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Enantioselective reduction and deracemisation using the non-conventional yeast *Pichia glucozyma* in water/organic solvent biphasic systems: preparation of (*S*)-1,2-diaryl-2-hydroxyethanones (benzoins)

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ABSTRACT

Water/organic solvent two-liquid-phase systems have been successfully applied in the synthesis of enantiomerically pure (*S*)-benzoin through two different methodologies catalysed by whole cells from the non-conventional yeast *Pichia glucozyma*: the stereoselective monoreduction of benzil and the deracemisation of benzoin. The presence of the organic solvent influences the redox systems implied in the reactions, avoiding the formation of the corresponding diols, increasing the enantioselectivity and allowing the easy isolation of the products in high yields and excellent enantiomeric excesses. The use of both strategies has been extended to the preparation of different chiral benzoin derivatives.

1. Introduction

Homochiral benzoins (1,2-diaryl-2-hydroxyethanone structures) can be chemically obtained through benzoin condensation, one of the most traditional C–C bond forming reactions, catalysed by chiral thiazolium and triazolium salts[1,2] or chiral metallophosphites.[3] Alternatively, biocatalytic methods can offer an attractive platform by exploiting different enzymatic reactions,[4] such as benzoin condensation catalysed by thiamine-diphosphate-dependent enzymes,[5–8] dynamic kinetic resolution of racemic benzoins,[9,10] enantioselective reduction of α-diketones,[11–16] or deracemisation of racemic benzoins using redox processes catalysed by whole cells.[17]

Optically pure benzoin is considered a useful building block in the synthesis of different drugs and fine chemicals due to its functional nature.[4,18–20] More concretely, benzoin can be used for the preparation of 2-amino-1,2-diphenylethanol which, in its optically pure form, has recently received widespread attention due to the use of these amino alcohols as chiral auxiliaries in asymmetric synthesis,[21] chiral stationary phases for HPLC applications[22] and as ligands in asymmetric catalysis.[23]

Microbial dehydrogenases bound to whole cells have been used for the stereoselective reductions of aromatic α-diketones, with formation of the desired chiral α-hydroxyketone as major product, and in some cases to a full reduction to the corresponding diol, most likely because the whole cell contains other competing ketoreductases. In fact, the reduction of benzil (**1**) was firstly reported with the most classical bio-reduction biocatalyst (baker's yeast, whole cells from *Saccharomyces cerevisiae*), although the reaction proceeded with no enantioselectivity.[24] More recent studies with this same biocatalyst show the production of (*R*)-benzoin ((*R*)-**2**) with 50% enantiomeric excess, while the use of organic co-solvents led to higher enantiopurity values.[13] Other microorganisms have also been described for this same process with more success,[11,12,14,15] but often these biotransformations are hampered by the low rates reported. The monoreduction of different 1,2-diarylethanediones (including benzil) with lyophilised

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whole cells from *Pichia glucozyma* CBS 5766 proved to be fast and highly regio- and enantioselective in most of the cases; (*S*)-benzoin (*S*)-**2** was obtained with total conversion within 3 h, but with a moderate enantioselectivity (ee 75%).[16] It should be noted that this yeast has shown interesting performances in the stereoselective reduction of different aromatic ketones.[25,26]

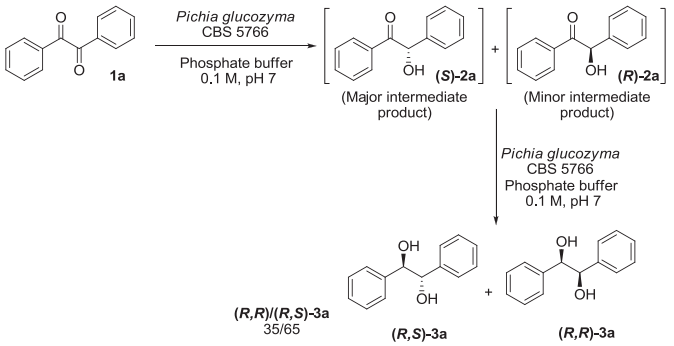
Another biocatalytic approach for obtaining homochiral benzoin is the deracemisation of racemic benzoin. In this way, *Rhizopus oryzae* was able to catalyse the chirality inversion of benzoins, to afford both enantiomers depending on the pH of the medium.[17] The formation of both enantiomers using different reaction conditions suggests the occurrence of ketoreductases acting on benzoin/benzil with different enantioselectivity, displaying the highest activity under different pH conditions.

As a part of our ongoing research focused on the biocatalytic synthesis of α-hydroxyketones,[16,9] in this work we have studied two biocatalytical methods for producing (*S*)-benzoin using a new organic/aqueous biphasic system: the first one is the enantioselective bio-reduction of benzil, while the second one is the deracemisation of racemic benzoin. It has been previously described that the use of two-liquid-phase systems increases the solubility of organic substrates, allowing the achievement of higher conversions, as well as an easier isolation of the products;[27,28] moreover, it is known that the use of organic co-solvents in microbial reductions considerably reduces the rate of undesired reactions, including reduction catalysed by enzymes with different stereoselectivity.[27–30] Both biotransformations are catalysed by enzymatic redox systems occurring in the non-conventional yeast *P. glucozyma*.

