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3-(Hydroxy(phenyl)methyl)azetidin-2-ones obtained via catalytic asymmetric hydrogenation or by biotransformation

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ABSTRACT

The catalytic asymmetric reduction of ethyl-2-(benzamidomethyl)-3-oxo-phenylpropanoate was realized with high enantiomeric and diastereoisomeric excesses via biotransformation using whole cells of different yeasts and asymmetric hydrogenation with Ru(II) complexes prepared from different chiral diphosphine ligands.

With these combined approaches it was possible to prepare both enantiomers of the *syn*-stereoisomers in almost enantiomerically pure form; one of the enantiomers of the *anti*-stereoisomer was obtained in high ee with selected yeast while the other enantiomer of the *anti* was prepared in low ee and de. With three of the four epimers we were able to prepare the corresponding azetidinones.

1. Introduction

Over the last few years, the importance of β-amino acids has increased since these molecules are interesting building blocks for the preparation of unnatural peptides and bioactive compounds, more specifically, β-hydroxy-β-amino acids have proven to be versatile precursors for drug syntheses and effective methods for their stereoselective preparation are crucial for the synthesis of the corresponding azetin-2-ones and consequently of new types of β-lactam rings.[1] The carbapenem family (imipenem, meropenem, ertapenem and other derivatives) show a wide range of activities toward many pathogens, whether Gram-positive or negative; this activity is combined with a good resistance and a poor affinity with β-lactamases.[2] Chemocatalysis and biocatalysis are complementary approaches for the stereoselective preparation of β-hydroxy-β-amino acids starting from the corresponding β-keto-β-amino acids.

Ruthenium [Ru(II)] in combination with chiral diphosphines has played a central role in asymmetric hydrogenation; *Saccharomyces cerevisieae* has also played the same role in the asymmetric reduction of keto groups.[3]

Asymmetric catalysis utilizing metal complexes has proven to be a powerful tool for generating pure enantiomers and a major objective for academic and industrial research. Biocatalytical methods have been used for the preparation of enantiomerically pure β-hydroxy-β-amino acids by using microbial whole cells bearing reductases/dehydrogenases.[4]

The role played by a substituent at the α-position to the carbonyl group[5] prompted us to extend the investigation to substrates that are able to behave as either an electron withdrawing or electron-donor group. In order to evaluate the influence of factors, such as the hindrance of the substituent, the stereoelectronic properties of the molecules, and the presence of a keto-enolic equilibrium especially in the α-substituted β-ketoesters, we have focused our research toward the reduction of ethyl-2-(benzamidomethyl)-3-oxo-phenylpropanoate, because in the literature, although the synthesis of the 2-aminomethyl-3-hydroxy-3-phenylpropanoic acid has been reported,[6] it has not been obtained by chemo or biocatalytic asymmetric reduction.

Herein we report the preparation and characterization of 3-hydroxy(phenyl)methyl azetidin-2-ones which, to the best of our knowledge, have not been prepared and investigated before. The reduction of the substrate, ethyl-2-(benzamidomethyl)-3-oxo-phenyl propanoate **4**, was expected to proceed with high enantiomeric and diastereoisomeric excess, this is the reason as to why we have studied, in detail, Ru(II) complexes with different chiral chelating diphosphines and different types of yeasts to obtain all of the epimers necessary for the synthesis of all the possible stereoisomers of 3-hydroxy(phenyl)methyl azetidin-2-ones **10**.

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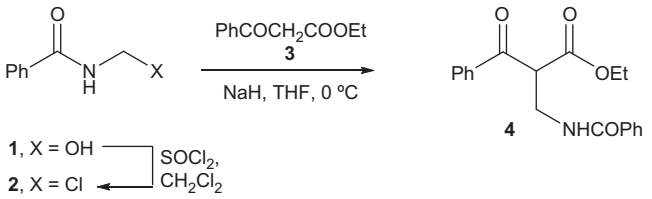
2. Results and discussion

We first chose a phenyl group as the substituents at the α-position of carbonyl group since good results were obtained in the reduction of ethyl-3-oxo-3-phenyl propanoate either with yeasts or with Ru(II)-chiral diphosphines complexes.[7,8]

The synthesis of ethyl-2-(benzamidomethyl)-3-oxo-phenylpropanoate **4** was carried out in good yield via a procedure modified to that developed by Takasago Co. for the synthesis of ethyl 2-(benzamidomethyl)-3-oxobutanoatoate (Scheme 1).



Scheme 1. Synthesis of ethyl-2-(benzamidomethyl)-3-oxo-phenylpropanoate **4**.

