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Journal of Molecular Catalysis B: Enzymatic 38 (2006) 43–47
JOURNAL OF MOLECULAR CATALYSIS B: ENZYMATIC
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Preparative enantioselective synthesis of benzoins and (*R*)-2-hydroxy-1-phenylpropanone using benzaldehyde lyase

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Received 9 September 2005; received in revised form 2 November 2005; accepted 9 November 2005

Abstract

A detailed study of the reaction parameters on the enzymatic activity and stability of benzaldehyde lyase (BAL) catalysed carboligation is presented, like the influence of the cosolvent (DMSO), the role of the cofactor ThDP, the pH of the reaction medium, and the substrate ratio in the case of cross condensation. Surprisingly, an alkaline reaction medium of pH 9.5 accelerates the BAL-catalysed condensation significantly. Under these conditions several (*R*)-benzoins were formed with high productivity of 240 g L^{-1} d^{-1} and high enantioselectivities (93–99% ee). For the synthesis of (*R*)-2-hydroxy-1-phenyl-propanone (2-HPP) by coupling benzaldehyde and acetaldehyde space-time-yields of 36 g L^{-1} d^{-1} were obtained with a maximum 2-HPP concentration of 15–20 g L^{-1} (97% ee) in 10–15 h.

Keywords: Benzaldehyde lyase; Acyloin condensation; Thiamine-diphosphate; Enzymatic carboligation; Aldehyde

1. Introduction

Benzaldehyde lyase (BAL; E.C. 4.1.2.38), a thiamine-diphosphate (ThDP)- and Mg^{2+}-dependent enzyme from *Pseudomonas fluorescens* Biovar I, was described more than a decade ago as a catalyst in the cleavage of benzoin and anisoin linkages [1]. In recent years its further synthetic capabilities for producing 2-hydroxy ketones, i.e. benzoins or 2-hydroxy-1-phenylpropanones (2-HPP) in very good yields and high enantioselectivities have been reported [2–10]. Recent studies have mainly focused on the study of the substrate spectrum of BAL-catalysed carboligations. Thus, the coupling of substituted aromatic benzaldehydes to produce benzoins was described, as well as the formation of 2-HPP derivatives by a cross acyloin condensation of an aromatic aldehyde and acetaldehyde [2,3]. In order to evaluate the industrial applicability of BAL-catalysed carboligations, a detailed study of the parameters influencing the activity and the stability of BAL is of pivotal importance. Addressing the challenges of process development issues, in the present paper we report on the influence of cofactors, cosolvents, and especially the pH-influence on the stability and activity of BAL. In addition, we disclose process conditions which allow the synthesis of optically active 2-hydroxy ketones with high reaction rates and high volumetric productivity.

2. Results and discussion

2.1. Benzoin condensation

For initial studies the BAL-catalysed syntheses of (*R*)-benzoins from the respective benzaldehyde derivatives were chosen as test reactions. (Scheme 1). These reactions, which have been described in detail previously [3,4,6] are usually performed in aqueous monophasic media.

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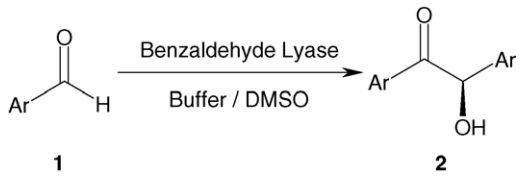
doi:10.1016/j.molcatb.2005.11.002



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Scheme 1. BAL-catalysed synthesis of benzoins (**2a**) by carboligation of benzaldehyde derivatives.

2.1.1. *Influence of cofactors*

The influence of the cofactors, ThDP and Mg^{2+}, on the stability of BAL in water and potassium phosphate buffer (KP_i) at pH 8 is shown in Fig. 1. Starting with a lyophilised enzyme preparation with a residual content of 0.015 mmol L^{-1} ThDP and Mg^{2+}, the enzyme looses its activity almost completely within 3 h in water. In the presence of potassium phosphate buffer, with or without Mg^{2+}, a slightly better stability is observed. Only in the presence of both cofactors, a significant enzymatic stabilisation is achieved. Moreover, the addition of DTT, a well-known stabiliser of hydrolases, has also a positive effect on the enzyme stability. A further increase of the cofactor concentration up to 1 mmol L^{-1} had no significant effect (data not shown).