2. Results and discussion

2.1. Benzil reduction

We firstly carried out the reduction of benzil (**1a**) catalysed by whole untreated cells of *P. glucozyma*. Thus, cells from growth cultures were only centrifuged and suspended in phosphate buffer; the reaction was started by the addition of **1a** previously dissolved in DMSO as described in Experimental section. Compound **1a** was reduced producing hydrobenzoin (**3a**) with transient formation of (*S*)-benzoin ((*S*)-**2a**) (Scheme 1).



Scheme 1. Benzil reduction catalysed by untreated whole cells from *P. glucozyma* CBS 5766 in phosphate buffer.

This benzil reduction reaction was monitored, in order to study the catalytic behaviour of the possible dehydrogenases contained in the cells (Fig. 1). Compound (*S*)-**2a** was initially produced with low enantiomeric excess (85% molar conversion and 45% ee after 1 h); the enantiomeric excess was gradually increased as (*S*)-**2a** was being further reduced to **3a**, indicating that the dehydrogenase acting on **2** has a strong stereopreference for the reduction of the

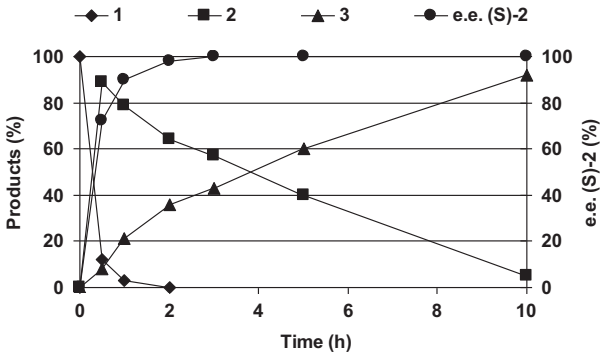


Fig. 1. Reaction progress of the reduction of **1a** catalysed by untreated whole cells from *P. glucozyma* CBS 5766 in phosphate buffer.

R-enantiomer. The reduction to **3a** was completed after 10 h and the isolated diol resulted a 35:65 mixture of (*R*,*R*)-**3a** and (*R*,*S*)-**3a**.

These results suggest that different dehydrogenases active on **1a** and **2a** are present in the whole cells. The overall activity of these enzymes can be influenced by the process of lyophilisation, which inactivates the enzymes active on **2a**, thus allowing its accumulation, as previously shown.[16,31] Other treatments at low temperature (such as maintenance at 4 °C or −20 °C for one month) gave similar effects, providing cells with lowered reducing activity towards **2a**; Table 1 summarizes the results obtained in the reduction of **1a** using cells after different treatments.

Table 1
Reduction of benzil (**1a**) catalysed by whole cells from *P. glucozyma* after different treatments

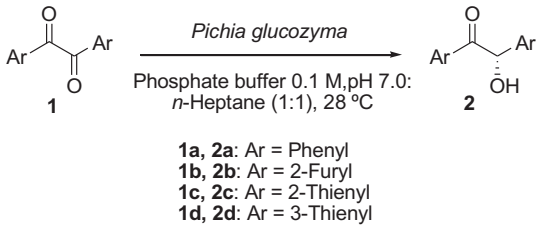
Cells	**2a** (%)	ee (*S*)-**2a** (%)	**3a** (%)	*t* (h)
Untreated	8	99	92	10
Lyophilised[16]	99	75	—	3
Maintained at 4 °C	85	88	15	3
Maintained at −20 °C	95	86	5	3

Cells maintained at −20 °C gave total conversion of the substrate; (*S*)-**2a** was formed in high yield (95%) and 86% ee, with minor amounts of **3a** (5%) observed after 3 h. Consequently, cells maintained at −20 °C were used in all the further experiments aimed at the synthesis of (*S*)-**2a** with high yields and enantioselectivity.

The biotransformation of **1a** was performed in a two-liquid-phase system composed of water and *n*-heptane (1:1); *n*-heptane (log *P*=4.0) was chosen since it allows high partition of the substrate into the organic phase and it is known to be often compatible with enzymatic activities.[28] Bio-reduction of **1a** using *P. glucozyma* in a two-liquid-phase system composed of water and *n*-heptane yielded (*S*)-**2a** (90% conversion, 99% ee) after 48 h.

This two-liquid-phase system was also tested in the reduction of other benzil derivatives (1,2-diaryl-1,2-ethanodione structures) (Table 2). In all cases only the corresponding α-hydroxyketones were obtained in high yields and excellent enantiomeric excesses, and no diols could be detected. All substrates and products were identified by comparison of HPLC retention times with racemic and optically active standards previously prepared following described methodologies.[9] In all cases the stereorecognition was similar and the absolute configurations of the products were assigned to be (*S*) except for those compounds containing a thienyl moiety (entries 3 and 4, Table 2), whose absolute configurations were (*R*) because of the preference of the sulfur atom.

