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Asymmetric synthesis of chiral 2-hydroxy ketones by coupled biocatalytic alkene oxidation and C–C bond formation

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ABSTRACT

Two different biocatalytic reactions – a C=C cleavage and a C—C forming reaction – were evaluated concerning their application in a reaction sequence. In the overall reaction, an aromatic alkene was converted to a chiral 2-hydroxy ketone. In the first step, the olefin *trans*-anethole was converted to *para*-anisaldehyde and acetaldehyde by an aqueous extract of the white rot fungus *Trametes hirsuta* G FCC 047. The selective oxidative cleavage of the carbon–carbon double bond was achieved using molecular oxygen as a substrate. In a second step *p*-anisaldehyde was ligated to acetaldehyde to yield either (*R*)- or (*S*)-2-hydroxy-1-(4-methoxyphenyl)-propanone. The reaction was catalyzed by the enantiocomplementary C—C bond forming enzymes benzaldehyde lyase and benzoylformate decarboxylase, respectively.

1. Introduction

The synthesis of enantiopure molecules is very important for the production of biologically active compounds. Furthermore, the proportion of chiral compounds of all fine chemicals will most probably increase in the future [1].

Chiral 2-hydroxy ketones are important building blocks or intermediates for the synthesis of several pharmaceutical agents, i.e. bupropion, nitidanin, cytoxazone, antidepressives and fungicides [2–4].

The enzymes benzaldehyde lyase (BAL) from *Pseudomonas putida* and benzoylformate decarboxylase (BFD) from *Pseudomonas fluorescens* are thiamine diphosphate-dependent lyases. BAL catalyses the cleavage of one enantiomer of aromatic 2-hydroxy ketones such as anisoin or benzoin, yielding the corresponding prochiral aldehydes [5] while BFD catalyses the non-oxidative decarboxylation of benzoyl formate to benzaldehyde [6].

The motivation for the evaluation of BAL and BFD in the reaction sequence is their ability to catalyse the enantiocomplementary syntheses of the (*R*)- and (*S*)-enantiomers of a chiral 2-hydroxy ketones with high yields and enantiomeric excesses by creating a C—C bond [7–11]. The chiral hydroxy group of the product can then be further processed to different functionalities while preserving the chirality centre. The creation of new carbon–carbon bonds are crucial reactions in synthetic organic chemistry [12–15].

The wood degrading white rot fungus *Trametes hirsuta* G FCC 047 is involved in the breakdown of lignocellulose compounds through oxidation by dioxygenase enzymes. There are some well-known or postulated mechanisms for the oxidation reaction of dioxygenases [16–18] but there is no other known biocatalytic mechanism that leads to the selective cleavage of double bonds and yields two aldehyde molecules as products [19,20].

A general difficulty is to oxidise compounds selectively in respect of both regio- and chemoselectivity and oxidation state. Aldehydes are easily over-oxidised to the corresponding carboxylic acid when chemical oxidation methods are applied. Furthermore, the dioxygenase from *T. hirsuta* needs only molecular oxygen as an oxidizing agent compared to the classical procedured that require heavy metal salts or other toxic reagents.

The focus of this work was to combine the dioxygenation reaction with the ligation to convert the double bond of a hydrocarbon backbone into a chiral center. This procedure, which involves selective oxidative cleavage and subsequent asymmetric C—C bond formation reactions is of general interest as it enables the conversion of a hydrocarbon structure to a chiral heteroaromatic compound with two different functional groups.

Approaches that combine the application of oxidoreductases and chemical C—C bond coupling reactions are i.e. the conversion of aldehydes to keto acids and subsequent reductive amination by leucine dehydrogenase to amino acids [21], the ligation of phenols and aromatic carboxylic acids to hydroxystilbenes followed by

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laccase-catalysed oxidative dimerisation reactions [22], or the oxidation of hydroquinones by laccases and subsequent Diels-Alder reactions [23].

To our knowledge however, the presented approach is the first example of a selective oxidative conversion of a C—C double bond to an asymmetric α-hydroxy ketone that comprised two different enzymatic reactions steps.