The reduction of **4** was performed using biocatalytic (whole cells of yeasts) and chemical methods (Ru(II)/phosphine complexes). A screen for the modification of **4** was carried out using the same library of yeasts previously used for the reduction of ethyl 2-(benzamidomethyl)-3-oxobutanoate.[5b]

The reduction of **4** into ethyl-2-(benzamidomethyl)-3-hydroxyphenylpropanoate **5** with good yields was obtained with *Kluyveromyces marxianus* CBS 1553 and *Pichia glucozyma* CBS 5766. We observed that only two yeasts (from a screen performed using 20 yeasts) gave good yields in the reduction of **4**, while twelve yeasts were able to reduce ethyl 2-(benzamidomethyl)-3-oxobutanoate; this is probably due to the additional steric hindrance caused by the *N*-(methyl)benzamide substituent, which causes mismatching between the two substituents at the α-position to the carbonyl group. The biotransformations with *K. marxianus* and *P. glucozyma* were optimized, following a strategy described in a previous work; the results are shown in Table 1. The type (glucose) and concentration (50 mg/mL, 280 mM) of co-substrate added for cofactor regeneration played a crucial role with regard to the high yields and stereoselectivity.

K. marxianus CBS 1553 gave (2*R*,3*R*)-**5** quantitatively and in enantiomerically and diastereoisomerically pure form (entry 2), while *P. glucozyma* CBS 5766 furnished the enriched diastereoisomer (2*R*,3*S*)-**5** (entry 1). It was possible to separate the pure stereoisomer (2*R*,3*S*)-**5** from (2*S*,3*S*)-**5** by flash chromatography.

Table 1
Biotransformation of **4** by yeasts

Entry	Catalyst	**5** (%)	ee (%)	de (%)
1	*Pichia glucozyma* CBS 5766	90	>99 (2*R*,3*S*)	70
2	*Kluyveromyces marxianus* CBS 1553	100	>99 (2*R*,3*R*)	98

Therefore, biocatalytic reduction gave access to enantiomerically pure (2*R*,3*R*)-**5** and (2*R*,3*S*)-**5**.

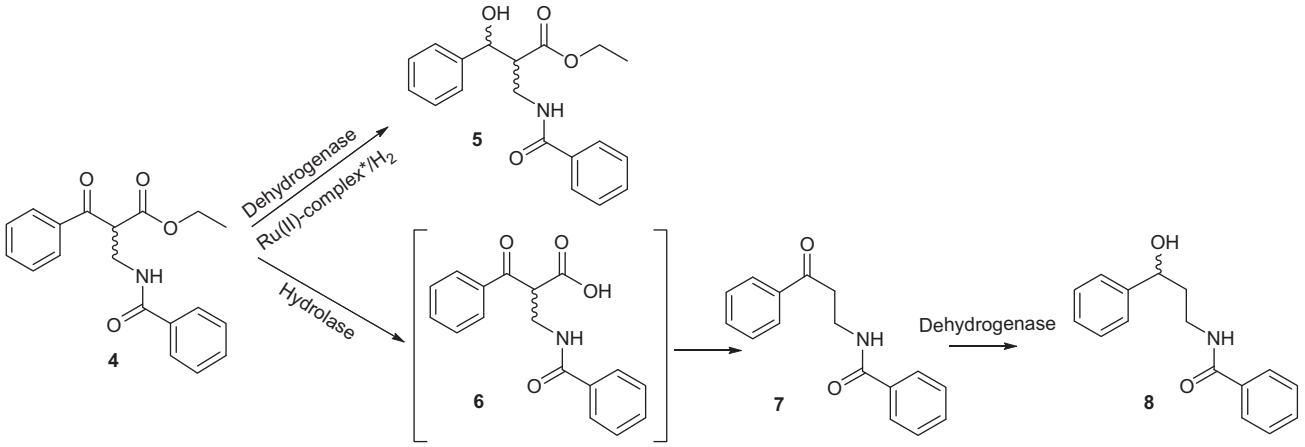
It is noteworthy that during the process of screening and optimization, it was observed that a few yeasts also gave *N*-(3-oxo-3-phenylpropyl)-benzamide **7** and *N*-(3-hydroxy-3-phenylpropyl)-benzamide **8** as by-products (Scheme 2).[5b]

When *P. glucozyma* CBS 5766 was used without any co-substrate **7** was obtained in high yield (85–90%) and could be used to give (*S*)-**8** with ee >99%. Compound (*S*)-**8** is an interesting building block in the synthesis of fluoxetine, an active principle of one of the most diffuse antidepressive drugs.[9] The formation of **7** is caused by the spontaneous decarboxylation of the intermediate carboxylic acid **6** produced by hydrolytic enzymes occurring in the whole cells. Compound **7** can then be enantioselectively reduced by dehydrogenase(s) to (*S*)-**8**. An alternative synthesis was carried out to confirm the structures of by-products **7** and **8**. Ketone **7** was obtained by hydrolysis of **4** catalyzed by lipase from *Candida cylindraceae* and racemic alcohol **8** was obtained by reduction with $NaBH_4$. Compound **8** had the same configuration of the product obtained by asymmetric hydrogenation with [$RuCl_2(DMF)_n$(−)-tetraMe-BITIOP]; the absolute configuration was assigned as (*S*) by comparison with the enantioselectivity shown by the same catalyst on ethyl-3-oxo-3-phenyl propanoate.[10]

The biocatalytic reduction gave the target molecule **5** almost enantiomerically pure; however for a detailed study of the biological activity of the azetidinone, it was necessary to have all epimers.