Table 2
Reduction of different benzoins catalysed by whole cells from *P. glucozyma* in a two-liquid-phase system[a]



Entry	Substrate	Product	*t* (h)	Conv. (%)	ee_p (%)
1	**1a**	(*S*)-**2a**	48	90	99
2	**1b**	(*S*)-**2b**	48	95	99
3	**1c**	(*R*)-**2c**	48	97	98
4	**1d**	(*R*)-**2d**	48	90	85

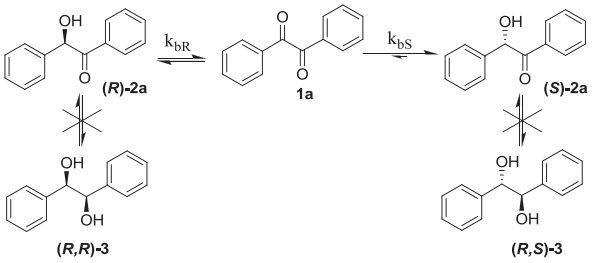
[a] Reaction conditions: cells from *P. glucozyma* (maintained at −20 °C, 20 g/L) were suspended in a 10 mL biphasic system phosphate buffer/*n*-heptane 1:1containing 5% glucose and they were incubated for 45 min at 28 °C. Then **1** (0.02 mmol) solved in DMSO (0.1 mL) was added. The mixture was shaken at 28 °C.

The biphasic system allowed not only the synthesis of optically active benzoins, without the formation of the corresponding diols, but also an easier isolation of the products. It was not necessary to carry out different extractions to isolate the products. The organic phase containing the final chiral compounds could be easily separated from water and products could be simply recovered for their purification and characterization.

2.2. Benzoin deracemisation

Biotransformation of **2** was first studied using untreated cells from *P. glucozyma*. The reduction of *rac*-**2a** in aqueous medium (phosphate buffer 0.1 M, pH 7) furnished **3a** with total conversion in 8 h. Isolated **3a** was a 50:50 mixture of (*R*,*R*)- and *meso*-stereoisomers. From the stereochemical outcome of this biotransformation, it can be assumed that the carbonyl group of **2a** should be asymmetrically reduced, forming a new stereocenter having the (*R*)-configuration. To confirm this hypothesis, the reduction of commercially available (*R*)-**2a** was carried out under the same reaction conditions. After 8 h the biotransformation reached 90% conversion: isolated **3a** showed a stereochemical composition of 75:25 of (*R*,*R*)-/*meso*-stereoisomers. This result is in apparent contradiction with the previous hypothesis and could be explained by considering a stereoinversion of the stereogenic centre of (*R*)-**2a**. It is known that stereoinversion of secondary alcohols can occur by concurrent tandem biocatalytic oxidation and reduction, whereby one enantiomer is oxidized to a carbonyl and then reduced to the opposite enantiomer.[32]

These observations led us to investigate the possibility of carrying out deracemisation of racemic **2a**. The biotransformation of *rac*-**2a** was performed in water and in the two-liquid-phase system composed of water and *n*-heptane using cells maintained at −20 °C. The biotransformation in water did not give any results, while the one performed in the two-liquid-phase system showed deracemisation with presence of (*S*)-**2a** (85%) as the only enantiomer. Compound **1a** was detected (10–15%) in the organic solvent during the progress of the reaction, pointing out the occurrence of an oxidative activity. In fact, highly hydrophobic **1a** partitions mostly in the organic phase, therefore allowing for its transient accumulation. The process of stereoinversion might be caused by the activity of different dehydrogenases: thus, one dehydrogenase would catalyse the oxidation of (*R*)-**2a** to **1a**, which is subsequently stereoselectively reduced to (*S*)-**2a** by another (*S*)-selective dehydrogenase with high affinity towards **1a**, as shown in Scheme 2. Moreover, the presence of the organic solvent completely prevents the formation of the diol.



Scheme 2. Proposed mechanism for **2a** deracemisation catalysed by whole cells from *P. glucozyma* maintained at −20 °C in an aqueous/organic biphasic system.

The deracemisation of *rac*-**2a** was also studied using organic solvents with different polarity values (Table 3). 2-MeTHF has emerged in last years as a more sustainable substitute of traditional THF and its chemical properties permit its use in many organic and biocatalytical processes.[33,34,10] It is less water miscible than THF, allowing an easier isolation of products. For these reasons we decided to test a biphasic system composed by this greener solvent. However, its polarity seemed to be too high, as no conversion was detected (entry 1, Table 3). Similar results were obtained when employing diisopropylether and toluene (entries 2 and 3, respectively, Table 3), which completely switched off the redox system of *P. glucozyma* active on (*R*,*S*)-**2a**.

Table 3
Results obtained in the deracemisation of (*R*,*S*)-**2a** catalysed by whole cells from *P. glucozyma* in a biphasic system composed by phosphate buffer and different organic solvents[a]

Entries	Solvent	log *P*	**2a** (%)	(*S*)-**2a** ee (%)
1	2-MeTHF	0.72	>99	—
2	Diisopropylether	1.9	90	<5
3	Toluene	2.5	92	<5
4	Hexane	3.5	83	>98
5	Heptane	4.0	85	>98
6	Isooctane	4.7	91	>98

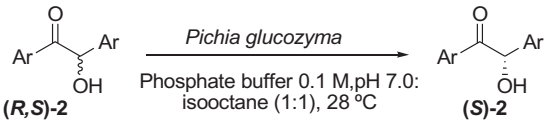
[a] Reaction conditions: cells from *P. glucozyma* (maintained at −20 °C, 20 g/L) were suspended in a 10 mL biphasic system phosphate buffer/organic solvent 1:1containing 5% glucose and they were incubated for 45 min at 28 °C. Then (*R*,*S*)-**2a** (0.02 mmol) solved in DMSO (0.1 mL) was added. The mixture was shaken at 28 °C for 48 h.