2.1.2. *DMSO as a cosolvent*

For a preparative large-scale application high substrate concentrations are generally desirable. This demand is difficult to meet with aromatic substrates, such as benzaldehyde, which are only poorly soluble in aqueous media. To overcome this problem, DMSO has been used as cosolvent, although DMSO leads often to some inconveniences concerning the product purification, enzyme inhibition, and deactivation. To minimise those drawbacks, the quantification of the influence of DMSO on BAL-stability was measured as time-dependent enzyme deactivation (Fig. 2). Unexpectedly, the stability of BAL increases with higher DMSO-content, reaching its optimum at about 30 vol.%. At concentrations of >30 vol.%, however, stability decreases.

When studying the enzymatic initial rate a linear increase of activity was observed up to this concentration (data not shown),

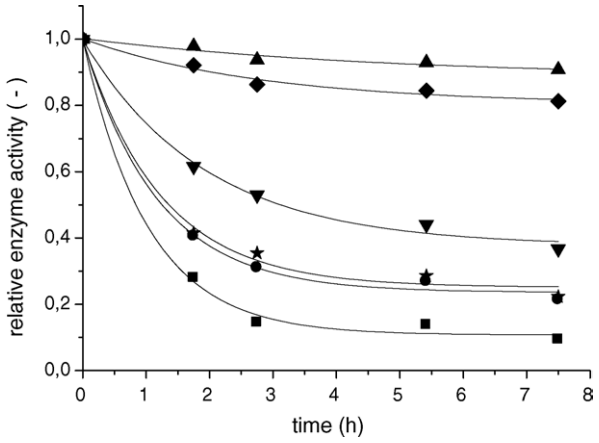


Fig. 1. Influence of cofactors on the stability of BAL (buffer pH 8.0, 0.5 mmol L^{-1} ThDP, 0.5 mmol L^{-1} Mg^{2+}, 1 mmol L^{-1} DTT, 0 °C), (■) water, (●) KPi, (⋆) $KPi_i + Mg^{2+}$, (▼) KPi_i + ThDP, (♦) $KPi_i + Mg^{2+}$ + ThDP, and (▲) $KPi_i + Mg^{2+}$ + ThDP + DTT.

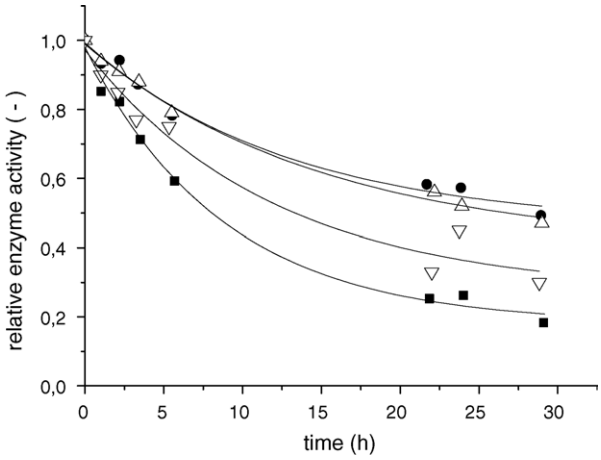


Fig. 2. Influence of the DMSO-concentration (vol.%) on the stability of BAL in 50 mM KP_i buffer (pH 8.0) with various concentrations of DMSO. (■) 0% DMSO, (●) 20% DMSO, (△) 30 vol.% DMSO, (▽) 40% DMSO and 0.5 mmol L^{-1} ThDP, 0.5 mmol L^{-1} Mg^{2+}, 20 °C.

indicating that the maximal BAL activity is not still achieved under these conditions. Exceeding the solubility limit of benzaldehyde (50 mmol L^{-1}), we found that BAL keeps its activity constant up to about 120–130 mmol L^{-1} in aqueous buffer and DMSO (30 vol.%) which can be regarded as the highest not deactivating concentration of benzaldehyde. Higher concentrations of benzaldehyde led to a progressive decrease of activity, so that at 200 mmol L^{-1} benzaldehyde no activity was detected any more.

