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Environmental Friendly Approach to α-Acyloxy carboxamides via a Chemoenzymatic Cascade

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A new, green route for the synthesis of α-acyloxy carboxamides from an alcohol, a carboxylic acid and an isocyanide was developed. The reaction comprises the aerobic oxidation of an alcohol to the corresponding aldehyde, catalyzed by the *Trametes versicolor* laccase /TEMPO system, followed by a *one pot* Passerini reaction in an aqueous surfactant medium. The influence of different types of surfactants on the reaction efficiency was investigated. The best results were obtained by employing dioctadecyldimethylammonium bromide (DODAB), a known vesicle-forming cationic surfactant. Importantly, apart from the metalloenzyme used, the described procedure toward α-acyloxy carboxamides is metal-free and does not reqiure hazardous organic solvents, what makes it environmentally friedly.

Introduction

α-Acyloxy carboxamides and α-hydroxy carboxamides, are the key building blocks for the synthesis of various natural products,[1-4] synthetic γ-lactones,[5] 2-furanones,[6] peptides,[7] peptidomimetics[8] and both enantiomers of α-amino acids.[9]

Various methods for the preparation of α-acyloxy carboxamides have been reported in the literature. Standard protocols usually require equimolar amounts of a coupling agent like *N*,*N*'-dicyclohexylcarbodiimide (DCC) and a catalyst like 4-(dimethylamino)pyridine (DMAP),[10] which is corrosive and very toxic.[11] Furthermore, these reactions require organic solvents like dichloromethane. Moreover, overall yields of multistep syntheses are moderate.

A convenient way for obtaining α-acyloxy carboxamides is to use a three component reaction between a carboxylic acid, a carbonyl compound and an isocyanide, first described by Passerini in 1921.[12] This Passerini-three component reaction (P-3CR) is usually performed in aprotic organic solvents, e.g. dichloromethane or toluene, which are toxic and carcinogenic.[13] To eliminate this inconvenience, the Passerini reaction can also be conducted under solvent free conditions.[14] However, in this case at least one substrate has to be in liquid state. Pirrung, Das Sarma and others have shown that for certain substrates the P-3CR can be performed efficiently also in water.[15] Furthermore, we have recently demonstrated that the application of aqueous suspensions of DODAB (dioctadecyldimethylammonium bromide), a vesicle-forming surfactant, can have a beneficial effect on the P-3CR, leading to a substantial increase in reaction yield.[16] Surfactant aggregates like vesicles or micelles enable to overcome the main disadvantage of water as a solvent, namely the low solubility of organic compounds. In addition, the presence of surfactant aggregates may even enhance reaction rates.[17] Therefore, the combination of the P-3CR with aqueous surfactant aggregates appears a valuable approach towards the development of an ideal, environmentally friendly synthesis. Furthermore, vesicle systems,[18] may well be suited for further combining the P-3CR with enzymatic transformations.

Aldehydes (as carbonyl compounds) are typical starting materials of the P-3CR, although they may be unstable during storage, leading to the formation of the corresponding alcohols and other impurities.[19] Moreover, the synthesis of aldehydes through oxidation of alcohols or reduction of carboxylic acids may be difficult since the reactions may not be selective and they may result in overoxidations or overreductions. One of the methods used to overcome this drawback is generating aldehydes *in situ*, in the reaction mixture,[20] particularly as a first step of a reaction cascade (reduction of waste, which is generated during the isolation and purification of intermediates).[21]

In this context it was reported in the literature about a P-3CR with primary alcohols using *o*-iodoxybenzoic acid (IBX) as an oxidant,[22] which is a potentially shock-sensitive explosive substance.[23] Furthermore, there were reports on oxidations with pure oxygen combined with the P-3CR, using the $CuCl_2$/TEMPO/$NaNO_2$ system[24] or a MNST (magnetic core-shell nanoparticles supported TEMPO)/TBN system,[25] in both cases with toluene as solvent.

Unfortunately, all the mentioned methods for the oxidation of alcohols[20] require hazardous reactants and toxic and expensive

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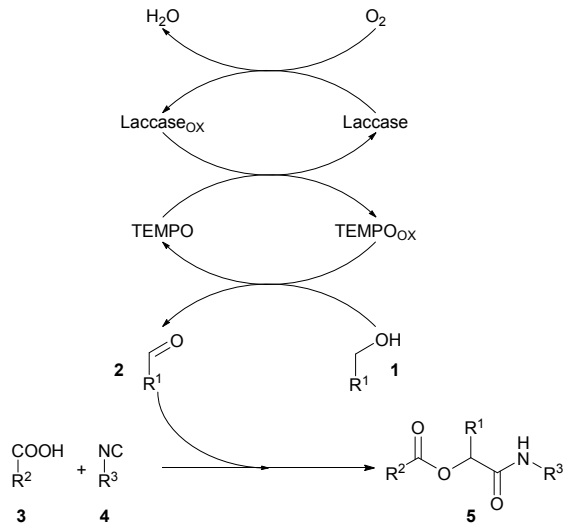
transition metals. Due to this fact, the products require tedious and expensive purification procedures, what make them unattractive for the pharmaceutical and cosmetic industries.[26] Especially since in the pharmaceutical industry, for which the pharmacopoeia limits of heavy metal contaminations are below 5 ppm, most of the classical chemical methods with metal catalysts are unacceptable.

