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To be cited as: *ChemCatChem* 10.1002/cctc.201700427

Link to VoR: http://dx.doi.org/10.1002/cctc.201700427

A Journal of
ChemPubSoc Europe

WILEY-VCH

www.chemcatchem.org

Enzyme-promoted Asymmetric Tandem Passerini Reaction

Anna Żądło-Dobrowolska, Dominik Koszelewski, Daniel Paprocki, Arleta Madej, Monika Wilk, Ryszard Ostaszewski[a]*

Abstract: A versatile and highly efficient three-step, one-pot, enzyme-promoted Passerini tandem reaction has been developed. The chemoenzymatic sequence involved simultaneous enzyme catalyzed hydrolysis, subsequent Passerini reaction and enzymatic kinetic resolution of the Passerini product was evaluated. This methodology combining the diversity served by multicomponent reactions with the selectivity of biocatalysts resulted in efficient synthesis of the target compounds with excellent enantiomeric excesses up to 99%. With a small set of substrates, large library of complex molecules was obtained within a short time using developed procedure.

Introduction

With the rise of biocatalysis, scientists inspired by nature, have designed multi-enzymatic cascades to mimic living organisms in building complex molecules from simple starting materials.[1] A lot of attempts have been made to reproduce such biomimetic-type processes *in vitro*.[2] Consequently, numerous multi-enzymatic cascades have been developed, ranging from the simple combination of oxidoreductases with a suitable cofactor regeneration system[3] to highly complex and specialized synthetic routes.[4] Since cascades represent a very promising approach, mainly due to the avoidance of purification steps and reduction of both waste and production costs, they are more readily employed by organic chemists.[5]

Compared with a cascade, where unstable intermediate is generated, multi-step syntheses, which could be carried out separately, is called tandem catalysis.[6] One of the most common challenges for its practicability is the combination of catalysts from different disciplines (biocatalysis, metal catalysis, or organocatalysis) with multicomponent reactions (MCRs).[7] To the best of our knowledge, literature recognized tandem processes are limited only to two sequential reactions what makes them inefficient in mimicking of the natural metabolic pathways.[8]

MCRs involve condensation of more than two reactants in the same pot to form a final product containing portions derived from each of the reacting molecules, ideally all atoms.[9] In this way, very high level of atom economy, efficiency and molecular diversity could be achieved in a simple one-pot fashion, avoiding time-consuming isolation and purification of intermediates.[10] Over the past two decades the field of MCRs has incredibly grown with the discovery and development of the new variations of classical MCRs.[11] Isocyanide-based MCRs, such as Ugi and Passerini reactions, have been of particular interest because of the large number of applications in medicinal chemistry and drug discovery,[12] combinatorial chemistry,[13] polymer chemistry,[14] natural product synthesis,[15] agrochemistry.[16] The Passerini three component reaction (P-3CR) involves the condensation of a carbonyl compound, a carboxylic acid and an isocyanide leading to an α-acyloxy carboxamide.[17] When carbonyl substrate different from formaldehyde is used, a new stereogenic center is generated. However P-3CR has been widely applied, the challenge of stereocontrol on a newly generated stereogenic center is currently very limited. Number of diastereoselective examples are known that use chiral auxiliaries or chiral substrates,[18] but only a limited success has been recorded in the field of enantioselective approach.[19] Several problems must be addressed, *e.g.* the majority of catalysts, especially metal complexes are toxic and expensive and the products require complex purification procedures. They cannot be used in the pharmaceutical and cosmetic industry, due to pharmacopoeia limits of heavy metal contaminations (below 5 ppm). Moreover, Lewis-acid-catalyzed P-3-CR could go through both the imidate and the epoxide intermediate providing two enantiomers of the α-acyloxy carboxamide, which led to the erosion in enantiomeric excess of the products.[20]

As an alternative to the commonly employed metalo-catalysis or organo-catalysis, a biocatalytic approach can be expected to be beneficial due to high selectivity of enzymes and mild reaction conditions, thereby fulfilling the general principles of green chemistry. The aim of the present work was to combine the selectivity of biocatalysts with the diversity offered by a Passerini reaction to obtain enantiomerically pure α-hydoxy carboxamide scaffolds. To address this challenge we performed studies toward coupling three reaction steps, involving enzyme catalyzed hydrolysis, subsequent Passerini reaction and enzymatic kinetic resolution (EKR), into a one-pot tandem process. Synthetically valuable α-hydroxy carboxamides, which could be easily transformed into the optically active natural and non-natural amino acids, were chosen as the target compounds.

