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Enzymatic tandem approach to Knoevenagel condensation of acetaldehyde with acidic methylene compounds in organic media

Dominik Koszelewski,* Daniel Paprocki, Arleta Madej, Filip Borys, Anna Brodzka and Ryszard Ostaszewski*

We would like to dedicate this paper to Professor Kurt Faber on occasion of his 65th birthday

Abstract: Enzyme-catalyzed Knoevenagel reaction with acetaldehyde is presented. A novel protocol for the synthesis of desired products through the tandem process based on the enzymatic hydrolysis and Knoevenagel reaction starting from acetaldehyde precursors is disclosed. The influence of the reaction conditions including organic solvent, enzyme type, and temperature on the reaction course was tested. Further this protocol was extended by second tandem chemoenzymatic transformations leading to epoxides.

Introduction

Knoevenagel condensation is one of the most important carbon–carbon bound-forming reactions, used in the synthesis of chemically, and pharmaceutically relevant compounds.[1] The Knoevenagel reaction products are also useful intermediates for further transformations, such as oxidative coupling reaction, Nazarov cyclization, Diels-Alder and Michael additions.[2] This atom-efficient reaction has been used to synthesize some interesting molecules, such as 4,7-dihydroisoxazolo[5,4-b]pyridines,[3] coumarins,[4] indanones,[5] indenes,[6] stilbenes,[7] and azepinones.[8] Generally, Knoevenagel condensation can be carried out in the presence of bases,[9] Lewis acids,[10] zeolites,[11] clays,[12] ionic liquids[13] or amino acids.[14] Additionally, several methods based on infra-red irradiation,[15] ultrasound irradiation, microwave,[16] and electrochemistry have also been reported. After more than a century, this typically base-catalyzed process is still an active research area, in the context of environmentally friendly routes including reactions in the absence of solvent.[17] Although the reaction can be carried out under catalyst-free conditions, the yields of the condensed products remain underwhelming.[18]

Unfortunately, many of these mentioned catalytic systems used in Knoevenagel condensation suffer from disadvantages such as inevitable side-products formed, excess starting materials needed, hazardous solvents, or harsh reaction conditions, not being environmentally friendly.[19]

Dr. D. Koszelewski, D. Paprocki, A. Madej, F. Borys, Dr. A. Brodzka, Prof. Ryszard Ostaszewski*
Institute of Organic Chemistry
Polish Academy of Sciences
Kasprzaka 44/52, 01-224, Warsaw, Poland
E-mail: dominik.koszelewski@icho.edu.pl;
ryszard.ostaszewski@icho.edu.pl

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

Recently, biocatalysts have attracted significant attention due to their high selectivity and mild conditions. Moreover, a new frontier, recognized as biocatalytic promiscuity, has emerged and largely extended the application of enzymes.[20] Enzyme catalytic promiscuity which refers to the ability of an enzyme to catalyze reactions which may vary from its natural role extending the application of enzymes. Furthermore, enzyme promiscuity provides environmentally friendly protocols for organic synthesis. Several elegant examples regarding the significance of enzymatic promiscuity have been published, such as three-component Hantzsch-type reaction,[21] perhydrolysis,[22] Markovnikov and anti-Markovnikov addition between thiols and vinyl esters,[23] Henry reactions,[24] Morita–Baylis–Hillman reaction,[25] Mannich reactions,[26] aldol additions,[27] or Michael additions.[28]

It was shown that some enzymes can mediate Knoevenagel condensation, but only limited examples have been reported up to date. In 2009, Yu *et al.* first reported that lipase from *Candida antarctica* B (CaL-B) catalyzes Knoevenagel condensation in acetonitrile/water medium, while a primary amine was required to form a schiff base in the course of the reaction and this protocol was limited to β-keto esters.[29] The proposed mechanism of CaL-B catalyzed Knoevenagel condensation was undermined by Bornscheuer and Evitt who pointed out that the ester substrates can be hydrolyzed by lipases providing carboxylic acid which mediates a non-enzymatic spontaneous condensation.[30] Experimental data provided by Li *et al*. proved that enzymatic Knoevenagel condensation does not require hydrolysis of ester substrates and can be classified as a true promiscuous catalysis.[31] While the Knoevenagel condensations are generally carried out with active methylene compounds such as β-ketoesters, cyanoacetates, malononitriles, the reaction with β-diketones is less reported. This is because the 1,3-diketones have an inherent tendency to form a stable cyclic enol, which makes it less reactive than other active methylene compounds. Hu *et al*. reported that papain promote the direct Knoevenagel reactions in DMSO/water between aromatic, hetero-aromatic and α,β-unsaturated aldehydes and 1,3-carbonyl providing corresponding products with moderate yield.[32] Also alkaline protease from *Bacillus licheniformis* was used as a biocatalyst in the Knoevenagel condensations of aromatic, hetero-aromatic and α,β-unsaturated aldehydes with less reactive acetylacetone or ethyl acetoacetate.[33] The biocatalytic promiscuous activity of protease from *Bacillus licheniformis* was sufficient for selected aromatic aldehydes but not for aliphatic one.[34]

It is well recognized that the Knoevenagel condensations are generally carried out with aromatic aldehydes as substrates.[35] Moreover, Wang *et al*. reported protocol based on lipase catalyzed condensation of the substituted salicylaldehyde

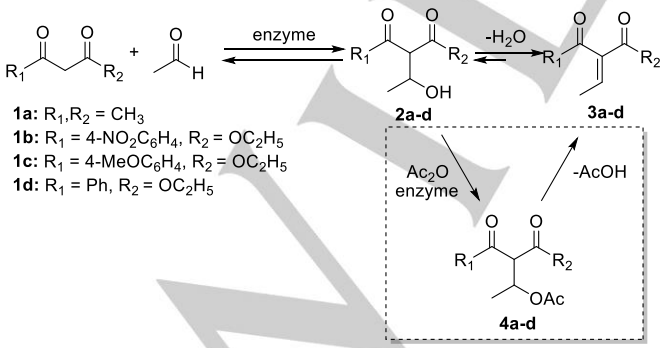
under microwave irradiation.[36] To the best of our knowledge, only limited examples with aliphatic aldehydes were reported and all of them are limited to α,β-unsaturated derivatives.[34,37] Therefore, in the field of enzymatic Knoevenagel condensation, there are still some drawbacks needed to be overcome.

The methods described above are predominantly limited to aromatic or branched aliphatic aldehydes and do not allow the preparation of ethylidene derivatives. Due to the electrons delocalization Knoevenagel condensation on the aromatic aldehydes provides products which are less prone to side reactions. However, despite numerous examples of Knoevenagel condensation those regarding application of acetaldehyde remain elusive. Application of acetaldehyde is complicated[38] by its low boiling point, below the ambient temperature, what imposes the use of either low temperature conditions with prolonged reaction time[39] or required sealed bomb.[40]

In the current study under application of the biocatalytic promiscuity in organic synthesis, we investigated the enzyme-catalyzed Knoevenagel condensation with aliphatic aldehyde, acetaldehyde. Additionally, we report our results on the novel approach to the synthesis of α,β-unsaturated compounds and epoxides *via* chemoenzyamatic tandem reaction in organic media.

Results and Discussion

Recently, Antonioletti *et al*. proposed a convenient protocol for the synthesis of 2-alkenyl-1,3-dicarbonyl compounds, starting from acetaldehyde.[39b] Initially we employed this strategy to prepare 3-ethylidene-pentane-2,4-dione (**3a**) and 2-benzoyl-but-2-enoic acid ethyl ester (**3d**). Unfortunately, reported method was difficult to reproduce providing after tedious and time consuming purification, products **3a** and **3d** with unacceptable low yields 3% and 5%, respectively (Scheme 1). Moreover, this protocol was inapplicable in case of preparation of products **3b** and **3c**. Therefore we turned our attention to enzymatic process that could lead to improved productivity of desired ethylidene derivatives **3**.