The olefin *trans*-anethole **1** was chosen as a substrate for the first reaction because of the best dioxygenase activity. The products, *para*-anisaldehyde **2a** and acetaldehyde **3** are subsequently ligated to the chiral hydroxy ketone **4a** (see Scheme 1) in the second step.

A general problem of synthetic procedures involving more than one reaction step is the product loss due to intermediate purification procedures. The main advantage of the developed reaction setup is that no intermediate purification is required.

The reaction mixture containing the intermediate compound **2a** is directly transferred to the reaction vessel where it is transformed to the final product **4a**.

2. Experimental

2.1. Materials

Lyophilised cells of the white-rot fungus *T. hirsute* G FCC 047 were prepared as previously described [19,20]. The enzymes benzoylformate decarboxylase from *P. putida* (E.C. 4.1.1.7) and benzaldehyde lyase from *P. fluorescens* biovar I (E.C. 4.1.2.38) were prepared as previously described [24,25]. Disruption of the cells and purification by metal ion affinity chromatography were performed as described in previous works [26]. Benzaldehyde was distilled before use. Other reagents and solvents were of analytical grade and applied without further purification. All solutions were prepared in deionised water.

2.2. Alkene oxidation

Lyophilised cells from *T. hirsute* G FCC 047 (100 mg) were rehydrated in bis–tris buffer (3.4 mL, 50 mM, pH 6 adjusted with HCl) on a rotary shaker (room temperature, 150 rpm) for 30 min and then centrifugated for 5 min (13,000 rpm). The substrate *t*-anethole (**1**, 30.0 μL, 50 mM) was transferred to a pressure-resistant reaction vessel, a Büchi "Tinyclave" (metallic-free glass reactor, 10 mL, Büchi, Flawil, Switzerland) and mixed with 0.6 mL (15%, v/v) of the cosolvent dimethylsulfoxide (DMSO). The supernatant from the cell extraction was added to the mixture and the reactor was aerated 5 times with pure oxygen gas. Prior to reaction the O_2 pressure was adjusted to 2 bar. After 24 h (72 h) of agitation at 150 rpm and 20 °C under constant oxygen pressure (2 bar), the reaction mixture was extracted with ethyl acetate (2 × 500 mL) and centrifugated after each extraction step (13,000 rpm, 3 min). The combined organic layers were dried over Na_2SO_4 and analysed by GC–MS on a 30 m × 0.53 mm HP-5 column (Agilent, Santa Clara, CA, USA) at a flow rate of 1 mL min^{-1}. Temperature program: 90 °C/19 min, gradient 50 °C min^{-1} to 180 °C/5 min, gradient 50–90 °C min^{-1}, injector temperature 250 °C, detector temperature 280 °C, $\tau_{(p\text{-anisaldehyde})}$ = 9.6 min, $\tau_{(t\text{-anethole})}$ = 11.4 min, $\tau_{(2\text{-hydroxy-1-(4-methoxyphenyl)-propanone})}$ = 21.6 min. The higher temperature is required to elute the compound **4a** when it is present in the reaction mixture.

2.2.1. Parallel alkene oxidations

Up to 7 different reaction conditions could be investigated simultaneously in a multi-vial pressure reactor made of PEEK provided by the workshop of Hamburg University of Technology [27,28]. The rehydration of the cells were performed as mentioned under Section 2.1, but in one single batch assay (700 mg lyophilised cells, 11.9 mL bis–tris buffer, 50 mM, pH 6). For the reactions, 1.7 mL of the cell extract were added to each reaction vessel (2.5 mL glass vial) containing 15 μL (50 mM) *t*-anethole and 0.3 mL (15 vol%) dimethyl sulfoxide. Reactions and analyses were performed as described in Section 2.2.

