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# Highly stereoselective reduction of haloketones using three new yeasts: application to the synthesis of (*S*)-adrenergic β-blockers related to propranolol

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**Abstract**—The stereoselective reduction of aryloxy-halo-2-propanones **1** or of 1-chloro-3(phthalimdyl)-propan-2-one **2** using baker's yeast usually displays poor yields and/or ees. Three new yeasts, *Saccharomyces bayanus* CECT 1317, *Yarrowia lipolytica* CECT 1240 and *Pichia mexicana* CECT 1015, were selected after a taxonomical screening looking for microorganisms active in the reduction of ketones. These strains have been used for the highly stereoselective reduction of **1** and **2**. This reduction is the key step in the stereoselective synthesis of (*S*)-adrenergic β-blockers related to the propranolol structure. *P. mexicana* (reduction of **1**) and *S. bayanus* (the reduction of **2**), gave ees greater than 90% and yields higher than 85% for the (*R*)-or (*S*)-halohydrins, respectively. This process constitutes an efficient alternative to the resolution of halohydrins carried out using lipases and can easily be scaled up.


## 1. Introduction

The use of whole cells as catalysts for the asymmetric reduction of ketones has attracted considerable interest.[1–4] The major advantage of using whole cells compared to the use of isolated reductases is the in vivo recycling of the red-ox co-enzymes (NADH or $FADH_2$). *Saccharomyces cerevisiae* (baker's yeast), a readily available and cheap catalyst, has been the most extensively studied microorganism in this process.[5,6] However, in many cases the use of baker's yeast gives products of unsatisfactory enantiomeric purity or in low yield. Several attempts have been made to optimize the enantiomeric excess and the yield of the products. Thus, for instance, the use of organic media,[7] addition of other compounds,[8] or the use of different carbon sources for the regeneration of the co-enzyme[9] have been shown to contribute to improvements in the process. Finally, immobilization techniques[10] for the reuse of the biocatalyst or the use of other microorganisms[11] have also been described for scaling up the process for the reduction of ketones.

The preparation of propranolol by the resolution of racemic halohydrin-precursor **3a** using lipases[12] or by the yeast-catalyzed reduction of 1-chloro-3-(1-naphthyloxy) propan-2-one, **1a**, has previously been described.[13] Following our work on the yeast reduction of haloketones, we herein report an efficient and highly enantioselective synthesis of halohydrins, **3** and **4**, precursors of adrenergic β-blockers (Scheme 1).

It is well known that, at least four ADH enzymes are active in *S. cerevisiae*[14,15] with different enantioselectivities. There are two D-enzymes and two L-enzymes with L-enzyme-1[15] and D-enzyme-2 being the most active.[6b,16,17] (Scheme 1). Therefore low ees are obtained in some cases when using unnatural substrates. To overcome the problems presented by the use of the baker's yeast, a taxonomic screening of 421 microorganisms was carried out.[18] Two different types of haloketones, **1** and **2**, were tested in order to explore a general method for the synthesis of these halohydrins using the stereoselective reduction of haloketones as the key step. The synthesis of the active adrenergic

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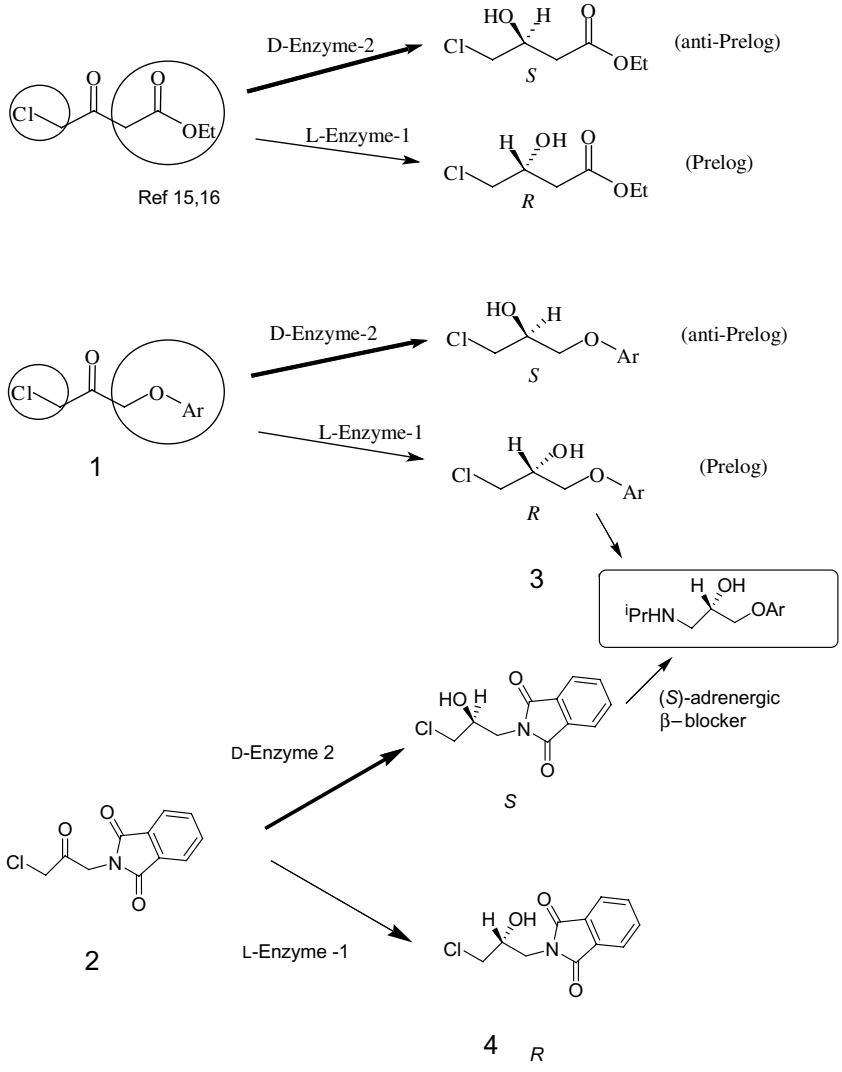