Only highly hydrophobic solvents (log *P*>3.5) allowed for deracemisation, obtaining best results with isooctane. The enantiomeric excess reached in the case of a biphasic system containing isooctane was higher than that obtained in *n*-heptane (entries 5 and 6, Table 2). This result is in accordance with other previously described, showing a similar correlation between the log *P* of the organic solvent and ADHs activities (the higher the better).[35]

Same reaction conditions were applied in the deracemisation of other benzoins (1,2-diaryl-2-hydroxyethanone structures). Results are shown in Table 4.

In most cases complete deracemisation was achieved, and enantiomerically pure benzoins were accumulated in the reaction medium for 48 h. To the best of our knowledge, this is the shortest benzoin deracemisation reaction, which leads to optically active products, easily isolated because of the use of a water/organic solvent biphasic system. Just in the case of substrate (*R*,*S*)-**2f** (entry 5, Table 4) low enantiomeric excess was achieved, probably because the bulkier substituents on the aryl moieties, which could interfere with the redox system action.

Table 4
Deracemisation of different benzoins catalysed by whole cells from *P. glucozyma* in a biphasic system phosphate buffer/isooctane, 1:1[a]



Entry	Substrate	Ar	Product	ee (%)
1	(*R*,*S*)-**2a**	Phenyl	(*S*)-**2a**	95
2	(*R*,*S*)-**2b**	2-Furyl	(*S*)-**2b**	99
3	(*R*,*S*)-**2d**	3-Thienyl	(*R*)-**2d**	99
4	(*R*,*S*)-**2e**	4-Methoxyphenyl	(*S*)-**2e**	91
5	(*R*,*S*)-**2f**	4-Ethoxyphenyl	(*S*)-**2f**	58

[a] Reaction conditions: cells from *P. glucozyma* (maintained at −20 °C, 20 g/L) were suspended in a 10 mL biphasic system phosphate buffer/isooctane 1:1containing 5% glucose and they were incubated for 45 min at 28 °C. Then (*R*,*S*)-**2** (0.02 mmol) solved in DMSO (0.1 mL) was added. The mixture was shaken at 28 °C for 48 h.

Enantiomerically pure products were identified by NMR spectroscopic analysis and HPLC data comparison with previously prepared chiral standards.[9] Optical rotations were measured and the absolute configurations were assigned to be *S* according to a correlation of the positive optical rotation values of these compounds with data from literature.[5,9] As well as in the reduction reaction, the deracemisation of the different benzoins leads to the formation of the (*S*) enantiomer, except for 3-thenoin, identified as the (*R*) enantiomer.

3. Conclusion

P. glucozyma CBS 5766 expresses different redox enzymes with activity on benzil and benzoin. Most of the activity towards benzoin is inactivated when cells are maintained at −20 °C, therefore the reduction of benzil occurs with accumulation of benzoin. The enantioselectivity of the reaction is increased when it is carried out in a two-liquid-phase system composed of water and a hydrophobic organic solvent (i.e., the use of *n*-heptane yielded (*S*)-**2a** with 90% conversion and 99% ee).

The presence of a hydrophobic solvent (i.e., heptane or isooctane) can be also exploited for the deracemisation of racemic benzoins. The simultaneous occurrence of oxidative activities towards racemic benzoin and enantioselective reduction of benzil allows for one-pot deracemisation.

In conclusion, this work shows how to achieve highly regio- and enantioselective reduction of benzil to (*S*)-benzoin and total deracemisation of racemic benzoin, as well as different benzoin derivatives, using the non-conventional yeast *P. glucozyma*.

4. Experimental section

4.1. General

Benzil (**1a**), *rac*-benzoin ((*R*,*S*)-**2a**), (*R*)-benzoin ((*R*)-**2a**), the three stereoisomers of hydrobenzoin ((*R*,*R*)-**3a**, (*R*,*S*)-**3a**, (*S*,*S*)-**3a**), 2-furoin ((*R*,*S*)-**2b**) and 4,4′-dimethoxybenzoin ((*R*,*S*)-**2e**) were purchased from Sigma Aldrich and used as-received. *P. glucozyma* CBS 5766 was obtained from Centraal Bureau voor Schimmelcultures (Utrecht, The Netherlands).

NMR spectra were recorded on a Bruker AC-250. Chemical shifts (δ) are reported in parts per million (ppm) relative to $CHCl_3$ (^{1}H: δ 7.27 ppm) and $CDCl_3$ (^{13}C: δ 77.0 ppm).

Column chromatography purifications were conducted on silica gel 60 (40–63 μm). TLC was carried out on aluminium sheets precoated with silica gel; the spots were visualized under UV light (λ=254 nm).

