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Communication

Stereoselective Chemoenzymatic Synthesis of Optically Active Aryl-Substituted Oxygen-Containing Heterocycles †

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† Dedicated to Professor Luigino Troisi on the occasion of his retirement.

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Abstract: A two-step stereoselective chemoenzymatic synthesis of optically active α-aryl-substituted oxygen heterocycles was developed, exploiting a whole-cell mediated asymmetric reduction of α-, β-, and γ-chloroalkyl arylketones followed by a stereospecific cyclization of the corresponding chlorohydrins into the target heterocycles. Among the various whole cells screened (baker's yeast, *Kluyveromyces marxianus* CBS 6556, *Saccharomyces cerevisiae* CBS 7336, *Lactobacillus reuteri* DSM 20016), baker's yeast was the one providing the best yields and the highest enantiomeric ratios (up to 95:5 er) in the bioreduction of the above ketones. The obtained optically active chlorohydrins could be almost quantitatively cyclized in a basic medium into the corresponding α-aryl-substituted cyclic ethers without any erosion of their enantiomeric integrity. In this respect, valuable, chiral non-racemic functionalized oxygen containing heterocycles (e.g., (*S*)-styrene oxide, (*S*)-2-phenyloxetane, (*S*)-2-phenyltetrahydrofuran), amenable to be further elaborated on, can be smoothly and successfully generated from their prochiral precursors.

Keywords: whole cell biocatalyst; baker's yeast; enantioselective bioreduction; oxiranes; oxetanes; tetrahydrofurans; halohydrins; chloroketones; oxygen-containing heterocycles; chemoenzymatic synthesis

1. Introduction

Oxygen-containing heterocycles are ubiquitous in natural products and biologically active compounds, and are also very common in many blockbuster pharmaceuticals [1,2]. The chemistry of saturated oxygen heterocycles is a topic of growing interest, and several papers dealing with more efficient methodologies for their preparation and their synthetic utility have been increasingly published. Epoxides, in particular, have been widely used in preparative chemistry [3,4] and in the asymmetric synthesis of fine chemicals and drugs (e.g., sertraline, nifenalol, Figure 1) [5–8] because of their versatility related to the ring strain. The oxetane skeleton is present in several

natural organic products (e.g., oxetanocin, taxol, mitrophorone), and represents a versatile building block for the construction of biologically active compounds (e.g., EDO, Figure 1), or other valuable heterocyclic compounds [9–11]. It is also of interest in medicinal chemistry for the isosteric replacement of both the carbonyl and the *gem*-dimethyl group [12–15]. Asymmetric syntheses of optically active tetrahydrofurans have also been extensively investigated in the last few decades [16] because of their presence in many natural products and biologically active compounds (e.g., Goniothalesdiol, Figure 1). The preparation of chiral tetrahydrofurans has been efficiently performed by asymmetric cycloetherifications of hydroxy olefins in the presence of organocatalysts [17] or transition metals [18], or by the catalytic asymmetric hydrogenation of substituted furans [19].

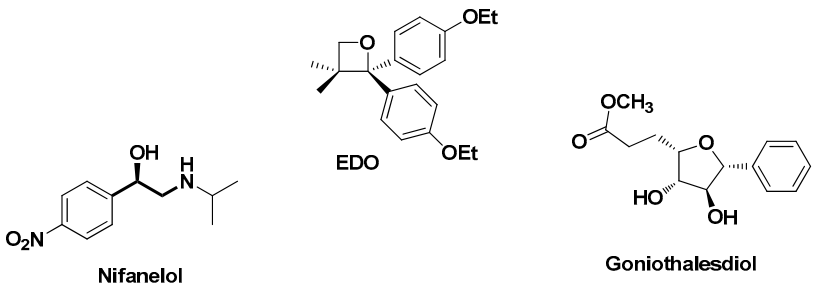
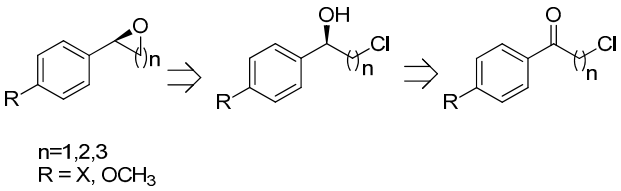


Figure 1. Drugs derived from optically active oxygen-containing heterocycles.

Optically active halohydrins have been successfully employed for the preparation of several chiral non-racemic oxygenated heterocycles (e.g., epoxides, oxetanes, tetrahydrofurans, pyrans).

Some general examples of stereoselective syntheses of halohydrins, as precursors of optically active cyclic ethers, are (a) the reduction of halogen-substituted ketones by means of hydrides complexed with chiral ligands (e.g., CBS-catalyst) [20,21]; (b) stereoselective hydrogenation processes run in the presence of Rh/Ru catalysts [22–24]; (c) microbial [25–28] or isolated enzymes-mediated [29] stereoselective reductions of α-halo-acetophenones; and (d) the kinetic resolution of racemic mixtures using dehalogenases (e.g., HheC from *Agrobacterium radiobacter AD1*) [30,31]. Our group recently focused on the development of new bio-catalyzed whole-cell biotransformations for the enantioselective preparation of chiral secondary alcohols, which are valuable precursor compounds for active pharmaceutic ingredients (APIs) [32–35].

Biocatalytic methodologies have received a great deal of attention for the asymmetric synthesis of biologically active molecules (also in industrial production) because of their high chemo-, regio-, and stereoselective performance under mild reaction conditions [36–38]. Building on these findings, herein we describe a chemoenzymatic synthetic strategy to prepare optically active epoxides, oxetanes, and tetrahydrofurans, which is based on the enantioselective bioreduction of α-, β-, and γ-haloketones in the presence of whole cell biocatalysts, followed by stereospecific cyclization of the corresponding enantio-enriched halohydrins (Scheme 1).