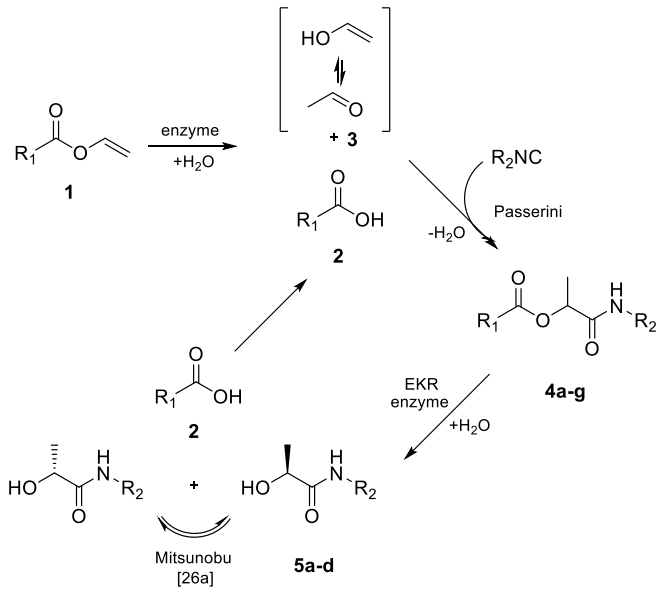
Results and Discussion

Biocatalytic retrosynthetic analysis suggested that the α-hydroxy carboxamide skeleton **5** could be prepared from the corresponding vinyl esters **1** *via* a sequence involving enzyme-catalyzed hydrolysis, Passerini reaction and EKR (Scheme 1). Designing our synthetic route we took into consideration the requirements assigned to multicatalyst asymmetric tandem reactions, such as possibility of coupling the wide spectrum of

[a] Dr A. Żądło-Dobrowolska, Dr D. Koszelewski, M. Sc. D. Paprocki, M. Sc. A. Madej, M. Sc. M. Wilk, Prof. R. Ostaszewski
Institute of Organic Chemistry
Polish Academy of Sciences
Kasprzaka 44/52, 01-224 Warsaw, Poland
E-mail: ryszard.ostaszewski@icho.edu.pl

Supporting information available: Experimental procedures, ^{1}H NMR, ^{13}C NMR spectra and chiral HPLC tracers.

substrates and reactions, catalysts compatibility and stereochemical control.[21]



4a R_1=Me; R_2=*p*-MeOBn
4b R_1=Et; R_2=*p*-MeOBn
4c R_1=Ph; R_2=*p*-MeOBn
4d R_1=$CH_3(CH2)_{10}$; R_2=*p*-MeOBn
4e R_1=Me; R_2=Bn
4f R_1=Me; R_2=*c*-Hex
4g R_1=Me; R_2=Hex

5a R_2=*p*-MeOBn
5b R_2=Bn
5c R_2=*c*-Hex
5d R_2=Hex

Scheme 1. Envisioned cascade process.

It is noteworthy that envisioned tandem process is a very complicated system consisting of 3 reaction steps. In the first step, enzyme catalyzes hydrolysis of vinyl ester **1**, what should lead to the corresponding carboxylic acid **2** and vinyl alcohol, which spontaneously tautomerises to acetaldehyde **3**,[22] Scheme 1. Vinyl esters are commonly used as acylating agents in enzymatic transformations.[23] In this step, water acts as a nucleophile and is compulsory for the initiation of enzymatic hydrolysis. Second step involves the condensation of previously generated carbonyl compound **3** and carboxylic acid **2** with an isocyanide present in the reaction mixture, leading to an α-acyloxy carboxamide **4**.[24] Finally, the third reaction step was designed based on previous studies, including pre- and post-modifications of MCR products.[25] According to previously reported protocol, in which hydrolytic enzymes were employed for the kinetic resolution of α-acyloxy carboxamides,[26] compound **4** may be enzymatically hydrolyzed releasing enantiomerically enriched α-hydroxy carboxamide **5** and carboxylic acid **2**. Although enzymatic kinetic resolution is always limited to a maximum yield of 50% of a single enantiomer, a careful selection of the enzyme should result in both enantimerically pure compounds **4** and **5**. If only one enantiomer of Passerini product is needed, compound **5** may be easily transformed with the inversion of configuration by Mitsunobu reaction.[26a] Moreover, the generation of carboxylic acid **2**, which can repeatedly react as a substrate, provides a possibility of self-regulation.

We started our investigation by performing simple transformations. At first, we investigated Passerini reaction in a phosphate buffer pH 7.4. As expected, the condensation of acetic acid, acetaldehyde and *p*-methoxybenzyl isocyanide afforded the desired compound **4a** with good yield (45%, Table 1, entry 1) after 24 hours. We also observed a small amount of a subsequent spontaneous hydrolysis product **5a**. Next, we have examined enzymatic kinetic resolution of racemic compound **4a** in a phosphate buffer pH 7.4. With the application of *Pseudomonas cepacia* lipase (PcL), enantiomerically enriched product (*S*)-**5a** was isolated with 43% yield and 90% *ee* (Table 1 entry 2).

Table 1. Enzyme screening.

Entry	Enzyme	*c* [%]	*ee* **4a** [%]	yield **5a** [%]	*ee* **5a** [%]	*E*
1	-	45[a]	nd	<5[a]	nd	nd
2	PcL [b]	nd	68	43	90	39
3	PcL	50	70	82	20	3
4	Novozym 435	50	25	56	20	2
5	Lipozym	44	*rac*	61	*rac*	1
6	PfL	47	44	77	15	2
7	CrL	47	*rac*	64	*rac*	1
8	WGL	33	25	73	26	2
9	PLE	28	5	61	3	1
10	PPL	32	*rac*	75	*rac*	1
11	CaL	48	38	79	13	2

Conditions: A reaction mixture containing vinyl acetate (0.5 mmol), *p*-methoxybenzyl isocyanide (0.5 mmol) and enzyme (20% by weight) in 20 mL of phosphate buffer (100 mM, pH 7.4) was stirred for 24 hours at rt. After this time two products **4a** and **5a** were isolated by column chromatography; Conversion refers to Passerini product **4a**, calculated as $((n_{4a}+n_{5a})/n_1)\cdot 100\%$; Yield **5a** refers to isolated product after enzymatic kinetic resolution, calculated as $((n_{5a})/n_{4a}+n_{5a})\cdot 100\%$; nd – non defined; [a] acetic acid (0.5 mmol) and acetaldehyde (0.5 mmol) were used instead of vinyl acetate; [b] Racemic **4a** was used as a starting material.

