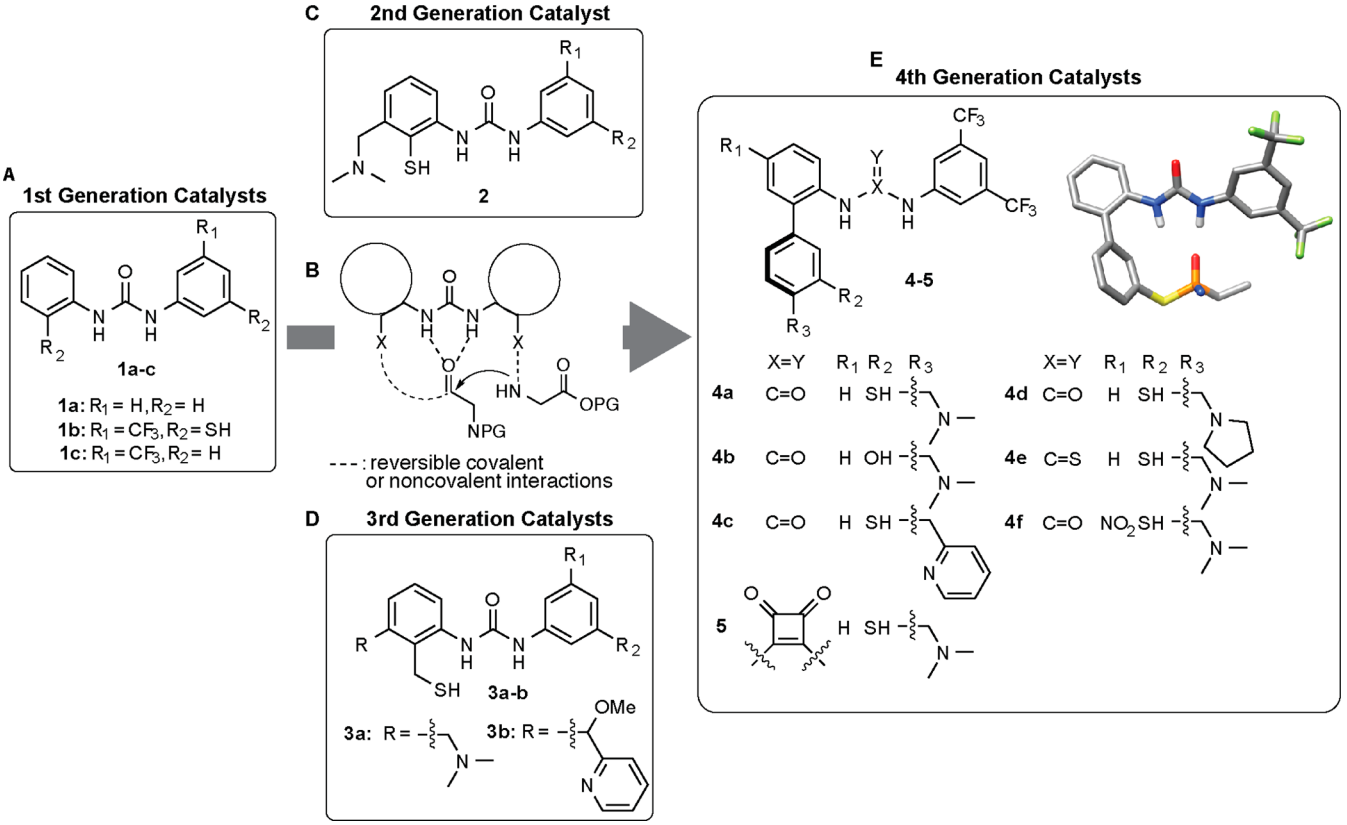

Figure 1. Iterative design of urea catalysts. (A) In our initial attempt, we tested the potential of simple bisphenyl urea catalysts. A thiol group was included to engage a thioester through a thioester-exchange reaction. The preliminary results suggested that of an optimal catalyst may require simultaneous coordination of the carboxy and amine reactants as well as activation of the carboxy group through hydrogen bonding. The idealized catalyst design that emerges from this hypothesis is shown in panel (B). PG = protecting group. (C, D) We developed several catalysts based on the bisphenyl urea scaffold to optimize placement of the thiol group and/or the amine base. None of these designs yielded an active catalyst. (E) Molecular modeling studies with a tetrahedral transition-state analogue suggest that a biphenyl group allows optimal hydrogen bonding of the thioester-exchanged intermediate. The modeling studies were performed with a phosphonamidothioate derivative as the tetrahedral intermediate. Several analogues of the biphenyl–phenylurea **4** were prepared to probe their potential to condense the model thioester and amine. A squaramide derivative **5** in which the urea-based hydrogen bonding module is replaced was also designed.