Scheme 1. A chemoenzymatic approach for the synthesis of optically active epoxides, oxetanes, and tetrahydrofurans via enantioselective bioreduction of halo-ketones with whole-cell biocatalysts.

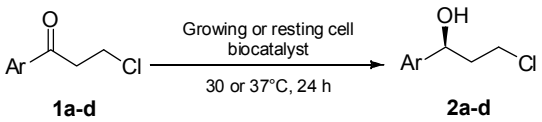
2. Results

2.1. Screening of Biocatalysts for the Stereoselective Reduction of 3-Chloro-1-Arylpropanones

Various microorganisms are known to express different alcohol dehydrogenases (ADHs), each one exhibiting a specific stereo-preference according to the species, the metabolic growth conditions and phase, and the substrate specificity. To date, different yeasts have proven to be effective for the synthesis of functionalized styrene oxides with high stereoselectivity [39], whereas whole-cell biocatalysts with different stereo-preferences (e.g., *Kluyveromyces marxianus*, *Lactobacillus reuteri*) have been successfully employed for the preparation of enantio-enriched secondary alcohols [32–35]. With the aim of identifying the best whole-cell biocatalyst able to reduce different chloroketones with high enantioselectivity, we started our study by screening various biocatalysts for the stereoselective reduction of 3-chloro-1-arylpropanones (Table 1).

In the presence of 0.1 g/L resting cells (RC) of baker's yeast, chlorohydrin (*S*)-**2a** could be isolated with a 42% yield and in up to a 94:6 enantiomeric ratio (er) (Table 1, entry 1) starting from 3-chloro-1-phenylpropanone (**1a**), whereas the reduction in the presence of *Saccharomyces cerevisiae* CBS 7336 (GC) furnished (*S*)-**2a** with a 48% chemical yield and lower er (75:25) (Table 1, entry 2). In the presence of growing cells (GC) of *Kluyveromyces marxianus* CBS 6556, a mixture of products was detected in the reaction crude after 24 h incubation at 30 °C, and (*S*)-**2a** was isolated with only a 31% yield and almost in a racemic form (58:42 er) (Table 1, entry 3). The same biotransformation run in the presence of *Lactobacillus reuteri* DSM 20016 (RC) whole cells did not afford the desired chlorohydrin, with the main reaction being instead the dehydroalogenation of the starting haloketone and the formation of other minor products (see Supporting Information), as observed for other biocatalysts [40].

Table 1. Screening of biocatalysts for the stereoselective reduction of 3-chloro-1-aryl-propanones [a].



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

Entry	Biocatalyst	Ar	Ketone 1	Product 2 (Yield %) [b]	Conversion %	er [c]	Abs. Conf. [d]
1	*Baker's yeast* (RC)	C_6H_5	**1a**	**2a** (42)	50	94:6	*S*
2	*Saccharomyces cerevisiae* (GC) [e]	C_6H_5	**1a**	**2a** (48)	55	75:25	*S*
3	*Kluyveromyces marxianus* (GC) [f]	C_6H_5	**1a**	**2a** (31) [g]	70	58:42	*S*
4	*Baker's yeast* (RC)	4-FC_6H_4	**1b**	**2b** (13)	15	63:37	*S*
5	*Baker's yeast* (RC)	4-BrC_6H_4	**1c**	**2c** (5) [h]	85	95:5	*S*
6	*Baker's yeast* (RC)	4-$MeOC_6H_4$	**1d**	**2d** (–)	12	ND [i]	ND [i]

[a] Typical reaction conditions: orbital incubator (200 rpm); temperature: 30 °C; (GC): inoculum after 24 h growth in a sterile medium containing glucose (1%), peptone (0.5%), yeast extract (0.3%), and malt extract (0.3%) in sterile water; (RC): 0.1 g/L of cell wet mass in 0.1 M KH_2PO_4 buffer (pH = 7.4) enriched with 1% glucose, halo-ketone (2 mM final concentration); [b] Isolated yield after column chromatography; [c] Enantiomeric ratio (er) determined by HPLC analysis; [d] Absolute configuration (abs. conf.) of halohydrins (**2a**–**d**) determined by comparing optical rotation sign and retention time (HPLC analysis) with known data; [e] CBS 7536; [f] CBS 6556; [g] Propiophenone (35%) and 1-phenylpropan-1-ol (33%) have been detected by ^{1}H NMR analysis of the reaction crude. [h] Propiophenone (75%) was isolated as the main product, together with 4-bromophenyloxetane (9%, er = 96:4); [i] ND means not determined because of the trace content.

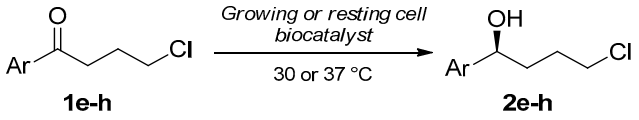
Electronic effects of substituents present on the aromatic ring were also investigated. Upon reduction of 3-chloro-1-(4-fluorophenyl)-1-propanone (**1b**) with baker's yeast (RC), the corresponding alcohol (*S*)-**2b** was formed with a 13% yield and 63:37 er only (Table 1, entry 4), whereas *Lactobacillus reuteri* DSM 20016 (RC) was ineffective (see Table S1, Supporting Information). 1-(4-Bromophenyl)-3-chloro-1-propanone (**1c**) mainly underwent a dechlorination reaction with baker's yeast (RC), furnishing the corresponding propiophenone as the main product (75% yield) together with a small amount of 4-bromophenyloxetane (9% yield), though highly enantio-enriched (96:4 er).

