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Chemoenzymatic synthesis of both enantiomers of 3-hydroxy- and 3-amino-3-phenylpropanoic acid

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ABSTRACT

Ethyl (*S*)-3-hydroxy-3-phenylpropionate (*S*)-**2** was obtained by the asymmetric reduction of ethyl 3-phenyl-3-oxopropionate **1** with the yeast *Saccharomyces cerevisiae* (ATCC 9080). The kinetic resolution of racemic ethyl 2-acetoxy-3-phenyl-propionate *rac*-**3** with the same microorganism, gave after hydrolysis ethyl (*R*)- and (*S*)-3-hydroxy-3-phenylpropionates (*R*)-**2** and (*S*)-**2** which were converted by a straightforward series of reactions to the enantiomers of 3-amino-3-phenyl-propionic acids (*S*)-**6** and (*R*)-**6**. The asymmetric reduction and hydrolytic kinetic resolution were also tested with several other whole cell systems under a variety of conditions.

1. Introduction

Enantiomerically pure β-hydroxy esters are important chiral building blocks for the synthesis of various pharmaceuticals such as fluoxetine,[1] β-lactam antibiotics,[2] Tuckolide (an HMGCoA reductase-inhibitor),[3] and dihydrokawain (a narcotic).[4] The amino derivative obtained from (*S*)-β-hydroxy ester is a building block of Taxol.[5] Ethyl 4-chloro-3-hydroxy butanoate is used in the preparation of L-carnitine, which is known as nutraceutical.[6] Chiral β-hydroxy esters are also used as starting materials for the preparation of enantiomerically pure β-blockers, that is, propranolol, alprenolol, and 1-(isopropylamino)-3-*para*-methoxyphenoxy-2-propanol.[7] The utility of sodium (*R*)-β-hydroxy butanoate as a cerebral function improving agent on cerebral hypoxia, anoxia, and ischemia in mice and rats has also been reported.[8] Both enantiomers of ethyl 3-hydroxy butanoate and ethyl 3-hydroxy pentanoate are important in the synthesis of pheromones.[9] β-Hydroxy esters also play an important role in many biological reactions inside the human body.[10]

The most commonly used methods for obtaining enantiomerically pure β-hydroxy esters are: (i) the reduction of the corresponding ketones; and (ii) the kinetic resolution of racemic β-hydroxy esters.

(i) The enantioselective reduction of β-keto esters with organometallics and by chemo-enzymatic procedures are known methods. Various microbial whole cells[11] and plant cells[12] are used for the chemoenzymatic synthesis of various chiral molecules. Engineered whole cells of baker's yeast have been reported to carry out a highly stereoselective synthesis of α-unsubstituted and α-alkyl-β-hydroxy esters.[13]

The microbial reduction of aliphatic β-keto esters using most often baker's yeast, a readily available and inexpensive reducing agent, was reported.[14] Depending on the reaction conditions, a wide range of yields and enantiomeric excesses of the product was observed.[15] For baker's yeast mediated bioreductions, the use of water miscible organic co-solvents is advantageous, while in water immiscible organic solvents, such as petroleum ether, the efficiency can even be further enhanced.[15]

Employing a wide range of microorganisms, such as yeasts (baker's yeast and *Rhodotorula* sp.), fungi (*Aspergillus, Geotrichum* and *Mortierella* sp.), or bacteria (*Lactobacillus* sp.) Bartod et al.[16] studied the reduction of β-keto esters. Freeze dried baker's yeast was used under fermenting and non-fermenting conditions. Bioconversions with other microorganisms were carried out using washed resting cells. Only two microorganisms (baker's yeast and *Mortierella isabellina*) were able to reduce β-keto esters to yield hydroxy esters. The addition of sucrose (fermenting conditions) was found to accelerate the baker's yeast mediated reaction.[17] With other microorganisms, low conversions and poor selectivities were observed and significantly more by-products were formed. Secondary reactions can appear at any stage, even from the beginning of the reaction.[18] A possible explanation could be that concurrent hydrolysis and decarboxylation of the ketoester occur. Side reactions can be suppressed by certain additives, but this may be at the expense of stereoselectivity.[19] For example ethyl chloroacetate is known to inhibit (*R*)-selective enzymes by allowing the expression of the

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(*S*)-selective enzymes,[20] which enhanced the enantiomeric excess for the cellular synthesis of (*S*)-hydroxy esters.

