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Preparation of halohydrin β-blocker precursors using yeast-catalysed reduction

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Abstract

The preparation of halohydrin β-blocker precursors using yeast-catalysed reduction of α-haloketones was performed. The influence in the yield and e.e. of several process variables was analysed. The (*S*)-enantioselectivity observed with *Saccharomyces cerevisiae* can be changed to (*R*)-enantioselectivity using methyl vinyl ketone as selective inhibitor (25 mM). Using resting fresh cells better yields and e.e.s are observed than using growing cells. *Yarrowia lipolytica* 1240 resting cells gave 87% yield of (*S*)-1-chloro-3(1-naphthyloxy)propan-2-ol (99% e.e.). *Pichia mexicana* 11105 resting cells gave 85% yield of (*R*)-1-chloro-3(1-naphthyloxy)propan-2-ol (precursor of propranolol) (95% e.e). The reduction process is applied to other α-haloketones, a lower e.e. being obtained the closer the size of the ketone substituents.

1. Introduction

The halohydrin **1** is the key intermediate in the preparation of homochiral β-blockers with the aryloxy-2-propanolamine structure. The resolution of (*RS*)-**1** using lipases, both by esterification or by deacylation of acylated (±)-**1** has been used as a standard alternative to prepare pure homochiral adrenergic β-blockers.[1] However, even the highly enantioselective lipase-catalysed process can only provide 50% of the starting racemate in optically active form. Alternatively, an enantiotope selective method, such as reduction of a prochiral ketone **2**, might quantitatively yield an optically active product with yields greater than 50%. The stereoselective reduction of prochiral ketones **2** has been carried out by using chiral complexes of Rh(I).[2] Knowing this process is expensive and very difficult to scale up, a cheap alternative is the use of redox enzymes from whole cells. In this way stereoselective reduction of ketones has been carried out with oxidoreductases from *Bacillus* sp. or *Staphylococcus* sp.,[3] or with alcohol dehydrogenases from *Alcaligenes eutrophus*,[4] *Zygosaccharomyces rouxii*, *Saccharomyces cerevisiae* (baker's yeast),[5] etc.

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In the in vitro enzymatic reductions of prochiral ketones to chiral alcohols, the recycling of redox cofactors is still difficult to achieve in a low cost and effective way. The use of whole cells offers the advantage that the cofactor is recycled in vivo and avoids the additional costs of the enzyme purification. So this biotechnological process has the potential to be scaled up.

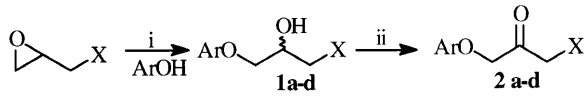
Some studies about the variables that control the biotransformation of prochiral ketones have been reported[5] due to the presence of several enzymes in the whole cell. In the case of the yeast *S. cerevisiae* four of these enzymes have been identified and purified.[6] These enzymes have opposite enantioselectivities[6] and can be inhibited by different substances, showing different activity–concentration of substrate profile.[7] These different behaviours are explained because they are enzymes involved in different metabolic pathways.[8]

In the present paper we have performed the stereoselective reduction of **2** to the halohydrin **1** with good yield and e.e. using different microorganism strains and experimental conditions.

2. Results and discussion

The 1-aryloxy-3-halopropan-2-ones **2a–d** were easily prepared by *o*-alkylation of the corresponding phenols with racemic epihalohydrin, followed by oxidation of **1a–d** (Table 1).

Table 1
Preparation of 1-aryloxy-3-halopropan-2-ones[a]



	Ar	X	(*RS*)-**1a–d** yield (%)[c]	**2a–d** yield (%)[c]
A	1-Naphthyl	Chloro	80	92
B	1-Naphthyl	Bromo[b]	82	70
C	Phenyl	Chloro	85	78
D	*p*-Acetamidophenyl	Chloro	90	95

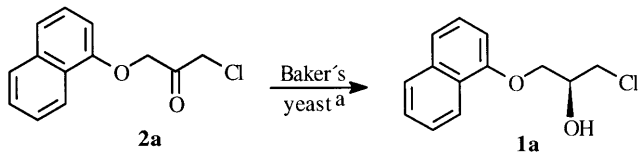
[a] Reagents: (i) (a) pyridine, (b) XH, $CHCl_3$; (ii) oxalyl chloride, DMSO, Et_3N.
[b] Pyridinium fluorochromate (PFC).
[c] Yields referred to isolated pure products.