In order to fulfil the requirements mentioned above, we report about a new chemoenzymatic protocol based on a one-pot oxidation-P-3CR procedure. The oxidation of a primary alcohol to the corresponding aldehyde is conducted enzymatically by oxygen from air (without usage of O_2, which can be potentially explosive), with the enzyme laccase and the mediator TEMPO. A schematic representation of this laccase/TEMPO system is shown in Scheme 1. After the oxidation, a carboxylic acid and an isocyanide are added, which initiate a P-3CR in the same "pot", providing an α-acyloxy carboxamide. All reactions proceed in an aqueous surfactant system, which excludes the use of organic solvents like toluene, and reactants like $NaNO_2$ and IBX, which are toxic, corrosive (IBX) and hazardous for the aquatic environment ($NaNO_2$).[27] Since, however, TEMPO and the surfactant are not fully renewable and appear as waste, the environmental (E) factor[28] is lower than in a surfactant-free system. Therefore, we also investigated the reusability of the catalytic system.



Scheme 1 Schematic representation of the laccase/TEMPO oxidation-P3CR system. First of all, the laccase is oxidizing the mediator (TEMPO) using aerobic oxygen as an oxidizing agent. Afterwards, the oxidized TEMPO is oxidizing a primary alcohol (**1**) to the corresponding aldehyde (**2**). Finally, the aldehyde participates together with a carboxylic acid (**3**) and an isocyanide (**4**) in the Passerini reaction to yield α-acyloxy carboxamide (**5**).

Results and discussion

First, we examined the influence of the vesicle forming surfactant dioctadecyldimethylammonium bromide (DODAB) on the aerobic oxidation of benzyl alcohol (**1a**) to benzaldehyde (**2a**) catalyzed by laccase from *Trametes versicolor*. We performed two experiments: (i) a mixture of **1a** (0.1 M), TEMPO (20 mol%) and enzyme (4 mg/ml) in phosphate buffer (pH=5.7, c=0.1 M) was stirred overnight at room temperature in the presence of DODAB (0.02 mmol); and (ii) the same mixture was stirred under the same conditions but without DODAB. In both cases, after extraction followed by TLC analysis, it was shown that only **2a** was obtained. These experiments demonstrated that the aerobic enzymatic oxidation of **1a** takes place in the presence of DODAB, what enabled further studies on the combination of the enzymatic transformation with the non-enzymatic P-3CR in the aqueous DODAB vesicle system, according to the previously developed procedure.[16]

As components for a model Passerini reaction, benzaldehyde (**2a**), benzoic acid (**3a**) and *p*-methoxybenzyl isocyanide (**4a**) were arbitrary selected. As shown before,[16] if the reaction is performed in phosphate buffer in the presence of 20 mol% DODAB, 2-(4-methoxybenzylamino)-2-oxo-1-phenylethyl benzoate **5a** is obtained with 92 % yield. With these results in mind, we performed the reaction of **1a**, **3a** and **4a** in the presence of laccase, TEMPO and DODAB in phosphate buffer (pH=5.7). Moreover, we investigated the influence of the enzyme, the mediator, and the reaction time on the reaction yield. We also performed the reaction with 4-hydroxy-1*H*-benzotriazole (HOBt) instead of TEMPO as a mediator and we optimized the reaction time. The results are summarized in Table 1.

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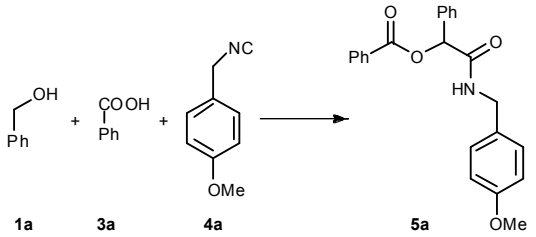
Table 1 Aerobic oxidation of benzyl alcohol (**1a**) by the *Trametes versicolor* laccase/TEMPO system combined with the Passerini reaction.[a]



1a **3a** **4a** **5a**

Entry	Mediator	Time (h)	Yield (%)
1	TEMPO	48	<5[b,c]
2	-----	48	<5[b]
3	TEMPO	48	27[d]
4	TEMPO	48	<5[b,e]
5	HOBt	48	<5[b]
6	TEMPO	24	47
7	TEMPO	48	64
8	TEMPO	72	64

[a] Reaction conditions: benzyl alcohol (**1a**, 0.5 mmol), benzoic acid (**3a**, 0.5 mmol), *p*-methoxybenzyl isocyanide (**4a**, 0.5 mmol), mediator (0.1 mmol), laccase from *Trametes versicolor* (20 mg), DODAB (0.1 mmol) were stirred at room temperature in phosphate buffer (5 mL, pH=5.7).
[b] Estimated by TLC, absence of spot of the product (**5a**), **1a** still clearly present in the reaction mixture.
[c] Reaction carried out without enzyme.
[d] Reaction carried out without DODAB.
[e] Reaction performed without benzoic acid (**3a**), with 1 mmol of benzyl alcohol (**1a**).

