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Model Studies on the First Enzyme-Catalyzed Ugi Reaction

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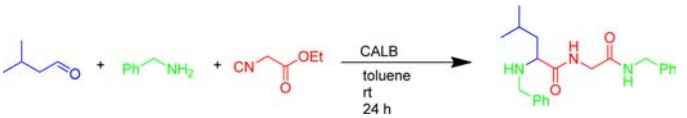
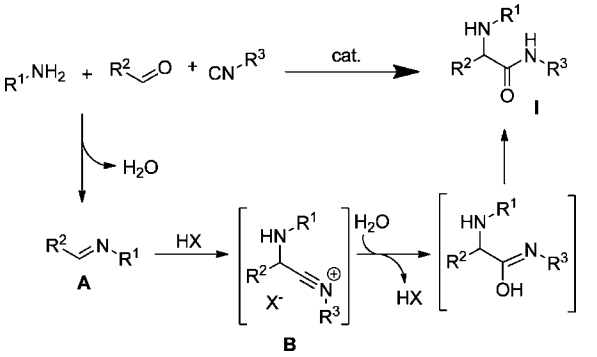
ABSTRACT



Multicomponent reactions are powerful tools for organic chemistry, and among them, the Ugi reaction provides remarkable improvement in many fields of organic chemistry such us combinatorial chemistry, medicinal chemistry, and peptide chemistry. A new, enzyme-catalyzed example of the Ugi three-component reaction is presented. The studies include the selection of an enzyme as well as determination of the scope and limitations of the newly described reaction. The presented method combines the enzyme promiscuity and multicomponent reaction advantages in the first one-pot formation of dipeptide 1.

Multicomponent reactions (MCRs) have great significance in the efficient synthesis of diverse compound libraries.[1−3] Among the MCRs, the Ugi four-component reaction (U-4-CR) has greatly contributed to modern synthetic methods.[4,5] The classic Ugi reaction is a condensation of a primary amine, a carbonyl compound, carboxylic acid, and isocyanide. This one-pot reaction results in α-amidoamide with water as the only byproduct.[6] Its simplicity and one-pot nature make the reaction a remarkable tool for many fields of chemistry such as heterocyclic chemistry,[7] medicinal chemistry,[8] and the chemistry of peptides and peptydomimetics.[9] Thus, interest in the further development of the Ugi reaction and other isocyanide-based multicomponent reactions (IMCRs) grew rapidly in recent years.[10,11] This led to the discovery of new, significant types of IMCRs. Among them, interesting examples of catalytic Ugi reactions leading to α-aminoamides were described. In these Ugi three-component reactions (U-3-CRs), a catalyst is required to activate an imine **A** in order to form nitrilium intermediate **B** (Scheme 1).

Scheme 1. Catalytic Ugi Three-Component Reaction



Pan and List have shown that such a reaction can be catalyzed by Brønsted acids. Among a wide spectrum of Brønsted acids examined, phenyl phosphinic acid has been found to be the best catalyst.[12] Further studies performed

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by other groups revealed that similar U-3-CRs can also be catalyzed by zinc chloride (R^1 = 2-hydroxyphenyl) and sulphonated cellulose in ethanol.[13,14]

The goal of the present paper is the presentation of enzymes as catalysts for U-3-CRs. We examined the ability of the lipases to catalyze the three-component Ugi reaction by activating the imine **A**. Lipases are widely utilized for numerous transformations, and they are known to exhibit unexpected promiscuities.[15−19] With regard to Ugi reactions, lipases were previously used for stereoselective hydrolysis of glutaric anhydrides that provided *in situ* an acidic substrate for U-4-CRs.[20] However, to the best of our knowledge, no Ugi reaction catalyzed by an enzyme has been reported so far.

We present, herein, the first example of an enzyme-catalyzed Ugi condensation of an amine, aldehyde, and isocyanide which leads to formation of dipeptide **1** (Scheme 2).

Scheme 2. Enzyme-Catalyzed Ugi Reaction

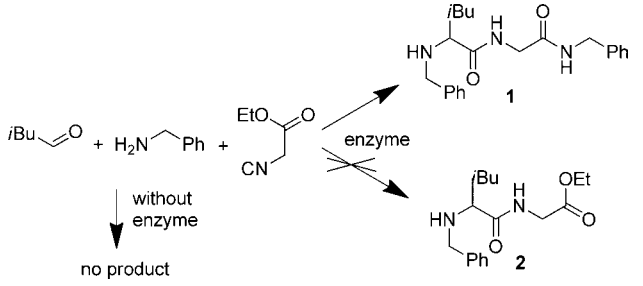

Table 1. Enzymatic Screening

entry	enzyme	yield [%][a]
1	Novozym 435	75
2	*Candida antarctica* lipase (native)	41
3	*Candida antarctica* lipase acrylic resin	41
4	*Candida cylindracea* lipase	15
5	Amano lipase PS	19
6	*Candida antarctica* lipase (deacivated)	0

[a] Isolated yields. Reaction conditions: amine (2 equiv), aldehyde (1 equiv), isocyanide (1 equiv, c = 0.02 M), enzyme (20%).[21]

