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The influence of the isocyanoesters structure on the course of enzymatic Ugi reactions

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ABSTRACT

The impact of isocyanoesters structure on enzymatic three-component Ugi reactions course has been determined. The significant promiscuous ability of enzyme in Ugi-type reaction switching between four (U-4CR) and three (U-3CR) components reactions depending on the size of used isocyanoester. The application of short-chain cyanoesters up to isocyanpropionate leading to product of three component reaction exclusively while longer isocyanobutyrate gives only the product of four component reaction. The limitation of studied enzymatic Ugi reaction is a substrate selectivity of lipases.

1. Introduction

Enzymes are extensively used in modern organic synthesis due to their high selectivity and mild reaction conditions [1,2]. These features have a great impact on the environmental factor (E factor) as the application of biocatalysts minimizes the amount of solvents and enhances the reactions rate what reduce the cost of reactants [3]. The biocatalysts are believed to be fairly specific, which would mean decrease number of side reactions leading to side products which lower the reaction atom economy. These side reactions hence lower the yield of the desired product (making the catalysis less efficient) and necessitate complicated downstream processing. However, there are many exceptions of this universally accepted enzyme specificity [4]. In recent years, a new frontier, known as biocatalytic promiscuity, has emerged and largely extended the application of enzymes [5]. Enzyme catalytic promiscuity refers to the ability of an enzyme to catalyze reactions vary from its natural role extending the application of enzymes. Several elegant examples regarding the significance of enzymatic promiscuity have been reported, such as Hantzsch-type reaction [6], perhydrolysis [7], Markovnikov and anti-Markovnikov addition between thiols and vinyl esters [8], 1,2-addition of thiols to imines [9], Henry reactions [10], Morita–Baylis–Hillman reaction [11], Mannich reactions [12], aldol additions [13], Michael additions [14] or domino type reaction leading to spirooxazino derivatives [15]. Although, mentioned examples of promiscuous behavior of biocatalysts significantly extend the classical synthetic methods and contribute to the knowledge about their action in chemical transformations, the exploration of biomolecules in it is limited to reactions involving general acid and general base catalysis by the functional groups available on the side-chains of amino acids strategically placed on their surface [16]. Moreover, in numerous reports, enzymes only initiate the "promiscuous" reaction providing reagents *via* their native activity, e.g. hydrolysis of β-keto esters followed by decarboxylative aldol and Knoevenagel reactions [17]. Additionally, those reports pertain to reactions that occur spontaneously leading to insoluble compounds what shifts reaction equilibria to the favorable product side and challenge promiscuous activity of used enzymes [9,17,18].

Multicomponent reactions (MCRs), which can form complex bioactive molecules from simple starting materials have attracted increasing attention in recent years [19]. Among the MCRs, the Ugi four-component reaction (U-4CR) has greatly contributed to modern synthetic methods [20]. The classical U-4CR involves the condensation of a primary amine, carbonyl compound, isocyanide and carboxylic acid leading to the formation of α-acylamino amides [21]. Further efforts to explore the efficient catalysts for Ugi reaction were continued resulting in a three-component version of this condensation (U-3CR) [22]. The reaction between aldehydes, isocyanides, and primary amines catalyzed both by Brønsted and Lewis acids provides α-amino amides with almost 100% atom-economy [22]. Amine and carbonyl components can be replaced by the corresponding imine leading to substituted pyrrolidine and piperidine derivatives [23]. These studies exploited enzyme promiscuity in the MCR, which significantly expanded the synthetic methodology for new peptide scaffolds. Recently, our group reported the first example of an enzyme-catalyzed Ugi 3-component reaction in

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organic medium with ethyl and benzyl isocyanoacetates as the substrates [24]. In subsequent studies under U-3CR using cyclic imines as the substrates, we have provided data which proved the proposed mechanism of the lipase action [25]. In general, the serine residue in the catalytic triad of the biocatalyst is acylated with an ester group of isocyanide. This assumption is based on the classic lipase-catalyzed ester hydrolysis mechanism [26]. Moreover, the provided mechanism shows that water addition stimulates the rate of enzymatic transformation [24,25]. The addition of water is known to be crucial for enzyme activity in organic solvents, which can be explained by the change in the tertiary structure of the protein [27]. Although we have provided the reasonable mechanism of lipase-catalyzed U-3CR, some aspects of it still remained unclear what stimulated us to further studies. The principal limitations in exploiting enzymes as catalyst are faced with limited grade of substrate acceptance [28]. A key factor for classifying specific lipases is the presence of a lid domain, which proved to be one of the most flexible part of enzymes [29]. Moreover, crystal structures of lipases revealed large variations in shapes of their active sites environments [30]. Recent review pointed out to the conclusion that the size of particular enzyme active site and its ability to provide additional conformation is crucial [31]. Probing and modulation of promiscuous enzyme activity is still a challenge. Thus, examine biocatalysts' active site by using structurally various substrates may provide valuable information on enzyme selectivity [32]. For example, the study on the specific activity of *Candidia antarctica* lipase A toward acylation of methyl shikimate with various vinyl esters revealed higher selectivity of enzyme with short-chain vinyl esters [33]. Vaysse and Ly studied chain-length selectivity of several lipases in hydrolysis, esterification and alcoholysis of saturated fatty acids [34]. Analogous research regarding the application of lipase B from *Candidia antarctica* (CaLB) has been reported for the synthesis of aryl-aliphatic glycolipids [35]. Another aspects of lipases substrate specificity rely on their ability to distinguish different structural feature of acyl group. Therefore, a wide variety of acyl acceptors were employed in enzymatic transesterification for biodiesel production [36]. Nelson et al. described extensive studies regarding lipases chemoselectivity in transesterification of triglycerides with C1-C4 aliphatic alcohols [37]. Our previous report showed the application of enzyme as catalyst in MCRs as a phenomenon capability for new peptide scaffolds. Herein, we demonstrate the selectivity of chosen enzymes in respect to the structure of isocyanoesters structure toward two types of Ugi reactions.