**Scheme 1.**

β-blockers, with the (*S*)-1-aminoalkyl-3-aryloxy-2-propranol structure, can easily be carried out from both haloketones (Scheme 1). Several groups were introduced in the aromatic ring to explore whether electronic/steric factors influence the reduction of the ketones.

## 2. Results and discussion

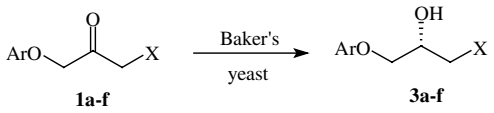
### 2.1. Reduction of haloketones using *S. cerevisiae*

The 1-aryloxy-3-halopropan-2-ones **1a**–**f**, were easily prepared by O-alkylation of the corresponding phenols with racemic epichlorohydrin followed by oxidation, as we have recently reported.[13] The reduction of haloketones **1a**–**f** was performed under the same conditions, which gave the best selectivity with **1a**.[13] The results of the bioreductions with *S. cerevisiae* (reference microorganism) are reported in Table 1.

In all cases, an (*S*)-stereopreference was observed. Therefore, this stereoselective reduction gave the opposite enantiomer (Scheme 1) to that used for the preparation of the (*S*)-adrenergic β-blocker after substitution of Cl by $NH_2$-*i*-Pr or $NH_2$-*t*-Bu (Scheme 1). The stereoselectivity of the process increases with the size of the aryl moiety, **1a**, **1b** and **1f**, while the compounds with small substituents in the aryl moiety **1d**, **1e** and **1c** (nonsubstituted) were transformed with low stereoselectivities. It is noteworthy that the electronic effects associated with the substituents on the aromatic ring are apparently irrelevant to the reduction yield or ee achieved in the reduction. We can see that the reduction of **1c** (nonsubstituted), **1d** (with a slightly electron-donating group) and **1e** (with an electron acceptor group) show similar yields and the same stereoselectivities, 18%, 22% and 18%, respectively. Therefore, we consider that the most important aspect is the size of the group.

In this reduction, the hydride is stereoselectively transferred from NADH to the-*Re*- face of **1**, giving (*S*)-3 as the main stereomer but only giving a moderated to low ee (Table 1). It is well known that, at least four ADH enzymes are active in *S. cerevisiae* with different

**Table 1.** Microbial reduction of 1-aryloxy-3-halopropan-2-ones **1a–f** in water[a]




**1a-f** **3a-f**

| Ketone | Ar | X | Yield (%)[b] | Configuration of **3a-f**[c] | Ee (%)[d] |
|---|---|---|---|---|---|
| **1a** | 1-Naphthyl | Cl | 52 | $S > R$[e] | 55 |
| **1b** | 1-Naphthyl | Br | 41 | $S > R$[e] | 50 |
| **1c** | Phenyl | Cl | 54 | $S > R$[e] | 18 |
| **1d** | *p*-Acetamidophenyl | Cl | 58 | $S > R$[e] | 22 |
| **1e** | *p*-Nitrophenyl | Cl | 60 | $S > R$[e] | 18 |
| **1f** | 2,5-Dimethylphenyl | Cl | 88 | $S > R$[e] | 89 |

[a] Reductions were carried out using 6.5 g of *S. cerevisiae* type II, 4 g of sucrose and 0.42 mmol of substrate in 25 mL of reaction media (0.1 M sodium phosphate buffer, pH 8.0). Incubations were performed at 30 °C for 48 h.
[b] Yields refer to isolated pure products.
[c] Absolute configuration of **3a–d** were assigned on the basis of their specific rotation.[12,13]
[d] Ee determined by chiral phase HPLC.
[e] Absolute configuration of **3e** was assigned on the basis of its conversion to **3d** via 'in situ' reduction and acetylation of the nitro function with Pd/C in $AcOH/Ac_2O$.[19]

enantioselectivities.[14,15] In the microorganisms, L-enzyme-1[15] and D-enzyme-2 were the most active ones.[6b,16,17]. Therefore, we can say that our main alcohol (*S*-isomer) is produced by means of the D-enzyme-2 (Scheme 1), as this enzyme is more active than the L-enzyme-1[14].