Then, to initially examine the feasibility of the envisaged tandem process, vinyl acetate and *p*-methoxybenzyl isocyanide were used as model reactants for the screening of a suitable biocatalyst. In the classical procedure highly volatile (boiling point 20 °C) and irritant acetaldehyde is required. Our elegant protocol engages vinyl acetate (boiling point 72 °C) which is in situ hydrolyzed by enzyme providing both substrates for P-3-CR;

aldehyde and carboxylic acid. It is noteworthy that reaction without enzyme did not run. Nine, various enzymes were investigated in a three-step one-pot tandem reaction; *Pseudomonas cepacia* lipase (PcL), *Pseudomonas fluorescens* lipase (PfL), Novozym 435, Lipozym (immobilized lipase from *Mucor miehei*) (MmL), *Candida rugose* lipase (CrL), wheat germ lipase (WGL), porcine liver esterase (PLE), porcine pancreas lipase (PPL) and *Candida antarctica* lipase (CaL). On the basis of our hypothesis, acetic acid and acetaldehyde formed during enzymatic hydrolysis of vinyl acetate, should smoothly react with isocyanide present in the reaction mixture. Results shown in Table 1, named as yield **5a**, were calculated only for EKR step.

To our delight, all examined biocatalysts were active in the first hydrolysis step (Table 1, entries 3-11) leading to the desired acetic acid and acetaldehyde, which immediately formed product **4a** with moderate to good yields (23-50%). Under the examined conditions, α-acyloxy carboxamides **4a** underwent subsequent enzyme catalyzed hydrolysis providing enantiomerically enriched product (*S*)-**5a**. Finally, *Pseudomonas cepacia* lipase was found to be the best biocatalyst regarding the enantioselectivity (*E*) and the yield (Table 1, entry 3) and was consequently used in further experiments. Enantioselectivity values were calculated according to Chen *et al.* equation.[27] It is important to notice that the same enzyme maintained first hydrolysis step and catalyzes enantioselectively the EKR, what solves the compatibility problem.

Comparison of the amount of product (*S*)-**5a** obtained for a whole tandem reaction, starting from **1** (82% yield, 19% *ee*, Table 1, entry 3) with a simple transformation, starting from racemic **4a** (43% yield, 90% *ee*, see Table 1, entry 2), revealed that formation of acetic acid shifts the reaction equilibrium toward products. However, such acceleration by acetic acid influenced negatively on enantioselectivity. Moreover, acetic acid formed during second hydrolysis step might react again as a substrate in the P-3-CR, what resulted in 5% increase in conversion (Table 1, entry 1 vs 3 and 4). This phenomena based on acceleration of the Passerini reaction by the excess of carboxylic acid is known.[28] The examined tandem process may mimic metabolic pathways, which must be finely regulated to maintain a constant homeostasis. In intrinsic regulation, the metabolic pathway self-regulates to respond to changes in the levels of substrates or products.[29] In our example, the overall sequence is regulated by the amount of acetic acid. This type of regulation remains out of reach in typical chemical tandems performed *in vitro*.

Due to the high complexity of the studied tandem process further optimization studies were undertake. Up to date, many enzymes were shown to be catalytically active in a variety of organic solvents.[30] Their catalytic efficiency and enantioselectivity in mixed aqueous-organic media are often higher than in aqueous media alone.[31] Moreover, it is known that Passerini reaction proceeds more productively in organic solvents than in aqueous solutions.[25] For those reasons, we decided to examine the influence of the organic solvent on the reaction efficiency, Table 2.

A small amount of water (0.5% by volume) was crucial for enzymatic hydrolysis, as well as for the stabilization of the tertiary structure of proteins.[32] As shown in Table 2, Passerini reaction does not proceed in ethers (Table 2, entries 5-6) and proceeds poorly without solvent (Table 2, entry 7). Among the screened solvents, dichloromethane (Table 2, entry 3) was found to be the most suitable for the examined tandem reaction leading to the product (*S*)-**5a** with 94% enantiomeric excess. Reaction performed in toluene or hexane resulted in higher yields but much lower enantioselectivities (Table 2, entries 2 and 4).

Table 2. Solvent influence on the enzyme-driven Passerini reaction.