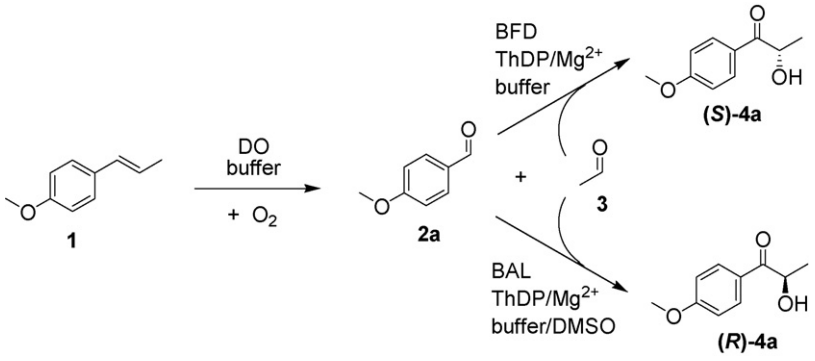
2.3. Aldehyde ligation—enzyme activity assay

The reactions were performed in tempered round-bottomed flasks at 25 and 30 °C for benzoylformate decarboxylase (BFD) and benzaldehyde lyase (BAL), respectively—see Scheme 2.

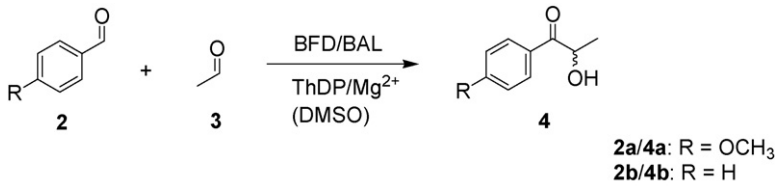
For the ligation assays with benzoylformate decarboxylase (BFD) the maximum possible soluble amount of donor aldehyde was added (anisaldehyde: 2 g L^{-1} [26]): 20 mM *p*-anisaldehyde **2a**, R = OCH_3, or 50 mM benzaldehyde **2b**, R = H, were coupled with 500 mM of the acceptor compound acetaldehyde **3** to yield (*S*)-2-hydroxy-1-(4-methoxyphenyl)-propanone **4a** and (*S*)-2-hydroxy-1-phenyl-propanone **4b**, respectively. With benzaldehyde lyase (BAL) 10 mM of *p*-anisaldehyde **2a**, R = OCH_3, or benzaldehyde **2b**, R = H, and 60 mM acetaldehyde **3** were applied to yield either (*R*)-2-hydroxy-1-(4-methoxyphenyl)-propanone **4a** or (*R*)-2-hydroxy-1-phenyl-propanone **4b**.

For the determination of the activities of BAL and BFD the ligation reaction of **2b** and **3** to **4b** was chosen (see Scheme 2).

Acetaldehyde was added in excess prior to the condensation reaction due to its volatility. The substrates were dissolved in 9.5 mL buffer solution. The buffer contains 50 mM triethanolamine (TEA), 0.5 mM thiamine diphosphate (ThDP) and 1 mM $MgSO_4$, pH 8, adjusted with H_3PO_4. For the reaction involving BAL only, 30% (v/v)



Scheme 1. Reaction sequence of the oxidative cleavage and the C—C bond coupling reactions. DO, Dioxygenase; BFD, benzoylformate decarboxylase; BAL, benzaldehyde lyase.



Scheme 2. Ligation reaction catalysed by BFD and BAL.

DMSO are added after pH adjustment. The reactions were started by adding 10 U of BAL (20 U mg^{-1}) or 10 U of BFD enzyme (10 U mg^{-1}) in 0.5 mL of the corresponding TEA buffer.