The enantioselective reduction of **2** would allow us to obtain a new non-racemic chiral building block, **4**, that can be used to prepare, by conventional reactions, the (*S*)-adrenergic β-blockers containing the aryloxy-2-propanolamine structure (Scheme 1). The obtained results are shown in Table 2.

The results obtained in the reduction of haloketone **2** showed excellent conversion rates at lower reaction times when compared to **1** (48 h, Table 1). Indeed, at 6 h (entry 3) the reduction was complete (isolated product). Nevertheless, the enantiomeric excess was very poor and (*R*)-enantiopreference was observed. Therefore we assume that both main enzymes in *S. cerevisiae* exhibit similar activities with this substrate, with the L-enzyme-1 (giving the (*R*)-isomer) being slightly more active than the D-enzyme-2 (Scheme 1). The low ee obtained makes this reaction of no further interest to be carried out on a preparative scale.

In attempts to improve the enantioselectivity of the reduction process, different changes in the experimental procedure were introduced: Increasing the amount of baker's yeast (entry 5); using selective inhibitors of the baker's yeast isoenzymes (entries 6–9) or using organic solvents (data not shown). However, we were not able to improve the results in any of the conditions tested.

## 2.2. Screening for new microorganisms

The low ee or the unfavourable stereochemistry of the halohydrin produced (Tables 1 and 2), led us to look for other microorganisms with active alcohol dehydrogenase (ADH) activity. The primary screening was performed with 421 strains (148 filamentous fungi, 71 bacteria, 33 marine fungi, 64 yeasts, 60 basidiomycetes and 45 actinomycetes) in order to look for microorganisms selective in the reduction of simple cycloalkanones using the maximum biological diversity to select the strains. Cyclohexanone was selected as a model substrate for the screening.[18] 216 strains were active in the reduction of cyclohexanone. In Table 3 we show the most interesting strains and the result obtained with baker's yeast, used as the reference. These microorganisms were selected because the oxidation of cyclohexanol

**Table 2.** Effect of the reaction conditions on the selectivity of the reduction of 1-chloro-3-(phthalimidyl)-propan-2-one, **2**[a]

| Entry | *S. cerevisiae* (g) | Additive | Time (h) | Yield (%)[b] | Ee (%) |
|---|---|---|---|---|---|
| 1 | 2.24 | — | 48 | 98 | 15 |
| 2 | 2.24 | — | 24 | 96 | 13 |
| 3 | 2.24 | — | 6 | 98 | 16 |
| 4 | 2.24 | — | 3 | 70 | 16 |
| 5 | 6.24 | — | 24 | 99 | 13 |
| 6 | 2.24 | 0.3 M methyl-vinyl-ketone | 24 | 85 | 13 |
| 7 | 2.24 | 0.6 M methyl-vinyl-ketone | 24 | 85 | 13 |
| 8 | 2.24 | 0.3 M ethyl chloroacetate | 24 | 60 | 12 |
| 9 | 2.24 | 0.6 M ethyl chloroacetate | 24 | 43 | 12 |

[a] Reductions were carried out using *S. cerevisiae* type II, 4 g of sucrose and 0.42 mmol of substrate in 25 mL of reaction media (0.1 M sodium phosphate buffer, pH 8.0). Incubations were performed at 30 °C for 48 h.
[b] Yields referred to the isolated pure products.

**Table 3.** Microorganisms selected from the cyclohexanone reduction ([cyclohexanone] = 10 mM)

| Microbial group | Strain | Reductase activity (%) | Oxidase activity (%) |
|---|---|---|---|
| Yeast | *Y. lipolytica* CECT 1240 | 54 | 0 |
| Yeast | *P. mexicana* CECT 11015 | 77 | 0 |
| Yeast | *Saccharomyces bayanus* CECT 1969 | 27 | 0 |
| Yeast | *S. cerevisiae* CECT 1317 | 52 | 0 |
| Yeast | *S. cerevisiae* (Type II, Sigma) | 13 | 0 |
| Yeast | *Schizosaccharomyces octosporus* | 87 | 0 |
| Actinomycete | *Actinoplanes* sp | 52 | 0.9 |
| Actinomycete | *Nocardia uniformis* subsp. *Tsuyamanensis* | 37 | 0 |
| Marine fungus | *Dactylospora haliotrepha* | 78 | 0 |
| Ascomycete | *Diplogelasinospora grovesii* | 85 | 0 |
| Ascomycete | *Rhizomucor variabilis* | 78 | 0 |
| Ascomycete | *Gongronella butleri* | 100 | 0 |
| Ascomycete | *Neosartorya hiratsukae* | 71 | 0 |
| Ascomycete | *Absidia glauca* | 81 | 0 |
| Ascomycete | *Pyrenochaeta oryzae* | 67 | 0 |
| Ascomycete | *Monascus kaoliang* | 89 | 0 |
| Ascomycete | *Echinosporangium transversale* | 58 | 2.3 |

(as the substrate) was lower than 5% and the reduction of cyclohexanone was greater than 50%.

