DOI: 10.1002/ejoc.201500676

Enzymatic Synergism in the Synthesis of β-Keto Esters

Catalina Wiśniewska,[a] Dominik Koszelewski,[a] Małgorzata Zysk,[a] Szymon Kłossowski,[a] Anna Żądło,[a] Anna Brodzka,[a] and Ryszard Ostaszewski*[a]

Keywords: Synthetic methods / Biotransformations / Enzyme catalysis / Transesterification / Alcohols / Esters

Reaction of alcohols with ethyl and *tert*-butyl acetoacetate catalyzed by a combination of commercially available enzymes is shown to be a convenient method for the preparation of a range of acetoacetic acid derivatives. Systematic studies proved that the combination of two or more enzymes enhances the yield of the reaction. Application of the selected enzyme mixture for enzymatic transesterification of various β-keto esters provided the respective products in excellent yields up to 96 % and quantitative within 24 and 48 hours, respectively. The presented methodology is simple and mild, and can be used to prepare acetoacetates from primary and secondary alcohols.

Introduction

β-Keto esters constitute a main class of organic building blocks, and they are used for the efficient synthesis of a range of complex natural products such as kermesic and carminic acid esters, whic are commonly used as food-coloring additives.[1] The importance of β-keto esters stems from the facile ability of such compounds to form bonds at all four of the carbon atoms that make up their structural unit; these consist of two different electrophilic carbonyl carbon atoms and two nucleophilic carbon atoms, which can react selectively under appropriate conditions. β-Keto esters are commonly used in the pharmaceutical, agrochemical, chemical, and polymer industries. They are suitable substrates for the preparation of pyridazines,[2] functionalized ketenimines,[3] aminopyrazole derivatives, and other heterocycles.[4] Additionally, they have been successfully applied in cycloaddition reactions of halogenated quinines.[5] Among the above organic compounds, β-keto esters are of particular interest because their chelate complexes with metals have found application as multifunctional additives in various lubricating compositions, including those based on oils of biological origin, as potential antiviral drugs, and as catalysts for various stereoselective reactions.[6] Additionally, β-keto esters are substrates for the Biginelli[7] reaction, providing 3,4-dihydropyrimidin-2(1*H*)-ones, which show antibacterial, and antiviral activities and they can be used as a calcium channel blockers, mitotic kinesine inhibitors, and adrenergic receptor antagonists.[8]

In view of their medicinal importance, synthetic studies on β-keto esters have attracted considerable interest. Several chemical protocols have been explored to provide routes to such compounds. There are a few procedures for the synthesis of β-keto esters based on transesterification reaction of ethyl or methyl acetoacetate with alcohols and, in general, these reactions are catalyzed by protic or Lewis acids.[9] Some of the drawbacks of homogeneous transesterification include troublesome isolation of the product and large volumes of waste generated during workup, and the catalysts are not always recyclable. Furthermore, selectivity is often low, resulting in the formation of unwanted mixtures of products. More recently, various catalysts have been reported to effect transesterification. Most of these methods are not general and are equilibrium-driven reactions in which the use of an excess of one of the reactants is mandatory to obtain the product in good yield. Transesterification of β-keto esters is catalyzed by 4-(dimethylamino)pyridine (DMAP) in good yield, but the application of this catalyst is limited because of its toxicity and high price, and because of the requirement for high temperature.[10] Furthermore, the majority of protic acid catalysts are highly corrosive and, hence, not environmentally friendly. These observations have led to the development of various heterogeneous analogues for the transesterification of methyl and ethyl acetoacetates in particular. The methods that are commonly used employ titanium tetra-alkoxides, tin complexes or indium iodide.[11] A few other homogeneous[12] and heterogeneous catalysts are also known to be effective for this transformation.[13]

Depres and co-workers reported transesterification without a catalyst with propargyl alcohols, but the reaction time was very long (up to 12 days).[14]

Unfortunately, all the mentioned methods require high temperatures.[15] In addition to this, the majority of catalysts, especially metal complexes are toxic or expensive and the products require complex purification procedures, so they cannot be used in the pharmaceutical and cosmetic

[a] Institute of Organic Chemistry PAS,
Kasprzaka 44/52, 01-224 Warsaw, Poland
E-mail: ryszard.ostaszewski@icho.edu.pl
http://ww2.icho.edu.pl/z20/

Supporting information for this article is available on the WWW under http://dx.doi.org/10.1002/ejoc.201500676.

industry. Another synthetic method to access β-keto esters is an acid-catalyzed reaction of aldehyde and diazo ester.[16] A significant inconvenience of this method, however, is that compounds containing a diazo group tend to be explosive, unstable and toxic. For the synthesis of β-keto esters that are to be used in the pharmaceutical or food industry, regulations require that the procedures provide products without inorganic and organic impurities. Especially in the pharmaceutical industry, for which pharmacopoeia limits of heavy metal contaminations are below 5 ppm, most of the classical chemical methods with metal catalysts are unacceptable. The development of economic and environmentally responsible methodologies for β-keto ester interconversion remains a challenge, because conventional processes often lead to significant amounts of wastes and/or are performed under harsh reaction conditions. We thus turn our attention to enzymatic catalysis, because this approach allows pure products to be obtained in high yields under mild conditions, thereby fulfilling the general principles of green chemistry.[17]