The obtained results clearly prove, that the laccase/TEMPO system is crucial for the oxidation of benzyl alcohol (**1a**) to benzaldehyde (**2a**) (entries 1 and 2). If one of the two components was absent, the reaction did not take place. Comparison of entry 3 with entry 7 shows, that the presence of DODAB significantly increased the yield of **5a** from 27 % to 64 %. The observed improvement of the reaction yield may be caused by the increase of the solubility of the reacting molecules in the hydrophobic part of the vesicular aggregates as well as by electrostatic attractions between the cationic surface of the vesicles and the benzoic acid anions. Analysis of the reaction conducted without **3a** (entry 4) showed that overoxidation of **1a** to **3a** did not occur. Furthermore, without laccase there was no aerobic oxidation of **2a** (entry 1), which according to literature takes place, when pure water is used as reaction medium.[29] For the conditions of entry 5, i.e. for the use of HOBt instead of TEMPO, no reaction took place, what proves that TEMPO is an efficient and ideal mediator in this type of biocatalytic oxidation reactions. Entries 6-8 show that 48 hours is an optimal time for this reaction, after 24 hours the yield of **5a** was 47 % and finally raised to 64 %. Further reaction time prolongation did not impact the reaction yield.
Next, we focused on the optimizing of the buffer pH value. It is well recognized that enzymes are stable and active in a relatively narrow pH range. According to literature, laccase from *Trametes versicolor* is active in the pH range from 3 to 6.[30] We performed reactions of **1a**, **3a** and **4a** in the presence of laccase, TEMPO and DODAB in phosphate buffers in the pH range between 3 and 8. Appropriate buffers were prepared by mixing 0.1 M solutions of orthophosphoric acid, sodium dihydrogen phosphate and disodium hydrogen phosphate to adjust the pH to the desired value. In the optimal range of pH, between 5 and 6, additional experiments were performed. All results are shown in Fig. 1.

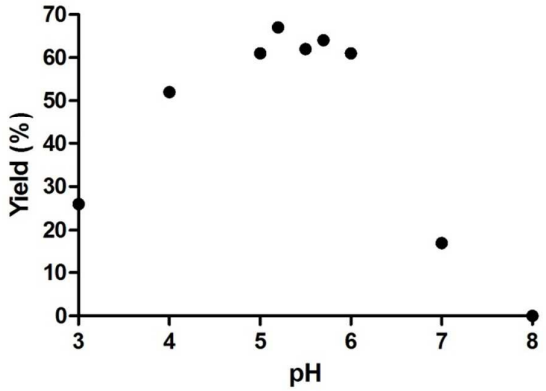


Fig. 1 Effect of pH on the isolated yield of **5a** of the model laccase/TEMPO oxidation – P-3CR system. Reaction conditions: benzyl alcohol (**1a,** 0.5 mmol), benzoic acid (**3a,** 0.5 mmol) and *p*-methoxybenzyl isocyanide (**4a**, 0.5 mmol), TEMPO (0.1 mmol), laccase (20 mg), DODAB (0.1 mmol) in 5 mL of phosphate buffer for 48 h at room temperature.

Although the optimal pH for the model laccase/TEMPO oxidation – P-3CR system was found to be 5.2, in the range from pH = 5 to 6 the yield of **5a** varied only slightly. Decreasing the pH value below 5, the yield of **5a** dropped, probably due to the acid catalyzed hydrolysis of the isocyanide.[31] At pH>6, the yield of **5a** also dropped, which is probably due to a decrease in laccase activity.[30]
In a next step of our investigations, we focused on a variation of the surfactant type. From our previous studies we already know, that DODAB was the best among the surfactants and other additives tested for performing the Passerini reaction in aqueous systems.[16] Afterwards, we checked whether DODAB is also the most suitable surfactant for the enzymatic transformation of **1a** to **2a**, preceding by the Passerini reaction. To investigate this point, we performed reactions of **1a**, **3a** and **4a** in the presence of laccase, TEMPO and different additives (20 mol%). The formation of **5a** was analyzed (Table 2).