The reduction of **2** using two chiral commercial chemical reductants such as (−)-diisopinocampheyl borane chloride or (*R*)-alpine-borane gave moderate yields of **1**, such as 18 or 36% with very low e.e. 16 or 8% (*S*>*R*).

The effect of the reaction conditions on enantiotope selectivity of the baker's yeast (*S. cerevisiae*, type II, Sigma) reduction of 1-chloro-3-(1-naphthyloxy)propan-2-one **2a** was investigated (Table 2).

We can observe that an increase in the amount of baker's yeast used increases the yield but the e.e. value remains nearly constant (entries 5, 7 and 9). This result indicates that we are in the lineal zone 'amount of biocatalyst versus yield', useful for catalytic studies. Small variations in e.e. and yield are observed using phosphate buffers with different pH (entries 9, 10 and 11). This behaviour is different from the one observed in the reduction of β-keto esters described by Hunt et al.[5a] using *Z. rouxii* or of ketones using alcohol dehydrogenase from *Thermoanaerobium*

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



2a **1a**

Entry	Temperature (°C)	*S. cerevisiae* (g)	Time (h)	pH	Yield[b] (%)	e.e. (%)[c] $S>R$
1	18	6.5	1	7	6	60
2	25	1.68	7	7	14	51
3	25	1.68	24	7	25	49
4	25	6.5	1	7	9	68
5	30	1.68	6	7	18	40
6	30	1.68	24	7	29	39
7	30	3.36	24	7	31	44
8	30	3.36	48	7	44	42
9	30	6.5	24	7	45	45
10	30	6.5	24	6	56	45
11	30	6.5	24	8	52	55

[a] Reaction conditions: **2a**, 100 mg; media (0.1 M sodium phosphate buffer), 25 ml; saccharose 4 g.
[b] Yields referred to isolated pure products.
[c] Absolute configurations and e.e. determined by chiral phase HPLC. The absolute configuration was assigned on the basis of the specific rotation.[1b,c]

Brokii,[9] where a clear variation was observed. This behaviour may be explained because *S. cerevisiae* has four enzymes with different pH profile and different thermal stability.[6]

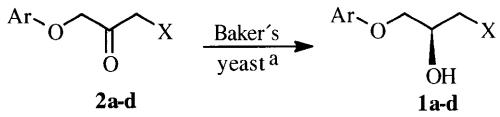
An increase in the reaction time produces an increase in the yield (entries 2 and 3) but a very small diminution in the e.e. value.

The stereoselectivity of the process increases with the pH value of the phosphate buffer (entries 10 and 11) (Table 2). The ability to switch the stereoselectivity by changing pH has been observed in the oxidation of 1,2-cyclohexanediol by *Gluconobacter oxidants*, resulting from catalysis by two enzymes of opposite enantioselectivity with different optimum pH.[10] Hunt et al.[5a] described the same phenomenon with the yeast *Pichia capsulata* in the reduction of 4-halo-2-ketoesters but with smaller variation than those described by Adlercreutz.[10] The increase in the enantioselectivity with pH has been analysed by De Souza,[11] who indicates that the production of NAD(P)H increases with the pH because at pH<7 the baker's yeast cells experience oxidative stress.