The asymmetric reduction of the C=O bond has been realized in many ways, but the most versatile catalysts remain those based on Ru(II) complexes.[11,12] We investigated the ligands described in Figure 1. Some of them are commercially available, such as BDPP, BINAP and Tol-BINAP,[13] while others have been developed in our laboratory.[5a,10,14] Some of these diphosphines are characterized by the presence of a stereogenic sp^3 carbon (Zedphos, XilylZedphos, Prolophos, BDPP); others by atropoisomeric chirality (tetraMe-BITIOP, BINAP, Tol-BINAP and DIOPHEP), or by a contemporary presence of both elements of chirality (Isaphos).

Most ligands, i.e. those characterized by atropoisomeric chirality and those with sp^3 stereogenic carbons (Table 2, entries



Scheme 2. Asymmetric reduction of **4**.

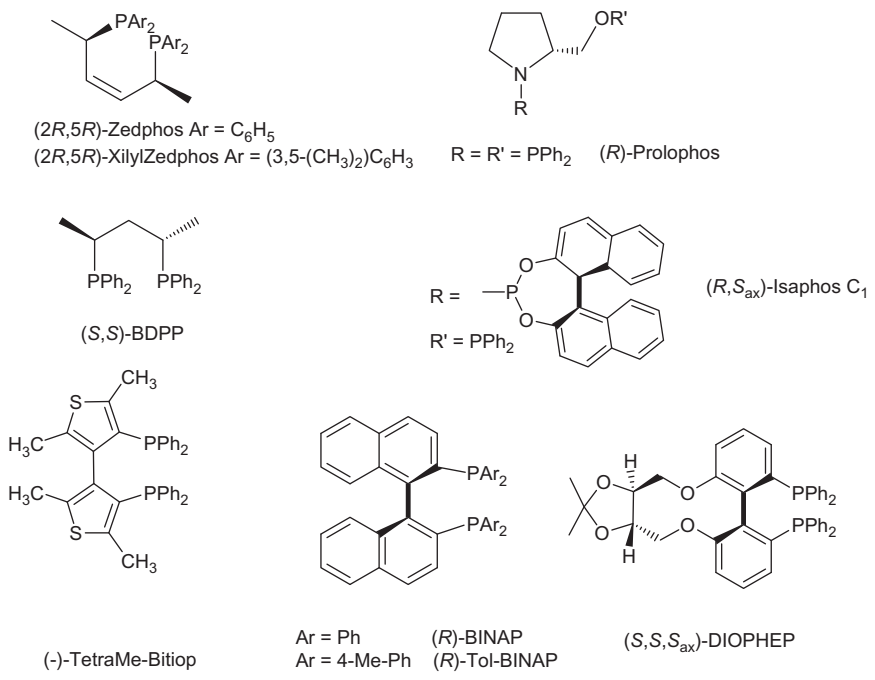


Figure 1. Chiral chelating diphosphines used in asymmetric hydrogenation of **4**.

Table 2
Asymmetric hydrogenation of **4**[a]

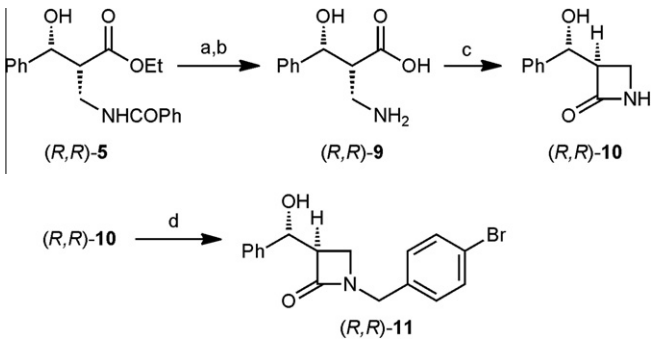
Entry	Ligands	ee[b] (%)	de (%)
1	(*S*)-BINAP	35 (2*S*,3*S*)	90
2	(*R*)-BINAP	32 (2*R*,3*R*)	93
3	(*S*)-Tol-BINAP	58 (2*S*,3*S*)	94
4	(−)-TetraMe-BITIOP	>99(2*R*,3*R*)	97
5	(+)-TetraMe-BITIOP	>99(2*S*,3*S*)	95
6	(*S*,*S*,S_{ax})-DIOPHEP	67 (2*S*,3*S*)	85
7	(*R*,*R*)-BDPP	50 (2*R*,3*R*)	80
8	(*S*,*S*)-BDPP	67 (2*S*,3*S*)	88
9	(2*R*,5*R*)-Zedphos	68 (2*R*,3*R*)	80
10	(2*R*,5*R*)-Xylil Zedphos	63 (2*R*,3*R*)	84
11	(*R*)-Prolophos[c]	65 (2*R*,3*R*)	52
12	(*R*,R_{ax})-Isaphos C_1[c]	80 (2*S*,3*R*)	50
13	(*R*,S_{ax})-Isaphos C_1[c]	70 (2*S*,3*R*)	62