2. Results and discussion

The influence of chain-length of fatty acids on enzyme selectivity has been described in a number of reports [38]. However, the impact of isocyanoesters structure on the enzymatic Ugi reaction course has been never tested. Therefore, we study the selectivity of certain lipases using various isocyanoesters (**1a-h**) in U-3CR (Table 1). Moreover, the performed studies reveal the unexpected ability of enzymes to switch their primary function upon the addition of phenylacetic acid what manifests in different synthetic outcome.

Reaction conditions were established based on our previous studies [25]. An isovaleric aldehyde and benzylamine were selected arbitrarily and used as model substrates together with seven different isocyanoesters (**1b-h**) (Table 1). We have tested the effect of the chain-length of isocyanoesters as well as the impact of ester group on the reaction course. Two native lipases (from *Candida cylindarcea* (CcL), *Pseudomonas cepacia* (PcL)) and one immobilized on acrylic resin (lipase B from *Candida Antarctica*) (Novozym 435) which were previously recognized as the most active in U-3CR were subjected to the studies [25]. Collected results were compared with those previously obtained for ethyl cyanoacetate (**1a**) (Table 1, entries 1, 13 and 21) [24]. The obtained results revealed a substantial substrate selectivity which strongly depends on the chain-length of the used isocyanoester **1** (Table 1). Among tested enzymes, the highest activity has been observed for immobilized lipase B from *Candida antarctica* (Novozym 435, CaLB). The application of ethyl 3-isocyanpropionate (**1b**) in the presence of CaLB gave product **2b** with 57% yield, exclusively (Table 1, entry 2). Increasing the distance between the isocyanide and the ester group from three to four carbons resulted in a decrease in the reaction yield to 44% (Table 1, entry 6). A similar behavior in enzymatic activity regarding the chain-length of the used isocyanoesters (**1b-c**) has been observed for *Candida cylindarcea* lipase (Table 1, entries 14–15), while *Pseudomonas cepacia* lipase exhibited slightly higher activity with ethyl 4-isocyanobutanoate (**1c**) than with ethyl 4-isocyanopropionate (**1b**) providing desired products **2c** and **2b** with 5% and 3% of yield, respectively (Table 1, entries 22 and 23).

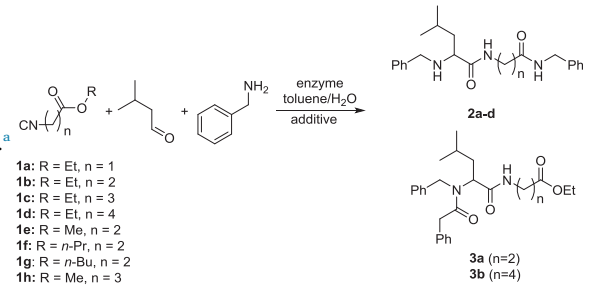
When ethyl 5-isocyanopentanoate (**1d**) was applied as a substrate, the formation of the expected product **2d** has not been observed for all used lipases, even after the extension of reaction time up to 7 days (Table 1, entries 7, 16 and 24). As a proof of catalytic role of enzyme, the reaction with **1b** was conducted in the presence of thermally deactivated Novozym 435. In this case, the formation of the product **2b** was not observed (Table 1, entry 4). The reaction of ethyl 4-isocyanopropionate (**1b**) with phenylacetic acid and thermally deactivated Novozym 435 resulted exclusively in the formation of U-4CR product **3a** (n = 2) with 28% of yield (Table 1, entry 5). However, the U-4CR product **3b** (n = 4) was provided in reaction with ethyl 5-isocyanopentanoate (**1d**), phenylacetic acid and active lipase B from *Candida antarctica* with 23% of yield (Table 1, entry 8). Extraordinary and unexpected attribute of enzymatic transformation manifested upon the addition of phenylacetic acid to reaction mixture with ethyl isocyanopropionate (**1b**) resulted in U-3CR product **2b** with 50% yield, exclusively (Table 1, entry 3). Thus result indicates that the enzyme fully controls the course of the multicomponent reaction, and despite of the presence of phenylacetic acid only U-3CR product was formed. To the best of our knowledge this is the first example of the such control in promiscuous reaction provided by lipases. It is also important to note that studied transformation represents double promiscuous activity fused in sequential process; multicomponent reaction followed by aminolysis [39]. The second enzymatic promiscuous reaction stimulates the three component Ugi condensation. Based on this conclusion, we presented a plausible reaction mechanism for three different types of Ugi reaction (Scheme 1).

The obtained results are in accordance with previously proposed mechanism [24,25] in which reaction proceeds directly in active site of enzyme. The first step of the biotransformation is analogical to traditional lipase-catalyzed ester hydrolysis: serine resided in the catalytic triad is acylated with an ester group of isocyanoester. At the same time, the iminium intermediate is activated by the acidic aspartate residue in the active site of enzyme. Then, an activated electrophilic iminium intermediate undergoes isocyanoester addition leading to nitrilium adduct. The next steps are consistent with three-component Ugi reaction mechanism [22]. The lower reaction efficiency with long-chain isocyanoesters (**1b** and **1c**) as well as no reaction with ethyl 5-isocyanopentanoate (**1d**) can be explained by the substrate selectivity of enzyme which cannot accept those isocyanoesters [40].