Entry	Solvent	Conversion [%]	*ee* **4a** [%]	Yield **5a** [%]	*ee* **5a** [%]	*E*
1	PBS	50	70	82	20	3
2	Toluene	41	36	24	14	1
3	DCM	44	*rac*	5	94	34
4	Hexane	38	5	13	32	2
5	Et_2O	0	nd	0	nd	nd
6	TBME	0	nd	0	nd	nd
7	none	73	*rac*	7	13	1

Conditions: A reaction mixture containing vinyl acetate (0.5 mmol), *p*-methoxybenzyl isocyanide (0.5 mmol) in 20 mL of organic solvent (0.5% v/v), and the enzyme (20% by weight) was stirred for 96 hours at rt. After this time two products **4a** and **5a** were isolated by column chromatography; Conversion refers to Passerini product **4a**, calculated as $((n_{4a}+n_{5a})/n_1)\cdot 100\%$; Yield **5a** refers to isolated product after enzymatic kinetic resolution, calculated as $((n_{5a})/n_{4a}+n_{5a})\cdot 100\%$; nd – non defined.

One of the major drawbacks in the implementation of one-pot reactions is the selection of the optimal operating conditions. When multiple reactions are run in the same pot, optimal conditions for each individual reaction are not always identical. To solve this problem, the optimization of the overall tandem was performed. The results of a survey of reaction conditions using *Pseudomonas cepacia* lipase as a catalyst and dichloromethane (DCM) as a solvent are summarized in Table 3.

Further investigations revealed that increase of the amount of isocyanide had a beneficial influence on the results. It was proved that isocyanide undergoes slow spontaneous hydrolysis in the presence of water.[26] Finally, increasing the molecular ratio of isocyanide to vinyl acetate (3:1, n/n) resulted in the product yield improvement from 5% to 21% with the simultaneous increase of the enantiomeric excess (Table 3, entry 3).

Next, we turned our attention to the optimization of the water content. It is noteworthy that 2 equivalents of water act as a nucleophile in the first and third reaction step and 1 equivalent is produced in the second step. The best result with 21% yield and >99% *ee* was achieved when 0.5% of water by volume was added into the reaction (Table 3, entry 3). Decreasing the

amount of water resulted in lower efficiency (Table 3, entry 4), while increasing the amount of water resulted in decreasing of enantiomeric excesses to 80% (Table 3, entries 6-8).

Table 3. Reaction optimization.

Entry	Water content [%]	Vinyl acetate [equiv.]	Isocyanide [equiv.]	*c* [%]	Yield (*S*)-**5a** [%]	*ee* (*S*)-**5a** [%]	*E*
1	0.5	1	1	44	5	94	34
2	0.5	3	1	79	5	91	22
3	0.5	1	3	80	21	>99	>200
4	0.2	1	3	39	5	>99	>200
5	1	1	3	64	14	>99	>200
6	2	1	3	55	11	98	112
7	5	1	3	44	14	99	>200
8	10	1	3	63	6	80	9

Conditions: A reaction mixture containing (0.5 or 1.5 mmol) vinyl acetate, (0.5 or 1.5 mmol) 4-methoxybenzyl isocyanide, in 20 mL of dichloromethane, 0.2-10% of water by volume, and the enzyme (20% by weight) was stirred for 96 hours at rt. After this time two products were isolated by column chromatography; Conversion refers to Passerini product, calculated as $((n_{4a}+n_{5a})/n_1)\cdot 100\%$; Yield **5a** refers to product after enzymatic kinetic resolution, calculated as $((n_{5a})/n_{4a}+n_{5a})\cdot 100\%$.

With the optimal conditions in hand, the generality of the reaction was further investigated using a set of vinyl esters and isocyanides. Since *Pseudomonas cepacia* lipase did not accept all employed compounds as substrates, Novozym 435 was also examined as a catalyst in the studied tandem reaction. It is well known that enzymes are highly selective and for each mixture of substrates the other biocatalyst might be suitable.[33]

Elaborated protocol is applicable to a wide range of vinyl esters and isocyanides, providing enantiopure α-hydroxy carboxamides, which are valuable building blocks for the peptide and peptidomimetics synthesis.[26] Application of our protocol enables to obtain the final product with good yield (up to 50%) and excellent enantiomeric excess (up to >99% *ee*) within 4 days at room temperature. Unsubstituted benzyl isocyanides and isocyanides bearing electron donating groups in the *para*-position were efficiently transformed by both enzymes with up to 50% conversion and high enantioselectivity (E>200) towards the enantiomerically pure products (*S*)-**5a** and (*S*)-**5b** (Table 4, entries 1,4,6 and 8). In order to assign the configuration of the obtained products, compounds (*S*)-**5a**-**c** were synthesized from commercially available, optically pure *L*-lactic acid, by conversion into the *S*-configured α-hydroxy carboxamides **5a**-**c** (supplementary data). We would like to emphasize that benzyl isocyanides can be replaced by cyclohexyl and hexyl isocyanide producing the corresponding products (*S*)-**5c** and **5d** in moderate yields (table 4, entries 9 and 10). The selectivity of the reaction decreased when benzyl isocyanide was exchanged for aliphatic isocyanide (Table 4, entries 8 vs 10), however aliphatic vinyl esters were better enzyme substrates than aromatic esters (Table 4, entries 1-4 vs 5). Application of vinyl acetate led to the product (*S*)-**5a** in 21% yield (Table 4, entry 1), whereas the elongation of the alkyl chain resulted in a lower yield, 5% for vinyl propionate (Table 4, entry 3-4) and 9% for vinyl laurate (Table 4, entry 6). Due to the high selectivity of the enzyme, in some cases, the third step of the tandem reaction was catalyzed by *Pseudomonas cepacia* lipase (Table 4, entries 5 and 10) or by Novozym 435, exclusively (Table 4, entries 6 and 9). This observation proves that careful selection of the enzyme is necessary whenever the structure of substrate is changed.