Jeromin and Welsch reported the synthesis of acetoacetate esters catalyzed by lipase, applying diketene as the acyl donor. Although this study was informative, it was narrow in scope.[18] Typically, β-keto esters are prepared by decomposing highly reactive and unstable diketene with various alcohols.[19] Although this methodology is atom economic, the corrosiveness and handling difficulties of diketene make it less attractive practically. The lachrymatory properties of diketene together with concerns regarding its toxicity and shipping have resulted in a need for alternative acetoacetylation technologies. One such "diketene-free" approach that was described by Clemens, Hyatt, and Kato involves the thermal reaction of 2,2,6-trimethyl-4*H*-dioxin-4-one with nucleophiles to produce acetoacetic acid derivatives in good yield.[20]

To our knowledge, there are only a few published procedures involving a single enzyme as a catalyst for the transesterification of β-keto esters; unfortunately, no straightforward and general approach has been developed.

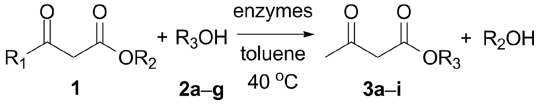
Janda and co-workers reported an elegant enzymatic protocol for the transesterification of β-keto esters catalyzed by immobilized *Candida antarctica* lipase B (CAL-B) performed at 40 °C. However, although the products were obtained with high yields, the reaction must be conducted under low pressure (10 Torr), which required special equipment.[21] Catalytic activity of Novozym 435 was also evaluated in the systems involving nonactivated acyl donors, and enhanced using microwave irradiation.[22] One of the major drawbacks of this relatively new technology remains the cost of the equipment. Furthermore, the microwave field is usually nonuniform and localized superheating occurs.[23] Finally, biocatalytic transesterification of β-keto esters in ionic liquid (ILs) has been reported, but the sensitivity and instability of the catalysts remain a serious problem.[24]

We recently reported the utilization of an enzyme mixture for the enzyme-catalyzed esterification of a range of carboxylic acids, providing the respective esters in excellent yields.[25] Although there are a few examples of enzymatic synergy in degrading plant polymers by glucosidases and xylanases in aqueous medium,[26] this was, to our knowledge, the first evidence of the cooperation between multiple enzymes in organic solvents. Our previous work on enzyme-catalyzed esterification of carboxylic acids prompted us to explore the application of the developed methodology in a consecutive way, using β-keto esters as the substrates. The aim of the presented work was to find a green alternative to traditional chemical reactions, without use of high temperature or low pressure, by employing enzymes as catalysts. The development of a method that allows transesterification under neutral conditions should heighten the synthetic potential of the reaction.

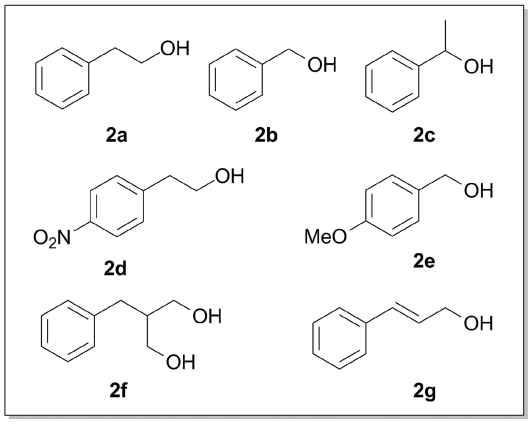
Results and Discussion

As was shown previously, a mixture of enzymes can catalyze the esterification of carboxylic acids and is much more efficient than the use of a single enzyme. Given that the products were obtained with good to excellent yields, we decided to use this method for the synthesis of β-acetoacetates as another class of compounds.

Screening of biocatalysts was performed with 32 commercially available enzymes in the model reaction conducted in toluene at 40 °C. 2-Phenylethanol (**2a**) was chosen for initial optimization study and reacted with ethyl acetoacetate for 24 hours to give 2-phenylethyl acetoacetate (**3a**) (Scheme 1).



R_1 = CH_3, 4-$MeOC_6H_4$
R_2 = Et, *t*Bu



Scheme 1. Transesterification of ethyl/*tert*-butyl β-keto esters using a range of alcohols.

To speed up the screening process, 32 enzymes were divided into eight reaction mixtures (vials); each group consisted of four different enzymes. It was observed that only one set – containing lipases from *Candida antarctica* (Novozym 435), *Rhizopus niveus*, lipoprotein lipase from *Pseudomonas sp.* and protease from papaine – catalyzed the

model reaction efficiently. An excess of starting β-keto ester **1a** (R^1 = CH_3, R^2 = Et) was required to achieve high productivity in 24 hours (Table 1, entries 1–4). Given that ethyl acetoacetate is relatively inexpensive, it was used in fourfold excess in further experiments. The yield of isolated product **3a** was 92% (entry 4). The order of enzyme addition had no impact on the final result. Ester **3a** was not observed in other groups. When the reaction was performed under reduced pressure[21] no significant change in reaction efficiency was observed, and the product **3a** was obtained in 87% yield.