Scheme 1. Classical *vs* acetic anhydride promoted lipase-catalysed Knoevenagel condensation (in dashed lines).

Initial studies under enzyme catalyzed Knoevenagel condensation were conducted using acetaldehyde (1 mmol) and acetylacetone (**1a**) (1 mmol) in *tert*-butyl alcohol as a model reaction (Scheme 1). The reaction were performed at 20 °C for 120 h in sealed glass vials. Two lipases (200 mg each), one native from porcine pancreas (PPL) and second from *Candida antarctica* B immobilized in acrylic resign (Novozym 435). After completion, the reaction mixture was evaporated. The crude product was purified by column chromatography (ethyl acetate/hexanes). Obtained results are summarized in Table 1.

Table 1. Lipase-catalysed Knoevenagel condensation using acetaldehyde under various conditions.

Entry	Enzyme/conditions[a]	Substrate	Yield [%][d]
1	-	**1a**	<1
2	PPL	**1a**	6
3	Novozym 435	**1a**	11
4	Novozym 435, Ac_2O	**1a**	16
5	Novozym 435,Ac_2O,Na_2CO_3	**1a**	8
6	Novozym 435, Ac_2O	**1b**	33
7	Novozym 435, Ac_2O[c]	**1b**	9
8	Novozym 435, Ac_2O	**1c**	25
9	Novozym 435, Ac_2O	**1d**	28

[a] Reaction conditions: **1** (1 mmol), acetaldehyde (1 mmol), *tert*-butyl alcohol (5 mL) and enzyme (200 mg) at 20 °C for 120 h. [b] According to the Ref. 41. [c] Reaction temperature 30 °C. [d] Isolated yield equal conversion.

It was found that both selected lipases can catalyze the studied reaction (Table 1, entries 2 and 3) providing desired product **3a** with 6% and 11%, respectively. Among of the selected enzymes, Novozym 435 exhibited the highest catalytic performance under studied conditions (Table 1, entry 3). Therefore, Novozym 435 was consequently used throughout the further studies. Furthermore, the denatured Novozym 435 (denatured by heating) or BSA (Table S1, entries 6 and 7, Supporting Information) was used as catalyst in this reaction and the result was similar to the control (Table 1, entry 1). The promiscuous activity of these enzymes toward Knoevenagel condensation with α,β-unsaturated aldehydes performed in the same reaction medium, *tert*-butyl alcohol was recently reported by Wang *et al*..[37]

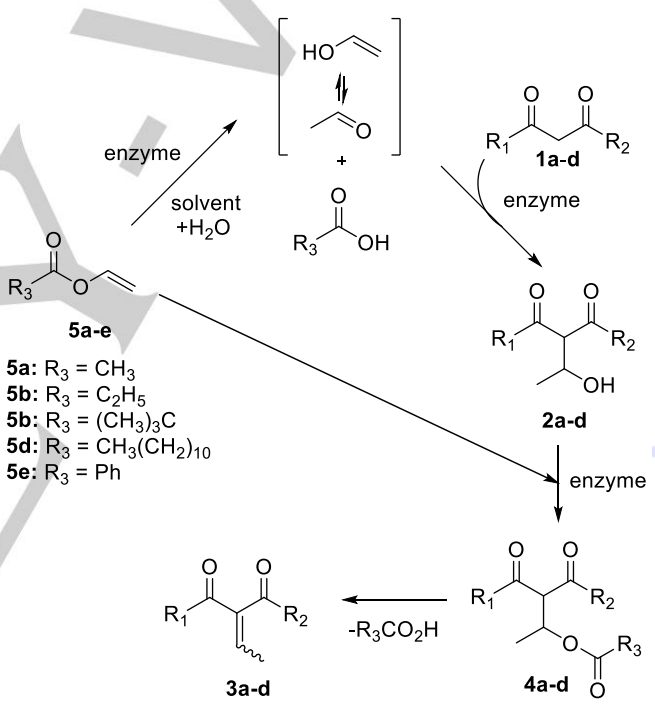
It is known that water content strongly affects the catalytic behavior of an enzyme in non-aqueous media.[42] Thus, we investigated the effect of water content, from 5 to 20% (v/v), on the yield of the Knoevenagel condensation catalyzed by Novozym 435 (Table S1, entries 2-4, Supporting Information). Previous reports demonstrated that water was essential in the enzymatic reactions of carbon–carbon bond formation.[32] We found that even 5%v/v addition of water affected in yield reduction (Table S1, entry 2, Supporting Information) what is in agreement with literature data for the Knoevenagel condensation of aromatic aldehydes with acyclic active methylene compounds catalyzed by lipoprotein lipase, where the highest yields were obtained without the addition of water.[33]

Obtained results regarding water content causes us to look closer on the mechanism of the studied process. Due to the general reaction mechanism of Knoevenagel condensation intermediate **2** shown on the Scheme 1 undergoes dehydration forming product **3** with carbon–carbon double. Based on this observation more efforts were taken to improve overall procedure. The active site of lipase functions in Knoevenagel condensation as a nucleophile, which condenses the acidic proton of the active methylene group. The better recognized promiscuous role of the lipases in organic synthesis is the formation, of esters and amides. An essential step towards utilizing the full potential of the lipases was their application in organic solvents. Based on the hypothesis that intermediate **4** obtained in the reaction with acetic anhydride should also provide desired product **3** additional experimental were performed to confirm this assumption (Scheme 1).

Next, the series of experiments with acetylacetone (**1a**), acetaldehyde, and acetic acid anhydride were performed. Acetic anhydride is activated acyl-donor which is commonly used in enzyme-catalyzed acylation of alcohols and amines.[43] Additionally, due its activated character it shifts the equilibrium constant in favor of acylation. Moreover, Dumas *et al*. showed that condensation of selected active methylene compounds with acetaldehyde was efficiently promoted by a catalytic amount of lithium bromide in the presence of acetic anhydride.[41] Unfortunately, this catalytic system turned out to be useless in case of using acetylacetone (**1a**) together with acetaldehyde (Table SI1, entry 5, Supplementary Information). To our delight when the Novozym 435 was introduced together with acetic acid anhydride the desired product **3a** was obtained with 16% yield (Table 1, entry 4). Careful, analysis of mas spectra of the reaction mixture shown the attendance of the intermediate **4a** which may undergo further elimination reaction providing unsaturated product **3a** (Supplementary Information). Observed enhancement in the studied reaction course can be explain by the fact that -OAc is better leaving group than -OH one, and therefore has desirable influence on the elimination step.[44] In the absence of enzyme formation of the expected product **3a** has not been observed. Moreover, application of sodium carbonate which competes to the anhydride reduced the yield of the product **3a** to 8% (Table 1, entry 5). Subsequently, some other active methylene compounds ethyl 4-aryloylacetates (**1b**-**d**) were applied to expand upon this Novozym 435 catalyzed condensation reaction to figure out the generality and scope of its biocatalytic promiscuity. The obtained products were characterized by NMR. It was found that selected ethyl 4-aryloylacetates (**1b**-**d**) effectively participate in lipase catalyzed Knoevenagel reaction to give the corresponding products **3b**-**d** (Scheme 1). To the best of our knowledge the application of lipases in this type of Knoevenagel condensation has not been reported so far for the 4-aryloylacetates. The results indicated that the ethyl 4-aryloylacetates (**1b**) with electron-withdrawing group at the *para*-positon is the most suitable substrate providing corresponding product (**3b**) with 33% isolated yield (Table 1, entry 6). Next, the effect of temperature on the reaction rate was studied. However, when the temperature rose to 30 °C the adverse effect on reaction yield was noticed (Table 1, entry 7). In case of ethyl 4-aryloylacetates with electron-donating group (**1c**) or non-substituted one (**1d**) corresponding products were obtained with slightly lower yields, 25% and 28%, respectively (Table 1, entries 8 and 9). It is worth pointing out that parallel synthetic trials using literature strictly chemical protocols failed in the synthesis of compounds (**3b**-**d**). Additionally, satisfactory Z/E selectivity (4.3:1) was obtained for the products from active methylene compounds (**1b**-**d**).