[a] Reaction conditions: solvent: $MeOH/CH_2Cl_2$ = 50:50; reaction time 60 h; conversion >99%; substrate/$[RuCl_2(DMF)_n(PP^*)]$ = 100:1; substrate concentration = 0.030 M; T = 60 °C; P = 50 atm H_2. The ee and de values were determined by chiral HPLC with a Daicel Chiralpak AD (90:10 = hexane/isopropanol, flow = 0.6 mL/min).
[b] Stereochemistry of the prevailing diastereoisomer.
[c] Conversion = 80%.

1–11) essentially only gave the *syn* diastereoisomers. However, good results were obtained with (+)- and (−)-tetraMe-BITIOP. The Ru(II) complex with (−)-tetraMe-BITIOP afforded (2*R*,3*R*)-ethyl-2-(benzamidomethyl)-3-hydroxy-phenylpropanoate **5**, the same enantiomer obtained with *K. marxianus*, in 97% de and almost enantiomerically pure (entry 4); (+)-tetraMe-BITIOP afforded the opposite enantiomer (2*S*,3*S*)-**5** (entry 5).

The ligands with mixed chirality (*R*,R_{ax})-Isaphos C_1 and (*R*,S_{ax})-Isaphos C_1 mainly gave the *anti*-diastereoisomers (entries 12 and 13). The prevailing enantiomer was (2*S*,3*R*)-**5** in both cases, the opposite enantiomer obtained by *P. glucozyma*; these results showed the predominant role of the sp^3 carbon atom, which controlled the whole stereochemistry of the catalytic reaction.

Phenylpropanoate (2*R*,3*R*)-**5** was transformed into phenyl-substituted azetidinones **10** via hydrolysis of **5** followed by cyclization of the amino acid **9** thus obtained (Scheme 3). The hydrolysis was carried out by heating at phenylpropanoate **5** 90 °C in the presence of 10% HCl. After aqueous work-up, the β-amino acid (2*R*,3*R*)-**9** was isolated by neutralization of the hydrochloride salt with Et_3N. The cyclization of the β-amino acid (2*R*,3*R*)-**9** to azetidinone (*R*,*R*)-**10** occurred smoothly by heating at 65 °C in the presence of 2,2′-dipyridyl disulfide and PPh_3 in acetonitrile at 65 °C. The same procedure was used to generate azetidinone (*S*,*S*)-**10** from (*S*,*S*)-**5**.



Scheme 3. Synthetic elaboration of phenylpropanoates to azetidinones. Reagents and conditions: (a) 10% HCl, 90 °C; (b) Et_3N, CH_3CN; (c) PPh_3, PySSPy, CH_3CN, 65 °C, 6 h; (d) NaH, 4-Br–C_6H_4–CH_2Br, THF–DMF, 25 °C, 20 h.

On the other hand, when (2*R*,3*S*)-**5** was subjected to the same protocol, azetidinone (*R*,*R*)-**10** was isolated as the main product along with minor amounts of the expected (*R*,*S*)-**10**. This behavior could be tentatively explained by the better stability of the *syn*-amino acid (2*R*,3*R*)-**9** with respect to the *anti*-(2*R*,3*S*)-**9**.

The absolute configuration was determined by single-crystal structure determination of *N*-(*p*-bromobenzyl)-**11** which was synthesized by N-alkylation of azetidinone (*R*,*R*)-**10** with *p*-bromobenzyl bromide (Fig. 2). The introduction of a bromine atom inserted into the molecule an anomalous X-ray scatterer in order to determine the absolute configuration.

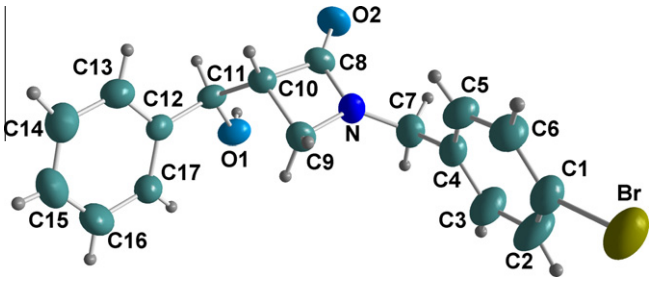


Figure 2. A perspective view of the (*R*)-1-(4-bromobenzyl)-3-((*R*)-hydroxy-(phenyl)methyl)azetidin-2-one **11**.