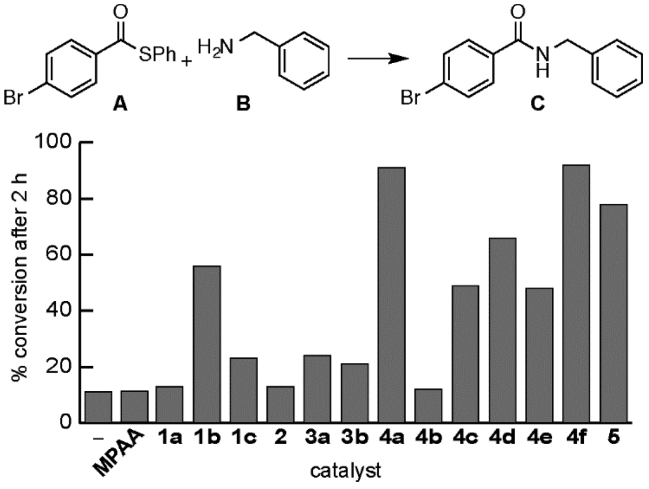

Figure 2. Conversion of thioester **A** to amide **C** in the presence of the urea catalysts after 2 h. Thioester **A** (10 mM), amine **B** (20 mM), and catalyst (1 mM) were incubated in acetonitrile at room temperature. Reaction progress was analyzed by HPLC. MPAA = 4-mercaptophenylacetic acid. Plots showing time-dependent reaction progress for each catalyst are included as Figure S2.

by a scaffold that can simultaneously complex both substrates, as observed for enzymes. This hypothesis led us to consider potential approaches for inducing a reversible catalyst–amine complex formation. The common approach for engaging the nucleophile in urea-based organocatalysts is to employ a basic amine that can participate in hydrogen-bonding interactions. We appended an amine to the urea scaffold to investigate the effect of a tethered base (Figure 1C). Unfortunately, urea **2** bearing this modification led to a decrease in catalytic activity as compared to **1b**, compelling us to re-evaluate our catalyst design. Molecular-modeling studies, performed using Macromodel software,[9] suggested that positioning of the thiol group *ortho* to the urea functionality is potentially not optimal for coordination of the thioester to the urea and complexation with the tertiary amine. Based on these modeling studies, we predicted that extension of the thiol functionality by a methylene unit might allow better coordination. Ureas **3a** and **3b** which feature an *o*-benzylthiol group and a tertiary or an aromatic amine base, were synthesized and evaluated. Neither of these designs delivered better performance than urea **1b** (Figures 1D and 2).

We next explored a larger shift away from the original urea scaffold. Molecular-modeling studies with a transition-state analogue to capture the tetrahedral thioester exchange intermediate suggests that positioning of the thiol group on a biphenyl moiety should lead to better organization of the reactive

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complex (Figure 1E). Gratifyingly, this change, which resulted in urea **4a**, led to a significant improvement in the observed rate (Figure 2). We found that greater than 90% of thioester **A** was converted to amide **C** within 2 h in the presence of this catalyst.

We prepared several analogues of **4a** to improve on this result and to analyze the key components of this trifunctional catalyst. Modification of the thiol group to alcohol (**4b**) resulted in a large decrease in the reaction rate, supporting the hypothesis that thioester exchange is a critical component of the reaction (Figures 1 and 2). Substitution of the dimethylamino group with a pyridyl (**4c**) or pyrrolidinyl (**4d**) group caused an appreciable decrease in product formation. Thiophenylureas are better hydrogen bond donors than phenylureas and have been shown to be generally superior as organocatalysts.[5b] We prepared thiourea **4e** to test the potential of this enhanced hydrogen-bond donor on reactivity; however, we found that the thiourea thiol can participate in thioester exchange with the substrate, potentially reducing the effectiveness of this catalyst.