Table 1. Transesterification of ethyl acetoacetate (**1a**; R^1 = Me, R^2 = Et) with 2-phenylethanol (**2a**) catalyzed by combinations of enzymes.[a]

Entry	**1** (equiv.)	Enzyme	Yield [%][b]
1	1	A, B, C, D	63
2	2	A, B, C, D	76
3	3	A, B, C, D	85
4	4	A, B, C, D	92
5	4	A	< 1
6	4	B	< 1
7	4	C	< 1
8	4	D	< 1
9	4	A, B	< 1
10	4	A, C	< 1
11	4	A, D	48
12	4	B, C	< 1
13	4	B, D	31
14	4	C, D	< 1
15	4	A, B, C	< 1
16	4	A, B, D	< 5
17	4	A, C, D	22
18	4	B, C, D	< 5

[a] For a typical procedure, see the Exp. Section; A: Novozym 435, B: protease from from *Carica papaya*, C: lipase from *Rhizopus niveus*, D: lipoprotein lipase from *Pseudomonas sp.* [b] Isolated yield after 24 h.

To find out which enzyme catalyzes the model reaction, a series of experiments with each enzyme alone was performed (Table 1). For each enzyme separately the yield of isolated product was lower than 1% (entries 5–8), which is consistent with results obtained in esterification of carboxylic acids.[25] To identify the catalytic effect of enzyme pairs, a second set of experiments was performed. According to the data included in Table 1, under standard conditions, product **3a** was obtained for some of these two-enzyme mixtures (entries 9–14). Two combinations of two enzymes gave the product in substantially higher yield (up to 48%; entries 11 and 13). However, this yield was still much lower in respect to the yield obtained with the four-enzyme mixture. Therefore, a further set of experiments was performed with mixtures of three different enzymes (entries 15–18). Only, one combination of enzymes successfully provided product **3a** in 22% yield (entry 17). Two other enzyme mixtures provided the product in less than 5% yield (entries 16 and 18). The presence of two enzymes: Novozym 435 and lipoprotein lipase from *Pseudomonas sp.* seem to be crucial for productivity enhancement. Changing immobilized Novozym 435 to native lipase from *Candida antarctica* led to a significant drop in yield to less than 5%. To examine the catalytic effect of the enzymes used, thermally deactivated *Candida antarctica* present in Novozym 435 was applied together with native lipoprotein lipase from *Pseudomonas sp.* and vice versa; however, under these conditions, only traces of product **3a** were obtained.

Moreover, the enzyme mixture could be reused without loss of activity by first washing it with ethyl ether and then drying in a dessicator. The preparation could be employed in more than five 48 hour cycles: the mixture of enzymes retained 80% of the initial activity after five repetitive uses in transesterification of ethyl acetoacetate with **2a**.

Furthermore, this strategy could be used to create a β-keto ester library. Both primary and secondary alcohols were appropriate substrates for the enzyme mixture, as was allylic alcohol (Table 2). These results then directed our attention to possible donors of acetoacetate group. Experiments with 2,2,6-trimethyl-4*H*-1,3-dioxine-4-one and *tert*-butyl acetoacetate were performed; however, only with *tert*-butyl acetoacetate was the corresponding product **3a** obtained with high isolated yield (88%; entry 2). The reaction with 2,2,6-trimethyl-4*H*-1,3-dioxine-4-one was not catalyzed by enzymes, which is probably because the acetylketene intermediate in transesterification can only be generated thermally.[27]

Table 2. Transesterification of acetoacetates with a range of alcohols.[a]

Entry	R^1	R^2	Alcohol **2**	Yield [%][b]	Yield [%][c]
1	CH_3	Et	**2a**	92	> 99
2	CH_3	*t*Bu	**2a**	88	> 99
3	CH_3	Et	**2b**	80	98
4	CH_3	*t*Bu	**2b**	76	96
5	CH_3	Et	**2c**	96	> 99
6	CH_3	*t*Bu	**2c**	72	94
7	CH_3	Et	**2d**	67	89
8	CH_3	*t*Bu	**2d**	86	> 99
9	CH_3	Et	**2e**	65	83
10	CH_3	*t*Bu	**2e**	84	> 99
11	CH_3	Et	**2f**	30[d]	72
12	CH_3	*t*Bu	**2f**	< 1	< 5
13	CH_3	Et	**2g**	74	92
14	CH_3	*t*Bu	**2g**	< 1	< 5
15	4-$MeOC_6H_4$	Et	**2a**	78	> 99
16	4-$MeOC_6H_4$	Et	**2b**	84	> 99

[a] For a typical procedure, see the experimental section; 4 equiv. of **1** was used. [b] Isolated yield after 24 h. [c] Isolated yield after 48 h. [d] Double acetylated product.