The expected chlorohydrin (*S*)-**2c** formed with a 5% yield only but with 95:5 er (Table 1, entry 5). Finally, the action of baker's yeast (RC) on 1-(4-methoxyphenyl)-3-chloro-1-propanone (**1d**) produced the corresponding propiophenone (5%) as the result of a dehalogenation reaction of the starting ketone. Of note, such a dehalogenation reaction took place at 37 °C also in the absence of yeast. Thus, the elimination reaction was found to be independent from the biocatalyst [41,42], different from the behavior of the other microorganisms [43].

2.2. Screening of Biocatalysts for the Stereoselective Reduction of 4-Chloro-1-Aryl-1-Butanones

Several 4-chloro-1-aryl-1-butanones **1e–h** were also incubated and screened with various whole-cell biocatalysts. Baker's yeast (RC) mediated bioreduction of **1e** took place with moderate yield (44%), affording the chlorohydrin (*S*)-**2e** with an excellent 95:5 er (Table 2, entry 1).

Table 2. Screening of biocatalysts for the stereoselective reduction of 4-chloro-1-aryl-1-butanones [a].



1e-h **2e-h**

Entry	Biocatayst	Ar	Substrate 1	Product 2 (Yield %) [b]	Conversion (%)	er [c]	Abs. Conf. [d]
1	*Baker's yeast* (RC)	C_6H_5	**1e**	**2e** (44)	49	95:5	*S*
2	*S. cerevisiae* (GC) [e]	C_6H_5	**1e**	**2e** (65)	70	49:51	*S*
3	*K. marxianus* (GC) [f]	C_6H_5	**1e**	**2e** (4)	7	42:58	*S*
4	*Baker's yeast* (RC)	4-FC_6H_4	**1f**	**2f** (–) [g]	40	ND [h]	ND [h]
5	*Baker's yeast* (RC)	4-BrC_6H_4	**1g**	**2g** [i]	– [i]	ND [h]	ND [h]
6	*Baker's yeast* (RC)	4-$CH_3OC_6H_4$	**1h**	**2h** (–) [j]	5	ND [h]	ND [h]

[a] Typical reaction conditions: orbital incubator (200 rpm); temperature: 30 °C; (GC): inoculum after 24 h cell growth in a sterile medium containing glucose (1%), peptone (0.5%), yeast extract (0.3%), and malt extract (0.3%) in sterile water; (RC): 0.1 g/L of cell wet mass in 0.1 M KH_2PO_4 buffer (pH = 7.4) enriched with 1% glucose, haloketone (2 mM final concentration); [b] Isolated yield after column chromatography; [c] Enantiomeric ratio (er) determined by HPLC analysis; [d] Absolute configuration (abs. conf.) of halohydrins (**2e–h**) determined both by comparing optical rotation sign and retention time (HPLC analysis) with known data; [e] CBS 7336; [f] CBS 6556; [g] The corresponding butyrophenone (37%) has been detected by ^{1}H NMR analysis of the reaction crude; [h] ND means not determined because of the trace content; [i] No reaction. [j] Chlorohydrin **2h** (5%) has been detected by GC-MS analysis of the reaction crude.

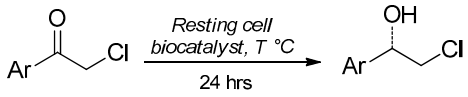
The yields increased up to 65% working with *Saccharomyces cerevisiae* CBS 7336 (GC), even if the corresponding halohydrin was isolated as a racemic mixture (49:51 er) (Table 2, entry 2). *Kluyveromyces marxianus* CBS 6556 (GC) reduced the halo-ketone **1e** both in low yield and enantioselectivity (Table 2, entry 3), whereas *Lactobacillus reuteri* DSM 20016 (RC) promoted the formation of 4-hydroxy-1-phenylbutanone as the only product, by the halogen substitution with a water molecule (Table S2, Supporting Information) [44]. As in the case of 1-arylpropanones, the baker's yeast performance was the best in terms of chemo- and stereo-selectivity. However, the reduction of different aryl-substituted γ-chloro-butyrophenones **1f–h** bearing electron-withdrawing and electron-donating groups proceeded sluggishly in water, presumably because of the poor solubility of the substrates in the used reaction medium or because of the lower intrinsic ketone reactivity, the main products being dehalogenated or hydroxy-substituted derivatives (Table 2 entries 4–6). Thus, the lower bioreduction reaction rates, corresponded to increasingly competitive dehalogenation reactions.

2.3. Screening of Biocatalysts for the Stereoselective Reduction of 2-Chloro-1-Acetophenones

The enantioselective reduction of functionalized α-haloacetophenones by baker's yeast is well-known [45], as well as the synthesis of optically active styrene oxides from haloketones by using isolated alcohol dehydrogenases (e.g., LkDHs from *Lactobacillus kefir*) [46]. Wild-type whole-cell biocatalysts are often preferred as biocatalysts over isolated and purified enzymes because they are

cheaper than isolated and purified enzymes, easy to handle, and have a continuous source of enzymes and efficient internal cofactor (e.g., NAD(P)H) regeneration systems [39,47]. Building on our recent studies on the *anti*-Prelog stereo-preference of *Lactobacillus reuteri* DSM 20016 in the bioreduction of acetophenones [32], we investigated the possibility of preparing both the enantiomers of chiral aryl-epoxides **3i**,**j** (Table 4) carrying out the biotransformations in the presence of either baker's yeast or *Lactobacillus reuteri* DSM 20016 whole cells, followed by cyclization in a basic medium of the corresponding halohydrins **2i**,**2j** (Table 3).