Table 4. Scope of limitations.

Entry	R_1	R_2	Product	Enzyme	Conversion [%]	Yield (*S*)-**5** [%]	*ee* (*S*)-**5** [%]	*E*
1	Me	*p*-MeOBn	**5a**	A	80	21	>99	>200
2	Me	*p*-MeOBn	**5a**	B	27	26	90	26
3	Et	*p*-MeOBn	**5a**	A	66	5	98	104
4	Et	*p*-MeOBn	**5a**	B	68	20	>99	>200
5	Ph	*p*-MeOBn	**5a**	A	13	15	92	28
6	$CH_3(CH_2)_{10}$	*p*-MeOBn	**5a**	B	65	9	99	>200
7	Me	Bn	**5e**	A	64	19	>99	>200
8	Me	Bn	**5e**	B	22	50	97	>200
9	Me	*c*-Hex	**5f**	B	21	33	7	1
10	Me	Hex	**5g**	A	58	5	nd	nd

Conditions: A reaction mixture containing vinyl ester (0.5 mmol), *p*-methoxybenzyl isocyanide (1.5 mmol) in 20 mL of dichloromethane, water (0.5% v/v), and the enzyme (20% by weight) was stirred for 96 hours in rt. After this time two products **4** and **5** were isolated by column chromatography; Conversion refers to

Passerini product **4**, calculated as $((n_{4a}+n_{5a})/n_1)\cdot100\%$; Yield **5a** refers to isolated product after enzymatic kinetic resolution, calculated as $((n_{5a})/n_{4a}+n_{5a})\cdot100\%$; enzyme A refers to *Pseudomonas cepacia* lipase.; B refers to Novozym 435.

Regardless of the substrates **4** structure, *S*-enatiomer of the corresponding α-hydroxy carboxamides **5a-c** was gained. Obtained results open a new field in enzyme-promoted multicomponent reactions. Further studies on the optimization and amplification of the chiral vinyl esters leading to products with two stereogenic centers are still underway. In spite of the described limitations the proposed approach diversifies already known methods and leads to the enantiomerically pure products.

Conclusions

In summary, we have developed a new, efficient, three-step one-pot tandem reaction. A wide range of aromatic and aliphatic vinyl esters were tolerated providing the corresponding α-hydroxy carboxamides in high yields and excellent enantiomeric excess (up to 99%). Access to enantiomerically pure products has been achieved using a chemoenzymatic sequence involving simultaneous enzyme catalyzed hydrolysis, subsequent Passerini reaction and enzymatic kinetic resolution of a Passerini reaction product. Since developed protocol requires only one enzyme, which catalyzes both hydrolysis steps, the reaction was run as a one-pot three-step process. To the best of our knowledge, this is the first chemoenzymatic tandem process, consisting of more than two reactions, which may be performed in organic solvents, as well as in water, without isolating the intermediates.

Experimental Section

General Methods. ^{1}H- and ^{13}C-NMR spectra were recorded in $CDCl_3$ solution. Chemical shifts are expressed in parts per million using TMS as an internal standard. TLC was done on Kieselgel 60 F254 aluminum sheets. All chemicals were commercial products of analytical grade. Commercial enzymes were purchased from Sigma–Aldrich and Novozymes.

General Screening Procedure in Phosphate Buffer. A vinyl ester (0.5 mmol) was added to the suspension of an enzyme (40 mg) in 20 mL of phosphate buffer (pH 7.4), followed by addition of an isocyanide (0.5 mmol). The mixture was stirred for 24 hours at room temperature. Reaction progress was controlled by TLC using hexanes:ethyl acetate (3:7, v/v) as an eluent. The mixture was extracted with ethyl acetate, organic layer was separated and dried with $MgSO_4$. Then solvent was evaporated in vacuum. The product was purified by column chromatography (silica gel, ethyl acetate/hexane).

General Screening Procedure in Organic Solvents. A vinyl ester (0.5 mmol) was added to the suspension of an enzyme (40 mg) in 20 mL of organic solvent, followed by addition of an isocyanide (1.5 mmol) and water (1% v/v). The mixture was stirred for 4 days at room temperature. Reaction progress was controlled by TLC using ethyl acetate/hexane (3:7, v/v) as an eluent. The enzyme and water was then filtered off on the funnel filled with Celite and $MgSO_4$. Then solvent was evaporated in vacuum. Crude product **5** was purified by column chromatography (ethyl acetate/hexane). The ^{1}H NMR data were in accordance with those recorded for racemates; (*S*)-(-)-**5a**: $[\alpha]_D$= -61.2 (c 1.0 $CHCl_3$, >99% ee), (*S*)-(-)-**5b**: $[\alpha]_D$= -29.8 (c 1.0 $CHCl_3$, >99% ee), (*S*)-(-)-**5c**: $[\alpha]_D$= -11.4 (c 1.0 $CHCl_3$, >99% ee).