Due to the operational difficulties related to low boiling temperature (20 °C) of acetaldehyde we were wondering if vinyl acetate (**5**, $R_3 = CH_3$) can be used as acetaldehyde precursor in enzyme-catalyzed Knoevenagel condensation. To address this challenge we performed studies toward coupling of the enzymatic hydrolysis with biocatalytic condensation as an chemoenzymatic tandem process (Scheme 2).

Recently, it has been recognized that lipases provide abnormal activity in aldol reaction with vinyl acetate.[45,46] Moreover, we have shown that vinyl acetate provides components for enzyme-promoted asymmetric tandem Passerini reaction.[47] Vinyl esters are often preferred as acyl donors as the resulting vinyl alcohol is rapidly converted to acetaldehyde thereby making the reaction faster and irreversible.



Scheme 2. Lipase-catalysed synthesis of 2-aryoyl-but-2-enoic acid ethyl ester **3** in chemoenzymatic tandem reaction.

Although a variety of two-component reactions have been developed in the study of biocatalytic promiscuity, three-component reactions, which can form multi-functional compounds, have never been reported. This is possibly due to the problem of side reactions caused by multi-reagents and the strict range of substrates in enzymatic reactions. In 2010, Lai *et al.* reported lipase-catalyzed tandem Knoevenagel condensation and esterification with alcohol co-solvents.[48]

For the preliminary studies on enzymatic tandem reaction, Novozym 435 was used (Scheme 2). A mixture of 4-nitrobenzoylacetate (**1b**) (1 mmol) and vinyl acetate (1 mmol) in organic solvent (5 mL) was stirred at 40 °C for 120 h. The reaction was terminated by filtering off the catalyst. Many reports have shown that organic media is one of the most important

factors influencing the enzyme catalytic performance.[31-34] Based on this fact fourteen common used organic solvents were investigated (Table 2).

Table 2. Effect of solvents on the Knoevenagel reaction between vinyl acetate (**5**, R_3=CH_3) and 4-nitrobenzoylacetate (**1b**).[a]

Entry	Solvent	log*P*	**3b** Yield [%][b]
1	Dimethyl sulfoxide	-1.4	6
2	*N*,*N*-Dimethylformamide	-1.01	<1
3	Acetonitrile	-0.33	45
4	Acetonitrile[c]	-0.33	18 (>99)[d]
5	Ethanol	-0.30	5
6	1,4-Dioxane	-0.27	9
7	Isopropyl alcohol	-0.07	12
8	*tert*-Butyl alcohol	0.35	44
9	*tert*-Butyl alcohol[c]	0.35	21 (>99)[d]
10	Tetrahydrofuran	0.44	23
11	Methyl *tert*-butyl ether	0.94	32
12	Methyl chloride	1.25	5
13	*iso*-Propylether	1.52	30
14	Chloroform	1.94	9
15	Toluene	2.30	13
16	Cyclohexane	3.44	11

Reaction conditions: **1a** (1 mmol), vinyl acetate (1 mmol), organic solvent (5 mL) and Novozym 435 (200 mg) at 40 °C for 120 h. [b] Isolated yield equal conversion. [c] Water was added (5% v/v). [d] Conversion in brackets.

The results indicated that acetonitrile and *tert*-butyl alcohol were the best medium for the studied model reaction (Table 1, entries 3 and 8), providing desired product **3b** with 44% and 45% yield. The *Z*/*E* selectivity remained the same (4.3:1) as was observed when acetaldehyde was used in condensation reaction. The results clearly indicated that the catalytic activity of immobilized *Candida antarctica* B lipase both in hydrolysis of vinyl acetate as well as condensation reaction was significantly influenced by the solvent (Table 2, entries 1-12), while no clear correlation between the value of log*P* and enzyme activity was observed, as is consistent with the literature. Additional experiments regarding enzyme loading and reaction time were undertaken. Further increasing the Novozym 435 loading or reaction time prolongation did not affected the reaction yield (data not shown). Thus, further experiments were performed for 120 hours using 200 mg of enzyme as the optimal amount. In the absence of enzyme formation of product **3b** was not observed. Next, the influence of water was tested on the course of studied cascade, mainly on the hydrolysis of vinyl acetate. It was revealed that addition of 5% v/v significantly reduced the yield of product **3b** to 18% and 21% in case of using acetonitrile and *tert*-butyl alcohol, respectively. It both cases, unwanted enzymatic hydrolysis and spontaneous decarboxylation of used 4-nitrobenzoylacetate (**1b**) occurred, providing 4-nitroacetophenone as the main product (Table 2, entries 4 and 9). Sufficient amount of water which is compulsory for the initiation of enzymatic hydrolysis of vinyl acetate is most likely derived from used enzyme preparation. All these facts collectively point out to a reaction sequence (Scheme 2) which involves: enzymatic cleavage of vinyl acetate to produce acetaldehyde even under anhydrous conditions, what good correlates with the literature data.[46a]

It's well known that the catalytic activity of a lipase depends mainly on the type and origin of the hydrolase. Thus, teen different kinds of enzymes were screened to catalyze studied tandem reaction (Table SI2, Supporting Information). In general, selected lipases (Table SI2, entries 1-5, Supporting Information) exhibited higher enzymatic promiscuity in studied tandem reaction than proteases (Table SI2, entries 8-10, Supporting Information), providing product **3b** with the yield up to 28% (Table SI2, entry 1, Supporting Information). No detectable product **3b** was obtained in case of using *Candida rugosa* lipase (Table SI2, entry 6, Supporting Information), bovine serum albumin (BSA) (Table SI2, entry 7, Supporting Information), or in the absence of enzyme (Table SI2, entry 8, Supporting Information) what proved that the specific tertiary structure of enzyme was essential to this reaction.

Table 3. Optimization of the enzymatic synthesis of **3b**-**d**.[a]

Entry	**1**	Solvent	**5** (equiv.)	T [°C]	Yield [%][b]
1	**1b**	Acetonitrile	**5a** (1.0)	30	39
2	**1b**	Acetonitrile	**5a** (1.0)	40	45
3	**1b**	Acetonitrile	**5a** (1.0)	50	44
4	**1b**	Acetonitrile	**5a** (1.0)	60	21
5	**1b**	Acetonitrile	**5a** (2.0)	40	48
6	**1b**	Acetonitrile	**5a** (3.0)	40	47
7	**1b**	*tert*-Butyl alcohol	**5a** (2.0)	30	44
8	**1b**	*tert*-Butyl alcohol	**5a** (2.0)	40	49
9	**1b**	*tert*-Butyl alcohol	**5a** (2.0)	50	52
10	**1b**	*tert*-Butyl alcohol	**5a** (2.0)	60	72
11	**1b**	*tert*-Butyl alcohol	**5a** (2.0)	70	61
12	**1b**	*tert*-Butyl alcohol	**5b** (2.0)	60	44
13	**1b**	*tert*-Butyl alcohol	**5c** (2.0)	60	3
14	**1b**	*tert*-Butyl alcohol	**5d** (2.0)	60	14
15	**1b**	*tert*-Butyl alcohol	**5e** (2.0)	60	16
16	**1a**	*tert*-Butyl alcohol	**5a** (2.0)	60	37
17	**1c**	*tert*-Butyl alcohol	**5a** (2.0)	60	48

18	**1d**	*tert*-Butyl alcohol	**5a** (2.0)	60	63

Reaction conditions: **1** (1 mmol), vinyl ester **5**, organic solvent (5 mL) and Novozym 435 (200 mg), 120 h. [b] Isolated yield equal conversion.