To explore the scope of the stereoselective reduction, several haloketones **2a–d** were used under the conditions which gave the best selectivity with **2a** (Table 3). In all cases, the e.e. was not very high, especially with **2c** and **2d**, the substrates with the smallest substituent, because the greater the difference in the size of substituents of carbonyl group the greater the stereoselectivity of the process.[5c,8]

In all cases, an (*S*)-stereopreference was observed. This disagrees with Prelog's rule of stereocontrol, generally described for the Baker's yeast reductions[8–10] with addition of hydride by the *Re*-face.

Table 3
Saccharomyces cerevisiae type II mediated biotransformation of 1-aryloxy-3-halopropan-2-ones **2a–d**[a]



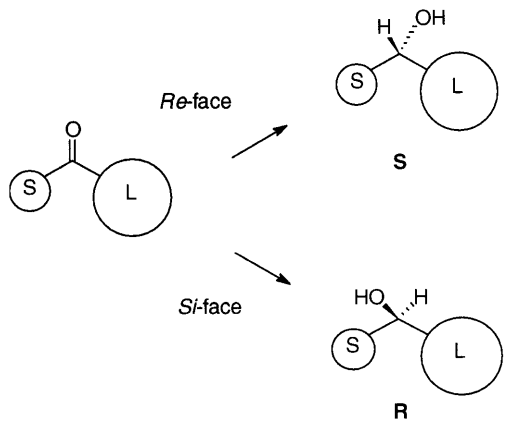
2a-d **1a-d**

Compound	Yield (%)[b]	Conf. of **1a–d**	e.e. (%)[c]
2a	52	*S*>*R*	55
2b	41	*S*>*R*	50
2c	54	*S*>*R*	18
2d	58	*S*>*R*	22

[a] Reaction conditions: **2a–d**, 100 mg; *S. cerevisiae* type II 6.5 g; media (0.1 M sodium phosphate buffer, pH 8) at 30°C, 25 ml; saccharose 4 g.

[b] Yields referred to isolated pure products.

[c] Absolute configurations and e.e. determined by chiral phase HPLC. The absolute configuration of **1b** was assigned on the basis of its conversion to propanolol using isopropylamine,[1b] **1c** and **1d** on the basis of their specific rotations.[1d]



Nevertheless, this statement is not exclusive because the stereocontrol of the process depends on the nature, the size and the electronic characteristics of the substituents of C=O bond, as described by several workers[5c,12,13] and to the presence of several isoenzymes with different stereoselectivity, pH and temperature profiles.[6] The polar characteristic of chlorine may be the origin of this enantioselectivity, as described by Waagen et al. for 1-hydroxy-2-propanones (giving *R*>*S* stereoselectivity as in our case) and for 1-aryloxy-2-propanones (giving *S*>*R*, the opposite stereoselectivity) or by Nakamura[13] (methyl ketones gave the Prelog (*S*)-alcohol and trifluormethyl ketones gave the anti-Prelog (*R*)-alcohol).

2.1. Selective poisoning of the reductases

Because (*S*)-**1a** will give the (*R*)-adrenergic-β-blocker, propranolol, we tried to change the enantioselectivity of the reduction process. In the literature the selective poisoning of Baker's yeast isoenzymes has been described using ethyl chloroacetate **3** or methyl vinyl ketone **4**. The results obtained by using 25 mM of inhibitor and 16.8 mM of substrate are shown in Table 4. The experiments were performed in optimum experimental conditions according to Table 2.

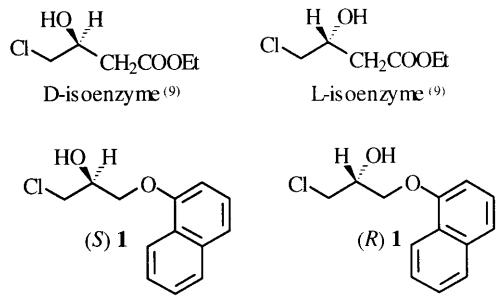
Table 4
Alteration of the enantioselectivity in the reduction of **2a** or **c** by the presence of some dehydrogenase inhibitors[a]