Due to the fact that only isocyanoesters undergo the studied reaction, the influence of the size of alkoxy group has been investigated. The reaction with methyl 3-isocyanopropanoate (**1e**) and methyl 3-isocyanobutanoate (**1h**) catalyzed by CaLB resulted in products **2b** and **2c** with reduced yields − 26% and 14%, respectively (Table 1, entries 9 and 12). Similar but not so meaningful decrease in catalytic activity of CaLB toward product **2b** was observed when propyl (**1f**) and butyl (**1g**) isocyanopropionates were applied (Table 1, entries 10 and 11). The results obtained for *Candida cylindracea* lipase with various isocyanoesters (**1b-h**) revealed that this enzyme exhibits higher activity with *n*-propyl isocyanopropionate (**1f**) than with ethyl isocyanopropionate (**1b**) providing product **2b** with 22% *vs* 10% (Table 1, entry 18). At the same time CcL shows no activity toward methyl and *n*-butyl isocyanopropionates (**1e** and **1g**) (Table 1, entries 17 and 19). As

Table 1

U-3CR with different isocyanoesters **1a-h** catalyzed by lipases.[a]



1a: R = Et, n = 1
1b: R = Et, n = 2
1c: R = Et, n = 3
1d: R = Et, n = 4
1e: R = Me, n = 2
1f: R = *n*-Pr, n = 2
1g: R = *n*-Bu, n = 2
1h: R = Me, n = 3

Entry	Enzyme	Isocyjanoester 1	Additive[d]	Time [h]	Product	Yield [%][c]	
						2	3
1[b]	CaLB	**1a**	–	24	**2a**	75	0
2	CaLB	**1b**	–	72	**2b**	57	0
3	CaLB	**1b**	$PhCH_2COOH$	72	**2b**	50	0
4	CaLB deactivated	**1b**	–	72	**2b**	0	0
5	CaLB deactivated	**1b**	$PhCH_2COOH$	72	**3a**	0	28
6	CaLB	**1c**	–	72	**2c**	44	0
7	CaLB	**1d**	–	168	**2d**	< 1	0
8	CaLB	**1d**	$PhCH_2COOH$	72	**3b**	0	23
9	CaLB	**1e**	–	72	**2b**	26	0
10	CaLB	**1f**	–	72	**2b**	41	0
11	CaLB	**1g**	–	72	**2b**	38	0
12	CaLB	**1h**	–	72	**2c**	14	0
13[b]	CcL	**1a**	–	24	**2a**	15	0
14	CcL	**1b**	–	72	**2b**	10	0
15	CcL	**1c**	–	72	**2c**	2	0
16	CcL	**1d**	–	168	**2d**	0	0
17	CcL	**1e**	–	72	**2b**	0	0
18	CcL	**1f**	–	72	**2b**	22	0
19	CcL	**1g**	–	72	**2b**	0	0
20	CcL	**1h**	–	72	**2c**	8	0
21[b]	PcL	**1a**	–	24	**2a**	19	0
22	PcL	**1b**	–	72	**2b**	3	0
23	PcL	**1c**	–	72	**2c**	5	0
24	PcL	**1d**	–	168	**2d**	0	0
25	PcL	**1e**	–	72	**2b**	21	0
26	PcL	**1f**	–	72	**2b**	15	0
27	PcL	**1g**	–	72	**2b**	6	0
28	PcL	**1h**	–	72	**2c**	8	0

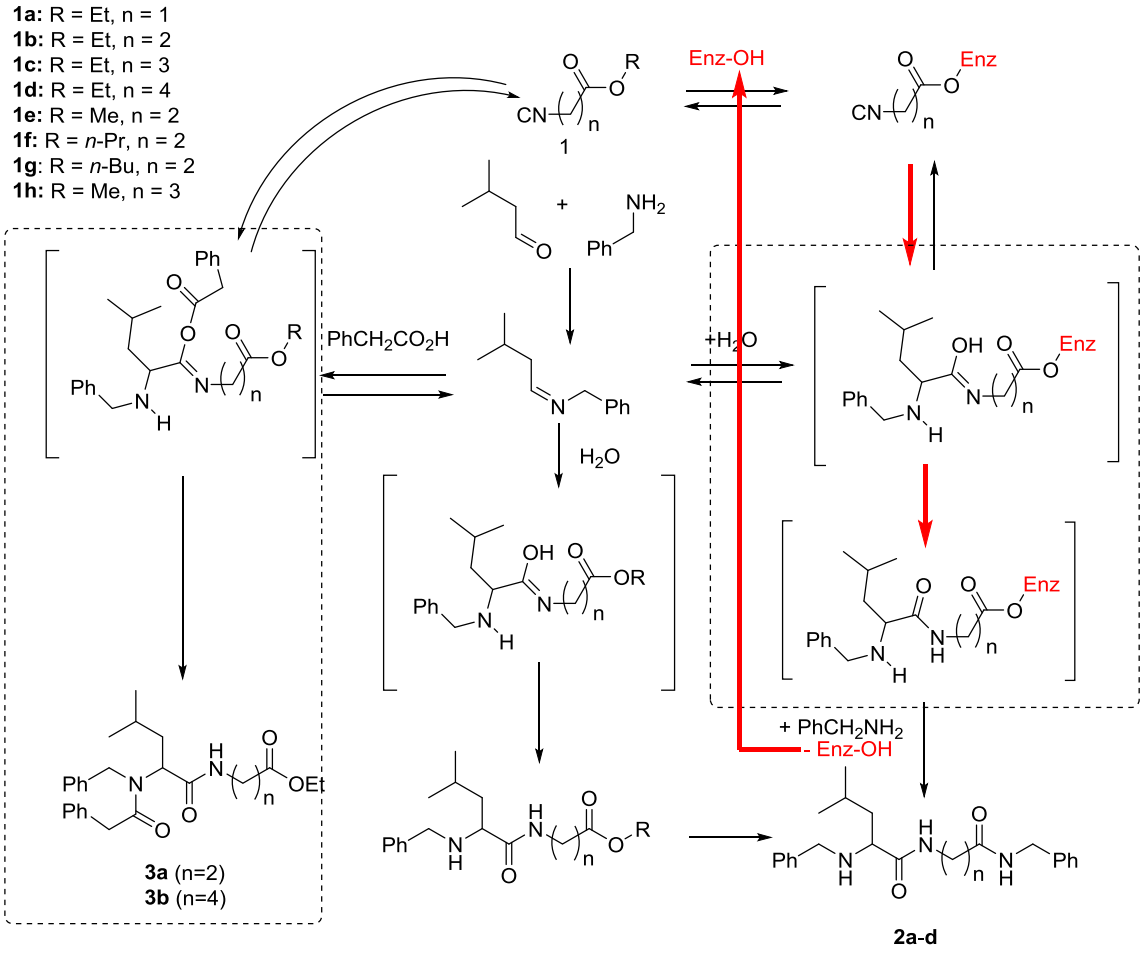
[a] Reaction conditions: benzylamine (1.0 mmol), isovaleric aldehyde (0.5 mmol), isocyanoester **1** (0.5 mmol), toluene (20 mL), water (0.1 mL), enzyme (20% by weight), ambient temperature.
[b] Ref. [24].
[c] Yield of isolated product.