In order to improve the reaction yields, other factors such as reaction temperature, type and amount of acetaldehyde precursor vinyl esters **5** were further investigated (Table 3). To evaluate the effect of temperature on the reaction performed in acetonitrile, the reaction was investigated at a temperature range from 30 °C to 60 °C. The reaction rate increased with increasing temperature from 30 °C to 50 °C (Table 3, entries 1-3); however the yield decreased significantly above 50 °C providing product **3b** with only 21% (Table 3, entry 4). The application of higher excess of vinyl acetate (3 and 4 equiv.) has scant impact on the reaction course, providing product **3b** with 48% and 47% yield, respectively (Table 3, entries 5 and 6). Analogous temperature studies with 2 equiv. of vinyl acetate were conducted in *tert*-butyl alcohol, which was the second suitable organic solvent for enzymatic tandem reaction catalyzed by Novozym 435 (Table 2, entry 8). The yield of product **3b** was increased from 44% for 30 °C (Table 3, entry 7) up to 72 % for 60 °C (Table 3, entries 8-10) and declined above 60 °C (Table 3, entry 11), which may be due to the conformational change of the active site of *Candida antarctica* B lipase caused by excessively high temperature. Thus, further experiments were performed in *tert*-butyl alcohol at optimal temperature 60 °C .

Next, we subsequently studied the effect of the acetaldehyde precursor on the rate of the studied tandem reaction (Scheme 2). Four different vinyl esters were used; vinyl propionate (**5b**, $R_3 = C_2H_5$), vinyl pivalate (**5c**, $R_3 = (CH_3)_3C$), vinyl laurate (**5d**, $R_3 = CH_3(CH_2)_{10}$), and vinyl benzoate (**5e**, R_3 = Ph). Thus, it was observed that the type of the used precursors has substantial influence on the reaction rate (Table 4, entries 12-15) and generally were less suitable than used vinyl acetate (**5a**). Product **3b** with the highest yield 44% was obtained with vinyl propionate (Table 3, entry 12). In case of long aliphatic chain vinyl laurate (**5d**) and aromatic vinyl benzoate (**5e**) product was obtained with lower but comparable yields 14% and 16%, respectively. For vinyl pivalate the product **3b** was obtained with only 3% yield. The funnel-like active site of *Candida antarctica* B lipase is known to be sterically restricted and accordingly the enzyme is not very efficient with two bulky substrates such as **1b** and vinyl pivalate.[49] During this performed studies applying substrate **1b** and vinyl acetate (**5a**), the enzyme was recycled for up to five cycles. There was no significant decrease in yield of product **3b** during the third run whereas the yield declined up to 62% after the completion of the fifth run (Figure 1). Decreased activity of Novozym 435 over five reaction cycles is largely due to leakage of *Candida antarctica* B lipase.

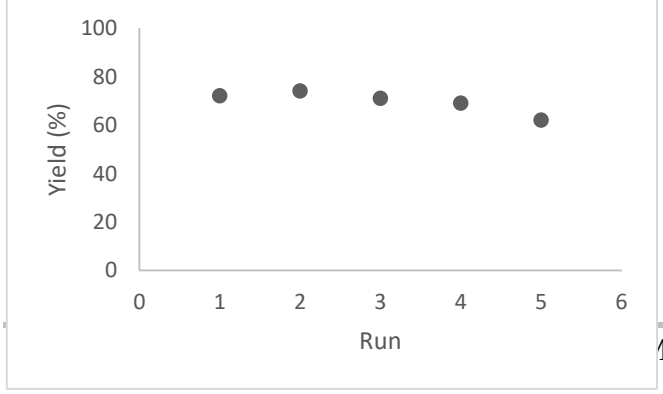
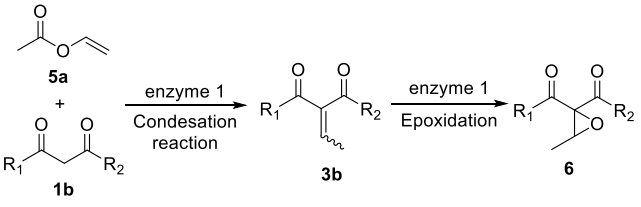


Figure 1. Recyclability of enzyme.

At this point, to validate our protocol additional experiments with acetylacetone (**1a**) and two different ethyl 4-aryloylacetates **1c** and **1d** were conducted under optimized conditions (Scheme 2), providing corresponding α,β-unsaturated products **3a**, **3c** and **3d** with 37%, 48% and 63% yield respectively, with good *E*/*Z* selectivity (4.3:1) (Table 3, entries 16-18). These compounds are versatile building blocks for synthesis of several bioactive compounds.[1i,1j] Moreover, when the ethyl 3-(4-methoxyphenyl)-3-oxopropanoate (**1c**) and ethyl benzoylacetate (**1d**) were subjected to the condensation reaction in methyl *tert*-butyl ether with Novozym 435, additionally to the expected α,β-unsaturated products **3c** and **3d**, intermediates **4c** and **4d** were obtained with 18% and 15% yield respectively (Scheme 2). Those compounds were again subjected to reaction with Novozym 435 providing corresponding products **3c** and **3d** quantitatively within 8 hours. Obtained results additionally to the mas spectra analysis of the reaction mixture (Supplementary Information) probate proposed mechanism of the enzymatic condensation proposed in Scheme 1 and show that formation of the intermediate **2** may be step limiting of the enzyme-catalyzed Knoevenagel reaction.

Inspired by the work of Yang *et al*. who show chemoenzymatic synthesis of α-cyano epoxides by a tandem Knoevenagel–epoxidation reaction catalyzed by *Candida antarctica* B lipase,[50] we extend our studies toward chemoenzymatic epoxides synthesis. Epoxides are important intermediates in the chemical industry due to their reactivity.[51] The synthesis of epoxides has gained more interest when enzymes began to be used as a catalyst with regards to the creation of environmentally friendly process. The use of biological catalysts like lipases has its own advantage such as high regioselectivity, which can lead mainly to high purity in epoxide production.[52]



R_1 = 4-$NO_2C_6H_4$, R_2 = OC_2H_5

Scheme 3. Lipase-catalysed synthesis of epoxides through a sequential-Knoevenagel–epoxidation reaction.

Referential epoxide **6** (Scheme 3, R_1 = 4-$O_2NC_6H_4$, R_2 = OC_2H_5) was obtained *via* reaction with *meta*-chloroperoxybenzoic acid (*m*-CPBA) with 56% yield. *m*-CPBA is a strong electrophilic reagent and thus highly reactive in the oxidation of alkenes, sulfides, selenides and amines; but a major drawback is the shock sensitivity and a detonative nature.[53] Based on the fact that Novozym 435 can generate *in situ* peroxyacids from the corresponding carboxylic acids[54] we set up experiment with 1.5 equiv. hydrogen peroxide urea adduct

(UHP) in acetonitrile or *tert*-butyl alcohol only traces of desired product **6** was observed (TLC) after 168 hours at 60 °C (Scheme 3). According to literature results about enzymatic epoxidation the best performance of the biocatalytic epoxidation was shown when peroxyacids were generated from ethyl acetate or phenylacetic acid.[55] Unfortunately, no beneficial impact was observed after addition of 5 equivalents of ethyl acetate. Additionally the yield of the product **3b** was reduced dramatically to 32%. For the comparison, when the enzymatic epoxidation was performed on **3b** using ethyl acetate and UHP at 60 °C the racemic epoxide **6** was obtained with 15% yield after 48 hours. Finally, when the phenylacetic acid and 1.5 equiv. of UHP were added sequentially after 120 hours to the reaction mixture in acetonitrile and left to react for additional 48 hours at 40 °C the desired epoxide **6** was isolated with 29% yield (Scheme 3).