Samples were taken by quenching 0.2 mL of the reaction solution with 0.2 mL acetonitrile in a glass vial and were subsequently analysed by HPLC on a 25 cm × 0.4 cm RP-8 column (Merck, Darmstadt, Germany): 40% acetonitrile in aqueous 0.2% TEA buffer, pH 3 adjusted with H_3PO_4, flow rate 1 mL min^{-1}, $\tau_{(HPP,\ MHPP)}$ = 5.2 min, $\tau_{(anisaldehyde)}$ = 7.7 min, $\tau_{(benzaldehyde)}$ = 8.0 min, $\tau_{(benzoin)}$ = 11.2 min, $\tau_{(anisoin)}$ = 11.6 min UV detection at 254 nm. Samples were centrifuged (5 min, 13,000 rpm) if they contained a precipitate. For the determination of the enantiomeric excess of 2-hydroxy-1-(4-methoxyphenyl)-propanone **4a** the corresponding sample was purified by analytical HPLC as described above. The product fraction was extracted with isohexane and analysed for enantiomeric excess by HPLC on a 25 cm × 0.46 cm CHIRALCEL OD-H column (Daicel, Tokyo, Japan): 2% 2-propanol in isohexane, 0.5 mL/min, $\tau_{((R)\text{-2-hydroxy-1-(4-methoxyphenyl)-propanone})}$ = 42.8 min, $\tau_{((S)\text{-2-hydroxy-1-(4-methoxyphenyl)-propanone HPP})}$ = 44.4 min, UV detection at 254 nm. The retention times of (*R*)- and (*S*) **4a** were determined by measuring the purified ligation products synthesised with BAL and BFD, respectively [8,26].

2.3.1. Coupled enzymatic reaction

The oxidative cleavage of *t*-anethole was performed for 72 h as described in Section 2.2 resulting in a concentration of approximately 40 mM *p*-anisaldehyde. 1 mL of this reaction mixture was transferred to a round-bottom flask containing 4 mL of the corresponding buffer solution (see Section 2.3) leading to an intermediate concentration of *p*-anisaldehyde of 8 mM and containing 5 U BAL or 10 U BFD, respectively. The activity refers to the initial reaction rate of the C—C bond formation of **2b** and **3** to **4b** if not otherwise stated (see Scheme 2). The reaction was started by adding acetaldehyde (17 μL, 60 mM, in the case of BAL-catalysis and 142 μL, 500 mM, for the BFD-catalysed reaction). Additional acetaldehyde is required for kinetic reasons. The reaction was monitored by HPLC. Enantiomeric excesses were also determined by HPLC as described above (Section 2.3).

3. Results and discussion

3.1. Enzymatic synthesis of p-anisaldehyde by regioselective oxidation

The conversion of the olefin **1** to the aldehydes **2a** and **3** is described in Scheme 1. According to the literature, a yield of 83% was obtained after 24 h reaction time [19,20] which was reproduced throughout this work. The reaction is only due to the biocatalytic conversion of olefin as no aldehyde could be detected in negative control assays.

The reaction system is complex and consists of four phases. It contains two non-miscible liquid phases as the substrate **1** is virtually insoluble in the buffer solution, the oxygen gas phase involved in the reaction and a solid phase which is formed during the reaction.

During the reaction a precipitate is formed, which contains product **2a** and substrate **1**.

The solubility of molecular oxygen in water is 2.4 mM (39 mg L^{-1}, at 20 °C and 1 bar gas pressure [30]). The diffusion of both oxygen and anethol to the active center of the dioxygenase is crucial for the reaction to take place. It is not known whether the reaction occurs at the liquid–liquid interface or if the hydrophobic molecule must diffuse into the aqueous layer. Thus, the mass transport of both substrates could limit the oxidation reaction.

To verify the effect of the concentration of the substrate **1** on the reaction rate, six parallel batch reactions were run and stopped at the same time, containing different amounts of **1** at the beginning of the reaction. The results are shown in Fig. 1.

In the range of 5–50 mM of **1**, no major changes in the rate of conversion can be seen. The overall conversion decreases when concentrations of 1 and 2 mM are applied.

3.2. BFD/BAL ligation assay

Coupling of the aromatic aldehyde benzaldehyde **2b** to acetaldehyde **3** is considered to be the standard reaction throughout this work to evaluate the activity of the C—C bond forming enzymes, see Scheme 2.

The reaction sequence involves *p*-anisaldehyde (R = OCH_3, **2a**) and produces (*R*)- or (*S*)-2-hydroxy-1-(4-methoxyphenyl)-propanone (**4a**), respectively (see Scheme 2).