it can be seen the elongation of the distance between the two functional groups in methyl isocyanoester (**1h**) resulted in activity recovery leading to desired **2c** with 8% yield (Table 1, entry 20). In case of *Pseudomonas cepacia* lipase, the product **2b** was obtained with the highest yield 21% for methyl isocyanopropionate (**1e**) while the application of its longer homolog (**1h**) resulted in substantial reduction in reaction yield of **2c** to 8% (Table 1, entries 25 and 28). Gradual decrease in enzymatic activity was observed for *n*-propyl and *n*-butyl isocyanoesters leading to corresponding product **2b** with 15% and 6% yields, respectively (Table 1, entries 26 and 27). Obtained results are consistent with literature data regarding the selectivity of enzymes in respect to esters alkoxy groups [41].

The first part of our research which revealed the modulation of enzyme promiscuous capability in the synthesis of various peptidomimetics **2** prompted us to extend our studies by application of imine as a substrate for enzymatic Ugi reaction. The results are presented in Table 2. The obtained cyclic amines are the key structural elements of many bioactive compounds, e.g. telaprevir and boceprevir, hepatitis C virus NS3 protease inhibitors [42]. The model reaction with 2-methyl-1-pyrroline, benzylamine and ethyl isocyanoacetate (**1a**) provided the desired product **4a** with 44% yield under previously established conditions [25]. Analogous experiments to those conducted for immobilized lipase B from *Candida antarctica* with different size-chain isocyanoesters **1b-d** have been performed. The yields of the corresponding products **4b-c** obtained with immobilized CaLB and native CcL and PcL are on the decline with the rise of the chain-size of the used isocyanoester **1a-d** (Table 2, entries 1–4, 9–12 and 17–20). Moreover, among tested isocyanoesters **1e-h** (Table 2, entries 5–8) only methyl and *n*-propyl isocyanoproponates (**1e** and **1f)** are accepted by CaLB, but provide product **4b** with only 8% and 2% yield, respectively (Table 2, entries 5 and 6). The lower or lack of enzyme activity may by caused by the nature of its active site which cannot accommodate branched substrates [40].

Candida cylindracea lipase exhibited much higher catalytic activity toward reaction with *n*-propyl and *n*-butyl 3-isocyanopropanoate (**1f** and **1g**) (Table 2, entries 14 and 15) than with methyl 3-isocyanopropanoate (**1e**) (Table 2, entry 14). For P*seudomonas cepacia* lipase a change in isocyanides ester moiety, did not affect the distinct change in reactions yields of the product **4b** (Table 2, entries 21–23). The obtained results are consistent with a data shown in Table 1. It is worth to mention that the application of methyl 4-isocyanobutanoate (**1h**) did not result in the formation of the desired product **4c** for all examined enzymes (Table 2, entries 8, 16 and 24).

The formation of nonracemic products was not observed under



Scheme 1. The proposed reaction mechanism.

studied reaction condition. In all cases the products **2** as well as **4** were obtained as a racemates. Nevertheless, the developed procedure provides an elegant, one-pot methodology for the synthesis of peptidomimetics scaffords, compared to traditional multistep way.

3. Conclusions

We have demonstrated that the structure of isocyanoesters has the great impact on the enzymatic Ugi reactions course and efficiency, depending on the used substrates, the U-3CR or U-4CR products are obtained. We have proven that the limitation of enzymatic Ugi reaction is the substrate selectivity of lipases. Specificity enzyme profile demonstrates notable differences at diversified of isocyanides. The selected enzymes indicated a preference for short-chain isocyanoesters (1 to 4-C) of substrates at enzymatic Ugi reactions. These results are consistent with recently proposed mechanism in which reaction proceeds directly in active site of enzyme. The obtained results are elegant examples of enzymatic promiscuity. Moreover, conducted experiments revealed the fact that the enzymatic reaction is faster that chemical Ugi reaction. This phenomena give us an opportunity for switching the course of one-pot Ugi reactions according to desired product.