None of the bacteria or the basidiomycetes tested in this assay displayed significant activity to be progressed for further characterization. Only one among the marine fungi, *Dactylospora haliotrepha*, showed interesting activity, according to previously indicated criteria. Two actinomycetes and five yeasts were selected for further studies in view of the high reductase activity observed. However, the group that displayed the highest number of active microorganisms was the filamentous fungi. A total of eight ascomycetes were selected, all displaying a very high reductase activity. All the selected microorganisms were more active than the commercial *S. cerevisae* (Type II from Sigma), used as a reference in many organic chemistry laboratories.

### 3. Reduction of 1a with other microorganisms

The reduction of **1a** was used as a model to explore the reductase activity of these new microorganisms in the reduction of aryloxy-halo-ketones using either growing cells or resting cells.

The enantioselectivity of the halohydrin obtained was variable and depended on the microorganism used in the reaction. The best ee values were obtained with the yeasts *Yarrowia lipolytica* and *Pichia mexicana*. These species produced higher ees than those obtained with the commercial baker's yeast from Sigma, under the same experimental conditions. Since the yields obtained with growing cells were very low for the reduction of **1a**, this reaction was performed using resting cells. It has been reported that the yields and ee values obtained under these conditions could be better than with growing cells, probably because of the intracellular levels of the cofactor and their availability for the reaction being higher in the resting conditions.[20] Indeed the results improved when compared to those in Table 3 in the case of the yeasts but not in the case of the ascomycetes, as we can see in the cases of *M. kaoliang* and *N. hiratsukae*. Therefore ascomycetes were discarded for further studies.

Both *S. cerevisiae* strains gave similar yields but opposite enantiopreference, either using growing or resting cells conditions. Pereira has indicated similar results, in other stereoselective reductions.[21] This author showed that strains of the same species, or species of the same genus, could display different stereospecificities in certain reactions. This fact could be explained by the different ratios of intracellular reductases in these strains. According to the data (Table 4), *Y. lipolytica* CECT 1240 and *P. mexicana* CECT 11015, acting as resting cells, were selected as the most interesting strains to perform the reaction under preparative conditions. These two yeasts produce the opposite enantiomers with very good yields and ees. Thus, *P. mexicana* gave the (*R*)-enantiomer, which is used for the (*S*)-adrenergic β-blocker (Scheme 1), whereas *Y. lipolytica* produced the (*S*)-enantiomer of the halohydrin.

Finally the reduction of the other substrates was carried out with the yeasts in resting cells conditions. The results obtained are shown in Table 5.

As expected, the stereochemistry in the reductions tested depends on the strain, and it is maintained for the different haloketones, **1a**, **1d** and **1f**, for each yeast. For substrates of type **1**, the enantioselectivity increases with the bulkiness of the substrate. We can obtain the (*S*)-halohydrins **3a** and **3f** in 99% ee with very good yields with *Y. lipolytica* CECT 1240, and the (*R*)-halohydrins **3a** and **3f** in 95% and 99% ee, respectively, with *P. mexicana* CECT 11015. These strains are the most interesting biocatalysts because the (*R*)-β-blocker (*Y. lipolytica* CECT 1240) or the (S)-β-blocker (*P. mexicana* CECT 11015) can easily be obtained by the substitution of Cl by $NH_2$-*i*Pr (Scheme 1). Additionally we can also obtain any (*S*)-adrenergic β-blocker using *S. bayanus* CECT 1969 and substrate **2**. With this strain we could obtain the corresponding (*S*)-halohydrin of the phthalimide compound, with 67% ee and 99% of con-

**Table 4.** Reduction of **1a** using growing and resting whole cells

| Microorganism | Fermenter conditions[a] | | | Resting cells[b] | | |
|---|---|---|---|---|---|---|
| | Yield[c] (%) | Enantiopref.[d] | Ee[c] (%) | Yield[c] (%) | Enantiopref.[d] | Ee[c] (%) |
| *M. kaoliang* | 55 | $R > S$ | 1 | 5 | $R > S$ | 10 |
| *N. hiratsukae* | 39 | $R > S$ | 6 | 10 | $R > S$ | 90 |
| *Y. lipolytica* CECT 1240 | 20 | $S \gg R$ | 99 | 87 | $S \ggg R$ | 99 |
| *P. mexicana* CECT 11015 | 19 | $R \gg S$ | 85 | 85 | $R \ggg S$ | 95 |
| *S. bayanus* CECT 1969 | 15 | $R > S$ | 52 | 40 | $R \gg S$ | 87 |
| *S. cerevisiae* (Type II) | 12 | $S > R$ | 70 | 30 | $S \gg R$ | 70 |
| *S. cerevisiae* CECT 1317 | 13 | $R > S$ | 70 | 27 | $R > S$ | 71 |

[a] Reductions were carried out with growing cells (48 h).
[b] Reductions were carried out using 0.19 mmol of substrate and 4 g of sucrose in 25 mL of reaction media (0.1 M sodium phosphate buffer, pH 8.0). Incubations were performed at 30 °C for 48 h.
[c] Molar conversion and ee determined by chiral HPLC.
[d] Absolute configuration of the halohydrin was assigned by specific rotation value. (*S*)-(+)-**1a** (99%) $[\alpha]_D^{25} = +9.3$ ($c = 1.9$ EtOH), (*R*)-(−)-**3a** (95%) $[\alpha]_D^{25} = -8.8$ ($c = 1.9$ EtOH).