The microreaction steps of the BAL-catalysed synthesis of the *R*-enantiomer and the BFD-catalysed production of the *S*-compound are different: the catalytic mechanism of benzaldehyde lyase is more complex as most of the 2-hydroxy ketone is not formed directly from the aldehydes, but follows the synthesis of an intermediate benzoin derivative which is the ligation product of two aromatic aldehydes [9]. These intermediates are *p*-anisoin when

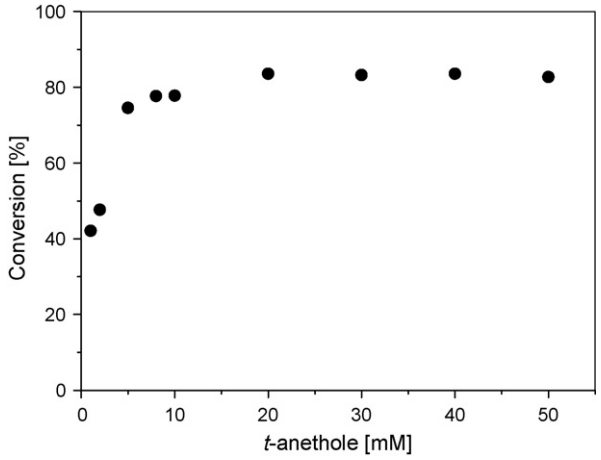


Fig. 1. Influence of the substrate concentration of **1** on the conversion of the oxidation (reaction time: 24 h). Extract of 50 mg *Trametes hirsuta*, 1.7 mL 50 mM bis–tris buffer, pH 6, 1–50 mM **1**, 0.3 mL DMSO, 2 bar O_2, shaking at 150 rpm, rotary stroke 33 mm.

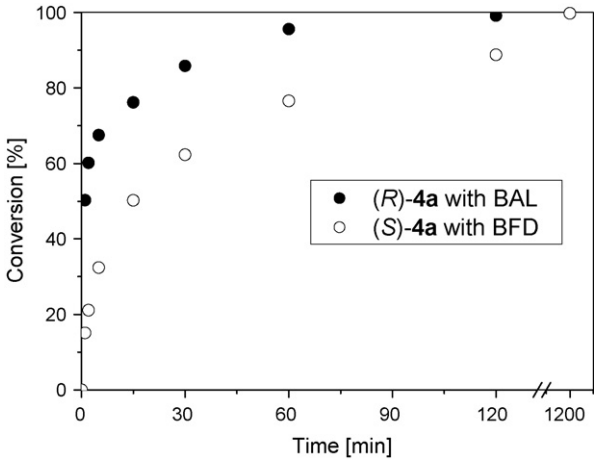


Fig. 2. Synthesis of (*R*)-and (*S*) **4a** catalysed by BAL and BFD, respectively. 9.5 mL 50 mM TEA buffer, pH 8, 0.5 mM ThDP, 1 mM $MgSO_4$. (*R*)-**4a**: 10 mM **2a**, 60 mM **3**, 30% (v/v) DMSO, 10 U BAL (0.2 mg), 25 °C. (*S*)-**4a**: 20 mM **2a**, 500 mM **3**, 10 U BFD (0.67 mg), 30 °C.