Molar conversions were determined by HPLC analysis using a Hypersil ODS column (250 mm×4.6 mm), a diode-array detector and a mixture of water/acetonitrile (6:4) as eluent with a flow rate of 0.8 mL/min.

Conversions and enantiomeric excesses of were routinely determined by HPLC using a Chiralcel® OD-H (Daicel Chemical Industries) analytical column (mobile phase of *n*-hexane/2-propanol, 90:10 at a flow rate of 1 mL/min, 25 °C). HPLC analyses: retention times of standards: (*R*)-**2a**, *t*=20.3 min, (*S*)-**2a**, *t*=14.6 min; (*R*)-**2b**, *t*=19.8 min, (*S*)-**2b**, *t*=16.6 min; (*R*)-**2c**, *t*=27.2 min, (*S*)-**2c**, *t*=21.8 min; (*R*)-**2d**, *t*=22.7 min, (*S*)-**2d**, *t*=17.9 min; (*R*)-**2e**, *t*=23.9 min, (*S*)-**2e**, *t*=21.3 min; (*R*)-**2f**, *t*=17.2 min, (*S*)-**2f**, *t*=16.5 min.

The stereochemical composition of hydrobenzoin was determined by HPLC using a Chiralcel® OJ column (mobile phase of *n*-hexane/2-propanol, 90:10 at a flow rate of 0.8 mL/min, 25 °C); the absolute configuration was assigned on the basis of the chromatographic behaviour of commercially available optically pure standards: (*R*,*R*)-**3**: 12.4 min, *meso*-**3**: 16.7 min.

4.2. Microorganisms, media and culture conditions

P. glucozyma CBS 5766 was cultured in 3.0 L fermenters with 1.0 L of malt broth pH 6.0 for 48 h, at 27 °C and agitation speed 100 rpm furnishing 8.15 g of dry weight biomass. The cells from submerged cultures were recovered by centrifugation and washed with 0.1 M phosphate buffer, pH 7.0 before further centrifugation. Whole wet cells were stored at 4 °C or at −20 °C.

4.3. Synthesis of 1,2-diarylethanediones employed as substrates of the reduction reactions. Synthesis of 1,2-di(furan-2-yl)ethane-1,2-dione (1b)

The synthesis of 1,2-diarylethanediones employed as substrates of the reduction reactions was carried out following the methodology previously described:[16] pyridinium chlorochromate (565 mg, 2.62 mmol) was suspended in CH_2Cl_2 (3.5 mL) in a 50 mL round bottom flask fitted with a reflux condenser. 2-Furoin ((*R*,*S*)-**2b**) (355 mg, 1.85 mmol) in CH_2Cl_2 (300 mL) was added and the mixture was stirred. After 6 h, 5 mL of ether was added and the supernatant decanted from the black residue. This solid was washed with ether (3×5 mL) and the combined organic solution was evaporated under vacuum. The resulting oil was purified by silica gel column chromatography (CH_2Cl_2), collecting 310 mg (1.63 mmol) of a yellow solid (88% yield): mp 161–162 °C (lit.[36] mp 160–162 °C).

Anal. Calcd for $C_{10}H_6O_4$: C, 63.16; H, 3.18. Found: C, 62.80; H, 3.00. ^{1}H NMR (250 MHz, $CDCl_3$) δ (ppm): 6.58 (2H, dd, *J*=3.7, 1.7 Hz), 7.59 (2H, dd, *J*=3.7, 0.7 Hz), 7.72 (2H, dd, *J*=1.7, 0.7 Hz). ^{13}C NMR (63 MHz, $CDCl_3$) δ (ppm): 113.5, 125.2, 149.8, 149.9, 177.2.

*4.3.1. Synthesis of 1,2-di(thiophene-2-yl)ethane-1,2-dione (**1c**).* Yellow solid, 348 mg (1.55 mmol) (84% yield): mp 80–81 °C (lit.[37] 81–82 °C). Anal. Calcd for $C_{10}H_6O_2S_2$: C, 54.03; H, 2.72; S, 28.85. Found: C, 53.78; H, 2.60; S, 28.50. ^{1}H NMR (250 MHz, $CDCl_3$) δ (ppm): 7.24 (2H, dd, *J*=4.8, 3.9 Hz), 7.88 (2H, dd, *J*=4.8, 1.0 Hz), 8.10 (2H, dd, *J*=3.9, 1.0 Hz). ^{13}C NMR (63 MHz, $CDCl_3$) δ (ppm): 129.1, 137.7, 137.9, 139.0, 182.8.

*4.3.2. Synthesis of 1,2-di(thiophene-3-yl)ethane-1,2-dione (**1d**).* Yellow solid, 370 mg (1.66 mmol) (89% yield): mp 79–80 °C (lit.[38] 79–80 °C). Anal. Calcd for $C_{10}H_6O_2S_2$: C, 54.03; H, 2.72; S, 28.85. Found: C, 53.70; H, 2.50; S, 28.50. ^{1}H NMR (250 MHz, $CDCl_3$) δ (ppm): 7.24 (2H, dd, *J*=4.9, 4.0 Hz), 7.88 (2H, dd, *J*=4.9, 1.2 Hz),

8.00 (2H, dd, *J*=4.0, 1.2 Hz). ^{13}C NMR (63 MHz, $CDCl_3$) δ (ppm): 129.1, 137.7, 137.9, 139.0, 182.8.