Table 3. Screening of biocatalysts for the stereoselective reduction of 2-chloro-1-arylethanones.



1i,j **2i,j**

Entry	Biocatayst	Ar	Substrate	Chlorohydrin 2 (Yield %) [a]	Conversion (%)	Er [b]	Abs. Conf. [c]
1	*Baker's yeast* [d]	C_6H_5	**1i**	**2i** (53)	55	90:10	*R*
2	*Baker's yeast*	4-ClC_6H_4	**1j**	**2j** (64)	70	63:37	*R*
3	*L. reuteri (RC)* [e]	4-ClC_6H_4	**1j**	**2j** (28)	30	96:4	*S*

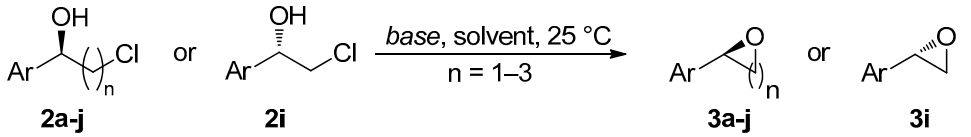
[a] Isolated yield after column chromatography; [b] Enantiomeric ratio (er) determined by HPLC analysis; [c] Absolute configuration (abs. conf.) of halohydrins determined by comparing optical rotation sign with known data; [d] Typical reaction conditions: orbital incubator: 200 rpm; temperature: 30 °C; haloketone (2 mM final concentration) was added to a 0.1 g/L of cell wet mass suspended in tap water (RC); [e] Typical reaction conditions: cells were suspended in PBS at pH 7.4 supplemented with 1% glucose; then, ketone was added at the final concentration of 1 g/L (50 mL total volume), anaerobiosis; temperature: 37 °C; orbital incubator: 200 rpm; [e] DSM 20016.

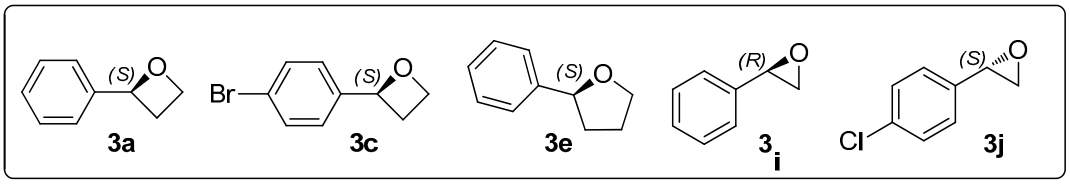
Baker's yeast successfully reduced α-chloroacetophenone **1i** and α-chloro-*p*-chloroacetophenone **1j** providing the expected chlorohydrins (*R*)-**2i** and (*R*)-**2j** with 53% and 64% yields, respectively, and with up to 90:10 er after 24 h incubation at 30 °C (Table 3, entries 1, 2). On the other hand, the *anti*-Prelog stereo-preference of *Lactobacillus reuteri* DSM 20016 [10,32] furnished (*S*)-**2j** with a 28% yield but with a higher stereoselectivity (96:4 er) in comparison with baker's yeast (Table 3, entry 3). Thus, baker's yeast and *Lactobacillus reuteri* DSM 20016 behave as two complementary whole cell biocatalysts for the synthesis of optically active 2-chloro-1-arylethanols because of their ADHs opposite stereo-preference, though with their own substrate specificity (Table 3, entries 2, 3).

2.4. Synthesis of Optically Active 2-Aryloxetanes, 2-Phenyltetrahydrofurans, 2-Arylepoxides

Stereospecific cyclization in basic conditions (*t*-BuOK/THF or NaOH/*i*PrOH, room temperature) of enantio-enriched chlorohydrins **2a**, **2c**, **2e**, **2i**, and **2j** obtained from baker's yeast (*vide supra*) took place smoothly, providing almost quantitatively the corresponding (*S*)-2-aryloxetanes **3a**,**c**, (*S*)-2-phenyltetrahydrofuran (**3e**), and (*S*)-styrene oxide (**3i**) with high er (up to 96:4) (Table 4, entries 1–4). On the other hand, (*R*)-*p*-chlorostyrene oxide **3j** was isolated with a 97% yield and with er = 96:4 further to the bioreduction of **1j** with *L. reuteri* DSM 20016 (Table 4, entry 5). Thus, two terminal enantiomeric arylepoxides could be synthesized exploiting the opposite stereo-preference of two cheap and complementary biocatalysts.

Table 4. Synthesis of optically active 2-aryloxygenated heterocycles **3** from halohydrins **2** [a].





Entry	Ar	Chlorohydrin 2 (er)	*n*	Product 3 (Yield %) [b]	er [c]	Abs. Conf. [d]
1	C_6H_5	(*S*)-**2a** (94:6)	2	**3a** (98)	95:5	*S*
2	4-BrC_6H_4	(*S*)-**2c** (95:5)	2	**3c** (98)	96:4	*S*
3	C_6H_5	(*S*)-**2e** (95:5)	3	**3e** (98)	95:5	*S*
4	C_6H_5	(*R*)-**2i** (90:10)	1 [e]	**3i** (95)	90:10	*R*
5	4-ClC_6H_4	(*S*)-**2j** (96:4)	1 [e]	**3j** (97)	96:4	*S*

[a] Typical reaction conditions: chlorohydrin **2** (1 mmol), *t*-BuOK (3 mmol), THF (5 mL), 25 °C, 4 h; [b] Isolated yield after column chromatography; [c] Enantiomeric ratio (er) determined by GC analysis; [d] Absolute configuration (abs. conf.) of cyclic ethers **3** determined by comparing optical rotation sign with known data; [e] NaOH (3 mL, 1 N) as the base and *i*-PrOH (2 mL) as the solvent were used instead of *t*-BuOK and THF.