3. Conclusion

The complementary use of biocatalysis with dehydrogenases present in whole cells of yeasts and organometallic catalysis with transition metal catalysts allowed for the preparation of three of the four epimers of ethyl-2-(benzamidomethyl)-3-hydroxy-phenylpropanoate **5**. Compound Ru(II)-(+)tetraMe-BITIOP gave (2*S*,3*S*)-**5**, Ru(II)-(−)tetraMe-BITIOP, *K. marxianus* CBS 1553 gave (2*R*,3*R*)-**5** and *P. glucozyma* CBS 5766 gave (2*R*,3*S*)-**5**. The phosphine ligands bearing an axial chirality and a stereogenic sp^3 carbon gave (2*S*,3*R*)-**5** but with lower stereoselectivity. The three epimers obtained as enantiomerically pure molecules were transformed into the corresponding azetininones.

4. Experimental

Catalytic reactions were performed in a 200 mL stainless steel autoclave equipped with temperature control and a magnetic stirrer. Unless otherwise stated, materials were obtained from commercial suppliers and used without further purification. The ruthenium catalysts are prepared according to the well established literature procedure.[15] Compounds **1** and **2** are prepared as reported in the literature.[16]

^{1}H NMR and ^{13}C NMR spectra were recorded on a Varian 200 MHz, a Bruker DRX Avance 300 MHz equipped with a non-reverse probe and also or on a Bruker DRX Avance 400 MHz. GC-MS spectra were recorded on Thermo Finningan MD 800 equipped with GC Trace (SE 52 column: length 25 m, ϕ int. 0.32 mm, film 0.4–0.45 μm) and MS analyses were performed by using a Thermo Finnigan (MA, USA) LCQ Advantage system MS spectrometer with an electrospray ionization source and an 'Ion Trap' mass analyzer. The MS spectra were obtained by direct infusion of a sample solution in a mixture MeOH/H_2O/AcOH 10:89:1 under ionization, ESI positive. HPLC analysis: Merck-Hitachi L-7100 equipped with Detector UV6000LP and Daicel Chiralcel OD or Chiralpak AD.

4.1. Preparation of ethyl-2-(benzamidomethyl)-3-oxo-phenylpropanoate 4

To a solution of NaH (250 mg, 1.04×10^{-2} mol), ethyl-3-oxo-3-phenyl propanoate **3** was added in anhydrous THF (50 mL) at 0 °C. The reaction mixture was stirred for 15 min, after which *N*-(chloromethyl)benzamide **2** in THF at 0 °C was added. The reaction mixture was stirred for 12 h while the temperature of the reaction was allowed to return to room temperature, then treated with $Na_2SO_4{\cdot}10H_2O$ and the reaction mixture stirred for 30 min. The mixture was filtered and concentrated in vacuo. Purification of the residue by flash chromatography on silica gel with cyclohexane/ethyl acetate (30:70) as eluents, gave the title compound (2.77 g, 82%) as a white oil. When the compound was stored in the freezer, it solidified. ^{1}H NMR($CDCl_3$): δ = 1.18 (t, 3H, J = 6.9), 3.93–3.97 (m, 2H), 4.09–4.16 (m, 2H), 4.19 (q, 2H, J = 6.9), 4.87 (dd, 1H, J =5.5), 6.73 (bs, 1H), 7.42–7.62 (m, aromatic), 7.71 (dd, aromatic, J_o = 1.5, J_m = 8.0), 8.10 (dd, aromatic, J_o = 2.2, J_m = 5.1); ^{13}C-NMR($CDCl_3$): δ = 14.1 (CH_3), 39.2 (CH_2–N), 53.7 (CH), 62.03 (CH_2), 127.1–135.9 (CH aromatic), 167.9 (C═O isomer), 169.2 (C═O isomer), 194.8 (C═O aromatic). HRMS of $C_{19}H_{19}NO_4$ (*m/z*): calcd 325,13, found 348.5 (MNa^+).

4.2. General procedure for the asymmetric hydrogenation

In a Schlenk tube sealed under argon, substrate **4** was added to the precatalyst followed by 20 mL of a choice solvent, the solution was stirred for 30 min and then transferred to an autoclave with a cannula.

The stainless steel autoclave (200 mL), equipped with temperature control and a magnetic stirrer, was purged five times with hydrogen, after the transfer of the reaction mixture, the autoclave was pressurized. At the end the autoclave was vented and the mixture was analyzed by GC–MS, NMR spectra and HPLC.