2.1.3. *pH-dependency*

BAL-catalysed carboligations are routinely performed at a neutral to slightly alkaline pH [3–6]. However, to our knowledge a detailed pH-study has not been published yet. Such pH-profile is depicted in Fig. 3. Remarkably, the highest carboligation activity was achieved at pH 9.5. At pH >10 a sharp decrease in activity is observed.

However, pH 9.5 might cause a lower stability of the enzyme. Thus, despite the high activity an unfavourable enzymatic overall performance would occur. Therefore the applicability of these conditions was investigated on a preparative scale.

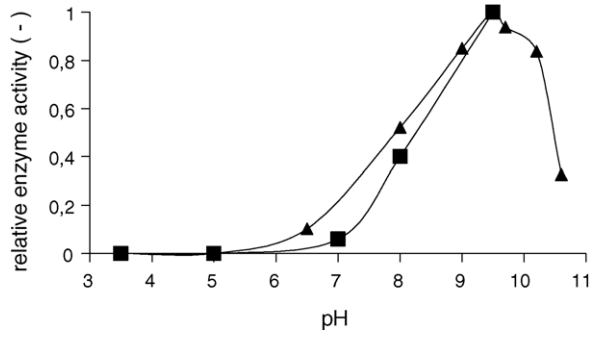


Fig. 3. Influence of the pH in the BAL-catalysed synthesis of benzoin (**2a**) (▲) and 2-HPP (**3**) (■). Relative initial activity corresponds to an activity of 10 μmol min^{-1} mL^{-1} in the case of benzoin (**2a**) and 5 μmol min^{-1} mL^{-1} in case of 2-HPP.

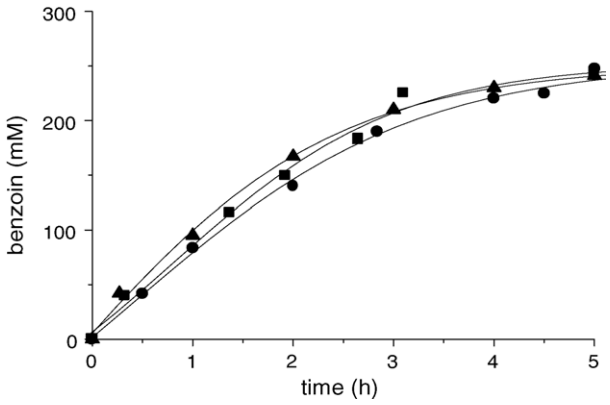
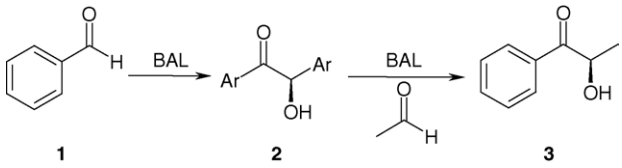


Fig. 4. Time-concentration-diagram of the BAL-catalysed synthesis of different benzoins at pH 9.5 (50 mM KP_i buffer, 30 vol.% DMSO, 350–500 U BAL), by repetitive additions of cycles of 5×100 mmol L^{-1} of aryl-aldehyde: (■) benzaldehyde (**1a**), (●) 4-methoxybenzaldehyde (**1b**) and (▲) furfural (**1c**).