3. Materials and Method

3.1. General Methods

^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker Avance 600 MHz (Bruker, Milan, Italy) or Varian Inova 400 MHz spectrometer (Agilent Technologies, Santa Clara, CA, USA) and chemical shifts are reported in parts per million (δ).^{19}F NMR spectra were recorded by using $CFCl_3$ as an internal standard. Absolute values of the coupling constants are reported. FT-IR spectra were recorded on a Perkin-Elmer 681 spectrometer (Perkin Elmer, Waltham, MA, USA). GC analyses were performed on a HP 6890 model Series II (Agilent Technologies, Santa Clara, CA, USA) by using a HP1 column (methyl siloxane; 30 m × 0.32 mm × 0.25 μm film thickness). Thin-layer chromatography (TLC) was carried out on pre-coated 0.25 mm thick plates of Kieselgel 60 F_{254}; visualisation was accomplished by UV light (254 nm) or by spraying a solution of 5% (*w*/*v*) ammonium molybdate and 0.2% (*w*/*v*) cerium(III) sulfate in 100 mL 17.6% (*w*/*v*) aq. sulfuric acid and heating to 200 °C until blue spots appeared. Column chromatography was conducted by using silica gel 60 with a particle size distribution of 40–63 μm and 230–400 ASTM. Petroleum ether refers to the 40–60 °C boiling fraction. GC-MS analyses were performed on a HP 5995C model (Agilent Technologies, Santa Clara, CA, USA) and elemental analyses on an Elemental Analyzer 1106-Carlo Erba-instrument (Carlo-Erba, Milan, Italy). MS-ESI analyses were performed on an Agilent 1100 LC/MSD trap system VL (Agilent Technologies, Santa Clara, CA, USA). Optical rotation values were measured at 25 °C using a Perkin Elmer 341 polarimeter (Perkin Elmer, Waltham, MA, USA) with a cell of 1 dm path length; the concentration (*c*) is expressed in g/100 mL. The enantiomeric ratios were determined by HPLC analysis using an Agilent 1100 chromatograph (Agilent Technologies, Waldbronn, Germany), equipped with a DAD detector, and Phenomenex LUX Cellulose-1 [Cellulose tris(3,5-dimethylphenylcarbamate)], LUX Cellulose-2 [Cellulose 2 tris(3-chloro-4-methylphenylcarbamate)], and LUX Cellulose-4 [Cellulose tris(4-chloro-3-methylphenylcarbamate)] columns (250 × 4.6 mm), or by GC-analyses performed on a Hewlett–Packard 6890 Series II chromatograph (Agilent Technologies, Inc., Wilmington, DE, USA) equipped with a Chirasil-DEX CB (250 × 0.25 μm) capillary column, column head pressure = 18 psi, He flow 2 mL/min, split ratio 100/1, *T* (oven) from 90 to 120 °C. All the chemicals and

solvents were of commercial grade and were further purified by distillation or crystallization prior to use. All optically active halohydrins **2a**–**j** and oxygen-containing heterocycles **3a**–**j** obtained by bioreductions of halo-ketones had analytical and spectroscopic data identical to those previously reported or to the commercially available compounds. Racemic mixtures (for HPLC references) were synthesized by $NaBH_4$ reduction in EtOH with 87%–96% yields according to the reported procedures [32–35].

3.2. Microorganism and Cultures

Saccharomyces cerevisiae CBS 7336 and *Kluyveromyces marxianus* CBS 6556 were obtained from public type culture collections (CBS, DSM, Delft, The Netherlands) under aerobic conditions in a medium containing 0.3% yeast extract, 0.3% malt extract, 0.5% peptone, and 1% glucose. Agar-agar (2%) was added to the same medium for cell preservation on agar slants.

Lactobacillus reuteri DSM 20016 was obtained from a DSMZ culture collection (Braunschweig, Germany) [48]. Cells were maintained at –80 °C in culture broth supplemented with 25% (w/v) glycerol. Pre-cultures and cultures were carried out in a classical MRS medium [49] (Oxoid) containing 20 g/L glucose, 10 g/L peptone, 8 g/L meat extract, 4 g/L yeast extract, 1 g/L Tween 80, 2 g/L di-potassium hydrogen phosphate, 5 g/L sodium acetate·$3H_2O$, 2 g/L tri-ammonium citrate, 0.2 g/L of magnesium sulfate·$7H_2O$, and 0.05 g/L manganese sulfate·$2H_2O$. Cells were incubated at 37 °C for 24 h, statically. Cell density was monitored using optical density at 620 nm (OD_{620}) with a Genesys TM 20 spectrophotometer (Thermo Fisher Scientific Inc., Waltham, MA, USA).

3.3. Blank Experiments

A 1 L flask containing 400 mL of the culture medium was stirred at 30 °C on an orbital shaker at 200 rpm. Halo-ketones **1a**–**j** (50 mg) were added. The reaction was monitored by TLC and stopped after 24 h. The content of the flask was extracted with Et_2O and analyzed by GC-MS or ^1H NMR analysis.