The results of the transesterification with various alcohols are summarized in Table 2. In the majority of cases, the products were obtained with excellent yields. For 2-phenylethanol (**2a**) and benzyl alcohol (**2b**) the corresponding products **3a** and **3b** were obtained with similar yields using ethyl or *tert*-butyl acetoacetate (entries 1–4). In case of chiral racemic alcohol **2c**, slightly higher yield was obtained by using ethyl ester (entries 5 and 6). For alcohols with functional groups in the phenyl ring (–OCH_3, –NO_2) the yields of products **3d** and **3e** were higher for *tert*-butyl acetoacetate (entries 7–10). For cinnamyl alcohol (**2g**) and 2-benzyl-1,3-propanediol (**2f**) the products **3f** and **3g** were obtained with good yield only with ethyl acetoacetate (en-

tries 11–14). In the reaction of 2-benzyl-1,3-propanediol (**2f**) with ethyl acetoacetate, 2-benzyl-1,3-propanediol diacetoacetate (**3f**) was obtained exclusively in 30% yield. When the reaction time was extended to 48 h, the corresponding products **3** were obtained with quantitative yields (Table 2). To diversify the scope of the products that can be obtained and to establish the generality of the developed procedure, ethyl 3-(4-methoxyphenyl)-3-oxopropionate was applied to the enzymatic reaction, leading to the formation of the corresponding esters **3h** and **3i** with quantitative yields in 48 h (entries 15 and 16).

The enzymatic transesterification of *tert*-butyl acetoacetate has, to our knowledge, not been reported. The application of esterases or lipases for the hydrolysis of esters of tertiary alcohols is hampered by the fact that most of the commercially available enzymes do not accept tertiary alcohols as substrates.[28] Only a limited group of enzymes possessing a special active site structure are able to hydrolyze esters of tertiary alcohols.[29] The results obtained in this study turned our attention to the mechanism of enzymatic transesterification of *tert*-butyl acetoacetate, which is the subject of ongoing studies.

Finally, we applied this transformation concept on a preparative scale. Thus, 160 mg of benzyl alcohol (**2b**) was transformed into ester **3i** with complete conversion, within 48 h, and with 98% yield of the isolated product. It should be noted that ester **3i** is an important prodrug moiety.[30]

The mixture of enzymes appeared to be an excellent catalyst system not only for the esterification of carboxylic acids,[25] but also for the transesterification of acetoacetates, and its use enables the synthesis of β-keto esters. It is clear from Table 2 that the conversion from ethyl/*tert*-butyl keto esters into higher homologues appears to be efficient and practical through this procedure. It should be pointed out that transesterification of β-keto esters with unsaturated alcohols is rather difficult because it is offset by facile decarboxylation rearrangement; however, by using this method, β-keto esters underwent such reactions smoothly.[31] The present procedure is quite general for a wide range of structurally varied alcohols. Neither high temperature, low pressure, nor toxic catalysts are required in the synthesis The procedure is very simple and provides the products with quantitative yields. We believe our methodology will be of ample use considering its mild conditions and simple procedure.

Conclusions

We have developed and demonstrated a viable new and highly efficient procedure for the synthesis of a non-commercial β-keto ester through transesterification in quantitative yields. Noteworthy merits of this protocol are the simple operation, mild reaction conditions, and easy work-up procedure, and no hazardous catalysts, or corrosive or toxic solvents are required.

We consider that this is a more practical method than the existing methodologies and expect that it should find widespread application in academic and industrial fields. Further research to widen the scope of this enhanced protocol is in progress.

Experimental Section

General Experimental Methods: All the chemicals were obtained from commercial sources. The solvents were of analytical grade. NMR spectra were recorded in $CDCl_3$ with TMS as an internal standard using 200 MHz spectrometers. The chemical shifts are reported in ppm (δ scale) and the coupling constants (J) are given in Hertz (Hz). Enzymes: CAL-B (Novozym 435) {lipase acrylic resin form *Candida antarctica*, Novozymes, 10 PLU/mg (PLU: propyl laurate)}; protease from *Carica papaya*, SIGMA {lyophilized powder, 12 U/mg (U: *N*-benzoyl-L-arginine ethyl ester)}; lipase from *Rhizopus niveus*, Sigma {lyophilized powder, 1.5 U/mg (U: olive oil)}; lipoprotein lipase from *Pseudomonas sp.*, Sigma {lyophilized powder, 50,000 U/mg (U: *p*-nitrophenyl butyrate)}. All enzymes were stored in compliance with providers' recommendations.

General Transesterification Procedure: Enzymatic reactions were performed in a vortex (Heidolph Promax 1020) equipped with incubator (Heidolph Inkubator 1000). All reactions were monitored by TLC on Merck silica gel Plates 60 F254, detector UV/Vis, mobile phase: hexanes/ethyl acetate (9:1, v/v).

Experimental Setup: A solution of alcohol (0.5 mmol), ethyl or *tert*-butyl acetoacetate (0.5, 1.0, 1.5 or 2 mmol) and 10 mg of each enzyme in toluene (3 mL) was placed in a 10 mL vial. The reaction mixture was agitated at 200 rpm for 24 or 48 h at 40 °C. Enzymes were removed by filtration, and toluene and remaining ethyl acetoacetate were removed under reduced pressure. The product was purified by silica gel column chromatography (ethyl acetate/hexanes). The spectroscopic data were in accordance with reported data.

Seven Other Sets of Enzymes Used in Screening:

1. Lipase from *Candida rugosa* (Sigma–Aldrich), lipase type II from porcine pancreas (Sigma–Aldrich), cellulase from *Aspergillus niger* (Sigma–Aldrich), acylase I from *Aspergillus. melleus* (Fluka).