(ii) Whole cell catalyzed kinetic resolution is another method for the synthesis of enantiomerically pure β-hydroxy esters from racemic β-hydroxy esters.[21] The limitation of this method is the maximum conversion of 50% for the desired enantiomer. The most common procedure is kinetic resolution in organic media mediated by hydrolases.[22] Hydrolysis of esters using whole cells in a water suspension is also known.[23]

These enantiomers of β-hydroxy esters can also be transformed into the corresponding β-amino acids that is, (*R*)- and (*S*)-3-amino-3-phenylpropanoic acids which are valuable building blocks for the synthesis of pharmaceutically active compounds. Their synthesis by hydrolase-catalyzed methods has been described.[24] (*R*)-β-Phenylalanine was produced from L-phenylalanine by phenylalanine 2,3-aminomutase from *Taxus canadensis* (*Tc*PAM),[25] whereas the phenylalanine 2,3-aminomutase from *Pantonea agglomerans* (*Pa*PAM) converted L-phenylalanine into (*S*)-β-phenylalanine.[26]

Herein we report the synthesis of both enantiomers of ethyl 3-hydroxy-3-phenylpropanoate with the yeast *Saccharomyces cerevisiae* (ATCC® 9080™). Ethyl 3-hydroxy-3-phenylpropanoate is an important intermediate in the synthesis of fluoxetine.[1] The β-hydroxy esters can also be transformed into the corresponding β-amino acids, that is, (*R*)- and (*S*)-3-amino-3-phenylpropanoic acids, which are the products of *Tc*PAM and *Pa*PAM reactions and are useful as valuable building blocks for the synthesis of biologically active compounds.

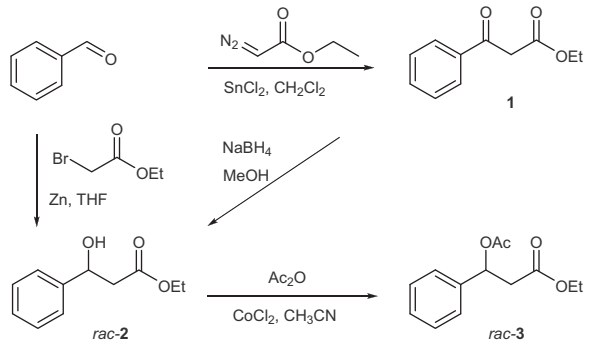
2. Results and discussion

2.1. Chemical synthesis of ethyl 3-hydroxy- and 3-acetoxy-3-phenylpropionate

There are several methods known for the direct synthesis of β-hydroxy acids. They can be produced by the Reformatsky reaction using β-bromo acids, and their methyl, ethyl, *tert*-butyl, or tetrahydropyranyl[27] esters, which provide mild acidic conditions for the starting materials. When such halogenated derivatives are not accessible, the synthesis of β-hydroxy esters can also be performed using the dianion of carboxylic acids obtained by the reaction between acids and extremely strong bases such as Li-diisopropylamide.[27] In this case, the carboxylic proton and one of the α-protons are both exchanged for lithium. In this way, several types of substituted α-phenyl-β-hydroxypropanoic acids are obtained. Other methods are based on a two step synthesis of the hydroxy esters. Herein, we first prepared ethyl 3-oxo-3-phenylpropanoate **1** by reacting benzaldehyde and ethyl diazoacetate in the presence of $SnCl_2$ as described in the literature[28] and used the product for a yeast mediated enantioselective reduction. This ketone **1** can also be reduced in order to produce the corresponding racemic β-hydroxy ester *rac*-**2**, which is also accessible by the Reformatsky reaction (Scheme 1). The latter compound was acylated under mild conditions with acetic anhydride in the presence of $CoCl_2$. In this way the use of acids or bases as catalysts promoting water elimination from the β-hydroxy esters can be avoided. The resulting diester *rac*-**3** was then used as a substrate for yeast mediated kinetic resolution by enantiomer selective hydrolysis.

2.2. Microbial synthesis of β-hydroxy- and β-acetoxyacid esters

The general biocatalytic pathways for the synthesis of both enantiomers of ethyl 3-hydroxy-3-phenylpropanoate (*R*)-**2** and (*S*)-**2** are shown in Scheme 2.



Scheme 1. Chemical synthesis of β-keto, β-hydroxy, and β-acetoxypropionic acid esters.