2.1.4. Preparative synthesis of benzoins

The enzymatic synthesis of different benzoins was further investigated at pH 9.5 in the presence of 30 vol.% DMSO. In order to obtain a high final product concentration, a fed batch process was applied by adding 100 mmol L^{-1} benzaldehyde in 5 cycles, respectively. The (*R*)-benzoin produced (**2a**) precipitated from the reaction medium. Notably, it was possible to use up to 500 mmol L^{-1} of aldehyde yielding 250 mmol L^{-1} benzoin in good yields and enantioselectivities (i.e., >99% ee in the case of benzoin (**2a**)). In Fig. 4 the time-concentration-dependency for the condensations of benzoins by adding the aromatic aldehydes in portions is depicted. As the pH drops about 0.5 units after the addition of 100 mmol L^{-1} of benzaldehyde the pH was kept constant at pH 9.5 by addition of NaOH after each substrate addition (Fig. 4). Considering the described enhancement of the BAL-catalysed carboligation rates at alkaline pH the syntheses of different substituted benzoins (starting from benzaldehyde (**1a**), 4-methoxybenzaldehyde (**1b**) and furfural (**1c**)) were performed (Fig. 4; Table 1, entries 1–4). All these reactions proceeded with initial rates of about 80 mmol L^{-1} h^{-1} and can be raised up to a final product concentration, after several batches, of ca. 250 mmol L^{-1} (product). Thus, the final product concentration is about 10-fold increased compared to standard proceedings reported on the literature [2–10]. During the first two additions of the different aldehydes the reaction rates remained constant. After the forth addition a significant decrease in the reaction rate was observed, however a complete conversion to benzoin (**1a**) shows that the enzyme is not deactivated significantly under these conditions. This proves that the stability of the enzyme under these conditions is sufficient for a preparative synthesis of benzoins.



Scheme 2. BAL-catalysed synthesis of (*R*)-2-hydroxy-1-phenylpropanone by carboligation of benzaldehyde (donor substrate) and acetaldehyde (acceptor substrate).

2.2. Formation of 2-HPP

We further applied the above described procedure for the synthesis of (*R*)-2-hydroxy-1-phenylpropanone (2-HPP) from benzaldehyde and acetaldehyde [3]. During the formation of 2-HPP (**3**) benzoin (**2a**) is observed as an intermediate. However in the presence of excess acetaldehyde – as acceptor aldehyde – the reaction is shifted quantitatively to 2-HPP (Scheme 2) [3].

2.2.1. Substrate ratio

First, the substrate ratio was investigated to find out the optimal ratio of benzaldehyde and acetaldehyde for the synthesis of 2-HPP. As stated previously, a surplus of acetaldehyde (as acceptor) is crucial as the formation of 2-HPP proceeds via benzoin as an intermediate (Scheme 2) [3]. The best enzymatic activity with respect to 2-HPP formation was obtained in the presence of a six-fold surplus of acetaldehyde, as depicted in Fig. 5. It

Table 1
Synthesis of different benzoins and 2-HPP catalysed by benzaldehyde lyase (BAL) at pH 9.5[a]

Entry	*R*	Substrate additions	Product	Time (h)	Yield (isol.) (ee)[b]
1	Phenyl	5×100 mmol l^{-1}	**2a**	5	90% (>99%)
2	4-Methoxy-phenyl	1×100 mmol L^{-1}	**2b**	0.5	65%[c] (>99%)
3	4-Methoxy-phenyl	5×100 mmol L^{-1}	**2b**	5	70%[c] (76%)
4	2-Furyl	5×100 mmol L^{-1}	**2c**	5	85% (93%)
5	Phenyl (R^1)	100 mmol L^{-1} (R^1)	**3**	10	83% (97%)
	Methyl (R^2)	600 mmol L^{-1} (R^2)			
6	Phenyl (R^1)	130 mmol L^{-1} (R^1)	**3**	13	74% (97%)
	Methyl (R^2)	720 mmol L^{-1} (R^2)			
7	Phenyl (R^1)	150 mmol L^{-1} (R^1)	**3**	20–48	n.d.[d] (97%)
	Methyl (R^2)	750 mmol L^{-1} (R^2)			

[a] Aqueous buffer, 30 vol.% DMSO, 350–500 U BAL reaction, RT.
[b] Determined by HPLC (benzoins) and by GC (2-HPP). Absolute configuration: (*R*) according to previous literature data [3].
[c] Emulsion was formed during the work-up.
[d] Not determined; the conversion (measured by GC) remained constant at approximately 60% after 12 h.