3.4. Bioreduction of Haloketones *1a*,*e* by Yeasts Growing Cells: General Procedure

Cells preserved on agar slants at 4 °C were used to inoculate 250 mL flasks containing 100 mL of the culture medium. The flasks were incubated aerobically at 30 °C on an orbital shaker and stirred at 250 rpm. Flasks (250 mL) containing 100 mL of the culture medium were then inoculated with 5 mL of the 24-h-old suspension and incubated in the same conditions for 24 h. Flasks (1 L) containing 400 mL of the culture medium were then inoculated with 5 mL of the latter suspension and incubated for 24 h. The optical density was checked at 620 nm for all cultures before adding halo-ketones **1a**,**e** (100 mg) previously dissolved in 1 mL of EtOH. The progress of the reactions was monitored by TLC and/or GC and stopped after 24 h, as indicated in Tables 1 and 2. The content of the flask was then centrifuged and the supernatant extracted with EtOAc. All the reactions were repeated at least twice without any detectable bias in the results. Silica gel column chromatography of the reaction crude, using hexane and EtOAc (90:10 or 80:20) as the eluents yielded the desired halohydrins (**2a**,**e**) (Tables 1 and 2).

3.5. Baker's Yeast Bioreductions General Procedure

Baker's yeast (15 g) was dispersed to give a smooth paste in tap water (250 mL). The substrate (100 mg) was added and stirred at 30 °C in an orbital shaker (200 rpm). The reaction progress was monitored by TLC. After 24 h (Tables 1–3), the reaction was stopped by centrifugation, decantation, and extraction by EtOAc or CH_2Cl_2. The extracts were dried over anhydrous Na_2SO_4 and the solvent was evaporated under reduced pressure. The residue was purified by silica gel column chromatography using hexane and EtOAc (10:1 or 8:2) as the eluents to yield the desired halohydrins (**2a**–**j**) (Tables 1–3).

3.6. Characterization Data of Compounds ***2a-c,e,i,j*** *and* ***3a,c,e,i,j***

(*S*)-3-Chloro-1-phenylpropan-1-ol (**2a**) [50]. 42% Yield (from baker's yeast), R_f 0.50 (2:8 ethyl acetate:hexane); $[\alpha]_D^{20}$ = –16.9 (*c* 1, $CHCl_3$), er [*S*]:[*R*] = 94:6 determined by HPLC [LUX Cellulose-1 column (hexane:2-propanol = 90:10), 0.8 mL/min], t_R [major (*S*)-enantiomer] = 11.9 min; t_R [minor (*R*)-enantiomer] = 12.8 min. ^{1}H NMR ($CDCl_3$, 600 MHz, 25 °C, δ): 7.38–7.25 (m, 5 H, aromatic protons), 4.97–4.95 (m, 1 H, C*H*OH), 3.77–3.73 (m, 1 H, C*H*HCl), 3.59–3.55 (m, 1 H, CH*H*Cl), 2.28–2.22 (m, 1 H, C*H*H), 2.13–2.08 (m, 1 H, CH*H*), 1.97–1.89 (bs, 1 H, O*H*, exchanges with D_2O). ^{13}C NMR (150 MHz, $CDCl_3$, 25 °C; δ): 143.7, 128.7, 127.9, 125.8, 71.3, 41.7, 41.4. GC-MS (70 eV) *m/z* (rel.int.): 172 [$(M + 2)^+$, 1], 170 (M^+, 3), 117(2), 115(2), 108(8), 107(100), 105(9), 79(49), 77(28), 31(8).

(*S*)-3-Chloro-1-(4′-fluorophenyl)propan-1-ol (**2b**) [51,52]. 13% Yield (from baker's yeast), R_f 0.40 (1:15 ethyl acetate:hexane); $[\alpha]_D^{20}$ = –8.13° (*c* 0.75, $CHCl_3$), er [*S*]:[*R*] = 63:37, determined by HPLC [LUX Cellulose-1 column (hexane:2-propanol 90:10), 0.8 mL/min], t_R [major (*S*)-enantiomer] = 9.8 min; t_R [minor (*R*)-enantiomer] = 10.5 min. ^{1}H NMR ($CDCl_3$, 400 MHz, 25 °C, δ): 7.36–7.33 (m, 2 H, aromatic protons), 7.07–7.03 (m, 2 H, aromatic protons), 4.96–4.93 (m, 1 H, C*H*OH), 3.77–3.70 (m, 1 H, C*H*HCl), 3.57–3.52 (m, 1 H, CH*H*Cl), 2.25–2.19 (m, 1 H, C*H*H), 2.10–2.03 (m, 1 H, CH*H*), 1.99–1.85 (bs, 1 H, O*H*, exchanges with D_2O). ^{13}C NMR ($CDCl_3$, 125 MHz, 25 °C, δ): 41.5, 41.6, 70.7, 115.5 (d, $^2J_{C–F}$ = 21.0 Hz, 127.4 (d, $^3J_{C–F}$ = 8.0 Hz), 139.4, 162.3 (d, $^1J_{C–F}$ = 246.0 Hz).^{19}F NMR (376 MHz, $CDCl_3$, δ): –114.53, (m). GC-MS (70 eV) *m/z* (rel.int.): 188 (M^+, 3), 126(8), 125(100), 123(11), 97(46), 96(7), 95(15), 77(14).