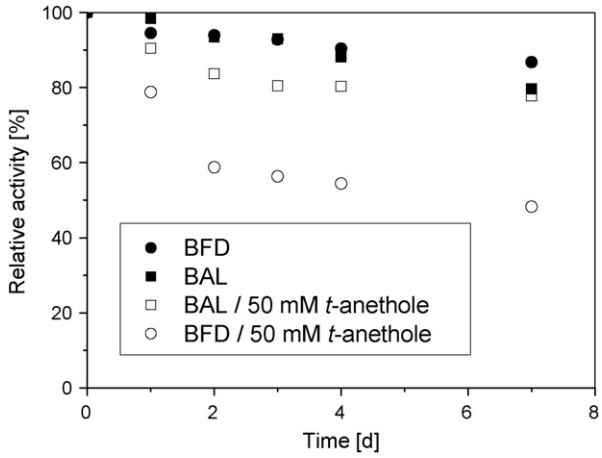


Fig. 4. Long-term stability of BAL and BFD in the reaction buffer in the presence and absence of *t*-anethole. Incubation assays: 5 mL 50 mM TEA buffer, pH 8, 0.5 mM ThDP, 1 mM $MgSO_4$, for BAL only: 30% (v/v) DMSO; activity assay: 9.5 mL 50 mM TEA buffer, pH 8, 0.5 mM ThDP, 1 mM $MgSO_4$, 0 or 50 mM **1**, 100 μL incubation solution of BFD, BFD/anethole, 20 mM **2a**, 500 mM **3**, 30 °C. BAL, BAL/anethole: 10 mM **2a**, 60 mM **3**, 30% (v/v) DMSO, 25 °C, 10 μL incubation solution.

2a is applied as substrate and benzoin in the case of **2b**. Due to the poor solubility of these compounds in water, 30% (v/v) of the organic cosolvent DMSO is added to avoid their precipitation during the BAL-catalysed reactions.

Complete conversion of **2a** and **3** to **4a** was obtained (Fig. 2), which is significantly higher than the data described in earlier reports [8,26,31,32]. In contrast to the literature the batches were continuously stirred and were carried out at pH 8. The reported results were obtained at pH 7 and without stirring by adding 6.75 U BFD to 1.5 mL and 20 h reaction time [26,32] and by adding three times 20 U BAL to 100 mL solution and 96 h reaction time [31].

The enantiomeric excesses were determined to be >99% for the (*R*)-enantiomer produced by BAL and 94% for the (*S*)-compound yielded by BFD, which agrees well with the literature data [7,23,28,29].

3.2.1. Evaluation of a coupled enzymatic chiral oxidation reaction

To evaluate the success of a one-pot enzymatic reaction sequence, the compatibility of BAL and BFD were investigated in view of residual substrate from the first reaction. For both enzymes, the initial ligase reaction rates in the presence of different *t*-anethole concentrations and their long-term stability in the presence of 50 mM *t*-anethole were measured.

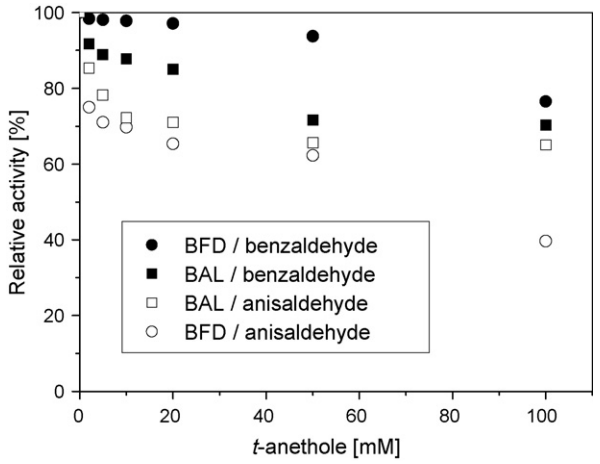


Fig. 3. Influence of the concentration of *t*-anethole on the initial rate activity of BAL and BFD, respectively. 9.5 mL 50 mM TEA buffer, pH 8, 0.5 mM ThDP, 1 mM $MgSO_4$, 1–50 mM **1**. BFD/benzaldehyde: 50 mM **2b**, 500 mM **3**, 10 U BFD, 30 °C. BFD/anisaldehyde: 20 mM **2a**, 500 mM **3**, 10 U BFD, 30 °C. BAL/benzaldehyde: 10 mM **2b**, 60 mM **3**, 30% (v/v) DMSO, 10 U BAL, 25 °C. BAL/anisaldehyde: 10 mM **2a**, 60 mM **3**, 30% (v/v) DMSO, 10 U BAL, 25 °C.