Compound	Inhibitor	**1** Yield[b] (%)	**1** (*R*) (%)	**1** (*S*) (%)	**1** (e.e. (%))	**1** Conf.[c]
2a	–	52	22	78	56	$S>R$
	3	28	47	53	6	$S>R$
	4	35	75	25	50	$R>S$
2c	–	54	41	59	18	$S>R$
	3	37	48	52	4	$S>R$
	4	41	79	21	58	$R>S$

[a] Reaction conditions: **2a** and **c** 100 mg; *S. cerevisiae* type II 6.5 g; media (0.1 M sodium phosphate buffer, pH 8) at 30°C, 25 ml; saccharose (4 g); inhibitor **3** or **4**, 25 mM.
[b] Yields referred to isolated pure products.
[c] Absolute configurations and e.e. determined by chiral phase HPLC.

Other experiments, with higher inhibitor concentrations than 25 mM were performed, but a strong reduction in the yield was observed. No reduction of **2** was observed at inhibitor concentration ≥ 50 mM.

We observed that the reduction of **2a** or **c** in the presence of ethyl chloroacetate **3** produces a reduction in the yield and an increase in the percentage of (*R*)-**1** and a moderated diminution in the amount of (*S*)-**1**. The same behaviour has been described by Egri et al.[5c] in the reduction of 1-acetoxy-3-phenoxypropan-2-one. When **4** is used the yield decreases, but the enantiopreference changes due to the dramatic reduction of the percentages of (*S*)-**1**. Taking into account the selective poisoning work carried out by Nakamura et al.[6] with pure isolated oxidoreductase enzymes from Baker's yeast, we can say that our main alcohol ((*S*)-isomer)) is produced by a D-isoenzyme (probably D-enzyme-2[9]) by means of an anti-Prelog pathway; and the (*R*)-isomer is produced by L-enzymes that should be less active than D-enzymes in our commercial strain (Scheme 1).



Scheme 1.

These D-enzymes that give (*S*)-**1** are in our case very sensitive to the poisoning by **3** and especially for **7**. The poisoning produces a reduction in the yield according to Nakamura et al.[6] and a strong diminution in the percentage of (*S*)-stereomer. Therefore the (*R*)-counterpart becomes the main alcohol changing the apparent stereoselectivity.

2.2. Screening with other yeasts

As an alternative we performed the reduction using other microorganisms. The reductions were directly performed in the growth cell culture medium or using fresh resting cells (Table 5). Fresh resting cells gave better yields and e.e. values than growing cells because of secondary metabolic reactions of growing cells that transform the substrate or the product.[14]

Table 5
Microbial reduction of **2a** to alcohol **1a** in water

Entry	Microorganism	Culture[a]			Fresh cells[b]		
		Yield[c] (%)	Conf.[d]	e.e.[d]	Yield (%)	Conf.[d]	e.e.[d]
1	*Saccharomyces cerevisiae* type II	15	*S*	80	30	*S*	83
2	*Saccharomyces cerevisiae* 1317	17	*R*	74	27	*R*	75
3	*Saccharomyces bayanus* 1969	18	*R*	54	40	*R*	88
4	*Yarrowia lipolytica* 1240	25	*S*	99	87	*S*	99
5	*Pichia mexicana* 11015	23	*R*	10	85	*R*	95

[a] Reductions carried out directly in the growth medium (48 h entry 1, 2, 4, 5 and 72 h entry 3). Reaction conditions: **2a**, 40 mg; 120 h.

[b] Reductions carried out with fresh cells suspended in a 0.1 M phosphate buffer (pH 7.0) at 28°C, in an aqueous reactor with a final volume of 20 ml; **2a**, 7.5 mg; substrate dissolved in benzene, saccharose 600 mg.

[c] Molar conversions determined after 48 h by HPLC on the crude mixture.

[d] Absolute configurations and e.e. determined by chiral phase HPLC and specific rotation. (*S*)-(+)-**1a** (99%) $[\alpha]_D^{25} = +9.3$ ($c = 1.9$, EtOH); (*R*)-(−)-**1a** (95%) $[\alpha]_D^{25} = -8.8$ ($c = 1.9$, EtOH).