2. Lipase from *Candida lipolytica* (Sigma–Aldrich), lipase from *Candida antarctica* (Sigma–Aldrich), lipase from wheat germ, Amano protease PS (Sigma–Aldrich).

3. Cellulase type VI from *Trichoderma viride* (Sigma–Aldrich), lysozyme from chicken egg, pectinase from *Rhizopus sp.*, Driselase *Basidiomycetes sp.* (Sigma–Aldrich).

4. Lipoprotein lipase from *Pseudomonas sp.* (Sigma–Aldrich), lipase from *Rhizopus niveus* (Sigma–Aldrich) papain crude powder (Merck), Novozym 435 (Novo Nordisk).

5. Amano lipase AK from *Pseudomonas fluorescens* (Sigma–Aldrich), lipase from *Pseudomonas cepacia*, lipase from hog pancreas (Fluka), Amano lipase PS from *Burkholderia cepacia* (Sigma–Aldrich).

6. Lipase from *Mucor javanicus* (Sigma–Aldrich), lipase from *Rhizopus arrhizus* (Sigma–Aldrich), lipase from *Mucor miehei* (Sigma–Aldrich), lipase from *Pseudomonas fluorescens* (Sigma–Aldrich).

7. Lipase from *Chromobacterium viscosum* (Sigma–Aldrich), protease from *Aspergillus oryzae* (Sigma–Aldrich), lipase from *Penicillium roqueforti* (Sigma–Aldrich), protease from *Bacillus amyloliquefaciens* (Sigma–Aldrich).

Synthesis of Benzyl 3-(4-Methoxyphenyl)-3-oxopropionate (3i): To a solution of benzyl alcohol (160 mg, 1.5 mmol) and ethyl acetoacetate (780 mg, 6 mmol) and 25 mg of each enzyme in toluene (10 mL) was placed in a 20 mL vial. The reaction mixture was agitated at 200 rpm for 48 h at 40 °C. Enzymes were removed by filtration and toluene was evaporated under reduced pressure. The crude product was purified by silica gel column chromatography (ethyl acetate/hexanes) providing ester **3i** (418 mg, 98%) as a colorless oil. The spectroscopic data were in accordance with reported data.

Analysis

2-Phenylethyl Acetoacetate (3a):[32] ^{1}H NMR ($CDCl_3$, 200 MHz): δ = 2.20 (s, 3 H), 2.96 (t, J = 7.0 Hz, 2 H), 3.43 (s, 2 H), 4.37 (t, J = 7.0 Hz, 2 H), 7.22–7.31 (m, 5 H) ppm. ^{13}C NMR ($CDCl_3$, 50 MHz): δ = 26.5, 31.4, 46.5, 62.2, 123.1, 124.9, 125.3, 133.82, 167.1, 200,1 ppm.

Benzyl Acetoacetate (3b):[33] ^{1}H NMR ($CDCl_3$, 200 MHz): δ = 2.24 (s, 3 H), 3.50 (s, 2 H), 5.18 (s, 2 H), 7.35–7.37 (m, 5 H) ppm. ^{13}C NMR ($CDCl_3$, 50 MHz): δ = 32.5, 71.6, 54.4, 132.6, 135.4, 136.3, 145.5, 175.2, 211.3 ppm.

1-Phenylethyl Acetoacetate (3c):[34] ^{1}H NMR ($CDCl_3$, 200 MHz): δ = 1.57 (d, J = 6.4 Hz, 3 H), 2.23 (s, 3 H), 3.46 (s, 2 H); 5.93 (q, J = 6.4 Hz, 1 H), 7.26–7.36 (m, 5 H) ppm. ^{13}C NMR ($CDCl_3$, 50 MHz): δ = 22.1, 30.3, 50.6, 73.7, 126.3, 128.3, 128.8, 139.0, 164.5, 198.6 ppm.

2-(4-Nitrophenyl)ethyl Acetoacetate (3d):[35] ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 2.23 (s, 3 H), 3.08 (t, J = 6.4 Hz, 2 H), 3.45 (s, 2 H), 4.41 (t, J = 6.4 Hz, 2 H), 6.88 (d, J = 9.2 Hz, 2 H), 7.30 (d, J = 9.2 Hz, 2 H) ppm. ^{13}C NMR ($CDCl_3$, 100 MHz): δ = 30.5, 34.9, 50.1, 64.9, 124.0, 129.9, 145.5, 167.1, 200.4 ppm.

4-Metoxybenzyl Acetoacetate (3e):[36] ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 2.23 (s, 3 H), 3.47 (s, 2 H), 3.81 (s, 3 H), 5.12 (s, 2 H), 6.88 (d, J = 8.8 Hz, 2 H), 7.30 (d, J = 8.8 Hz, 2 H) ppm. ^{13}C NMR ($CDCl_3$, 50 MHz): δ = 30.4, 50.4, 55.5, 67.2, 114.2, 127.6, 130.5, 154.3, 168.5, 200.0 ppm.