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Table 2 Effect of different additives on the model laccase/TEMPO oxidation – P-3CR system.[a]

Entry	Additive (20 mol%)	Yield (%)
1	----------	21
2	Montmorillonite K 10 (10 mg)	16
3	Sodium dodecyl sulphate (SDS)	6
4	Sodium bis(2-ethylhexyl) sulfosuccinate (AOT)	12
5	Cholesterol	22
6	Tween 80	23
7	Triton X-100	47
8	3-(*N*,*N*-Dimethylmyristylammonio) propanesulfonate	23
9	Didodecyldimethylammonium bromide	34
10	Dioctadecyldimethylammonium bromide (DODAB)	67

[a] Reaction conditions: benzyl alcohol (**1a,** 0.5 mmol), benzoic acid (**3a,** 0.5 mmol) and *p*-methoxybenzyl isocyanide (**4a**, 0.5 mmol), TEMPO (0.1 mmol), laccase (20 mg), DODAB (0.1 mmol) in 5 mL of phosphate buffer (pH=5.2, c=0.1M) for 48 h at room temperature.

For the reaction performed without any additive, **5a** was obtained with 21 % isolated yield. Addition of Montmorillonite K 10 (entry 2) resulted in a decrease of the yield to 16%. Entries 3 and 4 show, that the addition of anionic surfactants (SDS or AOT) caused a significant decrease in reaction yield, probably due to repulsive interactions between the benzoic acid anions (deprotonated form of **3a**) and the negatively charged surface of the micellar or vesicular aggregates formed. Addition of cholesterol (entry 5) had a negligible effect, similarly to the addition of the non-ionic surfactant Tween 80 (entry 6) or the zwitterionic surfactant 3-(*N*,*N*-dimethylmyristylammonio)propanesulfonate. In all these cases, the reaction yields were almost the same as without additive. In the presence of the non-ionic surfactant Triton X-100, the reaction yield was significantly increased, probably due to a positive effect of this neutral surfactant on the P-3CR, associated with an increased solubility of the reactants in the reaction medium. Addition of the two different cationic surfactants with the long aliphatic side chains; didodecyldimethylammonium bromide or DODAB (entries 9 and 10) enhanced the yield of **5a**. The best result was obtained in the presence of DODAB, which is known to form bilayers in aqueous solutions, present as dispersed vesicles,[32] the reaction yield was increased from 21 % without any additive (entry 1) to 67 % (entry 10). This indicates that DODAB is the most suitable additive among the ones checked for the Passerini reaction preceded by the laccase-catalyzed oxidation.

Next, the reaction was studied by varying the DODAB concentration, ranging from 5 to 50 mol%. For each condition, the critical concentration for aggregate formation (cac) was reached (for H_2O, 25°C, cac(DODAB) = $4.28*10^{-5}$ M).[33] The results are shown in Fig. 2. In the presence of 5 mol% DODAB the reaction yield remained comparable to the one without surfactant. Upon increasing the amount of DODAB from 5 to 20 mol%, the yield of **5a** increased up to 67%. A further elevation of the DODAB concentration from 20 to 50 mol%, did not improve the yield further. Based on these data, DODAB was used at a concentration of 20 mol % for all further experiments as optimal concentration.

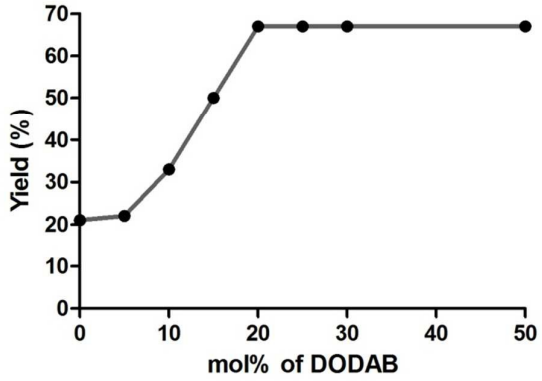

Fig. 2 Effect of DODAB on the isolated yield of **5a** for the model laccase/TEMPO oxidation – P-3CR system. Reaction conditions: benzyl alcohol (**1a,** 0.5 mmol), benzoic acid (**3a,** 0.5 mmol) and *p*-methoxybenzyl isocyanide (**4a**, 0.5 mmol), TEMPO (0.1 mmol), laccase (20 mg), DODAB in 5 mL of phosphate buffer (pH=5.2, c=0.1M) for 48 h at room temperature.

In order to try to improve the reaction yield to a level which is close to the yield of the enzyme-free reaction carried out with **2a**, **3a** and **4a** (92 %),[16] we performed a few experiments in which the amount of benzyl alcohol (**1a**) as one of the starting materials was changed. Usage of 2 equivalents of **1a** resulted in product **5a** with 76 % yield, and with 5 equivalents the yield of **5a** was 79 %. For a reaction carried out with 2 equivalents of isocyanide (**3a**) resulted in **5a** with 88 % yield. These experiments indicate that very high yields cannot be achieved with the laccase/TEMPO – P-3CR system due to the possible accompanying hydrolysis of **3a**. In order to overcome this problem the reaction was conducted stepwise: first 24 hours oxidation of **1a** with laccase, TEMPO and surfactant, followed by the Passerini reaction with **3a** and **4a** for another 24 hours, resulting in product **5a** with 86 % yield (Table 3, entry 1).