4. Experimental

4.1. General considerations

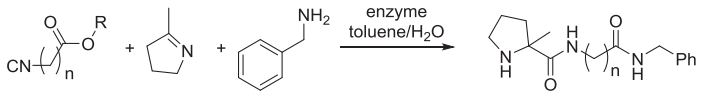
All the chemicals were obtained from commercial sources and the solvents were of analytical grade. ^{1}H and ^{13}C NMR spectra were recorded in $CDCl_3$ solution. Chemical shifts are expressed in ppm using TMS as an internal standard. TLC was done on Kieselgel 60 F254 aluminium sheets. Immobilized lipase B from *Candida antarctica* B (Novozym 435) was purchased from Novo Nordisk. Lipases from *Candida cylindarcea* (CcL), *Pseudomonas cepacia* (PcL) were provided from Sigma-Aldrich. Column chromatography was performed on Merck silica gel 60/230–400 mesh. Enzymatic reactions were performed in a vortex (Heidolph Promax 1020) equipped with incubator (Heidolph Inkubator 1000). Melting points are uncorrected. The elemental analyses were performed on CHN Perkin-Elmer 240 apparatus. To prove the ability of the established protocol each reaction was repeated at least three times.

4.1.1. General procedure for synthesis of ethyl isocyanonides *1b-h*

According to the literature procedure [43] corresponding aminoacids were esterified with ethanol and thionyl chloride in quantitative yields. Received hydrochlorides (1 equiv.) were suspended in corresponding: triethyl/ tripropyl/ tributyl orthoformates (9 equiv.) and heated to reflux under stirring for 6 h. Solvent was evaporated in vacuum and obtained product was used without further purifications. Corresponding formamides (9.8 mmol) were dissolved in dry DCM (16 mL) and *N*-methylmorpholine (4.5 mL) was added. Then the reaction mixture was cooled down to −40 °C and diphosgene (696 µL, in 8,2 mL of dry DCM) was dropped to the reaction mixture over a period of approximately 30 min. After the solution was stirred for 1 h, the cooling bath was removed. The mixture was allowed to reach room temperature and after 30 min cold water was added. Organic layer was separated, washed with 5% $NaHCO_3$ dried over $MgSO_4$ and concentrated in vacuum. The crude product was purified by column chromatography (silica gel, hexanes/ethyl acetate).

Table 2

Enzymatic U-3CR with 2-methyl 1-pyrroline and various isocyanoesters **1a-h**.[a]



1a: R = Et, n = 1
1b: R = Et, n = 2
1c: R = Et, n = 3
1d: R = Et, n = 4
1e: R = Me, n = 2
1f: R = *n*-Pr, n = 2
1g: R = *n*-Bu, n = 2
1h: R = Me, n = 3

Entry	Enzyme	Isocyanoester 1	Time [h]	Product	Yield [%][c]
1[b]	CaLB	**1a**	24	**4a**	44
2	CaLB	**1b**	72	**4b**	13
3	CaLB	**1c**	72	**4c**	6
4	CaLB	**1d**	168	**4d**	0
5	CaLB	**1e**	72	**4b**	8
6	CaLB	**1f**	72	**4b**	2
7	CaLB	**1g**	72	**4b**	0
8	CaLB	**1h**	72	**4c**	0
9	CcL	**1a**	24	**4a**	33
10	CcL	**1b**	72	**4b**	7
11	CcL	**1c**	72	**4c**	2
12	CcL	**1d**	168	**4d**	0
13	CcL	**1e**	72	**4b**	1
14	CcL	**1f**	72	**4b**	20
15	CcL	**1g**	72	**4b**	6
16	CcL	**1h**	72	**4c**	0
17	PcL	**1a**	24	**4a**	30
18	PcL	**1b**	72	**4b**	16
19	PcL	**1c**	72	**4c**	< 1
20	PcL	**1d**	168	**4d**	0
21	PcL	**1e**	72	**4b**	7
22	PcL	**1f**	72	**4b**	12
23	PcL	**1g**	72	**4b**	14
24	PcL	**1h**	72	**4c**	0

[a] Reaction conditions: 2-methyl-1-pyrroline (0.5 mmol), benzylamine (0.5 mmol), isocyanoester **1** (0.5 mmol), toluene (20 mL), water (0.1 mL), enzyme (20% by weight), ambient temperature.
[b] Ref. [24].
[c] Yield of isolated product.

*4.1.1.1. Ethyl 3-isocyanopropanoate (**1b**)*. Compound **1b** was obtained as a colorless oil (56%). ^{1}HNMR ($CDCl_3$, 400 MHz): δ 4.20 (q, 2H, J = 7.2 Hz, $-O\underline{\mathbf{CH_2}}CH_3$), 3.69–3.66 (m, 2H, $CN-\underline{\mathbf{CH_2}}-CH_2-$), 2.73–2.69 (m, 2H, $-CH_2-\underline{\mathbf{CH_2}}-(CO)-O-$), 1,28 (t, 3H, J = 7.2 Hz, $-CH_2\underline{\mathbf{CH_3}}$); ^{13}CNMR ($CDCl_3$, 100 MHz) δ 169.4, 157., 161.3, 61.3, 37.2, 34.2, 14.1; Anal calcd for: $C_6H_9NO_2$ + 1/3 H_2O: C: 55.03, H: 7.39, N: 10.69; found C: 55.22, H: 7.39, N: 10.83.