4.4. Synthesis of 1,2-diaryl-2-hydroxyethanones employed as substrates of the deracemisation reaction. Synthesis of 2-thenoin [2-hydroxy-1,2-di(thiophen-2-yl)ethanone] ((*R,S*)-2c)

The synthesis of the 1,2-diaryl-2-hydroxyethanones employed as standards and as substrates of the deracemisation reactions was carried out following the methodology previously described:[9] Thiamine hydrochloride (1.686 mg, 5 mmol) was dissolved in absolute ethanol (30 mL) and triethylamine (4.2 mL, 30 mmol) and 2-thiophenecarboxaldehyde (8.9 mL, 100 mmol) were added. The mixture was stirred at room temperature under argon atmosphere. After 24 h the product started to precipitate; the mixture was filtered and the solid was collected and washed with cold ethanol, yielding 10.75 g (48 mmol) of a white solid (48% yield): mp 107–108 °C (lit.[39] 107–108 °C).

Anal. Calcd for $C_{10}H_8O_2S_2$: C, 53.53; H, 3.59; S, 28.59. Found: C, 53.58; H, 3.62; S, 28.56. ^{1}H NMR (250 MHz, $CDCl_3$) δ (ppm): 4.41 (1H, s), 6.07 (1H, s), 7.01 (1H, dd, *J*=5.09, 3.50 Hz), 7.13 (1H, dd, *J*=3.50, 1.21 Hz), 7.15 (1H, dd, *J*=4.91, 3.80 Hz), 7.34 (1H, dd, *J*=5.09, 1.21 Hz), 7.75 (1H, dd, *J*=4.91, 1.10 Hz), 7.79 (1H, dd, *J*=3.80, 1.10 Hz). ^{13}C NMR (63 MHz, $CDCl_3$) δ (ppm): 71.3, 127.2, 127.3, 127.6, 128.8, 134.7, 135.8, 139.6, 142.4, 190.3.

*4.4.1. Synthesis of 3-thenoin [2-hydroxy-1,2-di(thiophen-3-yl)ethanone] ((R,S)-**2d**).* White solid, 9.18 g (41 mmol, 41% yield): mp 106–107 °C (lit.[40] 106–107 °C). Anal. Calcd for $C_{10}H_8O_2S_2$: C, 53.53; H, 3.59; S, 28.59. Found: C, 53.52; H, 3.92; S, 27.36. ^{1}H NMR (250 MHz, $CDCl_3$) δ (ppm): 4.34 (1H, d, *J*=6.03 Hz), 5.84 (1H, d, *J*=6.03 Hz), 6.99 (1H, dd, *J*=4.99, 1.21 Hz), 7.28 (1H, dd, *J*=4.99, 2.83 Hz), 7.29 (1H, dd, *J*=2.91, 0.69 Hz), 7.33 (1H, dd, *J*=2.83, 1.21 Hz), 7.51 (1H, dd, *J*=5.13, 0.69 Hz), 8.04 (1H, dd, *J*=2.13, 2.91 Hz). ^{13}C NMR (63 MHz, $CDCl_3$): δ 72.4, 124.2, 124.3, 125.3, 126.5, 127.2, 134.2, 138.5, 140.2, 192.4.

*4.4.2. Synthesis of 1,2-bis(4-ethoxyphenyl)-2-hydroxyethanone ((R,S)-**2f**).* White solid, 960 mg (3.2 mmol, 53% yield): mp 89–90 °C (lit.[41] 86 °C). Anal. Calcd for $C_{18}H_{20}O_4$: C, 71.98; H, 6.71. Found: C, 71.79; H, 6.70. ^{1}H NMR (250 MHz, $CDCl_3$) δ (ppm): 1.36 (3H, t, *J*=7.00 Hz), 1.39 (3H, t, *J*=7.10 Hz), 3.96 (2H, q, *J*=7.00 Hz), 4.03 (2H, q, *J*=7.10 Hz), 4.57 (1H, d, *J*=6.02 Hz), 5.83 (1H, d, *J*=6.02 Hz), 6.72–6.81 (2H, m), 6.82–6.93 (2H, m), 7.05–7.19 (1H, m), 7.20–7.38 (1H, m), 7.70–7.80 (1H, m), 7.83–7.95 (1H, m). ^{13}C NMR (63 MHz, $CDCl_3$) δ (ppm): 14.6, 14.8, 63.4, 63.8, 75.2, 114.3, 114.9, 128.9, 131.5, 136.1, 158.5, 163.4, 197.3.