From an environmental point of view, the reusability of the catalytic system is a critical issue. Therefore, we investigated whether the catalytic system based on the laccase/TEMPO oxidation and the DODAB vesicular suspension can be used more than once. The model reaction was carried out according to the optimized procedure, benzyl alcohol (**1a,** 0.5 mmol), TEMPO (0.1 mmol), DODAB (0.1 mmol) in 5 mL of phosphate buffer (pH=5.2, c=0.1M) were incubated with laccase (4 mg/mL) for 24 h at room temperature, then benzoic acid (**3a,** 0.5 mmol) and *p*-methoxybenzyl isocyanide (**4a**, 0.5 mmol) were added and the reaction mixture was stirred for additional 24 h. During the progress of the reaction, solid product **5a** formed and felt out in impure form from the reaction mixture. The product could be separated by filtration through a cotton pad, followed by purification using column chromatography.

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The filtrate containing DODAB, enzyme and TEMPO in phosphate buffer was employed for another run: **1a** was added first, then – after 24 h – **3a** and **4a** were added, and the reaction was carried out for additional 24h. After filtration through a cotton pad, the filtrate containing the surfactant, laccase and TEMPO was used for the reaction one more time. The results from these catalytic system reusability tests are shown in Table 3 (entries 2 and 3). The laccase/TEMPO and DODAB catalytic system could be reused one time with an appreciable decrease in yield from 86 % (entry 1) to 67 % (entry 2). Afterwards, there was a large drop in yield to 19 % (entry 3). This decrease in reaction yield may be due to a deactivation of the enzyme with time and/or a significant loss in laccase, TEMPO or DODAB during the filtration step.

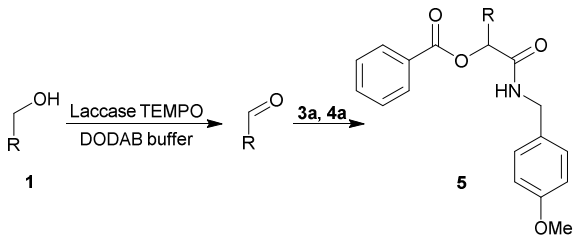
Table 3 Reusability of the catalysts for the laccase/TEMPO oxidation – P-3CR system[a].

Entry	Recycle	Yield (%)
1	Fresh catalysts	86
2	I recycle	67
3	II recycle	19

[a] Reaction conditions: **1a** (0.5 mmol), TEMPO (initially 0.1 mmol), laccase (initially 20 mg), DODAB (initially 0.1 mmol) in 5 mL of phosphate buffer (pH=5.2, c=0.1M) were mixed for 24 h at room temperature, then **3a** (0.5 mmol) and **4a** (0.5 mmol) were added and the reaction mixture was stirred for additional 24 h.

After the optimization of the reaction conditions for benzyl alcohol (C_6H_5-CH_2OH, **1a**), experiments with other alcohols (R-CH_2OH, **1**) were also carried out. Alcohol **1** was mixed with laccase, TEMPO and DODAB in phosphate buffer at room temperature, after 24 h **3a** and **4a** were added, and the isolated yields were determined after additional 24 h. The results are summarized in Table 4.

Table 4 Effect of different alcohols (**1**) on the laccase/TEMPO oxidation – P-3CR system.[a]



1 5

Entry	R	5	Yield (%)
1	C_6H_5	**5a**	86
2	*p*-$NO_2C_6H_4$	**5b**	78
3	*p*-FC_6H_4	**5c**	72
4	*p*-ClC_6H_4	**5d**	65
5	*p*-MeC_6H_4	**5e**	68
6	*p*-$OMeC_6H_4$	**5f**	62
7	$CH=CH_2$	----	0
8	$C_{11}H_{23}$	**5g**	11
9	$CH(C_2H_5)C_4H_9$	**5h**	30[b]
10	$CH=CHC_6H_5$	**5i**	81

[a] Reaction conditions: alcohol (**1,** 0.5 mmol), TEMPO (0.1 mmol), laccase (20 mg), DODAB (0.1 mmol) in 5 mL of phosphate buffer (pH=5.2, c=0.1 M) were mixed for 24 h at room temperature. Then benzoic acid (**3a,** 0.5 mmol) and *p*-methoxybenzyl isocyanide (**4a**, 0.5 mmol) were added and the reaction mixture was stirred for additional 24 h.
[b] Mixture of diastereoisomers, dr = 1:1, determined by ^{1}H NMR.

For the reactions carried out with derivatives of benzyl alcohol (entries 2-6), the resulting yields varied between 62 and 86 %, with the highest one obtaining for benzyl alcohol (**1a**). These results indicate that there is only a slight influence of the type of substituent in the benzene ring on the reaction yield. In case of allyl alcohol (entry 7), no reaction occurred. This could be due to the highly reactive and polymerizable acrolein, which is the product of the oxidation reaction. This is probably the reason why – to the best of our knowledge - acrolein has never been used successfully as a substrate in P-3CR. Reactions carried out with alkyl alcohols, dodecanol (entry 8) and 2-ethylhexanol (entry 9) resulted in products **5g** and **5h** with 11 % and 30 % yields respectively, what corresponds to literature data for aliphatic alcohols oxidized by the laccase/TEMPO system.[34] The reaction with the more demanding cinnamyl alcohol (entry 10) provided product **5i** with 81 % yield.