**Table 5.** Microbial reduction of haloketones **1** and **2** to the halohydrins, **3** and **4**, using resting cells[a]

| Yeast | **3a** | | | **3d** | | | **3f** | | | **4** | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Yield[b](%) | *E* | Ee[b] (%) | Yield[b] (%) | *E* | Ee[b] (%) | Yield[b] (%) | *E* | Ee[b] (%) | Yield[b] (%) | *E* | Ee[b] (%) |
| *S. cerevisiae* type II | 30 | *S* | 83 | 54 | *S* | 22 | 88 | *S* | 89 | 90 | *R* | 12 |
| *S. cerevisiae* CECT 1317 | 27 | *R* | 75 | 5 | — | — | 87 | *R* | 21 | 60 | *R* | 32 |
| *S. bayanus* CECT 1969 | 40 | *R* | 88 | 95 | *R* | 52 | 52 | *R* | 79 | 99 | *S* | 67 |
| *Y. lipolytica* CECT 1240 | 87 | *S* | 99 | 90 | *S* | 90 | 90 | *S* | 99 | 90 | *S* | 5 |
| *P. mexicana* CECT 11015 | 45 | *R* | 95 | 45 | *R* | 72 | 72 | *R* | 99 | 54 | *R* | 10 |

[a] Reductions were carried out using fresh cells re-suspended in 20 mL of reaction media (0.1 M sodium phosphate buffer, pH 7.0; 7.5 mg of **1a**, dissolved in benzene, and 600 mg of sucrose). Incubations were performed at 28 °C for 48 h.
[b] Molar conversions and ee determined after 48 h by chiral HPLC on the crude mixture.

version. These results clearly surpass the ee and yield values obtained in the resolution of racemic halohydrins **3** using lipases.[12]

## 4. Conclusion

From the data shown in Table 5, it can be deduced that *Y. lipolytica*, *P. mexicana* and *S. bayanus* are three interesting strains susceptible of progression to process optimization and scale-up studies. These strains produced the best conversions and enantiomeric excesses. In addition, both yeast strains present a high tolerance to the substrate, since the conversions and ees remain high in all the cases, especially for *Y. liypolytica*. The microorganisms from the *Saccharomyces* genus were shown to be very sensitive to the substrate concentration as can be deduced from the disparity of the yields and ee observed. Baker's yeast showed the worst global results among the microorganisms of this group, with the exception of *S. bayanus*. This strain presents a certain interest because it carries to the adequate stereomers in both cases, thus allowing us to achieve the (*S*)-adrenergic β-blockers with good yield and ee.

## 5. Experimental

### 5.1. Chemicals

1-Naphthol, epichlorohydrine, phenol, 4-acetamidophenol, pyridinium chlorochromate (PCC) and potassium phthalimide were purchased from Aldrich Chemical Company. *S. cerevisiae* (Baker's yeast) Type II was purchased from Sigma. *S. cerevisiae* CECT 1317, *S. cerevisiae* CECT 1969, *Y. lipolytica* CECT 1240 and *P. mexicana* CECT 11015 were kindly supplied by the Spanish Type Culture Collection (CECT, Valencia, Spain).

**5.1.1. Analytical methods.** The enantiomeric excess (ee) and the absolute configuration of **3** were determined by HPLC using a ConstaMetric 4100 system equipped with a chiral column (Chiracel OD), UV–vis detector and a Knauer chiral detector. Mobile phases: **3a**, **3c**, **3d** and **3e**, hexane–isopropanol–diethylamine = 70:30:0.1 (v/v/v), 0.5 mL min$^{-1}$; **3b**, hexane–isopropanol–diethylamine = 60:40:0.1 (v/v/v), 0.4 mL min$^{-1}$; **1f**, hexane–isopropanol–diethylamine = 80:20:0.1 (v/v/v), 1.0 mL min$^{-1}$. $^1$H and $^{13}$C NMR spectra were determined with a Bruker AC-250 MHz. All spectra were taken in $CDCl_3$ solution and

the chemical shift values expressed in ppm, using TMS as the internal standard. Optical rotations were measured with a Perkin Elmer 241 polarimeter. Optical density was measured on a Cecil 1021 spectrophotometer at 600 nm. Gas chromatographic analysis was performed using a Hewlett Packard Model 5890A GC equipped with a flame ionization detector and a TR-WAX X 60 m 0,25 mm, 0,25 μm capillary column from SUGELABOR (Spain). The oven temperature was programmed from 155 °C (1 min isotherm) to 175 °C at 4.0 °C $min^{-1}$ and then held at the upper limit for 6.5 min, with the final chromatogram time being 12.5 min. The column pressure was 40 psi, the flow rate of the carrier gas (helium) was 2.1 mL $min^{-1}$ (auxiliary gas was not necessary), and the split flow ratio was 20:1.