Conclusions

In summary, we have described here a first example of enzyme-catalyzed Knoevenagel reaction on acetaldehyde providing unsaturated products in aqueous free organic solvents. We disclosed a novel protocol for the synthesis of desired products through a tandem process based on the enzymatic hydrolysis of esters and Knoevenagel reaction starting from acetaldehyde precursors vinyl carboxylates. Further this protocol was extended by enzymatic epoxidation. All described enzymatic transformations were sequentially catalyzed by one enzyme immobilized *Candida antarctica* B lipase revealed it outstanding catalytic promiscuity. Moreover, application of vinyl carboxylates as a precursors of highly volatile acetaldehyde significantly simplifies the process providing products which cannot be obtained *via* chemical approach.

Experimental Section

General methods. 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 parts per million using TMS as an internal standard. TLC analyses were done on Kieselgel 60 F254 aluminum sheets. Lipases from porcine pancreas, *Pseudomonas cepacia*, *Pseudomonas fluorescens*, *Candida rugosa*, *Candida lipolytica* and *Candida cylindracea* were purchased from Sigma-Aldrich. Immobilized lipase from *Candida antarctica* B (Novozym 435) was purchased from Novo Nordisk. Lipase from *Carica papaya* was kindly provided by prof. Shau-Wei Tsai from Graduate Institute of Biochemical and Biomedical Engineering (GIBBE), Chang Gung University (CGU), Taoyuan City, Taiwan. Proteases from *Bacillus licheniformis* and *Bacillus acyloligefaciens* were purchased from Sigma-Aldrich. Proteinase K and bovine serum albumin were purchased from Sigma-Aldrich. *m*-CPBA (73% in water) was commercially available from Aldrich-Sigma. Column chromatographies were 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). To prove the ability of the established protocol each reaction was repeated at least three times. 3-Ethylidene-pentane-2,4-dione (**3a**) and 2-benzoyl-but-2-enoic acid ethyl ester (**3d**) were obtained with 3% and 5% yield respectively according to the literature procedure.[39b]

General procedure for enzyme-catalyzed Knoevenagel reaction with acetaldehyde. A mixture of acetaldehyde (1 mmol), enzyme (200 mg) and active methylene compound (1 mmol) in *tert*-butyl alcohol (5 mL, dried by anhydrous Na_2SO_4) was shaken at 200 rpm at 20 °C for 120 hours. The reaction was monitored by thin layer chromatography (eluent: ethyl acetate/hexanes, 2:8, v/v) and terminated by filtering off the catalyst. The residue was washed with ethyl acetate. The combined organic phase was dried with Na_2SO_4 (anhydrous) and concentrated under vacuum. The resulting residue was purified by column chromatography (silica gel, eluent: ethyl acetate/hexanes) to afford the desired ethylidene derivatives **3**.

General procedure for enzyme-catalyzed Knoevenagel reaction with vinyl acetate. A mixture of vinyl acetate (1 mmol), enzyme (200 mg) and active methylene compound (1 mmol) in *tert*-butyl alcohol (5 mL, dried by anhydrous Na_2SO_4) was shaken at 200 r/min at 40 °C for 120 hours. The reaction was monitored by thin layer chromatography (eluent: ethyl acetate/hexanes, 2:8, v/v) and terminated by filtering off the catalyst. The residue was washed with ethyl acetate. The combined organic phase was dried with Na_2SO_4 (anhydrous) and concentrated under vacuum. The resulting residue was purified by column chromatography (silica gel, eluent: ethyl acetate/hexanes) to afford the desired ethylidene derivatives **3**.

4-Nitroacetophenone: (Commercially Available) ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.65 (s, 3H), 8.08 (d, *J* = 8.8 Hz, 2H), 8.27 (d, *J* = 8.8 Hz, 2H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 26.9, 123.8, 129.2, 141.4, 150.3, 196.2. The ^{1}H and ^{13}C NMR data were in accordance with those reported in the literature.[56]

3-Ethylidene-pentane-2,4-dione (3a): Colorless oil. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.91 (d, *J* = 7.2 Hz, 3H), 2.30 (s, 3H), 2.31 (s, 3H); 6.79 (q, *J* = 7.2 Hz, 1H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 15.4, 25.9, 31.5, 141.8, 196.8, 203.3. The ^{1}H and ^{13}C NMR data were in accordance with those reported in the literature.[39b] LR ESIMS $(M+Na)^+$ calcd for $C_7H_{10}O_2Na$ 149.1, found 149.2.

2-(4-Nitrobenzoyl)-but-2-enoic acid ethyl ester (3b) Colorless oil. Two isomers. Ratio 4.3:1; ^{1}H NMR ($CDCl_3$, 400 MHz) major isomer: δ 1.14 (t. *J* = 7.2 Hz, 3H), 1.83 (d, *J* = 7.2 Hz, 3H), 4.12 (q, *J* = 7.2 Hz, 2H), 7.32 (q, *J* = 7.6 Hz, 1H), 7.98-8.01 (m, 2H), 8.25-8.31 (m, 2H); minor isomer: 1.15 (t. *J* = 7.2 Hz, 3H), 2.19 (d, *J* = 7.2 Hz, 3H), 4.14 (q, *J* = 7.2 Hz, 2H), 6.87 (q, *J* = 7.2 Hz, 1H), 7.85-7.88 (m, 2H), 8.25-8.31 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 192.7, 191.5, 164.7, 163.8, 150.5, 149.0, 145.5, 142.5, 141.3, 135.4, 133.9, 129.7, 129.5, 123.9, 123.6, 61.3, 61.1, 15.9, 15.4, 13.9, 13.8; HR ESIMS $(M-H)^-$ calcd for $C_{13}H_{12}NO_5$ 262.0715, found 262.0712; Elementary analysis calcd. for $C_{13}H_{13}NO_5$; C 59.31%, H 4.98% found C 59.42%, H 4.89%.

2-(4-Methoxybenzoyl)-but-2-enoic acid ethyl ester (3c) Colorless oil. Two isomers. Ratio 4.3:1; ^{1}H NMR ($CDCl_3$, 400 MHz) δ major isomer: δ 1.09 (t. *J* = 7.2 Hz, 3H), 1.83 (d, *J* = 7.6 Hz, 3H), 3.87 (s, 3H), 4.15 (q, *J* = 7.2 Hz, 2H), 6.93-6.96 (m, 2H), 7.34 (q, *J* = 7.6 Hz, 1H), 7.97-8.02 (m, 2H); minor isomer: 1.10 (t. *J* = 7.2 Hz, 3H), 2.19 (d, *J* = 7.2 Hz, 3H), 3.87 (s, 3H), 4.15 (q, *J* = 7.2 Hz, 2H), 6.10 (q, *J* = 7.6 Hz, 1H), 6.93-6.96 (m, 2H), 7.97-8.02 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 13.95, 14.0, 16.0, 15.5, 55.0, 55.1, 61.3, 61.4, 113.7, 114.0, 129.3, 131.1, 131.2, 164.0, 164.2, 196.5; HR ESIMS $(M+Na)^+$ calcd for $C_{14}H_{16}O_4Na$ 271.0946, found 271.0942; Elementary analysis calcd. for $C_{14}H_{16}O_4$; C 67.73%, H 6.50%, found C 67.69%, H 6.71%.