2.2.1. Enantioselective reduction of β-keto ester 1

First the enzymatic reduction of ethyl 3-oxo-3-phenylpropanoate **1** with *Saccharomyces cerevisiae* (ATCC® 9080™, originally deposited as *Saccharomyces carlsbergensis* Hansen) was carried out and compared with other methods already reported,[29] such as a reduction mediated by baker's yeast (*S. cerevisiae*) or *Daucus carota* (Table 1). In all cases, the enantioface-selective reduction of the prochiral ketone followed Prelog's rule yielding ethyl (*S*)-3-hydroxy-3-phenylpropanoate (*S*)-**2**. We carried out the microbial reductions both under fermenting and non-fermenting conditions and the influence of additives, such as ethyl bromoacetate or L-cysteine, on the yield and enantiomeric excess (ee_P) of the product was studied as shown in Table 1. In order to investigate the stereoselectivity of the microbial reduction of ethyl 3-oxo-3-phenylpropanoate **1**, analytical scale reactions were first performed under fermenting and non-fermenting conditions and with or without additives (Table 1). Samples were taken and analyzed by HPLC. Enantioselectivity was higher when the biocatalyst *S. cerevisiae* (ATCC® 9080™) yeast was used under fermenting conditions and supplemented with ethyl bromoacetate (Table 1, entry 8). In this way high ee values (>97%) and yields (>90%) of the isolated ethyl (*S*)-3-hydroxy-3-phenylpropanoate (*S*)-**2** could be realized.

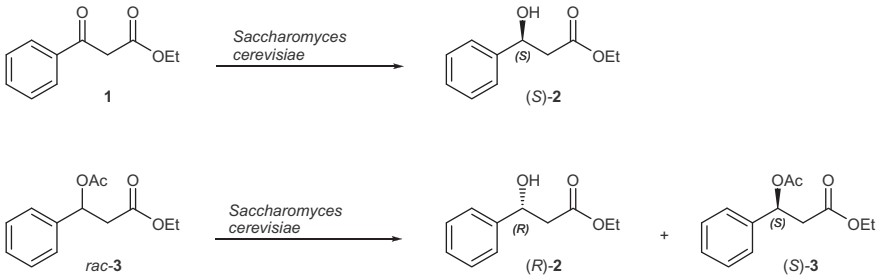
With these results in our hand, a preparative scale synthesis of ethyl (*S*)-3-hydroxy-3-phenylpropanoate (*S*)-**2** was carried out. Dilutions and substrate-biocatalyst ratios were the same as those that were found to be optimal in the case of the analytical scale reactions. The reactions were stopped when all of the substrate was consumed.

2.2.2. Kinetic resolution of racemic ethyl 3-acetoxy-3-phenylpropanoate *rac*-3

In addition to being widely used as biocatalysts for enantioselective reductions, yeast cells also contain hydrolases with a broad range of substrate acceptability and chemo-, regio-, and stereoselectivity. Therefore, we also extended our studies to explore the scope of yeasts for the enantioselective hydrolysis of racemic ethyl 3-acetoxy-3-phenylpropanoate *rac*-**3**.

As shown in Scheme 2, enantioselective hydrolysis of the acylated secondary alcohol followed Kazlauskas' rule yielding ethyl (*R*)-3-hydroxy-3-phenylpropanoate (*R*)-**2** and unreacted ethyl (*S*)-acetoxy-3-phenylpropanoate (*S*)-**3**.

The experiments were performed in aqueous media both under fermenting and non-fermenting conditions (Table 2). The enantioselective hydrolysis of the substrate with lyophilized cells in organic media was also tested. In every case, the hydrolases showed high chemoselectivity, that is, the carboxyethyl function of the substrate was not affected. The influence of the reaction conditions on the selectivity of the enzymatic hydrolysis of the racemic substrate is shown in Table 2.



Scheme 2. Yeast mediated synthesis of optically active β-hydroxyacid derivatives.