Conclusions

We have developed a new, environmentally friendly protocol which provides α-acyloxy carboxamides with high yields. This protocol comprises the enzymatic aerobic oxidation of an alcohol, followed by a multicomponent Passerini reaction in an

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aqueous surfactant medium. The elaborated protocol excludes employment of explosive pure oxygen, flammable and toxic organic solvents, and transition metals, what makes it attractive for the pharmaceutical industry. Moreover, the presented protocol reveals a very low E value. Further studies towards an application of the procedure for other multicomponent reactions, which require aldehydes as a substrate,[35] are in progress in our laboratory.

Acknowledgements

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Experimental

^{1}H NMR and ^{13}C NMR spectra were recorded with Varian 200 MHz and Bruker 400 MHz spectrometers, with TMS used as an internal standard or the signal of residual chloroform. High resolution mass spectrometry (HR:MS) spectra were recorded on an Mariner (PerSeptiveBiosystems) and Synapt G2:SHD apparatus. Melting points were determined with a model SMP-20 device (Büchi, Flawil, Swizerland). Laccase from *Trametes versicolor*, powder, light brown, ≥0.5 U/mg was purchased from Sigma Aldrich, product number 38429, lot number # BCBQ2928V. The following products were also from Sigma Aldrich: Dioctadecaldimethylammonium bromide (DODAB), purity ≥98.0 % (AT), product number 40165; dioctyl sulfosuccinate sodium salt (AOT = sodium bis (2-ethylhexyl) sulfosuccinate, purity 98 %), product number 323586; sodium dodecyl sulfate (SDS), purity ≥99.0 % (GC), product number L6026; cholesterol, purity ≥99.0 %, product number C3045; Triton X-100, product number T9284; and 3-(*N*,*N*-dimethylmyristylammonio)propanesulfonate, purity ≥99.0 %, product number T7763. Dilauryldimethylammonium bromide (= didodecyldimethylammonium bromide, purity >98.0 %), was purchased from TCI, product number D1974. Tween 80, was purchased from Schuchardt München (now Merck). *p*-Methoxybenzyl isocyanide (**4a**) was synthetized from *p*-mehoxybenzyl amine in a two-step synthesis (see Supporting Information). The remaining starting materials were purchased from Sigma Aldrich or TCI.

General procedure for the synthesis of compounds 5a-i. A mixture of the corresponding alcohol (0.5 mmol), laccase from *Trametes versicolor* (20 mg), TEMPO (0.1 mmol) and DODAB (0.1 mmol) was stirred in phosphate buffer (5 mL, pH=5,2 c=0,1M) at room temperature. After 24 h, benzoic acid (0.5 mmol) and *p*-methoxybenzyl isocyanide (0.5 mmol) were added and the reaction mixture was stirred for additional 24 h. Afterwards, the reaction mixture was extracted with dichloromethane (3×20 mL). The combined organic layers were dried with $MgSO_4$ and residuals of solvent were removed by distillation under reduced pressure. The crude products were purified by column chromatography on silica gel using hexane/AcOEt as eluent. NMR spectra are given in Supporting Information.

2-(4-Methoxybenzylamino)-2-oxo-1-phenylethyl benzoate **5a**. White powder; mp 141-142 °C; ^{1}H NMR (400 MHz; $CDCl_3$) δ_H3.77 (3H, s, C*H*$_3$O), 4.04-4.45 (2H, m, C*H*$_2$N), 6.36 (1H, s, C*H*), 6.42 (1H, br s, N*H*), 6.81-6.84 (2H, m, Ph), 7.13-7.15 (2H, m, Ph), 7.36-7.46 (5H, m, Ph), 7.53-7.60 (3H, m, Ph), 8.05-8.08 (2H, m, Ph); ^{13}C NMR (100 MHz; $CDCl_3$) δ_C 42.91, 55.28, 76.01, 114.15, 127.38, 128.60, 128.82, 128.95, 129.03, 129.23, 129.84, 130.13, 133.62, 135.55, 159.11, 165.02, 168.28; HRMS calcd. for $C_{23}H_{21}NO_4Na$ $[M+Na]^+$: 398.1368, found: 398.1366.