### 5.2. Preparation of racemic halohydrins 3

**5.2.1. Preparation of racemic 1-aryloxy-3-halopropan-2-ols 3a–f.** A mixture of phenol (0.02 mol), epichlorohydrin (0.1 mol) and pyridine (0.002 mol) was stirred at room temperature for 24 h. Then, the unreacted epichlorohydrin and pyridine were removed under vacuum. The mixture was cooled at which point $CHCl_3$ (10 mL) and 5 mL of HCl 35% were added and the mixture was stirred for 1 h at room temperature. Afterwards, 10 mL of water were added and the organic phase removed and washed again with 10 mL of water. The organic phase was dried over anhydrous $CaCl_2$ and concentrated under vacuum. Preparation of halohydrins **3a**–**d** has been previously described.[12,13]

**5.2.2. (*RS*)-1-Chloro-3-(*p*-nitrophenyloxy)propan-2-ol 3e.** Prepared as described above. Halohydrin was obtained in 77% yield after the usual work-up followed by chromatography (eluant $CH_2Cl_2$). $^1H$ NMR ($CDCl_3$, 250 MHz) $\delta$: 2.52 (d, 1H, OH), 3.74 (m, 2H, $CH_2$–Cl), 4.13 (m, 2H, $CH_2$–O), 4.37 (m, 1H, CH), 6.92 (d, 2H, Ar–H), 8.22 (d, 2H, Ar–H) ppm; $^{13}C$ NMR ($CDCl_3$) $\delta$: 45.8 ($CH_2$–Cl), 69.2 (CH–OH), 69.6 ($CH_2$–O), 114.6 ($C_2$, $C_6$, Ar), 126.1 ($C_3$, $C_5$, Ar), 142.0 ($C_1$, Ar), 163.0 ($C_4$, Ar), ppm. Elemental Anal. Calcd for $C_9H_{10}ClNO_4$: C, 46.72; H, 4.45; N, 6.03. Found: C, 46.70; H, 4.46; N 6.05.

**5.2.3. (*RS*)-1-Chloro-3-(2,5-dimethyl-phenyloxy)propan-2-ol 3f.** Prepared as described above. Halohydrin was obtained in 70% yield after the usual work-up followed by chromatography (eluant hexane/ethyl acetate 9:1). $^1H$ NMR ($CDCl_3$, 250 MHz) $\delta$: 2.16 (s, 3H, $CH_3$), 2.30 (s, 3H, $CH_3$), 2.56 (d, 1H, OH), 3.77 (m, 2H, $CH_2$–Cl), 4.06 (m, 2H, $CH_2$–O), 4.21 (m, 1H, CH), 6.64 (s, 1H, Ar–H), 6.99 (d, 1H, Ar–H), 7.01 (d, 1H, Ar–H), ppm; $^{13}C$ NMR ($CDCl_3$): 15.9 ($CH_3$), 21.4 ($CH_3$), 46.2 ($CH_2$–Cl), 68.4 (CH–OH), 70.1 ($CH_2$–O), 112.3 ($C_6$, Ar), 121.7 ($C_4$, Ar), 123.6 ($C_2$, Ar), 130.6 ($C_3$, Ar), 136.9 ($C_5$, Ar), 156.1 ($C_1$, Ar), ppm. Elemental Anal. Calcd for $C_{11}H_{15}ClO_2$: C, 61.53; H, 7.04. Found: C, 61.55; H, 7.06.

**5.2.4. (*RS*)-1-Chloro-3-phthalimide propan-2-ol 4.** A mixture of potassium phthalimide (0.054 mol), and epichlorohydrin (0.27 mol) in EtOH/$CH_2Cl_2$ (1:1) (50 mL) was stirred at room temperature for 8 h. Then, the unreacted epichlorohydrin was removed under vacuum. The mixture was cooled and $HCCl_3$ (20 mL) and 6 mL of HCl 35% then added, and the mixture stirred for 1 h at 0–5 °C. Afterwards, 10 mL of water were added and the organic phase removed and washed again with 10 mL of water. The organic phase was dried over anhydrous $CaCl_2$ and concentrated at vacuum. Halohydrin was obtained in 89% yield after the usual work-up followed by chromatography (eluant $CH_2Cl_2$). $^1H$ NMR ($CDCl_3$, 250 MHz) $\delta$: 2.86 (d, 1H, OH), 3.62 (m, 2H, $CH_2$–N), 3.90 (m, 2H, $CH_2$–Cl), 4.16 (m, 1H, CH–OH), 7.72 (m, 2H, Ar–H), 7.85 (m, 2H, Ar–H), ppm; $^{13}C$ NMR ($CDCl_3$) $\delta$: 41.6 ($CH_2$–N), 47.3 ($CH_2$–Cl), 69.8 (CH–OH), 123.6 ($2C_H$, Ar), 131.9 (2C, Ar), 134.4 ($2C_H$, Ar), 168.7 (2CO–N), ppm. Elemental Anal. Calcd for $C_{11}H_{10}ClNO_3$: C, 55.12; H, 4.22; N, 5.80. Found: C, 55.30; H, 4.38; N, 5.91.