Our model substrates were ethyl isocyanoacetate, isovaleric aldehyde, and benzylamine (1 equiv of each). As it is shown in Scheme 2, in the model reaction without enzyme, no formation of expected aminoamide **2** occurred. Also conversion of ethyl isocyanoacetate was rather poor, even after 4 days of stirring the substrates. On the other hand, in the presence of Novozym 435, we observed full conversion of ethyl isocyanoacetate after 24 h. Unexpectedly, under these reaction conditions, we did not observe formation of aminoamide **2**. It turned out that the compound with structure **1** was isolated as a main product (43% isolated yield). We also observed only the slight formation of 2-isocyano-*N*-(phenylmethyl)ethanamide (**3c**). Based on the chemical structure of the main product, we decided to change the molar ratio of the substrates. Applying 1:2:1 to the aldehyde/amine/isocyanide ratio, the reaction yield significantly increased up to 75% (Table 1, entry 1).

The wide spectrum of commercially available enzymes was screened as a biocatalyst for this reaction.[22] The results are summarized in Table 1. In general, *Candida antarctica* lipase (native and immobilized) as well as Amano PS lipase catalyzed the Ugi reaction. Among them, the best yield was obtained when using Novozym 435 (Table 1, entry 1). We also performed additional experiments in order to confirm that the enzyme activity, not the presence of a protein itself, is responsible for the formation of the product **1**. For this purpose, we used inactivated CALB, but no formation of compound **1** was observed (Table 1, entry 6).

The model reaction was carried out in toluene, but also other solvents were examined.

Table 2. Solvent Influence on Enzymatic Ugi Reaction

entry	solvent	yield [%][a]
1	toluene	75
2	chloroform	64
3	water	45
4	ethanol 96%	0
5	tetrahydrofuran	0

[a] Isolated yields.

The results presented in Table 2 show that initially chosen toluene is the most efficient solvent; however a high yield was also observed when chloroform was used as a solvent (Table 2, entry 2). Compound **1** was not formed in ethanol, despite the fact that it is considered to be a proper solvent for Ugi reactions (as a protic solvent). Even more significant is the observation that the reaction can also be perfomed in water (Table 2, entry 3). Water is often not compatible with the Ugi reaction due to isocyanide decomposition which occurs in the presence of acids in aqueous media. On the other hand, it is known that

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(22) For detailed list of enzymes, see the Supporing Information.
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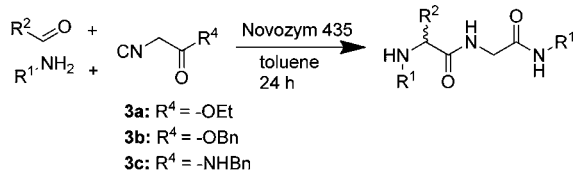
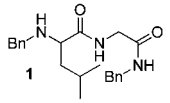
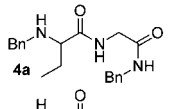
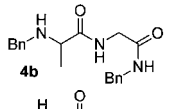
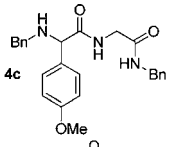
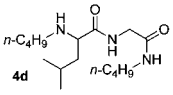
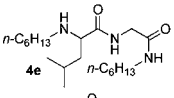
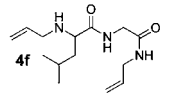
Table 3. Three-Component Ugi Reaction with Various Amines and Aldehydes



3a: R^4 = -OEt
3b: R^4 = -OBn
3c: R^4 = -NHBn

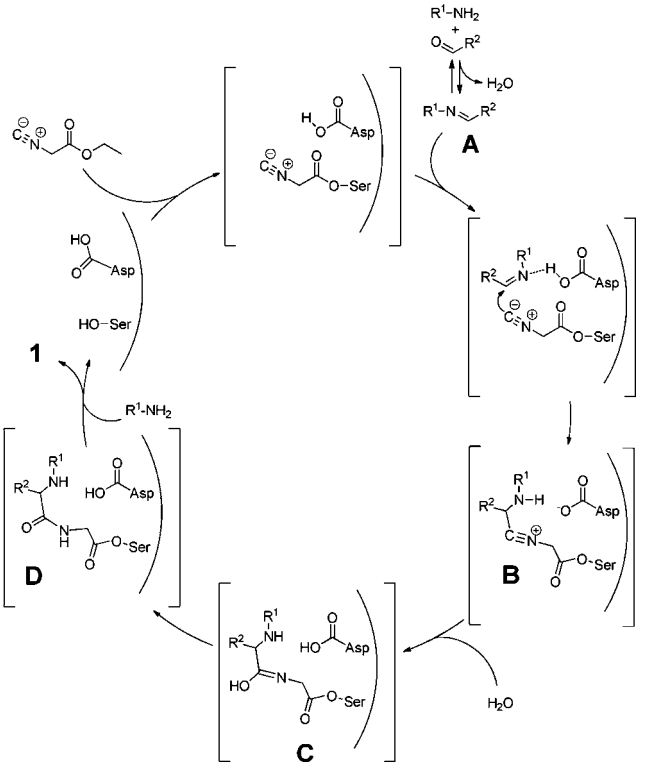
entry	structure of product	isocyanide	yield [%][a,d] stand. cond.[b]	yield [%][a,d] with water add.[c]
1	 **1**	**3a**	75	29
2		**3b**	78	0
3	 **4a**	**3a**	76	87
4	 **4b**	**3a**	2	63
5	 **4c**	**3a**	0	43
6	 **4d**	**3a**	10	0
7	 **4e**	**3a**	12	44
8	 **4f**	**3a**	21	49
9	R^1 = Bn-	R^2 = *n*-nonyl-	**3a**	no reaction
10	R^1 = Bn-	R^2 = *i*-Bu-	**3c**	no reaction
11	R^1 = Bn-	R^2 = *i*-Bu-	cyclohexyl isocyanide	no reaction