Table 1
Reduction of ethyl 3-oxo-3-phenylpropanoate **1** (5 mM) with various whole-cell biocatalysts (100 mg mL^{-1}) under different conditions in water at room temperature after 48 h

Entry	Microorganism	System	Additives	Yield (%)	ee_P (%)
1	Baker's yeast[a]	Non-fermenting	—	63	80
2	Baker's yeast[a]	Fermenting	—	71	88
3	Baker's yeast[a]	Fermenting	Ethyl bromoacetate	65	93
4	Baker's yeast[a]	Fermenting	L-Cysteine	72	86
5	*D. carota*[b]	—	—	47	94
6	*S. cerevisiae* (ATCC 9080)	Non-fermenting	—	78	92
7	*S. cerevisiae* (ATCC 9080)	Fermenting	—	85	96
8	*S. cerevisiae* (ATCC 9080)	Fermenting	Ethyl bromoacetate	92	97

[a] *Saccharomyces cerevisiae*, product of Budafok Ltd, from a local store.
[b] *Daucus carota*.

Table 2
Yeast mediated kinetic resolution of ethyl 3-acetoxy-3-phenylpropanoate (*rac*-**3**: 5 mM) with different strains (100 mg mL^{-1}) after 48 h

Entry	Microorganism	System	$Yield_{(S)\text{-}3}$ (%)	$ee_{(S)\text{-}3}$ (%)	$ee_{(R)\text{-}2}$ (%)
1	Baker's yeast[a]	Non-fermenting	47	81	89
2	Baker's yeast[a]	Fermenting	48	87	92
3	Baker's yeast[a]	Lyophilized cells in hexane	41	65	82
4	*S. cerevisiae* (ATCC 9080)	Non-fermenting	48	84	88
5	*S. cerevisiae* (ATCC 9080)	Fermenting	49	96	96
6	*S. cerevisiae* (ATCC 9080)	Lyophilized cells in hexane	45	72	85

[a] *Saccharomyces cerevisiae*, product of Budafok Ltd, from a local store.

2.3. Conversion of 3-hydroxy-3-phenylpropanoate enantiomers (*R*)-2 and (*S*)-2 into (*R*)-and (*S*)-3-amino-3-phenylpropanoate (*R*)-6 and (*S*)-6

Finally, the enantiomerically highly enriched ethyl (*R*)- and (*S*)-β-hydroxypropanoates (*R*)-**2** and (*S*)-**2** were chemically converted into the corresponding (*S*)- and (*R*)-3-amino-3-phenylpropanoic acids (*R*)-**6** and (*S*)-**6** as presented in Scheme 3. Several methods are known for the transformation of secondary alcohols into the corresponding amines without a significant loss of the enantiopurity.[30] Our investigation into various methods showed the best yields and enantiopurities for the product were obtained when the hydroxy group in (*R*)-**2** or (*S*)-**2** was transformed into azide (*S*)-**4** or (*R*)-**4**, respectively, by using diphenyl phosphorazide. This reaction proceeds via an S_N2 mechanism involving a stereospecific inversion of the configuration. Reducing the azide (*S*)-**4** or (*R*)-**4** using the Staudinger reaction resulted in the formation of the corresponding ethyl (*S*)- and (*R*)-3-amino-3-phenylpropanoates (*S*)-**5** or (*R*)-**5** with the same enantiopurities as determined for the starting ethyl (*R*)- or (*S*)-3-hydroxypropanoates (*R*)-**2** and (*S*)-**2**, respectively.