4.2.1. Ethyl 2-(benzamidomethyl)-3-hydroxy-3-phenylpropanoate *syn*-5

^{1}H NMR ($CDCl_3$): δ = 1.01 (t, 3H, J = 7.0 Hz), 2.93–3.01 (m, 1H, *syn* diastereoisomers), 3.61–3.69 (m, 2H), 3.98 (q, 2H, J = 7.0 Hz), 4.12–4.19 (m, 2H), 4.96 (d, 1H, J = 7.3 Hz), 6.72 (br s, 1H), 7.29–7.53 (m, aromatic), 7.77 (dd, aromatic, J_o = 1.5 Hz, J_m = 8.0 Hz); ^{13}C NMR ($CDCl_3$): δ = 14.0 (CH_3), 37.9 (CH_2–N, isomer), 53.0 (CH), 61.1 (CH_2), 72.6 (CH), 126.4–132.0 (CH aromatic), 173.5 (C═O amide). HRMS of $C_{19}H_{21}NO_4$ (*m/z*): calcd 327,15, found 350.4 (MNa^+). $[\alpha]_D^{20}$ (2*R*, 3*R*) = +33.2 (*c* 0.15, $CHCl_3$); $[\alpha]_D^{20}$ (2*S*, 3*S*) = −11.0 (*c* 0.18, $CHCl_3$); HPLC data: CHIRALPAK AD, eluent: hexane/2-propanol=90:10, flow = 0.6 mL/min, λ = 230 nm, rt: (2*R*,3*R*) = 35.1 min, (2*S*,3*S*) = 37.1 min.

4.3. Microorganisms: culture conditions

Strains from official collections or from our own collection (Microbiologia Industriale Milano) were routinely maintained on malt extract (8 mg/mL, agar 15 mg/mL, pH 5.5). To obtain cells for biocatalytic activity tests, the microorganisms were cultured in 500 mL Erlenmeyer flasks containing 100 mL of medium and incubated for 48 h at 28 °C on a reciprocal shaker (100 spm). The yeasts were grown on malt extract with 5 mg/mL Difco yeast extract. Fresh cells from submerged cultures were centrifuged (5000 rpm per 10 min) and washed with 0.1 M phosphate buffer pH 7 prior the use.

4.4. Bioreduction conditions

General procedure for the screening: reductions were carried out in 10 mL screw-capped test tubes with a reaction volume of 3 mL. The cells (20 mg/mL, dry weight) suspended in 0.1 M phosphate buffer pH 7 with or without 5% of glucose and 1 mg/mL of ethyl 2-(benzamidomethyl)-3-oxo-phenylpropanoate **4** as substrate. The substrate **4**, dissolved in DMSO, was added to the biotransformation system to give 1 mg/mL of substrate concentration and 2% of solvent. The reactions were carried out at 28 °C under magnetic stirring.

The production of (2*S*,3*R*)-**5** on a semi-preparative scale was carried out with *P. glucozyma* CBS 5766. The desired amount of cells was suspended in different 0.1 M phosphate buffers containing glucose and 1.3 g of substrate was added to reach the desired concentration (2 mg/mL); the suspensions obtained were magnetically stirred at 28 °C.

After 48 h, the biotransformation was stopped by centrifugation of the cellular suspension; the liquid fraction was extracted with ethyl acetate (3 × 300 mL). The organic extracts were dried over

Na_2SO_4 and the solvent was removed under reduced pressure. After flash chromatography on silica gel eluted with cyclohexane/ethyl acetate (3:7), 751 mg of pure products were obtained.

4.4.1. Ethyl 2-(benzamidomethyl)-3-hydroxy-3-phenylpropanoate (2*R*,3*S*)-5

^{1}H NMR ($CDCl_3$): δ = 1.01 (t, 3H, J = 7.0 Hz), 3.15–3.24 (m, 1H, *anti* diastereoisomers), 3.61–3.69 (m, 2H), 3.98 (q, 2H), 4.12–4.18 (m, 2H), 4.95 (d, 1H, J = 7.3 Hz), 6.72 (br s, 1H), 7.29–7.53 (m, aromatic), 7.77 (dd, aromatic, J_o = 1.5 Hz, J_m = 8.0 Hz); ^{13}C NMR ($CDCl_3$): δ = 14.0 (CH_3), 38.0 (CH_2–N), 53.0 (CH), 61.1 (CH_2), 72.6 (CH), 126.4–132.0 (CH aromatic), 173.5 (C=O amide). HRMS of $C_{19}H_{21}NO_4$ (*m/z*): calcd 327,15, found 350.4 (MNa^+). $[\alpha]_D^{20}$ (2*R*, 3*S*) = −11.3 (*c* 0.12, $CHCl_3$). HPLC data: CHIRALPAK AD, eluent: hexane/2-propanol = 90:10, flow=0.6 mL/min, λ = 230 nm, rt: (2*S*,3*R*) = 49.8 min, (2*R*,3*S*) = 68.8 min.