[a] Isolated yields. [b] Reaction conditions: amine (2 equiv), aldehyde mac_opb;1 equiv), isocyanide (1 equiv, c = 0.02 M), Novozym 435 (20%). [c] 0.5% water addition (v/v). [d] The reactions were stopped after 24 h.

lipase-catalyzed reactions are often more efficient in the interface.[23] Since no acids are used in the presented reaction, the addition of water should not affect the isocyanide stability. This conclusion prompted us to investigate the influence of water on the presented reaction.

We performed a series of enzyme-catalyzed Ugi reactions with and without the addition of water. The results are presented in Table 3. In many cases, the addition of water improved the reaction yield (Table 3, entries 3, 4, 7, and 8) or actually enabled the formation of product (Table 3, entry 5). On the other hand, in the other cases, the addition of water decreased the yield of the reaction (Table 3, entries 1, 2, and 6). Nevertheless, the results in Table 3 clearly show that the reaction is relatively diverse

Scheme 3. Possible Mechanism of the Presented Ugi Reaction



considering the amine and aldehyde components. The reaction occurs with either ethyl or benzyl isocyanoacetate with similar yields (Table 3, entries 1 and 2). However, the ester moiety is required, as compound **1** was not obtained when the ester group was replaced with an amide moiety (isocyanide **3c**) or alkyl group (cyclohexyl isocyanide).

This observation indicates that the ester group may be involved in enzyme acylation. Based on this conclusion, we presented a plausible explanation for the enzyme-catalyzed Ugi reaction (Scheme 3). We proposed that, in the fisrt step, the serine residue in the catalytic triad of the enzyme is acylated with an ester group of isocyanide. This assumption is based on the classic lipase-catalyzed ester hydrolysis mechanism.[23] Then, the imminium intermediate **A** is activated. It is possible that the acidic Asp residue in the active site of the enzyme is responsible for the activation of imine **A** as in the classic mechanism of the Ugi reaction presented in Scheme 1. The subsequent steps are also consistent with the U-3-CR mechanism described before.[12] An activated electrophilic imminium intermediate undergoes isocyanide addition leading to nitrilium adduct **B**. Addition of water results in intermediate **C** formation, followed by isomerization in adduct **D**. The reaction with another amine molecule finally leads to a cleaved dipeptide **1** and free enzyme (Scheme 3). The proposed mechanism suggests that water addition should improve, in general, the reaction yield. On the other hand, the experimental results indicate that this influence is not the same for all the substrates. This observation can be explained by the change of the tertiary structure of an enzyme. It is known

that the tertiary structure of enzymes in organic solvent strongly depends on the water content.[24] Therefore, for certain substrates, such a change in protein structure in the active site may result in a decreased reaction rate.

We observed that, under the conditions studied, the reactions are not stereoselective and the products are obtained as racemic mixtures.[25] The lack of stereoselectivity can be explained by the relatively high reactivity of the isocyanide moiety. Therefore, the fact that the isocyanide addition step (Scheme 3, A→B) proceeds rapidly but reversibly may explain the lack of stereoselectivity in the presented reactions.

In summary, we presented the first example of an enzymatically catalyzed multicomponent Ugi reaction. The developed procedure utilizes the enzyme promiscuity and advantages of the multicomponent reaction for the synthesis of dipeptides **1**, which have never been obtained in a one-pot procedure before. In contrast to previously described U-3-CR procedures (heating required), the enzymatic U-3-CR is carried out at room temperature and is catalyzed by commonly avaible enzymes. Furthermore, the reaction proceeds in either aqueous solution or organic solvents. This makes the reaction attractive for applied chemistry and inprints well within the green chemistry concept. The presented U-3-CR is relatively diverse and can be applied to the synthesis of dipeptides with unnatural amino acids (**4f**) in a single step. The products are obtained in moderate to excellent yields.

The presented studies revealed the unique and unpredictible behavior of the enzymes which, combined with multicomponent reactions, significanctly expanded the synthetic methodology leading to peptide scaffolds.

Supporting Information Available. Detailed experimental procedures, spectral data, and additional information are available.This material is available free of charge via the Internet at http://pubs.acs.org.

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The authors declare no competing financial interest.

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(25) For detailed description of the stereoselectivity studies, see the Supporting Information.