The stereochemistry is different in each strain but it is not altered by the physical situation of the biocatalyst—growing cells or resting cells. Resting cells of *Yarrowia lipolytica* 1240 yielded the corresponding (*S*)-halohydrin in 99% e.e, whereas *Pichia mexicana* 11015 gave the (*R*)-halohydrin in 95% e.e. These strains are the most interesting biocatalysts because the (*R*)-β-blocker (*Y. lipolytica* 1240) or the (*S*)-β-blocker (*P. mexicana*) can easily be obtained by substitution of Cl by NH-*i*Pr. This methodology overcomes the shortfalls in terms of the yield and/or the enantioselectivity obtained using lipase.[1b,c] Besides these strains improve on the results obtained with *S. cerevisiae* (Table 2).

Presently, a more detailed study is in progress in order to immobilise the resting cells to enable an industrial catalytic process to be scaled up.

3. Experimental

3.1. Chemicals

1-Naphthol, epichlorohydrin, epibromohydrin, phenol, 4-acetamidophenol, pyridinium fluorochromate (PFC) were purchased from Aldrich Chemical Company. *S. cerevisiae* (Baker's yeast) type II was purchased from Sigma. *S. cerevisiae* 1317, *S. cerevisiae* 1969, *Y. lipolytica* 1240 and *P. mexicana* 11015 were kindly supplied by Spanish Type Culture Collection (CECT; Valencia Spain).

3.2. Analytical methods

The enantiomeric excess (e.e.) and the absolute configuration of **1** 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: **1a**, **c**, and **d**, hexane–isopropanol–diethylamine = 70:30:0.1 (v/v/v), 0.5 ml min^{-1}; **1b**, hexane–isopropanol–diethylamine = 60:40:0.1 (v/v/v), 0.4 ml min^{-1}. ^1H and ^{13}C NMR spectra were determined on a Bruker AC-250 MHz spectrometer; all spectra were taken in $CDCl_3$ solution and chemical shift values are expressed in ppm values from TMS as internal standard. Optical rotations were measured on a Perkin–Elmer 241 polarimeter. Optical density was measured on a Cecil 1021 spectrophotometer at 600 nm.

3.3. Preparation of 1-aryloxy-3-halopropan-2-ols (rac)-**1a–d**

A mixture of phenol (0.02 mol), epichlorohydrin (0.1 mol) and pyridine (0.002 mol) were stirred at room temperature for 24 h. Then, the unreacted epichlorohydrin and pyridine were removed in vacuo. The mixture was cooled and $HCCl_3$ (10 ml) and 5 ml of HCl 35% were added, and the mixture stirred for 1 h at room temperature. Afterwards, 10 ml of water were added and the organic phase was removed and washed again with 10 ml of H_2O. The organic phase was dried over anhydrous $CaCl_2$ and concentrated in vacuo.

3.3.1. (RS)-1-Chloro-3-(1-naphthyloxy)-2-propanol **1a**

^1H NMR ($CDCl_3$, 250 MHz) δ: 2.86 (d, 1H, OH), 3.85 (m, 2H, CH_2–Cl), 4.23 (m, 2H, CH_2–O), 4.35 (m, 1H, CH), 6.82 (d, 1H, Ar–H), 7.36 (t, 1H, Ar–H), 7.49 (m, 3H, Ar–H), 7.80 (m, 1H, Ar–H), 8.18 (m, 1H, Ar–H) ppm; ^{13}C NMR ($CDCl_3$) δ: 46.5 (CH_2–Cl), 68.8 (CH–OH), 70.1 (CH_2–O), 105.2 (C_2, Ar), 121.2 (C_4, Ar), 121.7 (C_8, Ar), 125.5 (C_9, Ar), 125.6 (C_7, Ar), 125.9 (C_3, Ar), 126.7 (C_6, Ar), 127.7 (C_5, Ar), 134.6 (C_{10}, Ar), 153.9 (C_1, Ar) ppm. Elemental anal. calcd for $C_{13}H_{13}O_2Cl$: C, 67.97; H, 5.54. Found: C, 67.72; H, 5.55.