1[(4-Methoxybenzyl)amino]-1-oxopropan-2-yl acetate (4a)

White crystals; m.p. 122-123 °C; ^{1}H-NMR ($CDCl_3$, 400 MHz, ppm) δ 7.22-7.12 (m, 2H, Ar-H), 6.98-6.84 (m, 2H, Ar-H), 6.40 (br. s., 1H, -CONH-), 5.22 (q, 1H, *J*=6.9 Hz, -OC<u>H</u>(CO)CH_3-), 4.39 (d, 2H, *J*=5.6 Hz, -NHC<u>H_2</u>Ar-), 3.79 (s, 3H, -OC<u>H_3</u>), 2.10 (s, 3H, -COC<u>H_3</u>), 1.48 (d, 3H, *J*=6.9 Hz, -CHC<u>H_3</u>); ^{13}C-NMR ($CDCl_3$, 100 MHz, ppm) δ 170.2, 169.5, 159.1, 129.9, 129.0, 114.2, 70.7, 55.3, 42.7, 21.1, 17.9; HR-MS (ESI, [M + H^+] calcd for: $C_{13}H_{17}NO_4Na$, 274.1055, found, 274.1047; TLC: R_f = 0.45 (ethyl acetate/hexanes, 7:3 v/v).

2-Hydroxy-*N*-(4-methoxybenzyl)propanamide (5a)

White crystals; m.p. 78-79 °C; ^{1}H-NMR ($CDCl_3$, 400 MHz, ppm) δ 7.23-7.13 (m, 2H, Ar-H), 6.97-6.80 (m, 3H, Ar-H, -CONH-), 4.43-4.34 (m, 2H, -NHC<u>H_2</u>Ar-), 4.28-4.19 (m, 1H, -HOC<u>H</u>(CO)CH_3-), 3.80-3.76 (s, 3H, -OC<u>H_3</u>), 2.76 (br.s., 1H, -O<u>H</u>), 1.43 (d, 3H, *J*=6.9 Hz, -CHC<u>H_3</u>); ^{13}C-NMR ($CDCl_3$, 100 MHz, ppm) δ 170.2, 169.5, 159.1, 129.9, 129.0, 114.2, 70.7, 55.3, 42.7, 21.1, 17.9; HR-MS (ESI, [M + H^+] calcd for: $C_{11}H_{15}NO_3Na$, 232.0950, found, 232.0940; TLC: R_f = 0.15 (ethyl acetate/hexane, 7:3 v/v). 2(*S*)-hydroxy-*N*-(4-methoxybenzyl)propanamide ((*S*)-**5a**) was prepared from *L*-lactic acid (supplementary data); $[\alpha]_D$= -58.2 (c 1.0 $CHCl_3$), HPLC: Chiralcel OD-H; hexane/isopropanol (80:20), λ = 225 nm; 1.0 mL/min, retention time of the racemic compound (in min): t_R (*R*)= 9.6, t_R (*S*)= 11.9.

1[(4-Methoxybenzyl)amino]-1-oxopropan-2-yl propanoate (4b)

Yellow oil; ^{1}H-NMR ($CDCl_3$, 400 MHz, ppm) δ 7.19 (d, 2H, *J*=8.4 Hz, Ar-H), 6.86 (d, 2H, *J*=8.8 Hz, Ar-H), 6.31 (br. s., 1H, -CONHC<u>H_2</u>-), 5.24 (q, 1H, *J*=6.9 Hz, -OC<u>H</u>(CO)CH_3-), 4.39 (d, 2H, *J*=5.6 Hz, -NHC<u>H_2</u>Ar), 3.79 (s, 3H, -OCH_3), 2.36 (q, 2H, *J*=7.6 Hz, CH_3C<u>H_2</u>CO-), 1.48 (d, 3H, *J*=6.9 Hz, C<u>H_3</u>CH-), 1.13 (t, 3H, *J*=7.6 Hz); ^{13}C-NMR ($CDCl_3$, 100 MHz, ppm) δ 172.9, 170.3, 159.2, 129.9, 129.0, 114.2, 70.5, 55.3, 42.7, 27.6, 17.9, 9.0; HR-MS (ESI, [M + H^+] calcd for: $C_{14}H_{19}NO_4Na$, 288.1212, found, 288.1209; R_f = 0.58 (ethyl acetate/hexane, 7:3 v/v).

1[(4-Methoxybenzyl)amino]-1-oxopropan-2-yl benzoate (4c)

White crystals; m.p. 143 °C; ^{1}H-NMR ($CDCl_3$, 400 MHz, ppm) δ 8.12-7.99 (m, 2H, Ar-H), 7.69-7.52 (m, 1H, Ar-H), 7.52-7.40 (m, 2H, Ar-H), 7.29-7.16 (m, 2H, Ar-H), 6.94-6.81 (m, 2H, Ar-H), 6.38 (br. s., 1H, -CONH-), 5.51 (q, 1H, *J*=6.9 Hz, -OC<u>H</u>(CO)CH_3-), 4.42 (dd, 2H, J_1=5.9 Hz, J_2=3.2 Hz -NHC<u>H_2</u>Ar-), 3.78 (s, 3H, -OC<u>H_3</u>), 1.63 (d, 3H, *J*=6.9 Hz, -COC<u>H_3</u>); ^{13}C-NMR ($CDCl_3$, 100 MHz, ppm) δ 170.2, 165.3, 133.6, 129.9, 129.7, 129.4, 128.9, 128.6, 114.2, 71.1, 55.3, 42.7, 17.9; HR-MS (ESI, [M + H^+] calcd for: $C_{18}H_{19}NO_4Na$, 336.1212, found, 336.1205; R_f = 0.65 (ethyl acetate/hexane, 7:3 v/v).