4.5. Biotransformation conditions (phosphate buffer medium)

Whole cells were suspended in 10 mL of 0.1 M phosphate buffer pH 7.0 containing 5% glucose to reach the desired biomass concentration (20 g/L dry weight). After 45 min of incubation at 28 °C, the substrate **1** (reduction reaction) or **2** (deracemisation reaction) (0.02 mmol), dissolved in 0.1 mL of dimethylsulfoxide, was added. In experiments with two-liquid-phase systems, different solvents were added to reach the desired volumes before adding the substrate. The flasks were shaken on a reciprocal shaker (100 rpm) at 28 °C. Samples (0.5 mL) were taken at intervals, added with an equal volume of AcOEt containing 1-phenylethanol as an internal standard and centrifuged. The organic phase was dried over anhydrous Na_2SO_4 and analysed. Preparative biotransformations were carried out on 100 mL scale; when the reaction was over, 100 mL of AcOEt was added and the resulting mixture was shaken and centrifuged; the aqueous phase was extracted twice more with 70 mL of AcOEt. The organic phases were collected and dried over Na_2SO_4 and the solvent was evaporated. The crude residue was purified with flash chromatography: benzoin was recovered using hexane/AcOEt 9:1, while for hydrobenzoin hexane/AcOEt 7:3 was used.

4.6. Biotransformation conditions (biphasic system)

Whole cells stored at −20 °C were suspended in 10 mL of a biphasic system composed by 0.1 M phosphate buffer pH 7.0 (5 mL) and the organic solvent (5 mL), containing 5% glucose to reach the desired biomass concentration (20 g/L dry weight). After 45 min of incubation at 28 °C, the substrate **1** (reduction reaction) or **2** (deracemisation reaction) (0.02 mmol), dissolved in 0.1 mL of dimethylsulfoxide, was added. The flasks were shaken on a reciprocal shaker (100 spm) at 28 °C. Samples from the organic phase (0.5 mL) were taken at intervals, dried over Na_2SO_4 and evaporated. Products were dissolved in a mixture hexane/2-propanol (90:10) and analysed by HPLC.

Reactions were scaled to 20 mL volume in order to isolate and purify the enantiomerically pure products. NMR data of isolated chiral products were in accordance with those described for racemic benzoins. Enantiomeric excesses were determined by HPLC analysis and optical rotations were measured:

Reduction products: (*S*)-**2a**, $[\alpha]_D^{20}$ +30.4 (*c* 0.33, $CHCl_3$); (*S*)-**2b**, $[\alpha]_D^{20}$ +37.8 (*c* 0.36, $CHCl_3$); (*R*)-**2c**, $[\alpha]_D^{20}$ +31.4 (*c* 0.36, $CHCl_3$); (*R*)-**2d**, $[\alpha]_D^{20}$ +34.6 (*c* 0.33, $CHCl_3$).

Deracemisation products: (*S*)-**2a**, $[\alpha]_D^{20}$ +16.9 (*c* 0.26, $CHCl_3$); (*S*)-**2b**, $[\alpha]_D^{20}$ +55.2 (*c* 0.46, $CHCl_3$); (*R*)-**2d**, $[\alpha]_D^{20}$ +52.9 (*c* 0.46, $CHCl_3$); (*S*)-**2e**, $[\alpha]_D^{20}$ +34.0 (*c* 0.4, $CHCl_3$); (*S*)-**2f**, $[\alpha]_D^{20}$ +17.2 (*c* 0.4, $CHCl_3$).

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References and notes

1. Enders, D.; Kallfass, U. *Angew. Chem., Int. Ed.* **2002**, *41*, 1743–1745.
2. Enders, D.; Han, J. *Tetrahedron: Asymmetry* **2008**, *19*, 1367–1371.
3. Linghu, X.; Potnick, J. R.; Johnson, J. S. *J. Am. Chem. Soc.* **2004**, *126*, 3070–3071.
4. Hoyos, P.; Sinisterra, J. V.; Molinari, F.; Alcantara, A. R.; De Maria, P. D. *Acc. Chem. Res.* **2010**, *43*, 288–299.
5. Demir, A. S.; Şeşenoglu, Ö.; Eren, E.; Hosrik, B.; Pohl, M.; Janzen, E.; Kolter, D.; Feldmann, R.; Dünkelmann, P.; Müller, M. *Adv. Synth. Catal.* **2002**, *344*, 96–103.
6. Dünkelmann, P.; Kolter-Jung, D.; Nitsche, A.; Demir, A. S.; Siegert, P.; Lingen, B.; Baumann, M.; Pohl, M.; Müller, M. *J. Am. Chem. Soc.* **2002**, *124*, 12084–12085.
7. Demir, A. S.; Ayhan, P.; Sopaci, S. B. *Clean–Soil, Air, Water.* **2007**, *35*, 406–412.
8. Müller, M.; Gocke, D.; Pohl, M. *FEBS J.* **2009**, *276*, 2894–2904.
9. Hoyos, P.; Fernandez, M.; Sinisterra, J. V.; Alcantara, A. R. *J. Org. Chem.* **2006**, *71*, 7632–7637.
10. Hoyos, P.; Quezada, M. A.; Sinisterra, J. V.; Alcántara, A. R. *J. Mol. Catal. B: Enzym.* **2011**, *72*, 20–24.
11. Maruyama, R.; Nishizawa, M.; Itoi, Y.; Ito, S.; Inoue, M. *J. Biotechnol.* **2002**, *94*, 157–169.
12. Saito, T.; Maruyama, R.; Ono, S.; Yasukawa,, N.; Kodaira, K.; Nishizawa, M.; Ito, S.; Inoue, M. *Appl. Biochem. Biotechnol.* **2003**, *111*, 185–190.
13. Mahmoodi, N. O.; Mohammadi, H. G. *Monatsh. Chem.* **2003**, *134*, 1283–1288.
14. Demir, A. S.; Hamamci, H.; Ayhan, P.; Duygu, A. N.; Igdir, A. C.; Capanoglu, D. *Tetrahedron: Asymmetry* **2004**, *15*, 2579–2582.
15. Demir, A. S.; Ayhan, P.; Demirtas, U.; Erkllıç, U. *J. Mol. Catal. B: Enzym.* **2008**, *55*, 164–168.
16. Hoyos, P.; Sansottera, G.; Fernandez, M.; Molinari, F.; Sinisterra, J. V.; Alcantara, A. R. *Tetrahedron* **2008**, *64*, 7929–7936.
17. Demir, A. S.; Hamamci, H.; Sesenoglu, O.; Neslihanoglu, R.; Asikoglu, B.; Capanoglu, D. *Tetrahedron Lett.* **2002**, *43*, 6447–6449.
18. Okano, K. *Tetrahedron* **2011**, *67*, 2483–2512.
19. Tanaka, T.; Kawase, M.; Tani, S. *Bioorg. Med. Chem.* **2004**, *12*, 501–505.
20. Aoyagi, Y.; Agata, N.; Shibata, N.; Horiguchi, M.; Williams, R. M. *Tetrahedron Lett.* **2000**, *41*, 10159–10162.
21. Williams, R. M. *Aldrichimica Acta* **1992**, *25*, 11–25.
22. Yuki, Y.; Saigo, K.; Tachibana, K.; Hasegawa, M. *Chem. Lett.* **1986**, 1347–1350.
23. Brown, B.; Hegedus, L. S. *J. Org. Chem.* **1998**, *63*, 8012–8018.