3.3.2. (RS)-1-Bromo-3-(1-naphthyloxy)-2-propanol **1b**

^1H NMR ($CDCl_3$, 250 MHz) δ: 2.6 (d, 1H, OH), 3.75 (m, 2H, CH_2–Br), 4.23 (m, 2H, CH_2–O), 4.34 (m, 1H, CH), 6.81 (d, 1H, Ar–H), 7.36 (t, 1H, Ar–H), 7.49 (m, 3H, Ar–H), 7.80 (m, 1H, Ar–H), 8.18 (m, 1H, Ar–H) ppm; ^{13}C NMR ($CDCl_3$) δ: 35.6 (CH_2–Br), 69.4 (CH–OH), 69.7 (CH_2–O), 105.1 (C_2, Ar), 121.2 (C_4, Ar), 121.7 (C_8, Ar), 125.5 (C_9, Ar), 125.6 (C_7, Ar), 125.9 (C_3, Ar), 126.7 (C_6, Ar), 127.7 (C_5, Ar), 134.6 (C_{10}, Ar), 153.8 (C_1, Ar) ppm. Elemental anal. calcd for $C_{13}H_{13}O_2Br$: C, 55.55; H, 4.68. Found: C, 55.58; H, 4.75.

3.3.3. (RS)-1-Chloro-3-phenyloxy-2-propanol **1c**

^1H NMR ($CDCl_3$, 250 MHz) δ: 2.65 (d, 1H, OH), 3.74 (m, 2H, CH_2–Cl), 4.06 (m, 2H, CH_2–O), 4.19 (m, 1H, CH), 6.90 (d, 2H, Ar-H), 6.97 (t, 1H, Ar–H), 7.29 (m, 2H, Ar–H) ppm; ^{13}C NMR ($CDCl_3$) δ: 46.0 (CH_2–Cl), 68.4 (CH–OH), 69.9 (CH_2–O), 114.6 (C_2, C_6, Ar), 121.5 (C_4, Ar), 129.7 (C_3, C_5, Ar), 158.2 (C_1, Ar) ppm. Elemental anal. calcd for $C_9H_{11}O_2Cl$: C, 57.92; H, 5.94. Found: C, 57.82; H, 5.95.

3.3.4. (RS)-1-Chloro-3-(p-acetamidophenyloxy)-2-propanol **1d**

^1H NMR ($CDCl_3$, 250 MHz) δ: 2.14 (s, 3H, CH_3), 2.51 (d, 1H, OH), 3.73 (m, 2H, CH_2–Cl), 4.03 (m, 2H, CH_2–O), 4.19 (m, 1H, CH), 6.85 (d, 2H, Ar–H), 7.05 (s, 1H, NH), 7.37 (d, 2H,

Ar–H) ppm; ^{13}C NMR ($CDCl_3$) δ: 24.9 (CH_3), 46.0 (CH_2–Cl), 68.8 (CH–OH), 69.9 (CH_2–O), 115.0 (C_2, C_6, Ar), 129.7 (C_3, C_5, Ar), 132.7 (C_4, Ar), 154.0 (C_1, Ar), 167.6 (CONH) ppm. Elemental anal. calcd for $C_{11}H_{14}NO_3Cl$: C, 54.22; H, 5.87; N, 5.79. Found: C, 57.82; H, 5.95; N, 5.77.