2-(4-Methoxybenzylamino)-1-(4-nitrophenyl)-2-oxoethyl benzoate **5b**. White powder; mp 127-128 °C; ^{1}H NMR (400 MHz; $CDCl_3$) δ_H 3.78 (3H, s, C*H*$_3$O), 4.42-4.45 (2H, m, C*H*$_2$N), 6.44 (1H, s, C*H*), 6.61 (1H, br s, N*H*), 6.82-6.86 (2H, m, Ph), 7.13-7.16 (2H, m, Ph), 7.46-7.50 (2H, m, Ph), 7.61-7.65 (1H, m, Ph), 7.72- 7.75 (2H, m, Ph), 8.05-8.10 (2H, m, Ph), 8.21-8.25 (2H, m, Ph); ^{13}C NMR (100 MHz; $CDCl_3$) δ_C 43.10, 55.29, 74.79, 114.25, 123.89, 128.05, 128.45, 128.52, 128.83, 129.00, 129.36, 129.83, 134.13, 148.19, 159.27, 164.61, 167.05; HRMS calcd. for $C_{23}H_{20}N_2O_6Na$ $[M+Na]^+$: 443.1219, found: 443.1218.

2-(4-Methoxybenzylamino)-1-(4-fluorophenyl)-2-oxoethyl benzoate **5c**. White powder; mp 130-131 °C; ^{1}H NMR (400 MHz; $CDCl_3$) δ_H 3.78 (3H, s, C*H*$_3$O), 4.41-4.49 (2H, m, C*H*$_2$N), 6.34 (1H, s, C*H*), 6.48 (1H, br s, N*H*), 6.83-6.86 (2H, m, Ph), 7.05-7.10 (2H, m, Ph), 7.15-7.17 (2H, m, Ph), 7.44-7.48 (2H, m, Ph), 7.48- 7.55 (2H, m, Ph), 7.55-7.64 (1H, m, Ph), 8.02-8.07 (2H, m, Ph); ^{13}C NMR (100 MHz; $CDCl_3$) δ_C 42.93, 55.26, 75.24, 114.16, 115.67, 115.89, 128.63, 128.96, 129.28, 129.36, 129.70, 129.79, 131.49, 131.52, 133.72, 159.16, 164.30, 164.91, 168.07; HRMS calcd. for $C_{23}H_{20}NO_4FNa$ $[M+Na]^+$: 416.1274, found: 416.1276.

2-(4-Methoxybenzylamino)-1-(4-chlorophenyl)-2-oxoethyl benzoate **5d**. White powder; mp 149-150 °C; ^{1}H NMR (400 MHz; $CDCl_3$) δ_H 3.79 (3H, s, C*H*$_3$O), 4.41-4.45 (2H, m, C*H*$_2$N), 6.32 (1H, s, C*H*), 6.47 (1H, br s, N*H*), 6.83-6.85 (2H, m, Ph), 7.15-7.16 (2H, m, Ph), 7.35-7.37 (2H, m, Ph), 7.44-7.51 (4H, m, Ph), 7.56- 7.62 (1H, m, Ph), 8.01-8.07 (2H, m, Ph); ^{13}C NMR (100 MHz; $CDCl_3$) δ_C 42.96, 55.27, 75.22, 114.18, 128.66, 128.72, 128.97, 128.99, 129.65, 129.80, 133.78, 134.11, 135.03, 159.18, 164.85, 167.83; HRMS calcd. for $C_{23}H_{20}NO_4ClNa$ $[M+Na]^+$: 432.0979, found: 432.0980.

2-(4-methoxybenzylamino)-2-oxo-1-p-tolylethyl benzoate **5e**. White powder; mp 133-134 °C; ^{1}H NMR (400 MHz; $CDCl_3$) δ_H 2.36 (3H, s, PhC*H*$_3$), 3.78 (3H, s, C*H*$_3$O), 4.36-4.49 (2H, m, C*H*$_2$N), 6.33 (1H, s, C*H*), 6.40 (1H, br s, N*H*), 6.82-6.85 (2H, m, Ph), 7.15-7.19 (4H, m, Ph), 7.42-7.46 (4H, m, Ph), 7.56-7.59 (1H, m, Ph), 8.04-8.07 (2H, m, Ph); ^{13}C NMR (100 MHz; $CDCl_3$) δ_C 21.19, 42.87, 55.26, 75.92, 114.11, 127.38, 128.53, 128.94, 129.31, 129.49, 129.81, 129.87, 132.63, 133.52, 138.96, 159.108, 165.05, 168.38; HRMS calcd. for $C_{24}H_{23}NO_4Na$ $[M+Na]^+$: 412.1525, found: 412.1520.

2-(4-Methoxybenzylamino)-1-(4-methoxyphenyl)-2-oxoethyl benzoate **5f**. White powder; mp 151-152 °C; ^{1}H NMR (400 MHz; $CDCl_3$) δ_H 3.79 (3H, s, C*H*$_3$O), 3.81 (3H, s, C*H*$_3$O), 4.39-4.50 (2H, m, C*H*$_2$N), 6.32 (1H, s, C*H*), 6.40 (1H, br s, N*H*), 6.83-6.85 (2H, m, Ph), 6.90-6.93 (2H, m, Ph), 7.16-7.17 (2H, m, Ph), 7.42-

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7.49 (4H, m, Ph), 7.56- 7.61 (1H, m, Ph), 8.05-8.08 (2H, m, Ph); ^{13}C NMR (100 MHz; $CDCl_3$) δ_C 42.90, 55.27, 55.30, 75.70, 114.14, 114.25, 127.72, 128.54, 128.96, 128.98, 129.34, 129.80, 129.88, 133.52, 159.10, 160.17, 165.08, 168.48; HRMS calcd. for $C_{24}H_{23}NO_5Na$ $[M+Na]^+$: 428.1474, found: 428.1474.