Urea **4a** is built on the classical diphenyl urea catalyst with one of the aromatic rings modified with bis-trifluoromethyl groups. We tested if the addition of an electron-withdrawing group on the aromatic ring would enhance the observed activity of the catalyst. Urea **4f**, which features a nitro group on the aromatic ring *para* to the urea functionality, was found to provide a rate increase to that of **4a**. Lastly, we prepared a squaramide analogue of **4a** to evaluate a different hydrogen-bonding anion recognition scaffold that has shown promise for the construction of receptors and catalysts.[10]

For our model amide bond-forming reaction, squaramide **5** proved to be active but not as effective as **4a**. As a control, we also tested the potential of 4-mercaptophenylacetic acid (MPAA), a well-known additive in NCL reactions,[11] to accelerate amide bond formation from thioester **A**. As expected, this simple thiol did not have an effect on the observed rate of conversion (Figure 2).

The systematic design and evaluation of different scaffolds provided urea **4a** as a lead catalyst for amide bond formation between model thioester and amine substrates. As part of these optimization studies, we also gauged the performance of the catalyst in different solvents (Table S2). As expected on the basis of the mode of interactions, the catalyst proved to be more effective in nonpolar solvents. We also prepared and evaluated phenyl and *o*-nitrophenyl ester analogues of **A** and analyzed the potential of **4a** to catalyze their amide bond formation. We found that oxoesters are not good substrates for the urea catalyst likely due to their inability to participate in rapid thioester exchange (Table S3).

Next, we evaluated the suitability of the catalyst for amino acids protected with the standard Fmoc group. We began by analyzing the rate of alanine dipeptide formation. Condensation of 10 mM Fmoc-alanine phenylthioester with 20 mM alanine methyl ester in toluene leads to 5% formation of the Fmoc-Ala-Ala-OMe dipeptide after 24 h at 22 °C. In the presence of 10 mol % of **4a** under the same conditions, the reaction is completed in roughly 10 min (Table 1, entry 1). Encouraged by this finding, we screened the catalyst for the formation of dipeptides to determine the scope of the hydrogen-bonding catalyst to accept a diverse range of thioesters and amines (Table S1 and Figures S4–S16).

The rates of product formation were monitored by HPLC. β-Branched amino acid residues are often difficult to condense with activated carboxylic acids. In keeping with their known lower reactivity, we find that the reaction of alanine thioester with valine methyl ester requires roughly 7 h for completion (Table 1, entry 4); we observe a significant amount (12%) of hydrolysis of the thioester during the reaction. (We attribute any water present in the reaction to the hygroscopic nature of the hydrochloric acid salt of the amino acid methyl esters used directly from commercial sources.) Addition of 20 mol % catalyst doubles the reaction rate such that alanine-valine dipeptide may be formed over 3 h.

Table 1. Potential of Urea 4a To Catalyze Dipeptide Formation from Amino Acid Thioesters[a]

entry	dipeptide	catalyst (mol %)	time[b]
1	FmocAlaAlaOMe	10	10 min
2	FmocAlaAlaOMe	no catalyst	>20 days[c]
3	FmocAlaValOMe	no catalyst	>50 days[c]
4	FmocAlaValOMe	10	7 h[d]
5	FmocAlaValOMe	20	3 h[d]
6	FmocAlaSarOMe	10	4 h[d]
7	FmocAlaSarOMe	20	2 h[d]
8	FmocPheAlaOMe	10	10 min
9	FmocProAlaOMe	10	40 min
10	FmocProAlaOMe	no catalyst	>50 days[c]
11	FmocValAlaOMe	10	4.5 h
12	FmocValAlaOMe	20	2 h

[a]Reaction conditions: Fmoc-Xaa-SPh (10 μmol), amino acid methyl ester HCl salts (20 μmol), Et_3N (20 μmol), and catalyst **4a** in 1 mL of toluene. [b]Time for >98% conversion of Fmoc-Xaa-SPh based on analysis of HPLC trace of the crude reaction mixtures. [c]Estimated time based on reaction progress after 24 h. [d]Roughly 10–15% hydrolysis of Fmoc-Ala-SPh was observed for these entries.

We also analyzed the potential of the catalyst for secondary amine coupling (Table 1, entries 6 and 7). While sarcosine (*N*-methylglycine) condenses with alanine thioester within 4 h in the presence of 10 mol % catalyst, we observed no dipeptide formation with proline over 24 h (Table S1). As part of these explorations, we also varied the thioester to determine the effectiveness of the catalysts to potentially participate in thioester exchange with various amino acids (Table S1). We observed less variation in the observed rates with the thioester component than with the amine, suggesting that reaction of the amine with the thioester–**4a** intermediate is the slower step (*vide infra*). Phenylalanine, lysine, proline, and arginine thioesters condensed with alanine methylester in 10, 30, 40, and 60 min, respectively. However, β-branching on the thioester partner also diminishes the rate of the reaction on a similar level as observed with the β-branched amine partner (Table 1, entries 11 and 12). No epimerization was observed under the reaction conditions after comprehensive evaluations with catalyst **4a** (Figure S17).