**5.2.5. Preparation of 1-aryloxy-3-halopropan-2-one 1 a–f and 1-chloro-3-phthalimide propan-2-one 2.** The synthesis of haloketones **1a**–**d** has already been described.[12,13] The alcohols **3e**,**f** and for **2** were oxidized as follows: A mixture of pyridinium chlororochromate (4.83 mmol) and $CH_2Cl_2$ (20 mL) was stirred for 10 min at room temperature. Alcohol (3.22 mmol) in $CH_2Cl_2$ (10 mL) was then added. The solution was stirred for 24 h for **3e** and **3f**, and for 48 h **2**. The solution was decanted and washed with three portions of ether. The combined organic solution was passed through celite, the solvent removed and the product subjected to chromatography on silica gel.

**5.2.6. 1-Chloro-3-(*p*-nitrophenyloxy)propan-2-one 1e.** Prepared as described above. Haloketone was obtained in 70% yield after the usual work-up followed by chromatography (eluant $CHCl_3$). $^1H$ NMR ($CDCl_3$, 250 MHz) $\delta$: 4.34 (s, 2H, $CH_2$–Cl), 4.93 (s, 2H, $CH_2$–O), 6.97 (d, 2H, Ar–H), 8.23 (d, 2H, Ar–H) ppm; $^{13}C$ NMR ($CDCl_3$) $\delta$: 46.7 ($CH_2$–Cl), 71.2 ($CH_2$–O), 114.6 ($C_2$, $C_6$, Ar), 126.0 ($C_3$, $C_5$, Ar), 142.0 ($C_1$, Ar), 161.9 ($C_4$, Ar), 197.0 (CO) ppm. Elemental Anal. Calcd for $C_9H_8ClNO_4$: C, 47.12; H, 3.51; N, 6.10. Found: C, 47.60; H, 3.29; N 5.85.

**5.2.7. 1-Chloro-3-(2,5-dimthyl-phenyloxy)propan-2-one 1f.** Prepared as described above. Haloketone was obtained in 63% yield after the usual work-up followed by chromatography ($CH_2Cl_2$). $^1H$ NMR ($CDCl_3$, 250 MHz) $\delta$: 2.22 (s, 3H, $CH_3$), 2.29 (s, 3H, $CH_3$), 4.47 (s, 2H, $CH_2$–Cl), 4.70 (s, 2H, $CH_2$–O), 6.48 (s, 1H, Ar–H), 6.72 (d, 1H, Ar–H), 7.02 (d, 1H, Ar–H) ppm; $^{13}C$ NMR ($CDCl_3$) $\delta$: 15.9 ($CH_3$), 21.4 ($CH_3$), 47.1 ($CH_2$–Cl), 71.8 ($CH_2$–O), 111.7 ($C_6$, Ar), 122.5 ($C_4$, Ar), 123.6 ($C_2$, Ar), 131.0 ($C_3$, Ar), 137.1 ($C_5$, Ar), 153.3 ($C_1$, Ar), 199.5 (CO) ppm. Elemental Anal. Calcd for $C_{11}H_{13}ClO_2$: C, 62.12; H, 6.26. Found: C, 62.34; H, 6.30.

**5.2.8. 1-Chloro-3-phthalimide propan-2-one 2.** The haloketone was obtained in 100% yield after the usual work-up followed by chromatography (eluant $CH_2Cl_2$). $^1H$ NMR ($CDCl_3$, 250 MHz) $\delta$: 4.20 (s, 2H, $CH_2$–N), 4.75 (s, 2H, $CH_2$–Cl), 7.74 (m, 2H, Ar–H), 7.87 (m, 2H, Ar–H) ppm; $^{13}C$ NMR ($CDCl_3$) $\delta$: 44.8 ($CH_2$–N), 46.3 ($CH_2$–Cl), 123.8 ($2C_H$, Ar), 133.0 (2C, Ar), 134.4 ($2C_H$, Ar), 167.5 (2CO–N), 195 (CO) ppm. Elemental Anal. Calcd for $C_{11}H_8ClNO_3$: C, 55.68; H, 3.41; N, 5.93. Found: C, 55.80; H, 3.45; N, 6.12.

### 5.3. Determination of the enantiomeric excesses of halohydrins

The α-values for halohydrins **3a**–**d** have been previously published.[12,13]

**5.3.1. Determination of the enantiomeric excess of 3e.** It was assigned on the basis of its conversion to **3d** by in situ reduction and acetylation of the nitro group with Pd/C in $AcOH/Ac_2O$.[15] Spectroscopic and analytical data are identical to those reported for racemic samples.

**5.3.2. Determination of the enantiomeric excess of (*R*)-(−)-1-chloro-3-(2,5-dimethyl-phenyloxy)propan-2-ol 3f.** Conversion of (2*S*)-(+)-glycidyl tosylate, 99% ee into **3f** was carried out by O-alkylation of the phenol and NaH, followed by hydrolysis with HCl (35%) for 1 h at room temperature.[22] Alcohol (−)-**3f** was obtained in 93% ee. $[\alpha]_D^{25} = -7.2$ (*c* 0.46, EtOH). Spectroscopic and analytical data are identical to those reported for racemic samples.