2-Benzyl-1,3-propanediol Diacetoacetate (3f): ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 2.28 (s, 6 H), 2.32–2.39 (m, 1 H), 2.69 (d, J = 7.6 Hz, 2 H), 3.48 (s, 4 H), 4.11–4.21 (m, 4 H), 7.16–7.30 (m, 5 H) ppm. ^{13}C NMR ($CDCl_3$, 100 MHz): δ = 30.4, 34.5, 39.3, 50.1, 64.5, 126.7, 128.8, 129.2, 138.5, 167.1, 200.6 ppm. $C_{18}H_{22}O_6$ (334.37): calcd. C 64.66, H 6.63; found C 64.63, H 6.67. HRMS (ESI): m/z calcd. for $C_{18}H_{22}O_6Na$ $[M + Na]^+$ 357.1314; found 357.1316.

Cinnamyl Acetoacetate (3g):[37] ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 2.28 (s, 3 H), 3.50 (s, 2 H), 4.79 (dd, J = 1.2, 5.2 Hz, 2 H), 6.24–6.31 (m, 1 H), 6.66 (d, J = 8 Hz, 1 H), 7.26–7.4 (m, 5 H) ppm. ^{13}C NMR ($CDCl_3$, 100 MHz): δ = 30.4, 50.2, 66.1, 122.5, 126.8, 128.4, 128.8, 135.1, 136.1, 167.1, 200.6 ppm.

2-Phenylethyl 3-(4-Methoxyphenyl)-3-oxopropionate (3h): ^{1}H NMR ($CDCl_3$, 200 MHz): δ = 2.93 (t, J = 7.2 Hz, 2 H), 3.86 (s, 3 H), 3.92 (s, 2 H), 4.36 (t, J = 7.2 Hz, 2 H), 6.91 (d, J = 9.0 Hz, 2 H), 7.18–7.32 (m, 5 H), 7.88 (d, J = 9.0 Hz, 2 H) ppm. ^{13}C NMR ($CDCl_3$, 50 MHz): δ = 35.3, 46.2, 55.9, 66.2, 114.2, 127.0, 128.2, 128.9, 129.3, 129.5, 131.3, 137.9, 164.4, 168.1, 191.2 ppm. $C_{18}H_{18}O_4$ (298.34): calcd. C 72.47, H 6.08; found C 72.37, H 6.27. HRMS (ESI): m/z calcd. for $C_{18}H_{18}O_4Na$ $[M + Na]^+$ 321.1103; found 321.1104.

Benzyl 3-(4-Methoxyphenyl)-3-oxopropionate (3i): ^{1}H NMR ($CDCl_3$, 200 MHz): δ = 3.85 (s, 3 H), 3.97 (s, 2 H), 5.17 (s, 2 H), 6.90 (d, J = 8.8 Hz, 2 H), 7.31–7.37 (m, 5 H), 7.89 (d, J = 8.8 Hz, 2 H) ppm. ^{13}C NMR ($CDCl_3$, 50 MHz): δ = 46.2, 56.0, 67.6, 114.2, 127.4, 128.0, 128.7, 128.8, 129.1, 129.5, 131.3, 135.8, 164.4, 168.1, 191.2 ppm. $C_{17}H_{16}O_4$ (284.31): calcd. C 71.82, H 5.67; found C 71.78, H 5.55. HRMS (ESI): m/z calcd. for $C_{17}H_{16}O_4Na$ $[M + Na]^+$ 307.0946; found 307.0948.

Acknowledgments

This project was supported by Polpharma Starogard Gdański. This work was partially supported by the project "*Biotransformations for pharmaceutical and cosmetics industry*" grant number POIG.01.03.01-00-158/09-01, project number 2013/11/B/ST5/02199 and by the European Union (EU) in the frame of the COST Action CM1303.

[1] a) S. J. Bingham, J. H. P. Tyman, *J. Chem. Soc. Perkin Trans. 1* **1997**, 3637; b) G. Roberge, P. Brassard, *J. Chem. Soc. Perkin Trans. 1* **1978**, 1041–1046; c) V. Andrushko, N. Andrushko, *Stereoselective Synthesis of Drugs and Natural Products*, John Wiley & Sons, **2013**, p. 1363; d) M. Marigo, K. Juhl, K. A. Jørgensen, *Angew. Chem. Int. Ed.* **2003**, *42*, 1367–1369; *Angew. Chem.* **2003**, *115*, 1405; e) I. Shinkai, *The Structure, Reactions, Synthesis and Uses of Heterocyclic Compounds*, in: *Comprehensive Heterocyclic Chemistry II*, vol. 3 (Ed.: I. Shinkai), Pergamon, Oxford, UK, **1996**, p. 1–75.

[2] O. A. Attanasi, P. Filippone, C. Fiorucci, E. Foresti, F. Mantellini, *J. Org. Chem.* **1998**, *63*, 9880–9887.

[3] I. Yavari, F. Nourmohammadian, *J. Chem. Res.* **2000**, 218–219.