(*S*)-3-Chloro-1-(4′-bromophenyl)propan-1-ol (**2c**) [53,54]. 5% Yield (from baker's yeast), R_f 0.3 (1:10 ethyl acetate:hexane); $[\alpha]_D^{20}$ = −4.95° (*c* 0.75, $CHCl_3$), er [S]:[R] = 95:5, determined by GC with isotherm at 170 °C, t_R [minor (*R*)-enantiomer] = 43.0 min; t_R [major (*S*)-enantiomer] = 44.3 min. ^{1}H NMR ($CDCl_3$, 400 MHz, 25 °C, δ): 7.49–7.45 (m, 2 H, aromatic protons), 7.25–7.22 (m, 2 H, aromatic protons), 4.14–4.08 (m, 1 H, C*H*OH), 3.77–3.70 (m, 1 H, C*H*HCl), 3.57–3.52 (m, 1 H, CH*H*Cl), 2.22–2.15 (m, 1 H, C*H*H), 2.05–2.00 (m, 1 H, CH*H*), 2.00–1.85 (bs, 1 H, O*H*, exchanges with D_2O). ^{13}C NMR ($CDCl_3$, 150 MHz, 25 °C, δ): 142.7, 131.8, 131.7, 127.5, 121.7, 70.6, 41.5. GC-MS (70 eV) *m/z* (rel.int.): [$(M + 4)^+$, 2]; [$(M+2)^+$, 8]; (M^+, 6), 188 (7), 187 (91), 185 (100), 183 (5), 159 (13), 157 (17), 155 (5), 78 (21), 76 (5), 75 (5), 51 (8), 50 (5).

(*S*)-4-Chloro-1-phenylbutan-1-ol (**2e**) [55]: 44% Yield (from baker's yeast), R_f 0.3 (1:10 ethyl acetate: hexane); $[\alpha]_D^{20}$ = –26° (*c* 1, $CHCl_3$), er [*S*]:[*R*] = 95:5, determined by HPLC [LUX Cellulose-1 coloumn (hexane:2-propanol = 90:10), 0.5 mL/min], t_R [minor (*R*)-enantiomer] = 24.2 min; t_R [major (*R*)-enantiomer] = 25.7 min. ^{1}H NMR ($CDCl_3$, 400 MHz, 25 °C, δ): 7.40–7.27 (m, 5 H, aromatic protons), 4.74–4.71 (m, 1 H, C*H*OH), 3.60–3.53 (m, 2 H), 1.97–1.78 (m, 4 H), 1.85–1.80 (bs, 1 H, O*H*, exchanges with D_2O). ^{13}C NMR ($CDCl_3$, 100 MHz, 25 °C, δ): δ 29.1, 36.4, 45.2, 74.0, 125.9, 128.0, 128.7, 144.5. GC-MS (70 eV) *m/z* (rel.int.): 186 [$(M+2)^+$, 0.4], 184 (M^+, 3), 126 (8), 108 (6), 107 (100), 105 (17), 91 (4), 79 (42), 78 (6), 77 (28).

(*R*)-2-Chloro-1-phenylethanol (**2i**): 53% yield, R_f 0.4 (1:10 ethyl acetate:hexane); $[\alpha]_D^{20}$ = −40° (*c* 0.50, $CHCl_3$) from baker's yeast, er [*S*]:[*R*] = 90:10, determined by HPLC [LUX Cellulose-1 coloumn (hexane:2-propanol 90:10), 0.8 mL/min], t_R [minor (*S*)-enantiomer] = 13.7 min; t_R [major (*R*)-enantiomer] = 15.4 min. ^{1}H NMR ($CDCl_3$, 600 MHz, 25 °C, δ): 7.41–7.38 (m, 5 H, aromatic protons), 4.92–4.91 (m, 1 H, C*H*OH), 3.77–3.75 (m, 1 H, C*H*HCl), 3.68–3.65 (m, 1 H, CH*H*Cl), 2.20 (bs, 1 H, O*H*, exchanges with D_2O).

(*S*)-2-Chloro-1-(4′-chlorophenyl)ethanol (**2j**): 28% Yield, R_f 0.4 (2:8 ethyl acetate:hexane); $[\alpha]_D^{20}$ = +29° (*c* 0.3, $CHCl_3$) from *Lactobacillus reuteri*. er [*S*]:[*R*] = 96:4, determined by HPLC t_R [minor (*R*)-enantiomer] = 17.4 min; t_R [major (*S*)-enantiomer] = 17.8 min. ^{1}H NMR ($CDCl_3$, 600 MHz, 25 °C, δ): 7.36–7.32 (m, 2 H), 7.20–7.16 (m, 2 H), 4.89–4.87 (m, 1 H, C*H*OH), 3.72–3.70 (m, 1 H, C*H*HCl), 3.62–3.59 (m, 1 H, CH*H*Cl), 3.30–2.60 (bs, 1 H, O*H*, exchanges with D_2O). GC-MS (70 eV) *m/z* (rel.int.): 192[$(M + 2)^+$, 3], 190 (M^+, 5), 143 (32), 142 (8), 141 (100), 113 (14), 78 (6), 77 (55); 51 (8), 50 (5), 49 (3).

(*S*)-2-Phenyloxetane (**3a**) [56–58]: 98% Yield, $[\alpha]_D^{20} = -4.3°$ (*c* 1, $CHCl_3$), er [S]:[R] = 95:5 determined by GC with isotherm at 90 °C, t_R [major (*S*)-enantiomer] = 38.3 min; t_R [minor (*R*)-enantiomer] = 40.8 min. ^{1}H NMR ($CDCl_3$, 400 MHz, 25 °C, δ): 7.48–7.27 (m, 5 H, aromatic protons), 5.69–5.65 (m, 1 H), 4.86–4.81 (m, 1 H), 4.69–4.50 (m, 1 H, C*H*H), 3.07–2.98 (m, 1 H, CH*H*), 2.72–2.63 (m, 1 H, CH*H*).