*4.1.1.2. Ethyl 4-isocyanobutanoate (**1c**)*. Compound **1c** was obtained as a pale yellow oil (70%). ^{1}HNMR ($CDCl_3$, 400 MHz): δ 4.14 (q, 2H, J = 7.2 Hz, $-OC\underline{\mathbf{H_2}}CH_3$), 3.50–3.46 (m, 2H, $CN-C\underline{\mathbf{H_2}}-CH_2-$), 2.48 (t, 2H, J = 7.2 Hz, $-CH_2-C\underline{\mathbf{H_2}}-(CO)-O-$), 2.01–1.96 (m, 2H, $CN-CH_2C\underline{\mathbf{H_2}}-CH_2-(CO)-$), 1,26 (t, 3H, J = 7.2 Hz, $-CH_2C\underline{\mathbf{H_3}}$); ^{13}CNMR ($CDCl_3$, 100 MHz) δ 172.1, 156.9, 60.7, 40.8, 30.4, 24.3, 14.1; HR ESIMS: calcd for $C_7H_{11}NO_2Na$ $[M+Na]^+$ 164.0687, found 164.0687.

*4.1.1.3. Ethyl 5-isocyanopentanoate (**1d**)*. Compound **1d** was obtained as a yellow oil (78%). ^{1}HNMR ($CDCl_3$, 400 MHz): δ 4.13 (q, 2H, J = 7.2 Hz, $-OC\underline{\mathbf{H_2}}CH_3$), 3.42–3.39 (m, 2H, $CN-C\underline{\mathbf{H_2}}-CH_2-$), 2.34 (t, 2H, J = 7.2 Hz, $-CH_2-C\underline{\mathbf{H_2}}-(CO)-O-$), 1.77–1.74 (m, 4H, $CN-CH_2-C\underline{\mathbf{H_2}}C\underline{\mathbf{H_2}}-CH_2-(CO)-$), 1.25 (t, 3H, J = 7.2 Hz, $-CH_2C\underline{\mathbf{H_3}}$); ^{13}CNMR ($CDCl_3$, 100 MHz) δ 172.8, 156.4, 60.5, 41.2, 33.2, 28.5, 21.7, 14.2; HR ESIMS: calcd for $C_8H_{13}NO_2Na$ $[M+Na]^+$ 178.0844, found 178.0841.

*4.1.1.4. Methyl 3-isocyanoproanoate (**1e**)*. Compound **1e** was obtained as a pale yellow oil (68%). ^{1}HNMR ($CDCl_3$, 400 MHz): δ 3.75 (s, 3H, J = 7.2 Hz, $-OC\underline{\mathbf{H_3}}$), 3.69–3.68 (m, 2H, $CN-C\underline{\mathbf{H_2}}-CH_2-$), 2.74–2.73 (m, 2H, $-CH_2-C\underline{\mathbf{H_2}}-(CO)-O-$); ^{13}CNMR ($CDCl_3$, 100 MHz) δ 169.8, 157.7, 52.2, 37.1, 33.9, HR ESIMS: calcd for $C_5H_7NO_2Na$ $[M+Na]^+$ 136.0374, found, 136.0371

*4.1.1.5. Propyl 3-isocyanopropanoate (**1f**)*. Compound **1f** was obtained as a pale yellow oil (65%). ^{1}HNMR ($CDCl_3$, 400 MHz): δ 4.03 (t, 2H, J = 6.8 Hz, $-OC\underline{\mathbf{H_2}}-CH_2\ CH_3$), 3.63–3.60 (m, 2H, $CN-C\underline{\mathbf{H_2}}-CH_2-$), 2.67–2.63 (m, 2H, $-CH_2-C\underline{\mathbf{H_2}}-(CO)-O-$), 1.65–1.56 (m, 2H, $-O-CH_2-C\underline{\mathbf{H_2}}CH_3$), 0.88 (t, 3H, J = 7.2 Hz, $-CH_2C\underline{\mathbf{H_3}}$), ^{13}CNMR ($CDCl_3$, 100 MHz) δ 169.4, 157.4, 66.7, 37.2, 34.1, 21.8, 10.2 ppm.; HR ESIMS: calcd for $C_7H_{11}NO_2Na$ $[M+Na]^+$, 164.0687, found 164.0685.

*4.1.1.6. Butyl 3- isocyanopropanoate (**1g**)*. Compound **1g** was obtained as a pale yellow oil (62%). ^{1}HNMR ($CDCl_3$, 400 MHz): δ 4.10 (t, 2H, J = 6.8 Hz, $-OC\underline{\mathbf{H_2}}-CH_2-$), 3.66–3.62 (m, 2H, $CN-C\underline{\mathbf{H_2}}-CH_2-$), 2.69–2.66 (m, 2H, $-CH_2-C\underline{\mathbf{H_2}}-(CO)-O-$), 1.59 (t, 2H, J = 6.8 Hz, $-O-CH_2-C\underline{\mathbf{H_2}}-CH_2CH_3$), 1.35 (q, 2H, J = 7.6 Hz, $-O-CH_2-CH_2-C\underline{\mathbf{H_2}}CH_3$) 0.90 (t, 3H, J = 7.6 Hz, $-CH_2C\underline{\mathbf{H_3}}$), ^{13}CNMR ($CDCl_3$, 100 MHz) δ 169.4, 157.5, 7.1, 37.2, 34.2, 30.5, 19.0, 13.6; HR ESIMS: calcd for $C_8H_{13}NO_2Na$ $[M+Na]^+$ 178.0844, found 178.0841.

*4.1.1.7. Methyl 4-isocyanobutyrate (**1h**)*. Compound **1h** was obtained as a yellow oil (85%). ^{1}HNMR ($CDCl_3$, 400 MHz): δ 3.70 (s, 3H, J = 7.2 Hz, $-OC\underline{\mathbf{H_3}}$), 3.53–3.45 (m, 2H, $CN-C\underline{\mathbf{H_2}}-CH_2-$), 2.51 (t, 2H, J = 7.2 Hz, $-CH_2-C\underline{\mathbf{H_2}}-(CO)-O-$), 2.02–1.97 (m, 2H,

$CH_2-C\underline{H_2}-CH_2-$); ^{13}CNMR ($CDCl_3$, 100 MHz) δ 172.5, 156.9, 51.8, 40.8, 30.2, 24.3. The ^{1}H and ^{13}C NMR data were in accordance with those reported in the literature [44].