4.4.2. *N*-(3-Oxo-3-phenylpropyl)benzamide 7

N-(3-Oxo-3-phenylpropyl)benzamide **7** was obtained by biotransformation. At first, 2 mg/mL of ethyl-2-(benzamidomethyl)-3-oxo-phenylpropanoate **4** and 10 mg/mL of lipase from *Candida cylindracea* were added to a 0.1 M phosphate buffer pH 7. The biotransformation was carried out at 30 °C with magnetic stirring. After 72 h, the reaction was extracted three times with ethyl acetate. The collected organic phases were dried over Na_2SO_4 and reduced under vacuum. The crude extract was purified with preparative TLC on silica gel (Kiesel 60 with fluorescent indicator) ^{1}H NMR ($CDCl_3$): δ = 3.35 (t, 2H, J = 5.5 Hz), 3.89 (q, 2H), 6.86 (br s, 1H), 7.36–7.63 (m, aromatic), 7.76 (dd, aromatic, J_o = 1.5 Hz, J_m = 8.0 Hz), 7.97 (dd, aromatic, J_o = 2.2 Hz, J_m = 5.1 Hz); ^{13}C NMR ($CDCl_3$): δ = 35.1 (CH_2), 38.4 (CH_2,), 127.1–136.8 (CH aromatic), 167.5 (C=O carbonyl), 199.9 (C=O, amide). HRMS of $C_{16}H_{15}NO_2$ (*m/z*): calcd 253,11, found 253.0 (M^+).

4.4.3. *N*-(3-Hydroxy-3-phenylpropyl)benzamide 8

The *N*-(3-oxo-3-phenylpropyl)benzamide **7** was reduced by $NaBH_4$ (1:5) in ethanol. The reaction was maintained at room temperature with magnetic stirring. After 12 h, water and CH_2Cl_2 were added to give product **8**. The organic extract was dried over Na_2SO_4 and concentrated in vacuum. The product was purified via preparative TLC. ^{1}H NMR ($CDCl_3$): δ = 1.00 (q, 2H), 3.21 (br s, 1H), 3.42–3.54 (m, 1H), 3.81–3.95 (m, 1H), 4.80–4.88 (m, 1H), 6.78 (br s, 1H), 7.31–7.55 (m, aromatic), 7.76 (dd, aromatic J_o = 1.5 Hz, J_m = 8.0 Hz). ^{13}C NMR ($CDCl_3$): δ = 37.7 (CH_2), 38.8 (CH_2), 73.1 (CH), 125.8–144.3 (CH aromatic), 168.1 (C=O amide). HRMS of $C_{16}H_{17}NO_2$ (*m/z*): calcd 255.13, found 237.0 (M^+−18).

4.4.4. (*R*)-3-((*R*)-Hydroxy(phenyl)methyl)azetidin-2-one 10

A mixture of (*R*,*R*)-**5** (7.12 mmol, 2.53 g) and 10% HCl (12 mL) was heated at 90 °C for 24 h. After cooling to rt, AcOEt (15 mL) was added and the organic phase separated. The aqueous phase was evaporated to dryness to give 1.08 g of solid **9**·HCl. The latter (6 mmol, 1.49 g) was suspended in CH_3CN (20 mL) and Et_3N (6.4 mmol, 0.9 mL) was added dropwise. After stirring at rt for 24 h, the white precipitate was filtered and washed with cold CH_3CN. The amino acid (*R*,*R*)-**9** thus obtained (5 mmol, 0.98 g) was suspended in CH_3CN (100 mL), and PPh_3 (6 mmol, 1.57 g) and dipyridyl disulfide (6 mmol, 1.32 g) were added sequentially. After heating at 72 °C for 20 h, the crude reaction mixture was directly purified by column chromatography (Kieselgel Merck Typ 9385–400 mesh, 60 Å; eluant CH_2Cl_2/MeOH 96:4) to give 0.58 g of the title compound, 65% yield, dr (NMR) 93:7. A diastereoisomerically pure compound was obtained through crystallization (CH_2Cl_2–hexane). Mp 145 °C, $[\alpha]_D^{20}$ = +129.0 (*c* 0.6, $CHCl_3$). ^{1}H NMR ($CDCl_3$): δ = 7.38–7.26 (m, 5H), 5.66 (br s, 1H), 5.24 (d, 1H, J = 3.7 Hz), 3.63 (m, 1H), 3.50 (m, 1H), 3.24 (m, 1H). ^{13}C NMR 37.1 (CH_2), 58.5 (CH), 69.4 (CH), 125.6 (CH), 126.4 (CH), 127.8 (CH), 141.7 (C), 169.5 (C).

4.4.5. (*S*)-3-((*S*)-Hydroxy(phenyl)methyl)azetidin-2-one 10

The title compound has been obtained in 44% overall yield from (*S*,*S*)-**5** by following the same procedure described for (*R*,*R*)-**10**. NMR data identical with those reported above for (*R*,*R*)-**10**. $[\alpha]_D^{20}$ = −128.1 (*c* 0.6, $CHCl_3$).