1[(4-Methoxybenzyl)amino]-1-oxopropan-2-yl laurate (4d)

White crystals; m.p. 98-99 °C; ^{1}H-NMR ($CDCl_3$, 400 MHz, ppm) δ 7.25-7.19 (m, 2H, Ar-H), 7.04-7.87 (m, 2H, Ar-H), 6.33 (br. s., 1H, -CONH-),

5.27 (q, 1H, *J*=6.9 Hz, -OCH(CO)CH_3-), 4.42 (d, 2H, *J*=5.6 Hz, -NHCH_2Ar-), 3.82 (s, 3H, -OCH_3), 2.35 (t, 2H, *J*=7.6 Hz, -COC$H_2$$CH_2$-), 1.69-1.56 (m, 2H), 1.51 (d, 3H, *J*=6.9 Hz, -COCH_3), 1.34-1.25 (m, 16H), 0.95-0.85 (m, 3H, -CH_2CH_3); ^{13}C-NMR ($CDCl_3$, 100 MHz, ppm) δ 172.3, 170.3, 159.2,129.9, 129.0, 114.2, 70.4, 55.3, 42.7, 34.3, 31.9, 29.6, 29.4, 29.3, 29.2, 29.1, 24.9, 22.7, 17.9, 14.1; HR-MS (ESI, [M + H^+] calcd for: $C_{23}H_{37}NO_4Na$, 414.2620, found, 414.2621; TLC: R_f = 0.70 (ethyl acetate/hexane, 7:3 v/v).

1-(Benzylamino)-1-oxopropan-2-yl acetate (4e)

White crystals; m.p. 118-119 °C; ^{1}H-NMR ($CDCl_3$, 400 MHz, ppm) δ 7.34-7.17 (m, 5H, Ar-H), 6.30 (br. s., 1H, -CONH-), 5.18 (q, 1H, *J*=6.7 Hz, -OCH(CO)CH_3-), 4.41 (dd, 2H, J_1=5.9 Hz, J_2=2.0 Hz, -NHCH_2Ar-), 2.10-1.98 (m, 3H, -COCH_3), 1.44 (d, 3H, *J*=6.9 Hz, -CHCH_3); ^{13}C-NMR ($CDCl_3$, 100 MHz, ppm) δ 170.3, 169.4, 137.9, 128.8, 127.7, 127.6, 70.7, 43.2, 21.1, 17.9; HR-MS (ESI, [M + H^+] calcd for: $C_{12}H_{15}NO_3Na$, 244.0950, found, 244.0948; TLC: R_f = 0.52 (ethyl acetate/hexane, 7:3 v/v).

***N*-Benzyl-2-hydroxypropanamide (5b)**

Colorless oil; ^{1}H-NMR ($CDCl_3$, 400 MHz, ppm) δ 7.40-7.18 (m, 5H, Ar-H), 6.69 (br. s., 1H, -CONHCH_2-), 4.46 (d, 2H, *J*=5.9 Hz, -NHCH_2Ar-), 4.27 (q, 1H, *J*=6.8 Hz, -HOCH(CO)CH_3-), 3.23 (br. s., 1H), 1.55-1.40 (m, 3H, *J*=6.9 Hz, -CHCH_3); ^{13}C-NMR ($CDCl_3$, 100 MHz, ppm) δ 174.5, 137.9, 128.7, 127.7, 127.6, 68.5, 43.1, 21.3; TLC: R_f = 0.30 (ethyl acetate/hexane, 7:3 v/v); 2(*S*)-hydroxy-*N*-(benzyl)propanamide ((*S*)-**5b**) was prepared from *L*-lactic acid (supplementary data); $[\alpha]_D$= -28.5 (c 1.0 $CHCl_3$), HPLC: Chiralcel IA; hexane/isopropanol (95:5), λ = 210 nm; 1.0 mL/min, retention time of the racemic compound (in min): t_R (*S*)= 18.5, t_R (*R*)= 21.7.

1-(Cyclohexylamino)-1-oxopropan-2-yl acetate (4f)

Colorless oil; ^{1}H-NMR ($CDCl_3$, 400 MHz, ppm) δ 5.90 (br. s., 1H, -CONH-), 5.15 (q, 1H, *J*=6.9 Hz, -OCH(CO)CH_3-), 3.94-3.69 (m, 1H,-NHCH$(CH_2)_2$-), 2.26-2.05 (s, 3H, -CHCH_3), 1.91 (td, 2H, J_1=4.1 Hz, J_2=2.1 Hz) 1.76-1.53 (m, 4H) 1.49-1.41 (d, 3H, *J*=6.8 Hz), 1.40-1.29 (m, 2H), 1.20-1.12 (m, 2H); ^{13}C-NMR ($CDCl_3$, 100 MHz, ppm) δ 169.4, 169.3, 70.7, 47.9, 33.0, 25.5, 24.8, 21.1, 17.9; HR-MS (ESI, [M + H^+] calcd for: $C_{11}H_{19}NO_3Na$, 236.1263, found, 236.1257; TLC: R_f = 0.54 (ethyl acetate/hexane, 7:3 v/v).