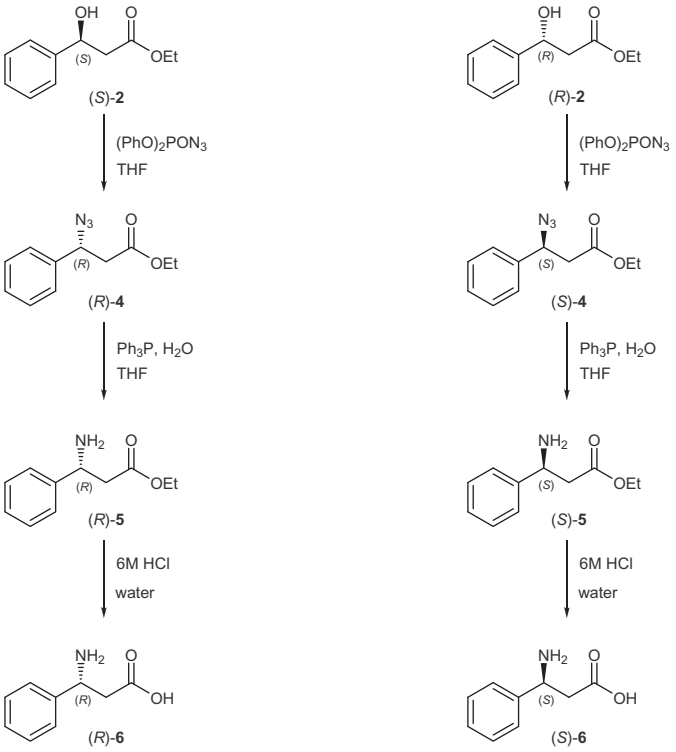
3. Conclusion

Herein the utility of *Saccharomyces cerevisiae* (ATCC® 9080™) as an enantioselective biocatalyst in the reduction of ethyl 3-oxo-3-phenylpropanoate **1** into ethyl (*S*)-3-hydroxy-3-phenylpropanoate (*S*)-**2** in good yield and with high enantiomeric purity has been demonstrated. In the presence of the same biocatalyst, highly enantio- and chemoselective kinetic resolution of ethyl 3-acetoxy-3-phenylpropanoate *rac*-**3** via enantioselective hydrolysis to the (*R*)-hydroxy-ester (*R*)-**2** was carried out. Both processes were optimized by varying the medium and by using additives. The enantiomerically enriched β-hydroxy esters (*S*)-**2** and (*R*)-**2** were converted into the corresponding (*R*)- and (*S*)-3-aminopropanoic acids (*R*)-**6** and (*S*)-**6** by a straightforward reaction sequence involving a stereospecific S_N2 reaction step with inversion of configuration.

4. Experimental

4.1. Reagents and solvents

Commercial chemicals and solvents were products of Aldrich or Fluka. All solvents were purified and dried by standard methods as required. Analytical data of all compounds were identical with those reported in the literature.[1,28,30] Thin layer chromatography (TLC) was carried out using Merck Kieselgel $60F_{254}$ sheets. Spots were visualized by treatment with 5% ethanolic phosphomolybdic acid and ninhydrin solution and heating. Preparative chromatographic separations were performed using column chromatography on Merck Kieselgel 60 (63–200 μm). The enantiomeric



Scheme 3. Transformation of (*S*)-**2** and (*R*)-**3** into the corresponding β-amino acid enantiomers.

composition of the chiral products was determined using high performance liquid chromatography (HPLC) with an Agilent 1200 instrument (25 °C, injection volume: 25 μL) or by Gas Chromatography (GC) with an Agilent 4890 instrument (H_2 as carrier gas, FID detector, injection volume: 1 μL) on columns filled with enantioselective stationary phases.

4.2. Strains used for biotransformations

Baker's yeast produced as wet cakes by Budafok Ltd, Hungary, was from a local store. Wet-cake cells of *Saccharomyces cerevisiae* (ATCC® 9080®; American Type Culture Collection, Manassas, VA; stored at −80 °C in 15% glycerol-water mixture) were prepared in-house. Cells were grown on agar plates (solid media agar, glucose, yeast extract, NH_4Cl, peptone, KH_2PO_4, NaCl, $MgCl_2 \times 6H2O$, $CaCl_2 \times 2H_2O$: 20, 15, 3, 2, 1, 0.1, 0.08, 0.07 and 0.01 g/L, respectively; in Petri dishes) at 30 °C for 24 h. A single colony from the agar plate was transferred into 50 mL of the growing media (pH 5; glucose, yeast extract, NH_4Cl, peptone, KH_2PO_4, NaCl, $MgCl_2 \times 6H2O$, $CaCl_2 \times 2H_2O$: 30, 10, 3, 2, 0.1, 0.08, 0.07 and 0.01 g/L, respectively) and was incubated and shaken at 200 rpm at 30 °C for 24 h. The whole seed-culture was used to inoculate the growing media (1.5 L) in a batch bioreactor (3 L). The fermentation was performed at pH 5 (adjusted with NaOH, 0.5 M) in the stirred (200 rpm) and aerated (2 L min^{-1}) bioreactor at 30 °C. The cell optical density (OD at 600 nm) was determined every 2 h. When the OD rose to 0.7–0.8, the fermentation was stopped and the resulting cell suspension was centrifuged (4000*g*, 5 °C, 30 min) to give 25–30 g wet-cake cells.