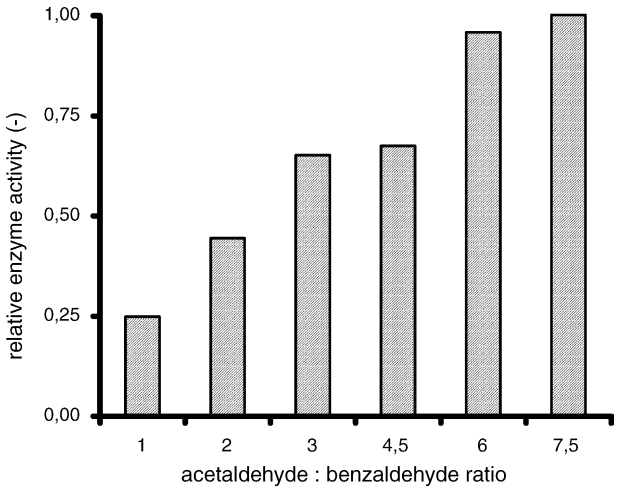


Fig. 5. Influence of excess of acetaldehyde – in presence of 20 mmol L^{-1} benzaldehyde – in the BAL-catalysed synthesis of 2-HPP, pH 7.0 (50 mM KP_i buffer, 20 vol.% DMSO) at room temperature. Relative enzyme activity corresponds to an activity of 0.13 μmol min^{-1} mL^{-1}.

has to be mentioned that this relationship is only exactly valid in the case of 20 mmol L^{-1} benzaldehyde. However, it provides a good estimation of the high surplus of acetaldehyde recommendable for the further improvement of the process with respect to higher substrate concentrations. If this substrate relationship was not maintained, a mixture of benzoin (**2a**) and 2-HPP (**3**) was obtained.

2.2.2. *pH-dependency and preparative synthesis*

Also in case of 2-HPP a drastic increase of activity was achieved by shifting the pH to 9.5 (Fig. 3). Due to the fact that 2-HPP does not precipitate in the reaction media, lower yields were achieved compared to benzoin synthesis. Thus, the addition of 100–130 mmol L^{-1} of benzaldehyde and 600–720 mmol L^{-1} of acetaldehyde (in one dose) at pH 9.5 led to high conversions (90–95%) in short reaction times (10–15 h) with high enantioselectivities (97% ee) (Table 1, entries 5–7). Under these conditions a space-time yield of about 36 g L^{-1} d^{-1} was achieved. Attempts to increase the productivity by adding an extra dose of benzaldehyde and acetaldehyde were unsuccessful, and mixtures of 2-HPP (**3**), benzoins (**2**) and remaining substrates were observed in different proportions (data not shown). It was considered that chiral 2-hydroxy ketones may easily racemise at basic pH. As depicted in Table 1, however only entry 4 (synthesis of anisoin) showed a low enantioselectivity (76% ee). This may be explained by a prolonged work-up of 72 h, due to the formation of a persistent emulsion of anisoin and DMSO. All other benzoin derivatives were formed with enantioselectivities in agreement with previously published results [3]. In the case of the synthesis of 2-HPP, a slightly lower enantioselecitivity (97% ee) than that one reported in the literature (>99% ee) was observed [3]. However, this different ee (measured by chiral GC in our case) was independent on the pH of the reaction studied, since it remained stable from pH 7 to 9.5, and the reaction time (up to 24 h). Thus, the slight ee difference observed in this case, compared to previous published data, which have been obtained by chiral HPLC (97% to >99% ee), are probably caused by thermal effects during GC.

We therefore conclude that the chiral 2-hydroxy ketones formed do not racemise under the reaction conditions applied (pH 9.5) within short reaction times (up to 24 h). Furthermore, no aldol reaction as a possible side reaction could be detected in the reaction of benzaldehyde with acetaldehyde at reaction times of up to 24 h.

The high activity-maximum at pH 9.5 of BAL-catalysed carboligations is the key aspect in our investigation for an optimised synthetic procedure. A neutral to slightly alkaline pH (7–8.5) for the asymmetric synthesis of 2-hydroxy ketones [2–4,6], or the anisoin cleavage [1], was applied previously for these reactions. Notably, one article describes the enhancement of the carboligation activity by increasing the pH value (pH-maximum ca. 9) in reactions catalysed by benzoylformate decarboxylase (BFD) from *Pseudomonas putida*, another ThDP-dependent enzyme [11]. Although the pH optimum with respect to the carboligation reaction catalysed by BFD and BAL is predominately at alkaline pH, pyruvate decarboxylase (PDC) from *Zymomonas mobilis* is most active at pH 7 [12]. These effects can be explained by the reaction mechanism of ThDP-dependent enzymes, which includes several protonation and deprotonation steps [13–15].