*4.1.2. General procedure for synthesis of compounds **2b-c***

Isovaleric aldehyde (1 equiv.) was added to the suspension of an enzyme (20% weight) in a mixture of toluene/water (20 mL/100 μL), followed by addition of benzylamine (2 equiv.). The mixture was stirred for 15 min. Then, isocyanoester **1** (1 equiv) was added and the reaction was gently vortexed at room temperature. The enzyme and water were filtered off through a funnel containing Celite and $MgSO_4$. The solvent was evaporated in vacuum and product **2** was purified by column chromatography (silica gel, hexanes/ethyl acetate).

*4.1.2.1. 2-Benzylamino-4-methyl-pentanoic acid (2-benzylcarbamoyl-ethyl)- amide (**2b**)*. Compound **2b** was obtained as a white solid, mp. 85–86 °C. ^{1}HNMR ($CDCl_3$, 400 MHz): δ 7.75 (bs, 1H, $-N\underline{H}$), 7.33–7.27 (m, 10*H*, 2x Ar−**H**), 6.19 (bs, 1H, $-N\underline{H}$), 4.40 (td, 2H, J_1 = 7.2 Hz, J_2 = 5.6 Hz), 3.73 (d, 1H, J = 13.2 Hz), 3.60–3.55 (m, 3H), 3.15–3.11 (m, 1H), 2.45 (t, 2H, J = 6 Hz), 2.01 (bs, 1H, −Bn−N**H**), 1.56–1.55 (m, 1H), 1.52–1.51 (m, 1H), 1.40–1.36 (m, 1H), 0.89 (d, 3H, J = 6.4 Hz, $-CH(C\underline{H_3})_2$), 0.81 ppm (d, 3H, J = 6.4 Hz, $-CH(C\underline{H_3})_2$); ^{13}CNMR ($CDCl_3$, 100 MHz) δ 175.1, 170.9, 139.2, 138.1, 129.0, 128.7, 128.6, 128.4, 128.2, 127.8, 127.5, 127.4, 125.3, 60.9, 52.9, 43.6, 42.8, 36.1, 35.1, 25.1, 23.2, 21.7 ppm.; HR ESIMS: calcd for $C_{23}H_{32}N_3O_2$ $[M+H]^+$ 382.2495, found 382.2492.

*4.1.2.2. 2-Benzylamino-4-methyl-pentanoic acid (3-benzylcarbamoyl-propyl)- amide (**2c**)*. Compound **2c** was obtained as a pale beige solid, m.p. 103–104 °C. ^{1}HNMR ($CDCl_3$, 400 MHz): δ 7.48 (bs, 1H, $-N\underline{H}$), 7.30–7.26 (m, 10*H*, 2x Ar−**H**), 6.76 (bs, 1H, $-N\underline{H}$), 4.43 (d, 2H, J = 5.6 Hz), 3.73 (d, 1H, J = 13.2 Hz), 3.64 (d, 1H, J = 13.2 Hz), 3.30–3.25 (m, 2H), 3.17–3.13 (m, 1H), 2.21–2.18 (m, 2H), 1.84–1.80 (m, 3H), 1.65–1.63 (m, 2H), 1.41–1.25 (m, 1H), 0.91 (d, 3H, J = 6.4 Hz), 0.80 (d, 3H, J = 6.4 Hz); ^{13}CNMR ($CDCl_3$, 100 MHz) δ 175.6, 172.4, 139.4, 138.6, 128.6, 128.6, 128.1, 127.8, 127.7, 127.4, 127.3, 61.0, 53.0, 43.5, 43.1, 38.1, 33.7, 26.4, 25.1, 23.3, 21.7; HR ESIMS: calcd for $C_{24}H_{34}N_3O_2$ $[M+H]^+$ 396.2651, found 396.2652.

*4.1.3. General procedure for synthesis of compounds **3a** and **3b***

The procedure is the same as for the synthesis of **2**, but the phenylacetic acid (1 equiv) was added before addition of the isocyanide.

*4.1.3.1. Ethyl 3-[2-(N-benzyl-2-phenylacetamido)-4-methyl-pentanamido]-propanoate (**3a**)*. Compound **3a** was obtained as a pale yellow oil. ^{1}HNMR ($CDCl_3$, 400 MHz): δ 7.33–7.11 (m, 10*H*, Ar−**H**), 6.80 (bs, 1H, $-N\underline{H}-$), 5.01–4.97 (m, 1H), 4.56 (s, 2H, Ph−$C\underline{H_2}$−C(O)−), 4.15–4.10 (m, 2H, −C(O)−$C\underline{H_2}-CH_3$), 3.61 (s, 2H, Ph−$C\underline{H_2}$−N−), 3.39 (q, 2H, J_1 = 6.8 Hz, J_2 = 6.4 Hz, $-CH_2-C\underline{H_2}$−C(O)−), 2.43–2.39 (m, 2H, −NH−$C\underline{H_2}-CH_2-$), 1.18–1.79 (m, 1H, $CH_2-C\underline{H}-(CH_3)_2$), 1.41–1.40 (m, 2H, −CH−$C\underline{H_2}$−CH−$(CH_3)_2$), 1.24 (t, 3H, J = 7.5 Hz, $-CH_2-C\underline{H_3}$), 0.83–0.78 (m, 6H,$-CH(C\underline{H_3})_2$); ^{13}CNMR ($CDCl_3$, 100 MHz) δ 174.0, 173.3, 171.8, 170.7, 137.6, 134.6, 128.8, 128.6, 127.4, 126.9, 126.0, 60.7, 56.1, 48.5, 41.3, 37.0, 34.9, 34.1, 25.1, 22.7, 22.3, 14.2; HR ESIMS: calcd for $C_{26}H_{34}N_2O_4Na$ $[M+H]^+$ 461.2416, found 461.2413.