4.3. Synthesis of the starting compounds

4.3.1. Ethyl 3-oxo-3-phenylpropanoate 1

Into a mixture of benzaldehyde (0.6 mol) and $SnCl_2$ (0.1 equiv) in freshly distilled dichloromethane (20 mL), a solution of ethyl diazoacetate in dichloromethane (1.2 equiv) was slowly added (10–12 h) at room temperature under argon. The reaction mixture was stirred for 3 days and every day an extra portion of $SnCl_2$ (0.1 equiv) was added into the reaction mixture. After the complete transformation of the substrate, THF (10 mL) was added to the reaction mixture. The solvent was evaporated in vacuum and the crude product was purified by preparative vacuum-chromatography using dichloromethane as eluent to obtain ethyl 3-oxo-3-phenylpropanoate **1** as a light colorless semisolid in 61% yield. The ^1H and ^{13}C NMR data were in accordance with those reported in the literature.[31]

4.3.2. Ethyl 3-hydroxy-3-phenylpropanoate *rac*-2

Method A: A solution of benzaldehyde (0.6 mol) in THF (10 mL) was added in small portions with slight warming into a refluxing mixture of zinc powder (40 g, 0.6 mol) and ethyl bromoacetate (83.5 g, 55.5 mL, 0.5 mol) in THF (100 mL), which was previously heated at reflux for 30 min. After completion of the reaction (approx. 1 h), the mixture was cooled to room temperature and filtered to remove the unreacted zinc. The solvent was evaporated in vacuo and the crude semisolid product was redissolved in CH_2Cl_2 (20 mL). The solution was cooled in an ice bath and treated with 10% sulfuric acid (10 mL) under vigorous stirring. After separation, the aqueous layer was extracted with CH_2Cl_2 (3 × 100 mL). The combined organic layer was washed with saturated $NaHCO_3$ solution (3 × 50 mL) and water (50 mL). The dried organic solution was concentrated in vacuo. The crude product was purified by preparative vacuum-chromatography using dichloromethane-methanol (9:1, v/v) as eluent yielding *rac*-**2** (53%) as a light colorless semisolid.

Method B: Into the ethanolic solution of ethyl 3-oxo-3-phenylpropanoate (**1**, 4.8 g, 25 mmol in 50 mL solvent), $NaBH_4$ (850 mg, 25 mmol) was added portionwise at room temperature. After the completion of the reaction (checked by TLC, approx. 2 h) the pH was adjusted to 6.5 with 5% HCl. The ethanol was evaporated in vacuo and the mixture was extracted with CH_2Cl_2 (3 × 200 mL). The combined organic layer was dried over anhydrous magnesium sulfate and evaporated in vacuo. The crude product was purified by preparative vacuum-chromatography using dichloromethane-methanol (9:1, v/v) as eluent to yield *rac*-**2** (87%) as a colorless semisolid. The ^1H and ^{13}C NMR data were in accordance with those reported in the literature.[32]

4.3.3. Synthesis of ethyl 3-acetoxy-3-phenylpropanoate *rac*-3

A mixture of the racemic ethyl 3-hydroxy-3-phenylpropanoate *rac*-**2** (50 mmol), acetic anhydride (60 mmol, 9.5 g, 9.55 mL), and anhydrous cobalt(II) chloride (32.4 g, 250 mmol) in acetonitrile (100 mL) was refluxed for 8 h. After cooling to room temperature, the cobalt(II) chloride was filtered off. The filtrate was evaporated in vacuo and the crude product was purified by preparative vacuum-chromatography using dichloromethane as eluent to obtain *rac*-**3** as a light colorless semisolid in 91% yield. The ^1H and ^{13}C NMR data were in accordance with those reported in the literature.[32]

4.4. Cellular biotransformations of ethyl 3-oxo-3-phenylpropanoate 1

4.4.1. Non-fermenting biotransformations on an analytical scale

Yeast (2.5 g) was suspended in 25 mL of water. After stirring for 15 min, ethyl 3-oxo-3-phenylpropanoate **1** (24 mg) dissolved in DMSO (0.5 mL) was added into the resulting cell suspension. Samples (100 μL) were taken every 12 h over 48 h and extracted with ethyl acetate. The combined organic layers were dried over anhydrous Na_2SO_4, the solvent was evaporated, the crude solid was

redissolved in *n*-hexane–isopropanol (1:1, v/v), and analyzed by HPLC.

4.4.2. Fermenting biotransformations on an analytical scale

A fresh wet cake of yeast (2.5 g) and sucrose (1 g) was added into water (25 mL). The resulting suspension was stirred for 30 min, followed by the addition of ethyl 3-oxo-3-phenylpropanoate **1** (24 mg) dissolved in DMSO (0.5 mL). Further experiments were performed as described in the previous section.