As shown in Fig. 3 the presence of **1** has a clear negative effect on the ligase activities of both enzymes in respect to the substrates **2a** and **2b** and increases with the concentration of **1**.

The ligation of **2b** to **4b** is less affected for both enzymes than the ligation of **2a** to **4a** which means that the reaction with the preferred standard ligation substrate appears to be less sensitive.

The long-term stability of the enzymes BAL and BFD was investigated in the presence of 50 mM of and shows a deactivating impact on both BAL and BFD (see Fig. 4).

The incubation assays with or without 50 mM **1** were carried out at room temperature using the same buffer solutions as applied in the ligation assays, but without substrates. The stability was determined by measuring the initial activity of BAL and BFD in respect to the ligation of **2a** and **3** to **4a**.

While BFD possesses a better long-term stability at the applied reaction conditions without addition of **1**, the deactivation caused by the presence of 50 mM **1** is significantly higher in the case of BFD than for BAL as seen in Table 1.

The presence of **1** reduces the half-life of BAL to about 62% and the half-life of BFD to about 15%.

The reduced activity and long-term stability in presence of **1** could result from a general effect of activity reduction because of the two-phase system. The very low solubility of *t*-anethole even in the presence of 30% (v/v) DMSO makes a two-phase system unavoidable in this setup. BFD is more affected than BAL in view of initial activity and long-term stability.

Table 1
Half-life times for the ligase activities of BAL and BFD with and without an additional deactivation caused by 50 mM of **1**

Incubation	Half-life BAL [d]	Half-life BFD [d]
Control	22	30
With 50 mM **1**	14	4.4

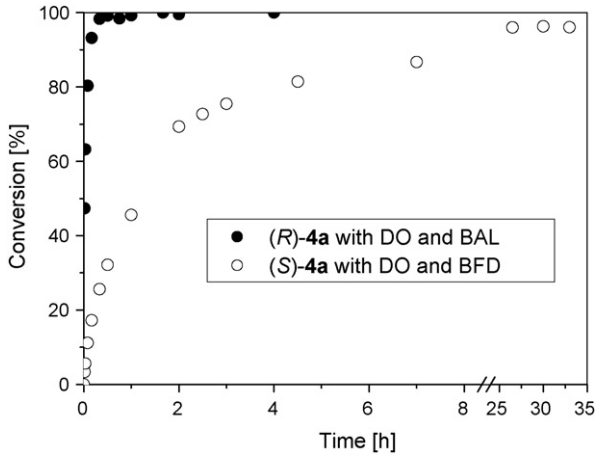


Fig. 5. Sequential synthesis of (*R*)-**4a** by DO and BAL and of (*S*)-**4a** by DO and BFD. 100 mg *Trametes hirsuta*, 3.4 mL 50 mM bis–tris buffer, pH 6, 50 mM **1**, 0.6 mL DMSO, 2 bar O_2, 150 rpm, 72 h. 1 mL each of mixture added to 4 mL TEA buffer, pH 8, 0.5 mM ThDP, 1 mM $MgSO_4$. (*S*)-**4a**: 500 mM **3**, 50 U BFD, 30 °C. (*R*)-**4a**: 60 mM **3**, 15% (v/v) DMSO, 10 U BAL, 25 °C.

3.3. Coupled biocatalytic synthesis of 2-hydroxy-1-(4-methoxyphenyl)-propanone

As the concurrent *in situ* coupling of the oxidative cleavage of **1** and subsequent ligation of the aldehydes was not successful, the reaction sequence was performed as a two-step procedure.

The dioxygenation reaction was carried out as described in Section 2.1. The reaction mixture was further reacted with the BAL or BFD to achieve the sequence as shown in Scheme 1. Hereby the reaction mixture from oxidation step was transferred to another reaction vessel to carry out the C—C bond coupling reaction. The addition of an excess of the substrate acetaldehyde is required due to its high K_M value in the case of the BFD-catalysed formation of *S*-**4** [22] and due to the lower selectivity of BAL for the synthesis of *R*-**4** in respect to the carboligation of aromatic aldehydes to benzoin derivatives [12].