*4.1.3.2. Ethyl 5-[2-(N-benzyl2-phenylacetamido)-4-methyl-pentanamido]-pentanoate (**3b**)*. Compound **3b** was obtained as a pale yellow oil. ^{1}HNMR ($CDCl_3$, 400 MHz) δ 7.34–7.10 (m, 10H, Ar−**H**), 6.51 (bs, 1H, $-N\underline{H}-$), 5.01–4.98 (m, 1H), 4.56 (s, 2H, Ph−$C\underline{H_2}$−C(O)−), 4.10–4.09 (m, 2H, −C(O)−$C\underline{H_2}-CH_3$), 3.61 (s, 2H, Ph−$C\underline{H_2}$−N−), 3.11 (q, 2H, J_1 = 6.8 Hz, J_2 = 6.4 Hz, $-CH_2-C\underline{H_2}$−C(O)−), 3.14–3.09 (m, 2H, −NH−$C\underline{H_2}-CH_2-$), 2.48 (t, 2H, C(O)−CH_2-CH_2-), 1.84–1.79 (m, 1H, $CH_2-C\underline{H}-(CH_3)_2$), 1.55–1.53 (m, 2H,−CH−$C\underline{H_2}$−CH−$(CH_3)_2$), 1.41–1.39 (m, 4H, $-CH_2-C\underline{H_2}-C\underline{H_2}-CH_2-$), 1.22 (t, 3H, J = 7.5 Hz, $-CH_2-C\underline{H_3}$), 0.82–0.78 (m, 6H,$-CH(C\underline{H_3})_2$); ^{13}CNMR ($CDCl_3$, 100 MHz) δ 173.5, 173.3, 170.6, 137.6, 128.6, 127.4, 127.0, 125.9, 60.3, 56.3, 48.4, 41.2, 38.8, 36.9, 33.8, 28.9, 25.2, 22.8, 22.3, 22.1, 14.2; HR ESIMS: calcd for $C_{28}H_{38}N_2O_4Na$ $[M+H]^+$ 489.2729, found 489.2713.

*4.1.4. General procedure for synthesis of compounds **4b-c***

2-Methyl-pyrroline (1 equiv.) was added to a suspension of an enzyme (20% weight) in a mixture of toluene/water (20 mL/100 μL), followed by the addition of benzylamine (1 equiv.) and isocyanoester **1** (1 equiv.). The mixture was stirred at room temperature. The enzyme and water were filtered off through a funnel containing Celite and $MgSO_4$. The solvent was then evaporated in vacuum. The product was purified by column chromatography (silica gel, DCM/methanol).

*4.1.4.1. N-[3-(benzylamino)-3-oxopropyl]-2-methylpyrrolidine-2-carboxamide (**4b**)*. Compound **4b** was obtained as a pale yellow oil. ^{1}HNMR ($CDCl_3$, 400 MHz) δ 8.32 (bs, 1H, $-N\underline{H}-$), 7.31–7.22 (m, 6H, Ar−**H**), 6.59 (bs, 1H, $-N\underline{H}-$), 4.40 (d, 2H, J = 5.6 Hz), 3.52–3.46 (m, 2H), 3.08–3.02 (m, 1H), 2.84–2.78 (m,1H), 2.45 (t, 2H, J = 12.4 Hz), 2.32 (bs, 1H,$-N\underline{H}-$), 2.14–2.10 (m, 1H), 1.70–1.67 (m, 4H), 1.33–1.28 (m, 3H, CH_3); ^{13}CNMR ($CDCl_3$, 100 MHz) δ 177.9, 170.9, 138.3, 128.6, 127.8, 127.4, 66.5, 467.0, 43.5, 37.6, 36.4, 35.3, 26.4, 25.7; HR ESIMS: calcd for $C_{16}H_{24}N_3O_2$ $[M+H]^+$ 290.1869, found 290.1872.

*4.1.4.2. N-[4-(benzylamino)-4-oxobutyl]-2-methylpyrrolidine-2-carboxamide (**4c**)*. Compound **4c** was obtained as a pale yellow oil. ^{1}HNMR ($CDCl_3$, 400 MHz):): δ 8.13 (bs,1H, $-N\underline{H}-$), 7.34–7.25 (m, 6H, Ar−**H**), 6.82 (bs, 1H, $-N\underline{H}-$), 4.45 (d, 2H, J = 5.6 Hz), 3.26 (q, 2H, J = 6.4 Hz), 3.09–3.07 (m, 1H), 2.84–2.81 (m, 1H), 2.23–2.18 (m, 3H), 1.87–1.80 (m, 3H), 1.74–1.60 (m, 3H), 1.39 (s, 1H); ^{13}CNMR ($CDCl_3$, 100 MHz) δ 172.7, 138.5, 128.6, 127.8, 127.3, 46.7, 43.6, 38.6, 37.4, 33.8, 29.7, 26.1, 25.9, 25.2; HR ESIMS: calcd for $C_{17}H_{26}N_3O_2$ $[M+H]^+$ 304.2025, found 304.2024.

5. Notes

The authors declare no competing financial interest.

Author contributions

The manuscript was written through contributions of all authors. / All authors have given approval to the final version of the manuscript. / All authors contributed equally.

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Appendix A. Supplementary material

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