4.4.3. Biotransformations under fermenting conditions in the presence of additives

Experiments were conducted as previously described. The additives (25 mg) were introduced into the suspension together with the sucrose.

4.5. Preparative synthesis of (*S*)-3-hydroxy-3-phenylpropanoate (*S*)-2 with *S. cerevisiae*

Into a suspension of cells (15 g) in water (150 mL), sucrose was added (3 g) and the mixture was stirred for 30 min. A solution of ethyl 3-phenyl-3-oxopropanoate **1** (144 mg, 0.75 mmol) dissolved in DMSO (2 mL) was added into the fermenting suspension. The reaction mixture was stirred at room temperature until the transformation of the substrate was completed (samples of 100 μL were taken and extracted by ethyl acetate, then analyzed with TLC). After completion of the reaction, the mixture was extracted with EtOAc (3 × 200 mL). The combined organic layer was dried over anhydrous Na_2SO_4, the solvent was removed in vacuo and the crude product obtained was purified by column chromatography on silica gel using dichloromethane to give (*S*)-**2** as a clear oil (75 mg, 86%). The ^1H and ^{13}C NMR spectra of the optically active (*S*)-**2** were indistinguishable from those of *rac*-**2**. $[\alpha]_D^{25} = -51.1$ (*c* 1.5, $CHCl_3$), ee = 96% by HPLC {lit.[33]: $[\alpha]_D^{25} = -43.8$ (*c* 1, $CHCl_3$), Ee > 99%}; HPLC (on Chiralpak IA/Chiralpack IB tandem, n-hexane/*i*-PrOH = 95:5, 1.0 mL/min, 218 nm), t_R (min): 22.4 [(*S*)-**2**], 23.1 [(*R*)-**2**].

4.6. Analytical and preparative scale kinetic resolutions of ethyl 3-acetoxy-3-phenylpropanoate *rac*-3

The same yeast cell-based conditions applied for the microbial reduction of **1** were used for the kinetic resolution of racemic ethyl 3-acetoxy-3-phenylpropanoate *rac*-**3**.

Samples of yeast suspension (ca. 5 mL, each; obtained by mixing 2.5 g of yeast cake in 20 mL of water) were transferred in freezer tubes and centrifuged for 5 min at 10,000*g*. The cell pellets were flash-frozen in liquid nitrogen (~1 min) and freeze-dried (overnight at 0.2 mbar). The yeast preparation lyophilized in this way was added to the solution of *rac*-**3** in hexane (5 mL). The progress and enantioselectivity of the reactions were followed by analyzing the samples by chiral GC.

The preparative scale kinetic resolution of racemic ethyl 3-acetoxy-3-phenylpropanoate *rac*-**3** (295 mg, 1.25 mmol) biotransformations was performed with 25 g of *S. cerevisiae* ATCC 9080 cells under the same conditions which were applied for the microbial reduction of **1** (see Section 4.5). The products were separated by silica gel chromatography (hexane:EtOAc, 9.5:0.5, v/v), resulting in the optically active alcohol (*R*)-**2** (119 mg, 49%) and acetate (*R*)-**3** (142 mg, 48%) as clear oils. The ^1H and ^{13}C NMR spectra of the optically active products (*R*)-**2** and (*S*)-**3** were indistinguishable from those of their racemates.

(*R*)-**2**: $[\alpha]_D^{25} = +51.0$ (*c* 1.5, $CHCl_3$), ee = 96% by HPLC {lit.[33]: $[\alpha]_D^{25} = +44.1$ (*c* 1, $CHCl_3$), Ee >99%}; HPLC, see Section 4.5.

(*S*)-**3**: $[\alpha]_D^{25} = -1.1$ (*c* 1.5, $CHCl_3$), ee = 96% by GC {lit.[34]: $[\alpha]_{546}^{17} = -1.2$ (neat)}; GC [on permethylated β-cyclodextrin, β-PM column (30 × 0.32 mm × 0.25 μM); carrier gas: nitrogen; oven temp.: 130 °C (100 min), 130 °C to 140 °C (1 °C min^{-1}, 140 °C (20 min); 140 °C to 150 °C (1 °C min^{-1}), 150 °C (20 min); FID], t_R (min): 141.0 [(*R*)-**3**], 147.2 [(*S*)-**3**].