By adding 4 mL of the corresponding buffer solutions (pH 8, see Section 2.3.1) to 1 mL of the reaction mixture of the first step it is assumed that the pH is roughly shifted from 6 towards the desired value of 8 due to the 4:1 ratio. However, the exact pH value could not be measured after mixing as the setup from the first reaction contains DMSO which is polar and aprotic.

After the first step 85% of the initial substrate **1** had been converted to anisaldehyde leaving a residual concentration of about 5 mM. By mixing 1 mL with 4 mL buffer solution the concentration of **2a** was reduced to 8.5 mM and the concentration of **1** to 1 mM. At these conditions, the ligase activities for the coupling of *p*-anisaldehyde and benzaldehyde are reduced by 15 and 25% for BAL and BFD, respectively, as shown in Fig. 3.

With both BAL and BFD, the reaction was successful, as 99 and 96% of the anisaldehyde were converted to give 84 and 81% of the final product, see Fig. 5 and Table 2.

Table 2
Conversion and enantiomeric excesses of the reaction sequence

Starting material	Dioxygenase	BFD		BAL	
	Conversion	Conversion	ee (*S*)	Conversion	ee (*R*)
2a	–	>99	94	>99	>99
1	85	96 (81)	92	> 99(84)	>99

All the values are given in percentage. The values in parenthesis refer to the overall conversion of the two reaction steps.

The rate of conversion only refers to the formation of **4a** in respect to total amount of **2** in the second step of the sequence.

The results for the ligation reactions (Scheme 2) and of the complete reaction sequence (Scheme 1) are shown in Table 2.

The sequential reaction involving BFD is significantly slower than the non-sequential ligation assay. This confirms the results shown in Figs. 3 and 4 and Table 1 indicating a higher sensibility of BFD towards the dioxygenase substrate *t*-anethole than BAL. However, both reactions were carried out successfully achieving conversions of at least 94% in the ligation reactions.

The final ee values of the reaction were >99% (*R*) for the sequence involving BAL and 92% (*S*) in the case of BFD. For both the BAL- and the BFD-catalysed reactions the ee values are comparable to the non-sequential reactions (Section 3.2).

4. Conclusion

An olefin was successfully converted into both enantiomers of a chiral 2-hydroxy ketone in a sequential biocatalytic approach, which formally resembles from a chemical point of view a dihydroxylation of the olefin and oxidation of one alcohol function to the corresponding ketone. This reaction constitutes of a stereoselective oxidation of a C—C double bond to an alcohol and a ketone group. The sequence involves a selective oxidative cleavage of a carbon–carbon double bond followed by a stereoselective ligation of the intermediate aldehydes. The sequential two-step reaction sequence was carried out without isolation or purification of the generated intermediates. This direct approach avoids losses caused by intermediary purification steps and thus contributes to increased overall yields and productivities of the investigated process.

The oxidation of *t*-anethole to *p*-anisaldehyde was achieved with dioxygenase from *Trametes hirsuta* as described. The conversion is apparently not affected by the concentration of the substrate *t*-anethole. Other factors like, i.e. mass transport limitation could influence the reaction velocity in this complex multi-phase system.

The ligation of *p*-anisaldehyde and acetaldehyde was achieved with almost full conversion, which has not been reported before, with both BAL and BFD. Although the ligase activity of the enzymes is reduced by *t*-anethole, the biocatalytic sequence was completed with an overall yield of 84% and 81% of (*R*)- and (*S*)-2-hydroxy-1-(4-methoxyphenyl)-propanone, with enantiomeric excesses of >99% (*R*) and 92% (*S*).

The evaluation of sequential enzymatic reactions can be a powerful tool to generate chiral building blocks from simple organic structures, even from pure hydrocarbon moieties. Nevertheless, the different optimal reaction conditions of the biocatalysts must be observed. Further work is required to get a better understanding of the mutual influences in this complex system in order to develop the sequential two-step approach to a one-pot process where both biocatalytic reactions are carried out simultaneously.

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