3. Conclusions

The reaction conditions for preparative BAL-catalysed carboligations were studied addressing the impact of cofactors and cosolvents. It was shown that 30 vol.% DMSO were optimal for the enzyme activity and stability. The catalytic activity of BAL can be drastically enhanced by increasing the pH of the reaction medium and the best enzymatic activity appears at pH 9.5 (10-fold increase compared to pH 7.0). Hereby a substrate concentration of 500 mmol L^{-1} benzaldehyde is applicable and space-time yields up to 240 g L^{-1} d^{-1} of (*R*)-benzoin (**2a**) were achieved. Several BAL-catalysed carboligations have been performed producing different benzoins and 2-HPP derivatives in high yields on a preparative scale and with high enantioselectivities up to 99% ee at reasonable reaction times.

4. Experimental

4.1. *Chemicals and biocatalyst*

All reagents were commercially available by Sigma–Aldrich and were used without further purification. Benzaldehyde lyase from *P. fluorescens* Biovar I was produced in *Escherichia coli* cells [6]. The preparation of BAL was done by sonication (ca. 3–5 min) of 2 g of *E. coli* cells, which were dissolved in 20 mL of phosphate buffer 50 mmol L^{-1}, with 2.5 mmol L^{-1} $MgSO_4$ and 0.3 mmol L^{-1} ThDP. The disrupted cells were centrifuged at 4000 rpm during 20 min at 4 °C. The pellet was removed and the supernatant was used as the free enzyme with cofactor. The biocatalytic characterisation was carried out as described in literature [3]. The volumetric activity of

this solution was determined to 100 U mL^{-1}. One unit (U) of activity is defined as the amount of enzyme which catalyses the cleavage of 1 μmol benzoin (1.5 mmol L^{-1}) into benzaldehyde in potassium phosphate buffer (20 mmol L^{-1}), pH 7.0, containing $MgSO_4$ (2.5 mmol L^{-1}), ThDP (0.15 mmol L^{-1}) and PEG 400 (15 vol.%).

4.2. Analytical performance

The syntheses of benzoin derivatives were followed by HPLC using a Chromasil C_{18} 5 μm column (250 × 4.6 mm), using a mobile phase composed of 40 vol.% water, 60 vol.% acetonitrile, and 1% (v/v) phosphoric acid, at 1 mL min^{-1} flow rate. The enantiomeric excess was determined by chiral HPLC phase, employing a column Chiracel OD, with a mobile phase composed of hexane/2-propanol (95:5) at a flow rate of 1 mL min^{-1}, using commercial racemates as standards. For the synthesis of 2-HPP, the reaction and the enantiomeric excess were followed by chiral phase GC, employing a Chirasil-DEX CB (Varian), 25 m × 0.32 mm, with a FID detector. The initial temperature (80 °C) was constant during 4 min, then increased with 40 °C min^{-1} until 135 °C and then a slope of 1 °C min^{-1} was set until 160 °C. Determination of absolute configuration was done by comparison to relative retention times described in [14].

4.3. Typical carboligation procedure with BAL

Different amounts of BAL (ca. 350–500 U) were added to a 50 mL mixture of phosphate buffer (50 mmol L^{-1}, with 2.5 mmol L^{-1} $MgSO_4$ and 0.3 mmol L^{-1} ThDP) and 30 vol.% of DMSO. The pH was adjusted to 9.5 by addition of NaOH (1 mol L^{-1}), then the substrates were added in one or several steps. It was observed that the pH dropped ca. 0.5 units after the addition of the aldehydes, and thus the pH was re-adjusted to 9.5 after each addition. For the synthesis of benzoins, several repetitive cycles of 100 mmol L^{-1} benzaldehyde were dosed when the substrate was almost consumed. For the synthesis of 2-HPP, only one initial addition was performed (100–130 mmol L^{-1} benzaldehyde and 600–720 mmol L^{-1} acetaldehyde). The reactors were magnetically stirred (up to 15 h) at room temperature. The amount of benzoins or 2-HPP was analysed by GC as described above. The determination of initial rate activities was performed by taking samples at 0, 5, 10, and 30 min, For the work-up, the reaction media were extracted with dichloromethane (4 × 50 mL). The organic phase was washed several times with water, to remove residual DMSO. After drying the organic layer with $MgSO_4$ and removing the solvent under reduced pressure, the products were achieved practically pure without need of further purification (^{1}H NMR).