4.7. Conversion of ethyl (*R*)- and (*S*)-3-hydroxypropanoates (*R*)-2 and (*S*)-2 into enantiomers of (*S*)- and (*R*)-β-phenylalanine (*S*)-6 and (*R*)-6

4.7.1. Ethyl (*S*)- and (*R*)-3-amino-3-phenylpropanoates (*S*)-5 and (*R*)-5

Into a cooled (0 °C) solution of optically active (*S*)- or (*R*)-ethyl 3-hydroxy-3-phenylpropanoate (*R*)- or (*S*)-**2** (136 mg, 0.7 mmol) and diphenyl phosphorazide (214 μL, 1 mmol) in dry THF (2 mL), neat DBU (150 μL, 1 mmol) was added under argon. The reaction mixture was stirred for 3 h at 0 °C, then slowly warmed to rt and stirred for 16 h. The resulting two-phase mixture was washed with water (2 × 10 mL) and 5% HCl (10 mL). The organic layer was concentrated in vacuum. The crude product was purified by vacuum-chromatography using petrol ether-ethyl acetate (9.5:0.5, v/v) as eluent to yield (*S*)- or (*R*)-ethyl 3-azido-3-phenylpropanoate (*S*)-**4** or (*R*)-**4** in 92% yield as a colorless semisolid which was used in the next step as such.

Into a solution of (*S*)- or (*R*)-**4** in THF (2 mL), water (18 μL, 1 mmol) and PPh_3 (155 mg, 0.6 mmol) were added. The mixture was refluxed overnight, cooled to rt, and then chromatographed without any work-up procedure using a mixture of CH_2Cl_2–EtOH (7:3, v/v) as eluent to yield (*S*)- or (*R*)-ethyl 3-amino-3-phenylpropanoate (*S*)- or (*R*)-**5** as a colorless semisolid (97 mg, 72%). ^1H NMR spectra of the optically active products were identical to those reported in the literature for (*S*)-**5**.[35]

(*S*)-**5**: $[\alpha]_D^{25} = -21.8$ (*c* 1, $CHCl_3$), ee = 96% by HPLC {lit.[35]: $[\alpha]_D = -22.9$ (*c* 1, $CHCl_3$), ee = 99.8%}; HPLC [on Chiracel OD-H column (4.6 × 250 mm), hexane/*i*-PrOH = 98:2, 1.0 mL/min, 218 nm], t_R (min): 34.3 [(*S*)-**5**], 40.5 [(*R*)-**5**].

(*R*)-**5**: $[\alpha]_D^{25} = +21.7$ (*c* 1, $CHCl_3$), ee = 96% by HPLC {lit.[36]: $[\alpha]_D = +21.1$ (*c* 1, $CHCl_3$), ee = 91%}; HPLC: see at (*S*)-**5**.

4.7.2. Synthesis of enantiomerically enriched (*S*)- or (*R*)-3-amino-3-phenylpropanoic acid (*S*)-6 or (*R*)-6

A mixture of ethyl (*S*)- or (*R*)-3-amino-3-phenylpropanoate (*S*)- or (*R*)-**5** (95 mg) in 6 M HCl solution (5 mL) was heated at reflux for 1 h. The solution was cooled to room temperature and the pH was adjusted to give a basic solution with conc. NH_3 solution. The crude product was purified by recrystallization from water to give (*S*)- or (*R*)-3-amino-3-phenylpropanoic acid (*S*)-**6** or (*R*)-**6** in 75% yield. The ^1H NMR and ^{13}C NMR data of the products were in accordance with those reported in the literature.[37]

(*S*)-**6**: $[\alpha]_D^{25} = -7.7$ (*c* 0.25, H_2O), ee = 96% by HPLC {lit.[38]: $[\alpha]_D^{25} = -8$ (*c* 0.27, H_2O), Ee >99%}; HPLC [on Chiracel IA column (4.6 × 250 mm), hexane/*i*-PrOH = 1:1, 1.0 mL/min, 218 nm], t_R (min): 27.8 [(*S*)-**6**], 33.1 [(*R*)-**6**]. t_R (min): 27.8 [(*S*)-**6**], 33.1 [(*R*)-**6**].

(*R*)-**6**: $[\alpha]_D^{25} = +7.6$ (*c* 0.25, H_2O), ee = 96% by HPLC {lit.[39]: $[\alpha]_D^{25} = +7$ (*c* 0.27, H_2O}; HPLC: see at (*S*)-**6**.

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