3.4. Preparation of 1-aryloxy-3-halopropan-2-ones ***2a–d***

The alcohols **1a**, **c** and **d** was oxidised as follows: A solution of freshly distilled oxalyl chloride (4.6 mmol) in CH_2Cl_2 (10 ml) was cooled to −70°C and DMSO (9.2 mmol) in CH_2Cl_2 (3 ml) was added over 5 min. The reaction mixture was stirred for a further 10 min at the same temperature and the alcohol (4.2 mmol) in CH_2Cl_2 (5 ml) was added over 5 min. After additional stirring for 15 min, Et_3N (0.3 ml) was added and the cooling bath was removed. At room temperature H_2O (10 ml) was added and the organic phase was separated from the water phase which was extracted with CH_2Cl_2 (3×25 ml). The organic phase was dried over anhydrous $CaCl_2$, concentrated under vacuum and chromatographed on silica gel.

3.4.1. 1-Chloro-3-(1-naphthyloxy)-2-propanone ***2a***

^{1}H NMR ($CDCl_3$, 250 MHz) δ: 4.53 (s, 2H, CH_2–Cl), 4.92 (s, 2H, CH_2–O), 6.68 (d, 1H, Ar–H), 7.35 (t, 1H, Ar–H), 7.51 (m, 3H, Ar–H), 7.81 (m, 1H, Ar–H), 8.25 (m, 1H, Ar–H) ppm; ^{13}C NMR ($CDCl_3$) δ: 47.0 (CH_2–Cl), 71.9 (CH_2–O), 104.9 (C_2, Ar), 121.6 (C_4, Ar), 122.0 (C_8, Ar), 125.2 (C_9, Ar), 125.6 (C_7, Ar), 125.9 (C_3, Ar), 126.9 (C_6, Ar), 127.8 (C_5, Ar), 134.7 (C_{10}, Ar), 153.1 (C_1, Ar), 199 (C=O) ppm. Elemental anal. calcd for $C_{13}H_{11}O_2Cl$: C, 66.53; H, 4.72. Found: C, 66.47; H, 5.75.

3.4.2. 1-Chloro-3-phenyloxy-2-propanone ***2c***

^{1}H NMR ($CDCl_3$, 250 MHz) δ: 4.41 (s, 2H, CH_2–Cl), 4.75 (s, 2H, CH_2–O), 6.89 (d, 2H, Ar–H), 7.0 (t, 1H, Ar–H), 7.31 (m, 2H, Ar–H) ppm; ^{13}C NMR ($CDCl_3$) δ: 46.0 (CH_2–Cl), 72.5 (CH_2–O), 114.3 (C_2, C_6, Ar), 121.5 (C_4, Ar), 129.6 (C_3, C_5, Ar), 157.2 (C_1, Ar), 198.4 (C=O) ppm. Elemental anal. calcd for $C_9H_9O_2Cl$: C, 58.63; H, 4.94. Found: C, 58.65; H, 4.95.

*3.4.3. 1-Chloro-3-(*p*-acetamidophenyloxy)-2-propanol* ***2d***

^{1}H NMR ($CDCl_3$, 250 MHz) δ: 2.15 (s, 3H, CH_3), 4.39 (m, 2H, CH_2–Cl), 4.73 (m, 2H, CH_2–O), 6.84 (d, 2H, Ar–H), 7.10 (s, 1H, NH), 7.41 (d, 2H, Ar–H) ppm; ^{13}C NMR ($CDCl_3$) δ: 25.1 (CH_3), 48.0 (CH_2–Cl), 72.8 (CH_2–O), 115.0 (C_2, C_6, Ar), 129.9 (C_3, C_5, Ar), 133.7 (C_4, Ar), 154.2 (C_1, Ar), 167.9 (CONH), 197.5 (C=O) ppm. Elemental anal. calcd for $C_{11}H_{12}NO_3Cl$: C, 54.72; H, 5.07; N, 5.84. Found: C, 54.75; H, 5.05; N, 5.74.

3.5. Preparation of 1-bromo-3-(1-naphthyloxy)-2-propanone ***2b***

The alcohol **1b** was oxidised as follows: A mixture of pyridinium fluorochromate (4.83 mmol) and CH_2Cl_2 (20 ml) was stirred for 10 min at room temperature. Alcohol **1b** (3.22 mmol) in CH_2Cl_2 (10 ml) was then added. The solution was stirred for 6 h. The solution was decanted and washed with three portions of ether. The combined organic solution was passed through Celite and the solvent was removed and chromatographed on silica gel.