24. Chêvenert, R.; Thiboutot, S. *Chem. Lett.* **1988**, 1191–1192.
25. Molinari, F.; Gandolfi, R.; Aragozzini, F.; Leon, R.; Prazeres, D. M. F. *Enzyme Microb. Technol.* **1999**, *25*, 729–735.
26. Husain, S. M.; Stillger, T.; Dünkelmann, P.; Lödige, M.; Walter, L.; Breitling, E.; Pohl, M.; Bürchner, M.; Krossing, I.; Müller, M.; Romano, D.; Molinari, F. *Adv. Synth. Catal.* **2011**, *353*, 2359–2362.
27. Mahmoodi, N.; Navrood, M. N. *Arkivoc* **2007**, *iii*, 37–45.
28. Cabral, J. M. S.; Aires-Barros, M. R.; Pinheiro, H.; Prazeres, D. M. F. *J. Biotechnol.* **1997**, *59*, 133–143.
29. D'Arrigo, P.; Fuganti, C.; Pedrocchi Fantoni, G.; Servi, S. *Tetrahedron* **1998**, *54*, 15017–15026.
30. Molinari, F.; Occhiato, E. G.; Aragozzini, F.; Guarna, A. *Tetrahedron: Asymmetry* **1998**, *9*, 1389–1394.
31. Carballeira, J. D.; Quezada, M. A.; Hoyos, P.; Simeó, Y.; Hernaiz, M. J.; Alcantara, A. R.; Sinisterra, J. V. *Biotechnol. Adv.* **2009**, *27*, 686–714.
32. Voss, C. V.; Gruber, C. C.; Kroutil, W. *Angew. Chem., Int. Ed.* **2008**, *47*, 741–745.
33. Aycock, D. F. *Org. Process Res. Dev.* **2007**, *11*, 156–159.
34. Shanmuganathan, S.; Natalia, D.; van den Wittenboer, A.; Kohlmann, C.; Greiner, L.; Dominguez de Maria, P. *Green Chem.* **2010**, *12*, 2240–2245.
35. de Gonzalo, G.; Lavandera, I.; Faber, K.; Kroutil, W. *Org. Lett.* **2007**, *9*, 2163–2166.
36. Wang, X.; Zhang, Y. *Tetrahedron* **2003**, *59*, 4201–4207.
37. Lukeš, V. r.; Breza, M.; Végh, D.; Hrdlovič, P.; Laurinc, V. *Synth. Met.* **2003**, *138*, 399–408.
38. Hyatt, J. L.; Stacy, V.; Wadkins, R. M.; Yoon, K. J. P.; Wierdl, M.; Edwards, C. C.; Zeller, M.; Hunter, A. D.; Danks, M. K.; Crundwell, G.; Potter, P. M. *J. Med. Chem.* **2005**, *48*, 5543–5550.
39. Bag, S.; Vaze, V. V.; Degani, M. S. *J. Chem. Res.* **2006**, *2006*, 267–269.
40. Roberts-Bleming, S. J.; Davies, G. L.; Kalaji, M.; Murphy, P. J.; Celli, A. M.; Donati, D.; Ponticelli, F. *J. Org. Chem.* **2003**, *68*, 7115–7118.
41. Weissberger, A.; Strasser, E.; Mainz, H.; Schwarze, W. *Justus Liebigs Ann. Chem.* **1930**, *478*, 112–128.