1-(4-Methoxybenzylamino)-1-oxotridecan-2-yl benzoate **5g**. White powder; mp 90-91 °C; ^{1}H NMR (400 MHz; $CDCl_3$) δ_H 0.87 (3H, t, *J* 7.2 Hz, C*H*$_3$CH$_2$), 1.19-1.36 (16H, br m, 8×C*H*$_2$), 1.36-1.48 (2H, m, C*H*$_2$CH$_2$), 1.97-2.03 (2H, m, C*H*$_2$CH), 3.78 (3H, s, C*H*$_3$O), 4.35-4.48 (2H, m, C*H*$_2$N), 5.44-5.47 (1H, m, C*H*), 6.30 (1H, br s, N*H*), 6.83-6.86 (2H, m, Ph), 7.16-7.18 (2H, m, Ph), 7.44-7.48 (2H, m, Ph), 7.74-7.61 (1H, m, Ph), 8.03-8.05 (2H, m, Ph); ^{13}C NMR (100 MHz; $CDCl_3$) δ_C 14.07, 22.65, 24.95, 29.24, 29.30, 29.38, 29.49, 29.58, 31.88, 32.01, 42.67, 55.26, 74.68, 114.12, 128.60, 128.91, 129.73, 129.99, 133.54, 159.08, 165.41, 169.80; HRMS calcd. for $C_{28}H_{39}NO_4Na$ $[M+Na]^+$: 476.2777, found: 476.2781.

3-ethyl-1-(4-methoxybenzylamino)-1-oxoheptan-2-yl benzoate **5h** – mixture of diasteromers. Colorless oil; ^{1}H NMR (400 MHz; $CDCl_3$) δ_H 0.87-0.89 (3H, m, C*H*$_3$CH$_2$), 0.95-0.99 (3H, m, C*H*$_3$CH$_2$), 1.16-1.43 (8H, br m, 4×C*H*$_2$), 2.07-2.15 (1H, m, C*H*(CH$_2$)$_2$), 3.78 (3H, s, C*H*$_3$O), 4.31-4.39 (1H, m, CH*H*N), 4.41-4.50 (2H, m, C*H*HN), 5.56-5.62 (1H, m, C*H*), 6.23-6.29 (1H, m, N*H*), 6.81-6.84 (2H, m, Ph), 7.16-7.18 (2H, m, Ph), 7.44-7.49 (2H, m, Ph), 7.58-7.60 (1H, m, Ph), 8.03-8.05 (2H, m, Ph); ^{13}C NMR (100 MHz; $CDCl_3$) δ_C 11.69, 11.71, 13.91, 13.98, 22.50, 22.74, 22.94, 23.13, 29.22, 29.28, 29.52, 42.06, 42.41, 42.68, 42.70, 55.25, 75.80, 76.07, 114.07, 128.64, 128.93, 128.96, 129.34, 129.75, 130.01, 133.56, 133.75, 159.03, 165.39, 169.64; HRMS calcd. for $C_{24}H_{31}NO_4Na$ $[M+Na]^+$: 420.2151, found: 420.2149.

(E)-1-(4-methoxybenzylamino)-1-oxo-4-phenylbut-3-en-2-yl benzoate **5i**. White powder; mp 126-127 °C; ^{1}H NMR (400 MHz; $CDCl_3$) δ_H 3.78 (3H, s, C*H*$_3$O), 4.44-4.47 (2H, m, C*H*$_2$N), 6.05 (1H, dd, *J* 1.2 Hz *J* 6.8 Hz, C*H*C*H*=C*H*), 6.43 (1H, br s, N*H*), 6.66 (1H, dd, *J* 6.8 Hz *J* 16 Hz, CHC*H*=CH), 6.83 (1H, dd, *J* 1.2 Hz *J* 16 Hz, C*H*Ph), 6.84-6.88 (2H, m, Ph), 7.20-7.22 (2H, m, Ph), 7.27-7.34 (4H, m, Ph), 7.40-7.42 (2H, m, Ph), 7.45- 7.49 (2H, m, Ph), 7.59-7.63 (1H, m, Ph), 8.08-8.11 (2H, m, Ph); ^{13}C NMR (100 MHz; $CDCl_3$) δ_C 42.93, 55.28, 74.86, 114.17, 122.49, 126.87, 128.43, 128.60, 128.62, 129.02, 129.22, 129.77, 129.83, 133.65, 134.87, 135.66, 159.16, 165.19, 168.04; HRMS calcd. for $C_{25}H_{23}NO_4Na$ $[M+Na]^+$: 424.1525, found: 424.1519.

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