3.5.1. 1-Bromo-3-(1-naphthyloxy)-2-propanone ***2b***

^{1}H NMR ($CDCl_3$, 250 MHz) δ: 4.26 (d, 2H, CH_2–Br), 4.94 (d, 2H, CH_2–O), 6.71 (d, 1H, Ar–H), 7.36 (t, 1H, Ar–H), 7.52 (m, 3H, Ar–H), 7.82 (m, 1H, Ar–H), 8.28 (m, 1H, Ar–H) ppm; ^{13}C NMR ($CDCl_3$) δ: 31.5 (CH_2–Br), 71.0 (CH_2–O), 104.7 (C_2, Ar), 121.5 (C_4, Ar), 121.7 (C_8, Ar), 125.3 (C_9, Ar), 125.4 (C_7, Ar), 125.7 (C_3, Ar), 126.7 (C_6, Ar), 127.5 (C_5, Ar), 134.5 (C_{10}, Ar), 153.6 (C_1, Ar), 198.8 (C=O) ppm. Elemental anal. calcd for $C_{13}H_{11}O_2Br$: C, 55.91; H, 4.03. Found: C, 55.88; H, 4.08.

3.6. Reduction of ketones with S. cerevisiae *type II (Sigma). General procedure*

A suspension of **2** (0.5 mmol), baker's yeast type II (6.5 g) and saccharose (4 g) in water/0.1 M phosphate buffer was stirred at 30°C for 24 h. After extraction with ether, the extract was washed with water, dried ($MgSO_4$) and evaporated to dryness. Chromatography of the residue on silica gel with dichloromethane as eluent yielded the alcohol **1**. ^{1}H and ^{13}C NMR spectra were identical with racemic samples.

3.7. Reduction with S. cerevisiae *type II in the presence of inhibitors*

A suspension of compound **2** (0.5 mmol), baker's yeast type II (6.5 g), ethyl chloroacetate, **3** or methyl vinyl ketone, **4** (16.8 mM) and saccharose (4 g) in water/0.1 M phosphate buffer was stirred at 30°C for 24 h. After extraction with ether, the extract was washed with water, dried ($MgSO_4$) and evaporated to dryness. Chromatography of the residue on silica gel with dichloromethane as eluent yielded the alcohol **1**. ^{1}H and ^{13}C NMR spectra were identical with racemic samples.

3.8. Microorganisms, media and culture conditions

All the yeasts were grown at 28°C on media with glucose (10 g), peptone (3 g), yeast extract (3 g) and 0.1 M phosphate buffer (pH 7.0) for 48–72 h. All the cultures were grown in 250 ml flasks containing 50 ml of medium. Microbial reductions were directly performed on the media of culture or with centrifuged and washed cells (see Table 5).

3.9. Biotransformation conditions

3.9.1. Reduction using the whole cells in the culture medium

The reduction of **2a** *Y. lipolytica* 1240 is reported as an example: A solution of ketone (40 mg) in benzene (200 μl) was added to a 250 ml flask containing 50 ml of culture of *Y. lipolytica* 1240 (48 h) (OD = 2.14, no of cells = 72.08×10^6 ml^{-1}). After 120 h ethyl acetate (5 ml) was added. The mixture was centrifuged and decanted to give pure (*R*)-(−)-1-chloro-3-(1-naphthyloxy)-2-propranol **1a** in 25% yield and 99% e.e.

3.9.2. Reduction using fresh resting cells

The reduction of **2a** using *Y. lipolytica* 1240 is reported as an example: A solution of ketone (7.5 mg) in benzene (200 μl) was added to a stirred suspension (20 ml) of fresh cells of *Y. lipolytica* 1240 resuspended in 0.1 M phosphate buffer (pH 7.0) at 28°C and saccharose (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 **1a** in 87% yield and 99% e.e.

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