2-Benzoyl-but-2-enoic acid ethyl ester (3d). Colorless oil. Two isomers. Ratio 4.3:1; ^{1}H NMR ($CDCl_3$, 400 MHz) major isomer: δ 1.10 (t. *J* = 7.2 Hz, 3H), 1.83 (d, *J* = 7.2 Hz, 3H), 4.13 (q, *J* = 7.2 Hz, 2H), 7.32 (q, *J* = 7.2 Hz, 1H), 7.32-7.61 (m, 3H), 7.71-8.12 (m, 2H); minor isomer: 1.15 (t. *J* = 7.2 Hz, 3H), 2.16 (d, *J* = 7.2 Hz, 3H), 4.12 (q, *J* = 7.2 Hz, 2H), 6.74 (q, *J* = 7.2 Hz, 1H), 7.32-7.61 (m, 2H), 7.71-8.12 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 194.4, 164.4, 146.6, 143.3, 136.7, 134.8, 133.6, 132.7, 128.9, 128.8, 128.7, 128.3, 61.0, 60.8, 15.6, 15.3, 13.9, 13.8. The ^{1}H and ^{13}C NMR data were in accordance with those reported in the literature.[39b]

Ethyl 3-(acetyloxy)-2-(4-methoxybenzoyl)butanoate (4c). Colorless oil. Two isomers. Ratio 1:1; ^{1}H NMR ($CDCl_3$, 400 MHz) one isomer: δ 1.14 (t. *J* = 7.2 Hz, 3H), 1.27 (d, *J* = 6.0 Hz, 3H), 1.88 (s, 3H), 3.87 (s, 3H), 4.15 (q, *J* = 7.2 Hz, 2H), 4.54 (m, 1H), 5.61-5.64 (m, 1H), 6.93-6.96 (m, 2H), 7.98-8.02 (m, 2H); second isomer: 1.14 (t. *J* = 7.2 Hz, 3H), 1.39 (d, *J* = 6.0 Hz, 3H), 2.00 (s, 3H), 3.87 (s, 3H), 4.15 (q, *J* = 7.2 Hz, 2H), 4.54 (m, 1H), 5.65-5.75 (m, 1H), 6.93-6.96 (m, 2H), 7.98-8.02 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 190.5, 169.7, 169.6, 167.2, 167.1, 164.2, 164.0, 131.2, 131.1, 129.3, 129.3, 114.0, 113.9, 69.9, 69.1, 61.6, 61.5, 59.2, 58.8, 55.5, 55.5, 21.0, 20.9, 18.5, 18.0, 13.9, 13.9; HR ESIMS $(M+Na)^+$ calcd for $C_{16}H_{20}O_6Na$ 331.1158, found 331.1157.

Ethyl 3-(acetyloxy)-2-benzoylbutanoate (4d). Colorless oil. Two isomers. Ratio 1:1; ^{1}H NMR ($CDCl_3$, 400 MHz) one isomer: δ 1.15 (t. *J* = 7.2 Hz, 3H), 1.29 (d, *J* = 6.0 Hz, 3H), 1.88 (s, 3H), 4.17 (q, *J* = 7.2 Hz, 2H), 4.59 (m, 1H), 5.64-5.66 (m, 1H), 7.46-7.50 (m, 2H), 7.57-7.60 (m, 1H), 7.98-7.8.02 (m, 2); second isomer: 1.16 (t. *J* = 7.2 Hz, 3H), 1.39 (d, *J* = 6.4 Hz, 3H), 1.99 (s, 3H), 4.17 (q, *J* = 7.2 Hz, 2H), 4.59 (m, 1H), 5.67-5.70 (m, 1H), 7.46-7.50 (m, 2H), 7.57-7.60 (m, 1H), 7.98-7.8.02 (m, 2); ^{13}C NMR (100 MHz, $CDCl_3$): δ 192.3, 192.2, 169.7, 167.0, 166.8, 136.3, 133.8, 133.6, 128.8, 128.7, 128.6, 69.0, 61.7, 61.6, 59.3, 59.0, 21.0, 18.5, 14.0, 13.9; HR ESIMS $(M+Na)^+$ calcd for $C_{15}H_{18}O_5Na$ 301.1051, found 301.1052.

Synthesis of ethyl 3-methyl-2-(4-nitrobenzoyl)oxirane-2-carboxylate (6). To a solution of 2-(4-nitrobenzoyl)-but-2-enoic acid ethyl ester (**3b**) (88 mg, 0.33 mmol) in dry DCM (10 mL), KOH (110 mg, 2.00 mmol) and *m*-CPBA (232 mg, 1.32 mmol) were successively added. After stirring for 16 hours the reaction was filtered using Celite, solvent was removed in vacuum. The resulting residue was purified by column chromatography (silica gel, eluent: ethyl acetate/hexanes) to afford the desired product **6** as a colorless liquid (51 mg, 0.18 mmol) with 56% yield; ^{1}H NMR ($CDCl_3$, 400 MHz) δ major isomer: δ 1.19 (t. *J* = 7.2 Hz, 3H), 1.30 (d, *J* = 5.6 Hz, 3H), 3.76 (q, *J* = 5.6 Hz, 1H), 4.22 (q, *J* = 7.2 Hz, 2H), 8.17 (d, *J* = 9.2 Hz, 2H), 8.33 (d, *J* = 9.2 Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 189.9, 166.6, 139.3, 130.2, 123.9, 70.0, 63.0, 62.9, 59.5, 15.1, 13.8; HR ESIMS $(M+Na)^+$ calcd for $C_{13}H_{13}NO_6Na$ 302.0641, found 302.0630; Elementary analysis calcd. for $C_{13}H_{13}NO_6$; C 55.92%, H 4.69%, N 5.02% found C 55.89, H 4.75%, N 4.96%.

General procedure for enzyme-catalyzed synthesis of ethyl 3-methyl-2-(4-nitrobenzoyl)oxirane-2-carboxylate (6). A mixture of the ethyl 3-(4-nitrophenyl)-3-oxopropanoate (237 mg, 1 mmol), vinyl acetate (3 mmol), and Novozym 435 (200 mg) in acetonitrile (5 mL) was shaken at 200 rpm at 40 °C for 120 hours. The reaction was monitored by TLC. Then, UHP (1.5 mmol) and phenylacetic acid (272 mg, 2.00 mmol) were added, and the mixture was stirred for 48 hours. Next, the mixture was filtered and the residue was washed with ethyl acetate. The combined organic phase was concentrated under vacuum. The resulting residue was purified by column chromatography (silica gel, eluent: ethyl acetate/hexanes) to afford the desired 3-methyl-2-(4-nitrobenzoyl)oxirane-2-carboxylate (**6**) with 29% yield.

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Keywords: Knoevenagel condensation • promiscuity • hydrolases • biocatalysis • tandem reaction

[1] a) E. Knoevenagel, *Chem. Ber*. **1894**, *27*, 2345−2346; b) E. Knoevenagel, *Chem. Ber*. **1898**, *31*, 2596−2619; c) J. J. Li, *Name Reactions*, 4th ed; Springer-Verlag: Berlin Heidelberg, **2009**; pp. 315−316; d) R. C. Larock, *In Comprehensive Organic Transformations*, 2nd ed.; John Wiley & Sons: Toronto, **1999**; e) Ebitani, K. *Comprehensive Organic Synthesis* (2nd Edition). Ed: Knochel, P.; Molander, G. A **2014**, *2*, pp. 571-605; f) Heravi, M.; Asadi, S.; Azarakhshi, F. Curr. Org. Synth. **2014**, *11*, 701-731; g) Vekariya, R.H. Patel, H.D. *Synth. Commun.* **2014**, *44*, 2756-2788; h) Voskressensky, L.G. Festa, A. A. Varlamov, A.V. *Tetrahedron* **2014**, *70*, 551-572; i) Bigi, F. Quarantelli, C. *Curr. Org. Synth*. **2012**, *9*, 31-39.

[2] a) T. Inokuchi, M. Okano, T. Miyamoto, *J. Org. Chem*. **2001**, *66*, 8059-8063; b) L. F. Tietze, *Chem. Rev*. **1996**, *96*, 115-136; c) K. Hackelöer, G. Schnakenburg, S. R. Waldvogel, *Eur. J. Org. Chem*. **2011**, 6314-6319; d) I. Walz, A. Bertogg, A. Togni, *Eur. J. Org. Chem*. **2007**, 2650-2658.

[3] T. Yamamori, Y. Hiramatu, K. Sakai, I. Adachi, *Tetrahedron* **1985**, *41*, 913-917.

[4] D. Bogdał, *J. Chem. Res. (S)* **1998**, 468-469.