[4] a) F. Mathey, F. Robin, F. Mercier, M. Spagnol, PCT Int. Appl., WO 98 00,375; *Chem. Abstr.* **1998**, *128*, 127603; b) K. Niigata, T. Kimura, S. Hayashibe, H. Shikama, T. Takasu, E. Hirasaki, Jpn. Kokai Tokkyo Koho, **1994**, JP 06,298,737; *Chem. Abstr.* **1995**, *122*, 13318; c) S. J. Binghama, J. H. P. Tymana, *Org. Prep. Proced. Int.* **2001**, *33*, 357–361; d) R. V. Ragavan, K. M. Kumar, V. Vijayakumar, S. Sarveswari, S. Ramaiah, A. Anbarasu, S. Karthikeyan, P. Giridharan, N. S. Kumari, *Org. Med. Chem. Lett.* **2013**, *3*, 6.

[5] M. Couturier, P. Brassard, *Synthesis* **1994**, 703.

[6] a) L. I. Koval, V. I. Dzyuba, O. L. Ilnitska, V. I. Pekhnyo, *Tetrahedron Lett.* **2008**, *49*, 1645–1647; b) D. W. Hutchinson, *Antiviral Res.* **1985**, *5*, 193–205; c) E. Tramontano, F. Esposito, R. Badas, R. Di Santo, R. Costi, P. La Colla, *Antiviral Res.* **2005**, *65*, 117–124.

[7] P. Biginelli, *Gazz. Chim. Ital.* **1893**, *23*, 360–416.

[8] a) H. Y. K. Kaan, V. Ulaganathan, O. Rath, H. Prokopcova, D. Dallinger, C. O. Kappe, F. Kozielski, *J. Med. Chem.* **2010**, *53*, 5676; b) C. O. Kappe, *Eur. J. Med. Chem.* **2000**, *35*, 1043–1052; c) M. Butters, C. D. Davies, M. C. Elliott, *Org. Biomol. Chem.* **2009**, *7*, 5001–5009; d) J. S. S. Sandhu, *ARKIVOC (Gainesville, FL, U.S.)* **2012**, *1*, 66–133.

[9] a) G. C. M. Kondaiah, L. A. Reddy, K. S. Babu, V. M. Gurav, K. G. Huge, R. Bandichhor, P. P. Reddy, A. Bhattacharya, R. V. Anand, *Tetrahedron Lett.* **2008**, *49*, 106–109; b) C. E. Rehberg, C. H. Fisher, *J. Am. Chem. Soc.* **1944**, *66*, 1203; c) C. E. Rehberg, W. A. Faucette, C. H. Fisher, *J. Am. Chem. Soc.* **1944**, *66*, 1723; d) C. E. Rehberg, *Organic Synthesis* Wiley, New York, **1955**, vol. 3, pp 146.

[10] a) F. T. Douglass, J. C. Amedio Jr., Y. K. Pate, *J. Org. Chem.* **1985**, *50*, 3619–3622; b) J. C. Gilbert, T. A. Kelly, *J. Org. Chem.* **1988**, *53*, 449–450.

[11] a) C.-T. Chen, J.-H. Kuo, C.-H. Ku, S.-S. Weng, C.-Y. Liu, *J. Org. Chem.* **2005**, *70*, 1328–1339; b) J. S. Witzeman, W. D. Nottingham, *J. Org. Chem.* **1991**, *56*, 1713–1718.

[12] a) P. Ilankumaran, J. G. Verkade, *J. Org. Chem.* **1999**, *64*, 3086; b) K. Wakasugi, T. Misaki, K. Yamada, Y. Tanabe, *Tetrahedron Lett.* **2000**, *41*, 5249.

[13] a) S. P. Chavan, K. Pasupathy, S. Shengule, V. Shinde, R. Anand, *ARKIVOC (Gainesville, FL, U.S.)* **2005**, *13*, 162–168; b) S. P. Chavan, P. K. Zubaidha, S. W. Dantale, A. Keshavaraja, A. V. Ramaswamy, T. Ravindranathan, *Tetrahedron Lett.* **1996**,

37, 233; c) B. S. Balaji, M. Sasidharan, R. Kumar, B. Chanda, *Chem. Commun.* **1996**, 707; d) B. S. Balaji, B. M. Chanda, *Tetrahedron* **1998**, *54*, 13237; e) D. Ponde, V. H. Deshpande, V. J. Bulbule, A. Sudalai, A. S. Gajare, *J. Org. Chem.* **1998**, *63*, 1058.

[14] Ch. Mottet, O. Hamelin, G. Garavel, J. P. Depres, A. E. Greene, *J. Org. Chem.* **1999**, *64*, 1380.

[15] L. I. Koval, V. I. Dzyuba, O. L. Ilnitska, V. I. Pekhnyo, *Tetrahedron Lett.* **2008**, *49*, 1645–1647.

[16] C. R. Holmquist, E. J. Roskamp, *J. Org. Chem.* **1989**, *54*, 3258–3260.

[17] M. Gupta, S. Paul, R. Gupta, *Curr. Sci.* **2010**, *99*, 1341–1360.

[18] a) K. Suginaka, Y. Hayashi, Y. Yamamoto, *Tetrahedron: Asymmetry* **1996**, *7*, 1153–1158; b) G. E. Jeromin, V. Welsch, *Tetrahedron Lett.* **1995**, *36*, 6663.