4.4.6. Attempted synthesis of (*R*)-3-((*S*)-hydroxy(phenyl)methyl) azetidin-2-one 10

When ester (2*R*,3*S*)-**5** was subjected to the same protocol described above for the conversion of (*R*,*R*)-**5** to the corresponding azetidinone, unexpectedly (*R*,*R*)-**10** was isolated in 28% overall yield along with a 3.4:1 mixture (12% overall yield) of the title compound with (*R*,*R*)-**10** (as determined by integration of the benzylic proton ^{1}H NMR ($CDCl_3$): (*R*,*R*)-**10**, 5.24 (d, 1H, J = 3.7 Hz); (*R*,*S*)-**10**, 5.00 (d, 1H, J = 7.6 Hz). HPLC data: CHIRALCEL OD, eluent: hexane/2-propanol = 90:10, flow = 0.8 mL/min, λ = 215 nm, rt: (2*S*,3*S*) = 21.5 min, (2*R*,3*R*) = 27.4 min, (2*R*,3*S*) = 30.3 min.

4.4.7. (*R*)-1-(4-bromobenzyl)-3-((*R*)-hydroxy(phenyl)methyl)azetidin-2-one 11

At first, 50% NaH (0.022 mmol, 11 mg) was added to a stirred solution of azetidinone (*R*,*R*)-**10a** (0.02 mmol, 37 mg) in a THF/DMF 6:1 mixture (3.5 mL) under a nitrogen atmosphere and at 0 °C. After 5 min, 4-bromobenzyl bromide (0.022 mmol, 55 mg) was added and the temperature was allowed to reach room temperature. After 20 h a few drops of saturated aqueous NH_4Cl were added and the solvent evaporated at reduced pressure. The residue was dissolved in CH_2Cl_2 (5 mL) and washed with water (1 mL). The organic phase was dried over Na_2SO_4, filtered and evaporated to dryness. The residue was purified by column chromatography (eluant AcOEt/PE 6:4) to give 35 mg of the title compound, yield 51% along with 13 mg of the azetidinone (*R*,*R*)-**10a**. Crystals for X-ray diffraction were obtained by crystallization with Et_2O. (*R*,*R*)-**11**—^{1}H NMR ($CDCl_3$): δ = 7.44 (d, 2H, J = 8.2 Hz), 7.33 (s, 5H), 7.07 (d, 2H, J = 8.0 Hz), 5.23 (s, 1H), 4.46 (d, 1H, J = 5.6 Hz), 4.22 (d, 1H, J = 5.6 Hz), 3.59 (br s, 1H), 3.31 (m, 1H), 3.04 (t, 1H, J = 5.3 Hz). ^{13}C NMR 40.3 (CH_2), 45.4 (CH_2), 56.9 (CH), 69.9 (CH), 122.1 (C), 125.6 (CH), 127.9 (CH), 128.6 (CH), 129.7 (CH), 131.9 (CH), 134.5 (C), 141.5 (C), 168.1(C).

4.4.8. X-ray structure determination of (*R*)-1-(4-bromobenzyl)-3-((*R*)-hydroxy(phenyl)methyl)azetidin-2-one 11

Crystal data. $C_{17}H_{16}BrNO_2$, M = 346.22, orthorhombic, a = 6.052(1), b = 8.895(2), c = 28.428(6) Å, U = 1530.4(5) Å^3, T = 294(2) K, space group $P2_12_12_1$ (no. 61), Z = 4, μ = (Mo-Kα) 2.690 mm^{-1}. 8889 reflections (3884 unique) were collected at room temperature in the range $2.40 \leqslant 2\theta \leqslant 29.55°$, employing a 0.35 × 0.09 × 0.04 mm crystal mounted on a Bruker Apex II CCD diffractometer. 2291 reflections (R_{int} = 0.0267), final R_1 [wR_2] values of 0.0314 [0.0667] on $I > 2\sigma(I)$ [all data], with GoF = 0.824 for 254 parameters.

Intensities were corrected for Lorentz-polarization effects and empirical absorption correction (SADABS).[17] The structure was solved by direct methods (SIR-97)[18] and refined on F_o^2 with the SHELXL-97[19] program (WINGX suite[20]) All the non-hydrogen atoms were refined with anisotropic thermal parameters, while hydrogen atoms, located on the ΔF maps, were treated isotropically.

The choice of the correct enantiomer was confirmed by the value of the Flack parameter [−0.010(8)]. The refinement of the wrong enantiomer resulted in a R index of 0.0628, significantly higher than that of the correct one.

Crystallographic data (excluding structure factors) have been deposited with the Cambridge Crystallographic Data Centre as

supplementary publication No. CCDC-785072. These data can be obtained free of charge via www.ccdc.cam.ac.uk/conts/retrieving.html (or from CCDC, 12 Union Road, Cambridge CB2 1EZ, UK; fax: +44 1223 336033; e-mail: deposit@ccdc.cam.ac.uk.

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