[5] a) H.-F. Cui, K.-Y. Dong, G.-W. Zhang, L. Wanga, J.-A. Ma, *Chem. Commun*. **2007**, 2284–2286; b) G. Sartori, R. Maggi, F. Bigi, C. Porta, X. Tao, G. L. Bernardi, S. Lanelli, M, Nardelli, *Tetrahedron*, **1995**, *51*, 12179-12192.

[6] C. D. Smith, G. Rosocha, L. Mui, R. A. Batey, *J. Org. Chem*. **2010**, *75*, 4716-4727.

[7] S. S. Al-Shihry, *Molecules* **2004**, *9*, 658.

[8] H. McNab, *Aldrichimica Acta* **2004**, *37*, 19.

[9] a) R. Tanikaga, N. Konya, K. Hamamura, A. Kaji, *Bull. Chem. Soc. Jpn*. **1988**, *61*, 2311-2316; b) R. Tanikaga, T. Tamura, Y. Nozaki, A. Kaji, *J. Chem. Soc., Chem. Commun*. **I984**, 87-88; c) E. Angeletti, C. Canepa, G. Martinetti, P. Venturello, *J. Chem. Soc. Perkn Trans. I* **1989**, 105-107.

[10] a) G. Bartoli, M. Bosco, A. Carlone, R. Dalpozzo, P. Galzerano, P. Melchiorre, L. Sambri, *Tetrahedron lett*. **2008**, *49*, 2555-2557; b) W. Lehnert, *Tetrahedron* **1974**, *30*, 301-305; c) B. Green, R. I. Crane, I. S. Khaidem, R. S. Leighton, S. S. Newaz, T. E. Smyser, *J. Org. Chem*. **1985**, *50*, 640-644; d) R. P. Shanthan R. V. Venkataratnam, *Tetrahedron Lett*. **1991**, *32*, 5821-5822; e) G. Bartoli, R. Beleggia, S. Giuli, A. Giuliani, E. Marcantoni, M. Massaccesi, M. Paletti, *Tetrahedron Lett*. **2006**, *47*, 6501-6504.

[11] a) S. Saravanamurugan, M. Palanichamy, M. Hartmann, V. Murugesan, *Appl. Catal. A* **2006**, *298*, 8-15; b) T. I. Reddy, R. S. Varma, *Tetrahedron Lett*. **1997**, *38*, 1721-1724.

[12] F. Bigi, L. Chesini, R. Maggi, G. Sartori, *J. Org. Chem.* **1999**, *64*, 1033-1035.

[13] a) B. C. Ranu, R. Jana, *Eur. J. Org. Chem*. **2006**, 3767-3770; b) R. V. Hangarge, D. V. Jarikote, M. S. Shingare, *Green Chem*. **2002**, *4*, 266-268; c) J. R. Harjani, S. J. Nara, M. M. Salunkhe, *Tetrahedron Lett*. **2002**, *43*, 1127–1130.

[14] a) Y.-H. He , Y. Hu, Z. Guan, *Synth. commun*. **2011**, *41*, 1617-1628; b) P. Goswami, B. Das, *Tetrahedron lett*. **2009**, *50*, 897-900,

[15] E. Obrador, M. Castro, J. Tamariz, G. Zepeda, R. Miranda, F. Delgado, *Synth. Commun*. **1998**, *28*, 4649.

[16] S. Kantevari, R. Bantu, L. Nagarapu, *J. Mol. Catal. A: Chem*. **2007**, *269*, 53-57.

[17] a) K. Tanaka, *Solvent-Free Organic Synthesis*; Wiley-VCH: Weinheim, **2003**. Chapter 3.2, pp 93–136; b) K. Tanaka, F. Toda, F. *Chem. Rev*. **2000**, *100*, 1025.

[18] a) R. Maggi, F. Bigi, S. Carloni, A. Mazzoc, *Green Chem*. **2001**, *173*; b) F. Bigi, M. L. Conforti, R. Maggi, A. Piccinno, G. Sartori, *Green Chem*. **2000**, 173; c) F. Bigi, S. Carloni, L. Ferrari, R. Maggi, A. Mazzacani, G. Sartori, *Tetrahedron Lett*. **2001**, *42*, 5203.

[19] a) Hangarge, R. V.; Sonwane, S. A.; Jarikote, D. V.; Shingare, M. S. *Green Chem*. **2001**, *3*, 310-312; b) Kaupp, G.; Naimi-Jamal, M. R.; Schmeyers, J. *Tetrahedron* **2003**, *59*, 3753-3760.

[20] a) Arora, B. Mukherjee, J. Gupta, M. N. *Sustainable Chem. Proc.* **2014**, *2*, 2-9; b) Bornscheuer, U. T. Kazlauskas, R. J. *Angew. Chem. Int. Ed*. **2004**, *43*, 6032-6040; c) Hult, K. Berglund, P. *Trends Biotechnol*. **2007**, *25*, 231-238; d) Kapoor, M. Gupta, M. N. *Process Biochem.* **2012**, *47*, 555-569; e) Miao, Y. Rahimi, M. Geertsema E.M. Poelarends, G.J. *Curr. Opin. Chem. Biol*. **2015**, *25*, 115-123; f) Lopez-Iglesias, M. Gotor-Fernandez, V. *Chem. Rec*. **2015**, *15*, 743-759.

[21] Wang, J.L.; Liu, B.K.; Yin, C.; Wu, Q.; Lin, X.F. *Tetrahedron* **2011**, *67*, 2689-2692.

[22] a) Zhou, P. Wang, X. Yang, B. Hollmann, F. Wang, Y. *RSC Adv*. **2017**, *7*, 12518-12523; b) Wang, X. P. Zhou, P. F. Li, Z. G. Yang, B. Hollmann, F. Wang, Y. H. *Sci Rep*. **2017**, *7*, 1-5; c) Ríos, M.Y. Salazar, E. Olivo, H.F. *Green Chem*. **2007**, *9*, 459-462; d) Kotlewska, A.J. van Rantwijk, F. Sheldona, R.A. Arends I.W.C.E. *Green Chem*. **2011**, *13*, 2154-2160; e) Yang, F. Wang, Z. Zhang, X. Jiang, L. Li, Y. Wang, L. *ChemCatChem* **2015**, *7*, 3450-3453; f) Yang, F. Zhang, X. Li, F. Wang, Z. Wang, L. *Green Chem*. **2016**, *18*, 3518-3521; g) Zhang, L. Li, F. Wang, C. Zheng, L. Wang, Z. Zhao, R. Wang, L. *Catalysts* **2017**, *7*, 115.

[23] Lou, F.-W. Liu, B.-K. Wu, Q. Lv, D.-S. Lin, X.-F. *Adv. Synth. Catal*. **2008**, *350*, 1959-1962.

[24] a) Tian, X. Zhang, S. Zheng, L. *J. Microbiol. Biotechnol*. **2016**, *26*, 80-88; b) Le, Z.-G. Guo, L.-T, Jiang, G.-F. Yang, X.-B. Liu, H.-Q. *Green Chem. Lett. Rev*. **2013**, *6*, 277-281.

[25] Tian, X. Zhang, S. Zheng, L. *Enzyme Microb Technol*. **2016**, *84*, 32-40.

[26] Li, K. He, T. Li, C. Feng, X.-W. Wang, N. Yu, X.-Q. *Green Chem*. **2009**, *11*, 777-779.

[27] Li, C. Feng, X.-W. Wang, N. Zhou, Y.-J. Yu, X.-Q. *Green Chem*. **2008**, *10*, 616-618.

[28] a) Torre, O. Alfonso, I. Gotor, V. *Chem. Commun*. **2004**, 1724-1725; b) Du, L.-H. Ling H.-M. Luo, X.-P. *RSC Adv*. **2014**, *4*, 7770-7773.

[29] Feng, X.-W. Li, C. Wang, N. Li, K. Zhang, W.W. Wang Z. Yu, X.-Q. *Green Chem*. **2009**, *11*, 1933-1936.