We analyzed the kinetics of the amidation reaction between Fmoc-valine thiophenylester (10 mM) and alanine methylester (100 mM) in toluene. These substrates were chosen because their dipeptide formation occurs over a sufficiently longer time period to allow precise measurement of product formation. Urea **4a** provides a 10000-fold rate acceleration for dipeptide formation over the uncatalyzed reaction (Table 2; details are included in the Supporting Information). We investigated the requirement for different components of the trifunctional catalyst with designed controls. Compounds **6**–**8**, in which the thiol, urea, or the tertiary amine groups are removed from **4a** (Table 2), were synthesized. Removal of either of these functional groups leads to a significant loss in the observed

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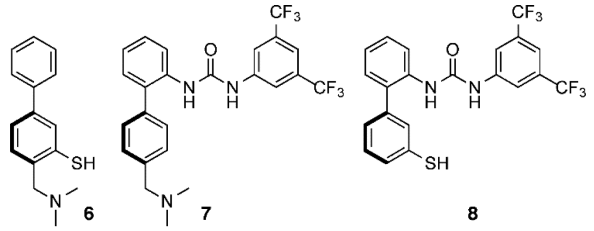
Table 2. Pseudo-First-Order Rate Constants for Dipeptide Formation with Urea 4a and Designed Controls 6−8



entry	catalyst	k (min^{-1})	k_{rel}
1	**4a**	5.22×10^{-2}	10000
2	**6**	1.50×10^{-5}	3
3	**7**	1.18×10^{-5}	2
4	**8**	2.98×10^{-5}	6
5	no catalyst	5.03×10^{-6}	1

activity, suggesting that the functional groups are participating in a cooperative mechanism as hypothesized.

We envision two key steps in the catalytic amide bond formation by **4a** (Figure S1). The first step involves a transthioesterification reaction between the thioester and **4a**. This step is postulated to be mediated by hydrogen bonding with the urea group. The catalyst·thioester complex then condenses with the amine leading to amide bond formation. We tested the dependence of the reaction on the concentration of the catalyst, thioester, and the amine moieties (SI). Analysis suggests that amide bond formation is slower than transthioesterification. Careful ^{19}F NMR studies implicate the tertiary amine in both the transthioesterification and the amide bond formation steps (SI). The postulated catalytic cycle supported by these extensive analyses is depicted in Figure 3.

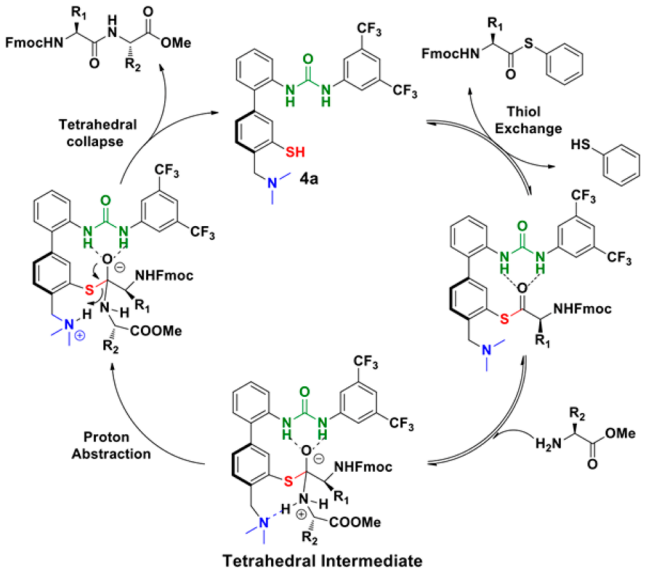


Figure 3. Proposed mechanism for amide bond formation catalyzed by **4a**.

In summary, we describe efforts to develop an organocatalyst for amide bond formation between thioesters and free amines. The catalyst design builds on urea-based hydrogen bonding scaffolds and the concept of covalent catalysis. The optimized trifunctional organocatalyst provides rate accelerations 10000-fold above the background reaction and mono- and difunctional derivatives. The catalyst is active on a diverse range of amino acid substrates. No epimerization of chiral amino acids was observed during reactions.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.orglett.7b02412.

Synthesis and characterization of catalysts, reaction screening, and mechanistic studies (PDF)

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Notes

The authors declare no competing financial interest.

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