**5.3.3. Determination of the enantiomeric excess of (*R*)-(+)-1-chloro-3-phthalimidyl propan-2-ol 4.** We followed essentially the same procedure described above, except that NaH was not used. Alcohol (+)-**4** was obtained in 93% ee $[\alpha]_D^{25} = +16.2$ (*c* 0.48, EtOH). Spectroscopic and analytical data are identical to those reported for racemic samples.

### 5.4. Reduction of ketones with *S. cerevisiae* type II (Sigma)

A suspension of **1** (0.42 mmol), baker's yeast type II (6.5 g), and sucrose (4 g) in water/0.1 M phosphate buffer (25 mL) was stirred at 30 °C for 48 h. After extraction with ether, the extract was washed with water, dried over anhydrous $MgSO_4$ and evaporated to dryness. Chromatography of the residue on silica gel with dichloromethane as the eluent, yielded alcohol **3**. $^1H$ and $^{13}C$ NMR spectra were identical to racemic samples.

### 5.5. Microorganisms media and culture conditions

The cell culture was developed using a rich culture medium suitable for each kind of microorganisms, referring to the ATTC, DSMZ and CETC catalogues when necessary. The microorganism collection was maintained as cell suspensions at −80°C in 50% glycerol. Cultures were started by adding an aliquot of 50 μL of each cell suspension to 20 mL of culture medium in a 100 mL flask. The selected culture media for each microbial group were

ABME (Actinomycetes): $CaCO_3$ 10 g/L, $FeSO_4.7H_2O$ 0.003 g/L, KCl 0.5 g/L, $MgSO_4.7H_2O$ 5 g/L, beef extract 5 g/L, malt extract 40 g/L.

HAGGS1 (Actinomycetes): Glycine 2 g/L, soybean flour 6 g/L, starch 20 g/L, glycerol 10 g/L, nutrient solution 10 mL/L ($FeSO_4.7H_2O$ 1 g/L, $MnSO_4.4H_2O$ 1 g/L, $CuCl_2$ 0.025 g/L, $CaCl_2$ 0.1 g/L, $H_3BO_3$ 0.056 g/L, $ZnSO_4.7H_2O$ 0.2 g/L, $(NH_4)_6Mo_7O_{24}.4H_2O$ 0.1 g/L.

LB (Bacteria): Tryptone 10 g/L, yeast extract 5 g/L, NaCl 5 g/L.

PDA (Basidiomycetes): Potato flour 22 g/L, dextrose 20 g/L. YM (Yeasts): Bacto yeast extract 3 g/L. Malt extract 3 g/L, bactopeptone 5 g/L, bactodextrose 10 g/L.

PDM (Marine fungi): Lyophilized potatoes 22 g/L, dextrose 20 g/L, NaCl 2 g/L. potato flour 22 g/L, dextrose 20 g/L.

Taxonomic identification of unicellular bacteria, actinomycetes and yeasts was performed by the Spanish Type Culture Collection (CETC, Valencia, Spain). The fungi were identified in the Basic Research Center of GlaxoSmithKline (Tres Cantos, Madrid, Spain) by using mature cultures on suitable media in order to ensure a good development of taxonomically relevant features.

### 5.6. Reaction conditions

The screening of the reductase activity for each microorganism was performed as follows: After an adequate culture time at 250 r.p.m. at 28 °C in an orbital shaker (48 h for yeasts and bacteria, and 72 h for fungi and actinomycetes), 20 μL (10 mM) of cyclohexanone were added to the culture flasks. The period of contact between the microorganism and the substrate was 72 h for all the microbial groups. The biotransformation was quenched by the addition of HCl solution until an acidic pH value was shown.

Then, the cell culture medium, after removal of cells, was extracted with ethyl acetate (5 mL ethyl acetate/20 mL cell culture medium) for 5 min. The organic phase was analyzed by GC. Gas chromatographic analysis was performed using a Hewlett Packard Model 5890A.

### 5.7. Reduction using whole cells in the culture medium

The reduction of **1a** using *Y. lipolytica* 1240 is reported as an example: A solution of the ketone (0.19 mmol) was

added into a 250 mL flask containing 50 mL of a *Y. lipolytica* 1240 culture (48 h) (Abs = 2.14 (DO at 260 nm), number of cells = $72.08 \times 10^6$ mL$^{-1}$). After 48 h, ethyl acetate (5 mL) was added. The mixture was centrifuged and decanted to give pure (*R*)-(−)-1-chloro-3-(1-naphthyloxy)-2-propanol **3a** in 25% yield and 99% ee.

### 5.8. Reduction using fresh resting cells

The reduction of **1a** using *P. mexicana* CECT 11015 is reported as an example: A solution of the ketone (0.19 mmol) in benzene (200 μL) was added to a stirred suspension (20 mL) of fresh *Y. lipolytica* CECT 1240 cells (Abs = 1.99 (DO at 660 nm), number of cells = $66.23 \times 10^6$ mL$^{-1}$) resuspended in a 0.1 M phosphate buffer (pH 7.0) at 28 °C and sucrose (600 mg). After 48 h, ethyl acetate (5 mL) was added. The mixture was centrifuged and decanted to give pure (*S*)-(+)-1-chloro-3-(1-naphthyloxy)-2-propanol **3a** in 85% yield and 95% ee.

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