[30] Evitt, A.S. Bornscheuer, U.T. *Green Chem*. **2011**, *13*, 1141-1142.

[31] Li, W. Li, R. Yu, X. Xu, X. Guo, Z. Tan, T. Fedosov, S.N. *Biochem. Engineering J.* **2015**, *101*, 99-107.

[32] Hu, W. Guan, Z. Deng, X. He, Y.-H. *Biochimie* **2012**, *94*, 656-661.

[33] Ding, Y. Ni, X. Gu, M. Li, S. Huang, H. Hu, Y. *Catal. Commun*. **2015**, *64*, 101-104.

[34] Xie, B.-H. Guan, Z. He, Y.-H. *Biocatal. Biotransform*. **2012**, *30*, 238-244.

[35] a) Ling, J. Hongwei, Y.U. *Chem. Res. Chin. Univ*. **2014**, *30*, 289-292; b) Borse, B.N. Shukla, S.R. Sonawane, Y.A. *Synth. Commun*. **2012**, *42*, 412-423; c) Ding, Y. Xiang, X. Gu, M. Xu, H. Huang, H. Hu, Y. *Bioprocess Biosyst*. *Eng*. **2016**, *39*, 125-131; d) Sonawane, Y.A. Phadtare, S.B. Borse, B.N. Jagtap, A.R. Shankarling, G.S. *Org. Lett*. **2010**, *12*, 1456-1459.

[36] Yang, F. Wang, Z. Wang, H. Wang, C. Wang, L. *RSC Adv*. **2015**, *5*, 57122-57126.

[37] Wang, Z. Wang, C.-Y. Wang, H.-R. Zhang, H. Su, Y.-L. Ji, T.-F. Wang, L. *Chinese Chem. Lett*. **2014**, *25*, 802-804.

[38] Georgieff, K. K. *J. Applied Polymer Sci*. **1966**, *10*, 1305-1313.

[39] a) W. Lehnert, *Tetrahedron* **1973**, *29*, 635; b) R. Antonioletti, P. Bovicelli, S. Malancona, *Tetrahedron* **2002**, *58*, 589; c) Ishida, A.; Yamashita, S.; Takamuku, S. *Bull. Chem. Soc. Jpn*. **1988**, *61*, 2229-2231; d) Magee, D. I. Ratshonka, S. McConaghy, J. Hood M. *Can. J. Chem*. **2012**, *90*, 450-463.

[40] a) Liu, H. N.; Auchus, R.; Walsh, C. T. *J. Am. Chem. Soc*. **1984**, *106*, 5335-5348.

[41] Sylla, M. Joseph, D. Chevallier, E. Camara, C. Dumas, F. *Synthesis* **2006**, 1045-1049.

[42] a) Kłossowski, S. Wiraszka, B. Berłożecki, Ostaszewski, R. *Org. Lett*. **2013**, *15*, 566-569; b) Żądło-Dobrowolska, A. Kłossowski, S. Koszelewski, D. Paprocki, D. Ostaszewski, R. *Chem. Eur. J.* **2016**, *22*, 16684-16689.

[43] a) Romeroa, M.D. Calvo, L. Alba, C. Daneshfar, A. Ghaziaskar, H.S. *Enzyme Microbial Technol*. **2005**, *37*, 42-48; b) Faber, K. Riva, S. *Synthesis* **1992**, 895-910; c) Hanefeld, U. *Org. Biomol. Chem*. **2003**, *1*, 2405-2415.

[44] Linstead, R.P. Owen, L.N. Webb, R.F. *J. Chem. Soc*. **1953**, 1218-1224.

[45] Majumder, A.B. Ramesh, N.G. Gupta, M.N. *Tetrahedron Lett*. **2009**, *50*, 5190-5193.

[46] a) Weber, H. K.; Weber, H.; Kazlauskas, R. J. *Tetrahedron: Asymmetry* **1999**, *10*, 2635-2638; b) Hogberg, H.-E.; Lindmerk, M.; Isaksson, D.; Sjodin, K.; Franssen, M. C. R.; Jogejan, H.; Wijnberg, J. B. P. A.; Groot, A. *Tetrahedron Lett*. **2000**, *41*, 3193-3196.

[47] Ostaszewski, R. Zadlo, A. Koszelewski, D. Paprocki, D. Madej, A. Wilka, M. ChemCatChem **2017**, 10.1002/cctc.201700427

[48] Lai, Y.F. Zheng, H. Chai, S.-J. Zhang, P.-F. Chen, X.-Z. *Green Chem*. **2010**, *12*, 1917-1918.

[49] Chênevert, R. Pelchat, N. Morin, P. *Tetrahedron: Asymm*. **2009**, *20*, 1191-1196.

[50] Yang, F. Zhang, X. Li, F. Wang, Z. Wang, L. *Eur. J. Org. Chem*. **2016**, 1251-1254.

[51] Lutz, J.T. In: M. Grayson (Ed.), *Encyclopedia of Chemical Technology*, *vol*. 9, Wiley, New York, **1980**.

[52] a) Méndez-Sánchez, D. Ríos-Lombardía, N. Gotor, V. Gotor-Fernández, V. *Tetrahedron*, **2014**, *70*, 1144–1148; b) gen. Klaas, M.R. Warwel, S. *Org. Lett*. **1999**, *1*, 1025-1026.

[53] a) Wang, Z. *Comprehensive Organic Name Reactions and Reagents*, John Wiley &Sons Inc., **2010**; b) Danheiser, S.D. Burke, R.L. *Handbook of Reagents for Organic Synthesis–Oxi-dizing and Reducing Agents*, *vol*. *26*, Wiley, California, **1999**.

[54] a) Tudorache, M. Gheorghe, A. Viana, A.S. Parvulescu, V.I. *J. Mol. Catal. B: Enzym*. **2016**, *134*, 9-15; b) Zanette, A.F. Zampakidi, I. Sotiroudis, G.T. Zoumpanioti, M. Leal, I.C.R. de Souza R.O.M.A. Cardozo-Filho, L. Xenakis, A. *J. Mol. Catal. B: Enzymatic* **2014**, *107*, 89-94.

[55] a) Abdulmalek, E. Arumugam, M. Mizan, H.N. Rahman, M.B.A. Basri, M. Salleh, A.B. *Hindawi Publishing Corporation The Scientific World Journal*, **2014**, *2014*, http://dx.doi.org/10.1155/2014/756418; b) Meyer, J. Holtmann, D. Ansorge-Schumacher, M.B. Kraume, M. Drews, A. *Biochem. Eng. J*. **2017**, *118*, 34-40.

[56] Moriyama, K. Takemura, M. Togo, H. *Org. Lett*. **2012**, *14*, 2414-2417.

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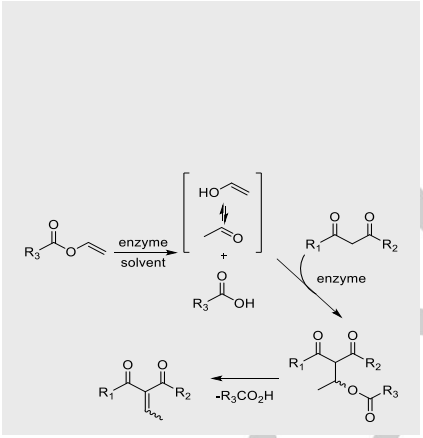
First example of enzyme-catalysed Knoevenagel reaction on aliphatic aldehyde acetaldehyde providing unsaturated products in aqueous free organic solvents has been described. The novel protocol for the synthesis of desired products through a tandem process based on the enzymatic hydrolysis of esters and Knoevenagel reaction starting from acetaldehyde precursors vinyl carboxylates was disclosed.



Dominik Koszelewski, Daniel Paprocki, Arleta Madej, Filip Borys, Anna Brodzka, Ryszard Ostaszewski**

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Enzymatic tandem approach to Knoevenagel condensation of acetaldehyde with acidic methylene compounds in organic media

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