***N*-Cyklohexyl-2-hydroxypropanamide (5c)**

Colorless oil; ^{1}H-NMR ($CDCl_3$, 400 MHz, ppm) δ 6.52 (br. s., 1H, -CONHCH_2-), 4.16 (m, 1H, J=6.4 Hz, HOCH(CO)CH_3-), 3.81-3.58 (m, 1H,-CH_2CH(NH)CH_2-), 3.47 (br. s., 1H, -OH), 1.89 (d, 2H), 1.77-1.65 (m, 2H), 1.65-1.54 (m, 1H), 1.43-1.29 (m, 5H), 1.23-1.07 (m, 3H); ^{13}C-NMR ($CDCl_3$, 100 MHz, ppm) δ 173.7, 68.3, 47.8, 33.0, 25.5, 24.8, 21.3; HR-MS (ESI, [M + H^+] calcd for: $C_9H_{17}NO_2Na$, 194.1157, found, 194.1152; TLC: R_f = 0.25 (ethyl acetate/hexanes, 7:3 v/v). *N*-cyklohexyl-2(*S*)-hydroxypropanamide ((*S*)-**5c**) was prepared from *L*-lactic acid (supplementary data); $[\alpha]_D$= -152.5 (c 1.0 $CHCl_3$), HPLC: Chiralcel IA; hexane/isopropanol (90:10), λ = 210 nm; 1.0 mL/min, retention time of the racemic compound (in min): t_R (*S*)= 6.0, t_R (*R*)= 8.0.

1-(Hexylamino)-1-oxopropan-2-yl acetate (4g)

Colorless oil; ^{1}H-NMR ($CDCl_3$, 400 MHz, ppm) δ 6.10 (br. s., 1H, -CONH-), 5.16 (q, 1H, *J*=6.8 Hz, -OCH(CO)CH_3-), 3.29-3.20 (m, 2H), 2.12 (s, 3H, -COCH_3), 1.61-1.47 (m, 2H), 1.44 (d, 3H, *J*=6.8 Hz), 1.35-1.23 (m, 6H), 0.87 (t, 3H, *J*=6.8 Hz) ; ^{13}C-NMR ($CDCl_3$, 100 MHz, ppm) δ170.2, 169.4, 70.7, 39.3, 31.4, 29.5, 26.5, 22.5, 21.1, 17.9, 13.9; HR-MS (ESI, [M + H^+] calcd for: $C_{11}H_{21}NO_3Na$, 238.1419, found, 238.1416; TLC: R_f = 0.63 (ethyl acetate/hexane, 3:7 v/v).

***N*-Hexyl-2-hydroxypropanamide (5d)**

Colourless oil; ^{1}H-NMR ($CDCl_3$, 400 MHz, ppm) δ 6.68 (br. s., 1H, -CONHCH_2-), 4.18 (q, 1H, J=6.9 Hz, -HOCH(CO)CH_3-), 3.58 (br. s., 1H, -OH), 3.37-3.16 (m, 2H, -NHC$H_2$$CH_2$-), 1.53-1.45 (m, 2H), 1.44-1.36 (d, 3H, *J*=6.8 Hz, -CHCH_3), 1.35-1.19 (m, 6H), 0.97-0.78 (m, 3H, -CH_2CH_3); ^{13}C-NMR ($CDCl_3$, 100 MHz, ppm) δ 174.7,68.3, 39.2, 31.4, 29.5, 26.5, 22.5, 21.3, 14.0; HR-MS (ESI, [M + H^+] calcd for: $C_9H_{19}NO_2Na$, 196.1313, found, 196.1310; TLC: R_f = 0.30 (hexane/ethyl acetate, 3:7 v/v).

Acknowledgements

This work was supported by the Polish National Science Center project Nos. 2014/14/M/ST5/00030.

Keywords: tandem • Passerini reaction • enantioselective • multicomponent reaction • enzymatic kinetic resolution

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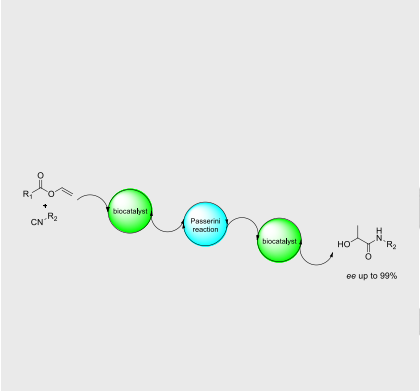
Access to enantiomerically pure α-hydroxy carboxamides has been achived using a chemoenzymatic tandem reaction involving enzyme catalyzed hydrolysis, subsequent Passerini reaction and enzymatic kinetic resolution. Reported protocol may be applied for a broad substrate scope, including various aromatic and aliphatic carboxylic vinyl esters and isocyanides.



*Anna Żądło-Dobrowolska, Dominik Koszelewski, Daniel Paprocki, Arleta Madej, Monika Wilk, Ryszard Ostaszewski**

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