[19] a) S. R. Wilson, M. F. Price, *J. Org. Chem.* **1984**, *49*, 722; b) Y. Tezuka, T. Ogura, S. Kawaguchi, *Bull. Chem. Soc. Jpn.* **1969**, *42*, 443.

[20] a) R. J. Clemene, J. A. Hyatt, *J. Org. Chem.* **1985**, *50*, 2431; b) M. Sato, N. Kanuma, T. Kato, *Chem. Pharm. Bull.* **1982**, *30*, 1315; c) M. Sato, H. Ogaeawara, E. Yohizumi, T. Kato, *Chem. Pharm. Bull.* **1983**, *31*, 1902; d) R. J. Clemens, J. S. Witzeman, *J. Am. Chem. Soc.* **1989**, *111*, 2186.

[21] A. Cordova, K. D. Janda, *J. Org. Chem.* **2001**, *66*, 1906–1909.

[22] M. Rissoa, M. Mazzini, S. Kroger, P. Saenz-Mendez, G. Seoane, D. Gamenara, *Green Chem. Lett. Rev.* **2012**, *5*, 539–543.

[23] M. Nüchter, B. Ondruschka, W. Bonrath, A. Gum, *Green Chem.* **2004**, *6*, 128–141.

[24] a) K. W. Kim, B. Song, M. Y. Choi, M. J. Kim, *Org. Lett.* **2001**, *3*, 1507; b) J. L. Kaar, A. M. Jesionowski, J. A. Berberich, R. Moulton, A. J. Russell, *J. Am. Chem. Soc.* **2003**, *125*, 4125; c) M. Persson, U. T. Bornscheuer, *J. Mol. Catal. B* **2003**, *22*, 21.

[25] M. Zysk, A. Zadlo, A. Brodzka, C. Wisniewska, R. Ostaszewski, *J. Mol. Catal. B* **2014**, *102*, 225–229.

[26] a) M. Wang, G.-L. Lai, Y. Nie, S. Geng, L. Liu, B. Zhu, Z. Shi, X.-L. Wu, *Front. Microbiol.* 6:509; DOI: 10.3389/fmicb.2015.00509; b) M. Qi, H.-S. Jun, C. W. Forsberg, *Appl. Environ. Microbiol.* **2007**, *73*, 6098–6105; c) J. Kopečnŷ, A. G. Williams, *Folia Microbiol.* **1988**, *33*, 208–212; d) J. P. L. F. Cairo, L. C. Oliveira, C. A. Uchima, T. M. Alvarez, A. P. S. Citadini, J. Cota, F. C. Leonardo, A. M. Costa-Leonardo, M. F. Carazzolle, F. F. Costa, G. A. G. Pereira, F. M. Squina, *Insect Biochem. Mol. Biol.* **2013**, *43*, 970–981; e) K. L. Kadam, E. C. Rydholm, J. D. McMillan, *Biotechnol. Prog.* **2004**, *20*, 698–705; f) L. Ji, J. Yang, H. Fan, Y. Yang, B. Li, X. Yu, N. Zhu, H. Yuan, *Biotechnol. Biofuels* **2014**, *7*, 1–12.

[27] A. D'Annibale, A. Pesce, S. Resta, C. Trogolo, *Tetrahedron Lett.* **1996**, *37*, 7429–7432.

[28] R. Kourist, U. T. Bornscheuer, *Appl. Microbiol. Biotechnol.* **2011**, *91*, 505–517.

[29] E. Henke, J. Pleiss, U. T. Bornscheuer, *Angew. Chem. Int. Ed.* **2002**, *41*, 3211–3213; *Angew. Chem.* **2002**, *114*, 3338.

[30] C.-Q. Sun, P. T. W. Cheng, J. Stevenson, T. Dejneka, B. Brown, T. C. Wang, J. A. Robl, M. A. Poss, *Tetrahedron Lett.* **2002**, *43*, 1161–1164.

[31] a) M. F. Carrol, *J. Chem. Soc.* **1940**, 704–706; b) W. Kimel, A. C. Cope, *J. Am. Chem. Soc.* **1943**, *65*, 1992–1998.

[32] S. Magens, M. Ertelt, A. Jatsch, B. Plietker, *Org. Lett.* **2008**, *10*, 53–56.

[33] S. Tayyari, F. Naghavi, S. Pojhan, R. W. McClurg, R. E. Sammelson, *J. Mol. Struct.* **2011**, *987*, 241–254.

[34] a) T. Hudlicky, T. Tsunoda, K. G. Gadamasetti, J. A. Murry, G. E. Keck, *J. Org. Chem.* **1991**, *56*, 3619–3623; b) A. Nudelman, R. Kelner, N. Broida, H. E. Gottlieb, *Synthesis* **1989**, *5*, 387–388.

[35] N. Iqbal, E. E. Knaus, *Arch. Pharm.* **1996**, *329*, 23–26.

[36] T. Ogawa, A. Nakazato, K. Tsuchida, K. Hatayama, *Chem. Pharm. Bull.* **1993**, *41*, 108–116.

[37] Y. Takekawa, K. Shishido, *J. Org. Chem.* **2001**, *66*, 8490–8503.

Received: May 27, 2015
Published Online: July 22, 2015