4.4. Analysis of products

(R)-1,2-diphenyl-2-hydroxy-ethan-1-one [(R)-benzoin] (**2a**): White solid. Yield (after work-up): 85–90% (ee > 99% *R*). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 4.55 (d, J = 6.1 Hz, 1H), 5.9 (d, J = 6.1 Hz, 1H), 7.2–7.9 (Ar, m, 10H).

(R)-1,2-difuryl-2-hydroxy-ethan-1-one [(R)-furoin] (**2b**): Yellow solid. Yield (after work-up): 85% (ee 93% *R*). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 5.8 (s, 1H), 6.3–6.5 (Ar, 3H), 7.2–7.6 (Ar, 3H).

(R)-1,2-di-(4-methoxy-phenyl)-2-hydroxy-ethan-1-one [(R)-anisoin] (**2c**): White solid. Yield (after work-up): 65–70% (ee 76–99% *R*). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 3.7 (s, 3H), 3.8 (s, 3H), 5.85 (s, 1H), 6.8 (m, Ar, 4H), 7.2 (m, Ar, 2H), 7.9 (m, Ar, 2H).

(R)-2-hydroxy-1-phenylpropan-1-one [(R)-(2-HPP)] (**3**): Colourless crystals. Yield (after work-up): 75–85% (ee 97% *R*). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.45 (d, J = 6.8 Hz, 3H), 3.7 (br, 1H), 5.15 (q, J = 6.8 Hz, 1H), 7.5–7.9 (m, Ar, 5H).

Acknowledgements

Dr. Pablo Domínguez de María gratefully acknowledges financial support of a Marie Curie Postdoctoral Industrial Fellowship. We thank Dr. O. May (Degussa) and Prof. M. Müller (Uni Freiburg, Germany) for fruitful discussions.

References

[1] B. González, R. Vicuña, J. Bacteriol. 171 (1989) 2401.
[2] P. Dünkelmann, D. Kolter-Jung, A. Nitsche, A.S. Demir, P. Siegert, B. Lingen, M. Baumann, M. Pohl, M. Müller, J. Am. Chem. Soc. 124 (2002) 12084.
[3] A.S. Demir, O. Sesenoglu, E. Eren, B. Hosrik, M. Pohl, E. Janzen, D. Kolter, R. Feldmann, P. Dünkelmann, M. Müller, Adv. Synth. Catal. 344 (2002) 96.
[4] A.S. Demir, O. Sesenoglu, P. Dünkelmann, M. Müller, Org. Lett. 5 (2003) 2047.
[5] M. Sánchez-González, J.P.N. Rosazza, Adv. Synth. Catal. 345 (2003) 819.
[6] M. Pohl, M. Müller, A.S. Demir, WO 2002002753 A2, 2002.
[7] M. Pohl, B. Lingen, M. Müller, Chem. Eur. J. 8 (2002) 5288.
[8] A.S. Demir, M. Pohl, E. Janzen, M. Müller, J. Chem. Soc., Perkin Trans. 1 (2001) 633.
[9] M. Pohl, G.A. Sprenger, M. Müller, Curr. Opin. Biotechnol. 15 (2004) 335.
[10] D. Kihumbu, T. Stillger, W. Hummel, A. Liese, Tetrahedron: Asymm. 13 (2002) 1069.
[11] H. Iding, T. Dünnwald, L. Greiner, A. Liese, M. Müller, P. Siegert, J. Grötzinger, A.S. Demir, M. Pohl, Chem. Eur. J. 6 (2000) 1483.
[12] G. Goetz, P. Iwan, B. Hauer, M. Breuer, M. Pohl, Biotechnol. Bioeng. 74 (2001) 317–325.
[13] R. Breslow, J. Am. Chem. Soc. 80 (1958) 3719.
[14] R. Kluger, Pure Appl. Chem. 69 (1997) 1957.
[15] F. Jordan, Y.H. Mariam, J. Am. Chem. Soc. 100 (1978) 2534.