(*S*)-2-(4-Bromophenyl)oxetane (**3c**) [59]: 9% yield, er [S]:[R] = 96:4, determined by GC with isotherm at 150 °C, t_R [minor (*R*)-enantiomer] = 22.5 min; t_R [major (*S*)-enantiomer] = 23.1 min. ^{1}H NMR ($CDCl_3$, 600 MHz, 25 °C, δ): 7.47–7.45 (m, 2 H, aromatic protons), 7.21–7.10 (m, 2 H, aromatic protons), 5.69–5.59 (m, 1 H), 4.83–4.78 (m, 1 H), 4.69–4.63 (m, 1 H), 2.93–2.50 (m, 2 H.

(*S*)-2-Phenyltetrahydrofuran (**3e**) [60]: 98% yield, $[\alpha]_D^{20} = -1.6°$ (*c* 0.50, $CHCl_3$), er [S]:[R] = 95:5 determined by GC with isotherm at 110 °C, t_R [major (*S*)-enantiomer] = 21.8 min; t_R [minor (*R*)-enantiomer] = 22.9 min. ^{1}H NMR ($CDCl_3$, 400 MHz, 25 °C, δ): 7.38–7.26 (m, 5 H, aromatic protons), 4.74–4.71 (m, 1 H), 4.60–3.53 (m, 2 H), 1.98–1.79 (m, 4 H).^{13}C NMR ($CDCl_3$, 150 MHz, 25 °C, δ): 144.3, 128.6, 127.8, 125.8, 73.9, 44.9, 36.2, 28.9.

(*R*)-Styrene oxide (**3i**) [61]: 95% yield, $[\alpha]_D^{20} = -25°$ (*c* 1, $CHCl_3$), er [R]:[S] = 90:10, determined by GC with isotherm at 100 °C, t_R [major (*R*)-enantiomer] = 11.7 min; t_R [minor (*S*)-enantiomer] = 12.3 min. ^{1}H NMR ($CDCl_3$, 600 MHz, 25 °C, δ): 7.31–7.22 (m, 5 H, aromatic protons), 3.83–81 (m, 1 H), 3.13–3.10 (m, 1 H); 2.91–2.87 (m, 1 H). ^{13}C NMR ($CDCl_3$, 150 MHz, 25 °C, δ): 51.0, 52.2, 125.4, 128.1, 128.4, 137.5.

(*S*)-4-Chlorostyrene oxide (**3j**) [62]: 97% yield, $[\alpha]_D^{20} = +23°$ (*c* 1, $CHCl_3$), er [R]:[S] = 4:96 determined by GC with isotherm at 100 °C, t_R [minor (*R*)-enantiomer] = 37.5 min; t_R [major (*S*)-enantiomer] = 39.2 min. ^{1}H NMR ($CDCl_3$, 600 MHz, 25 °C, δ): 7.30–7.27 (m, 2 H, aromatic protons), 7.19–7.17 (m, 2 H, aromatic protons), 3.81–3.79 (m, 1 H), 3.12–3.10 (m, 1 H), 2.73–2.71 (m, 1 H). ^{13}C NMR ($CDCl_3$, 150 MHz, 25 °C, δ): 51.3, 51.8, 126.8, 128.7, 133:9, 136.2.

4. Conclusions

In summary, stereo-defined aryl-substituted oxygen-containing heterocycles have been, for the first time, synthesized via a new chemoenzymatic approach based on the stereoselective whole-cell bioreduction of α-, β-, and γ-chloroalkyl arylketones into the corresponding chlorohydrins, followed by a final stereospecific cyclization. Among the different microorganisms screened (baker's yeast, *Kluyveromyces marxianus* CBS *6556*, *Saccharomyces cerevisiae* CBS 7336, *Lactobacillus reuteri* DSM 20016) baker's yeast was the most efficient in providing chlorohydrins with the best isolated yields ranging from 42% to 64% and the highest er up to 95:5. 3-Chloropropiophenone, 4-chlorobutyrophenone, 4-chloro-4′-bromopropiophenone, and 2-chloroacetophenone have been reduced with good to moderate enantioselectivities by baker's yeast, whereas *Lactobacillus reuteri* DSM 20016 proved to be the best microorganism in performing the bioreduction of 2-chloro-4′-chloroacetophenone with an (*S*) absolute configuration and er up to 96:4. All the optically active chlorohydrins were subsequently stereo-specifically and almost quantitatively converted into optically active *S*-configured 2-aryloxetanes, 2-phenyltetrahydrofuran, and 2-arylepoxides without any erosion of the starting er. (*R*)-*p*-Chlorostyrene oxide could be prepared with the opposite configuration and in up to 96:4 er compared to baker's yeast, by subjecting to cyclization the α-chlorohydrin obtained from *Lactobacillus reuteri* DSM 20016. Since the wild-type whole-cell biocatalysts selected (baker's yeast and *Lactobacillus reuteri* DSM 20016) are cheap and commercially available, this methodology is auspicious for setting up industrially relevant and cost-effective biotransformations for a large-scale production of oxygen-containing heterocycles, and thus for the stereo-selective preparation of chiral drugs [18]. It is noteworthy that the tested substrates were slightly soluble in the aqueous solvents used in the above-mentioned biotransformations. Hence it is very likely that the yield can be further increased by simple process engineering approaches such as the fed-batch supply of the substrate or the use of bioreactors with carefully controlled operational conditions.

Supplementary Materials: The following are available online at www.mdpi.com/2073-4344/7/2/37/s1, Table S1: Screening of biocatalysts for the stereoselective reduction of 3-chloro-1-aryl-propanones, Table S2: Screening of biocatalysts for the stereoselective reduction of 4-chloro-1-aryl-1-butanones.

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