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Article

Deracemization of *sec*-Alcohols through Sequential Application of *C. albicans* and Ketoreductases

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A biocatalytic cascade process was developed using immobilized cells of the wild type yeast *Candida albicans* CCT 0776 in calcium alginate beads and a commercially available ketoreductase. The aim was to promote deracemization by stereoinversion of (±)-1-arylethanols in high substrate concentration (above 100 mmol L^{-1}) to prepare the (*R*)-enantiomers of the alcohols (90-99%), with a high enantiomeric excess (83-99%) after 2 to 19 h. The (*R*)-1-(3-methoxyphenyl)ethanol, with 70% yield and 91% ee, obtained after 5 h was used to prepare (*S*)-1-(3-methoxyphenyl)-ethylamine with 60% yield and 91% ee after two steps, a key intermediate in the synthesis of (*S*)-rivastigmine.

Keywords: oxidative kinetic resolution, deracemization, stereoinversion, ketoreductase, alcohol dehydrogenase

Introduction

Biocatalytic processes using whole cells of microorganisms or isolated enzymes are recognized as useful tools in the preparation of important enantiopure compounds such as chiral alcohols or amines. These compounds are used as building blocks to synthesize natural products, pharmaceuticals, and agricultural chemicals[1-5] such as (*S*)-rivastigmine,[6] taxol,[7] (*S*)-fluoxetine,[8] beta-lactams antibiotics,[9,10] lipid A[11] and L-carnitine[12] and (*S*)-beta-blockers[13] as propranolol, metoprolol and (*S*)-carvedilol (Figure 1).

The use of enzymes was restricted to selective modification of racemic mixtures in kinetic resolution (KR) procedures, resulting in a maximum 50% yield of the enantiopure product[14-20] or reduction of prochiral compounds.[21-23] In recent years, multi-enzymatic methodologies have emerged as useful tools in the preparation of chiral compounds with a theoretical 100% yield. This is because enzymes largely operate under similar conditions, such as neutral pH and moderate temperature, and typically exhibit high substrate specificity.[2,24,25]

Oxidoreductases, such as alcohol dehydrogenases (ADHs, also known as ketoreductases (KREDs)), depend on their (costly) nicotinamide cofactor ($NAD(P)^+$) to catalyze enantioselective reduction of ketones (and aldehydes), as well as the stereoselective oxidation of alcohols.[1,26,27] The use of ADHs to promote deracemization of racemic secondary alcohols, in substrate concentration range of 25-80 mmol L^{-1}, has been studied using a single microorganism,[28-32] a consortium of two microorganisms,[33-35] whole cells of a natural or recombinant biocatalyst and an isolated ADH,[1,36] chemo-enzymatic[6,37] and enzymatic processes[38,39] with a view to obtaining enantiopure compounds exhibiting a 100% maximum yield, since racemic substrates are more readily available starting materials than prochiral substrates.[24]

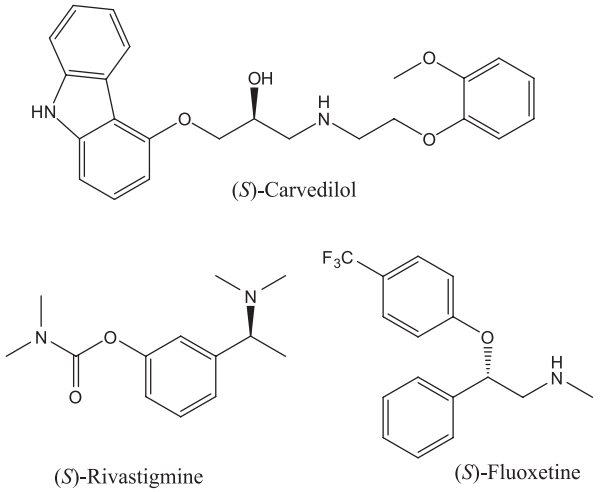

Figure 1. Examples of drugs derived from chiral secondary alcohol precursors.

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Biocatalytic deracemization of secondary alcohols, which means conversion of a racemate into an enantiopure compound mediated by enzymes, generally occurs via two distinct mechanisms: stereoinversion or cyclic deracemization. Deracemization via stereoinversion involves the stereoselective oxidation of an enantiomer into an intermediate ketone, which is subsequently reduced to the alcohol, with the opposite configuration to the enantiomer oxidized, obtaining a single enantiomer at the end of the process.[40] Cyclic deracemization systems, on the other hand, use a low(non)-selective irreversible reaction, such as partially selective reduction of a prochiral ketone, combined with stereospecific oxidation of an enantiomer. Thus, the enantiomer that is not oxidized accumulates after several cycles.[41-43] In this respect, the yeast *Candida* sp. is widely reported as an important source of oxidoreductases and mediates the synthesis of a broad range of chiral compounds.[44] In a previous study, we reported deracemization of (±)-1-phenylethanol via stereoinversion, initially using a sequential process with *Candida albicans* and *Lactobacillus brevis* to prepare the (*R*)-enantiomer. Inverting the sequence of these microorganisms produces an enantioenriched antipode.[14]

Here, we report efficient deracemization via stereoinversion of (±)-1-arylethanols, in high substrate concentration (above 100 mmol L^{-1}), using a cascade process with *Candida albicans* CCT 0776 and different commercial ketoreductases to produce enantioenriched (*R*)-alcohols. Additionally, we demonstrate a practical application of this deracemization procedure and the value of these compounds in synthesizing the key intermediate drug (*S*)-rivastigmine, used to treat Parkinson's and Alzheimer's disease.[45]

Experimental

General methods

The ketones **2a**-**i** and ADH from *Parvibaculum lavamentivorans* (ADH-PL) were purchased from Sigma-Aldrich® and the KREDs from Codexis®. All the other commercial reagents and solvents were purchased from Sigma-Aldrich® at the highest purity available and used as received. Nuclear magnetic resonance (NMR) spectra were recorded in an Avance spectrometer at 400 (^{1}H NMR) and 100 MHz (^{13}C NMR). Chemical shifts were reported in parts *per* million (ppm) and coupling constants (*J*) in hertz (Hz). Optical rotation was measured using a Carl Weiss POLAMAT A polarimeter. Gas chromatography (GC) analyses were performed in an Agilent® 6890 Series GC system equipped with a J&W Scientific DB1 silica capillary column (30 m × 0.25 mm i.d. × 0.25 m film thickness), using helium as the carrier gas (0.9 mL min^{-1}) and a split ratio of 1:50. The injector and detector temperatures were maintained at 230 and 280 °C, respectively. Column temperature was kept at 60 °C for 1 min, increased to 230 °C at a rate of 30 °C min^{-1}, raised again to 280 °C at a rate of 25 °C min^{-1} and then maintained constant for 2 min. One microliter of a 0.3 mg mL^{-1} solution of the compound or extracted reaction aliquots in ethyl acetate was injected. Chiral GC flame ionization detection (FID) analysis was conducted in an Agilent® 6850 Series GC system equipped with a Hydrodex®-β-3P chiral capillary column (25 m × 0.25 mm × 0.25 m). The carrier gas was hydrogen (1 mL min^{-1}) and injector and detector temperatures were set at 200 and 220 °C, respectively. Chiral high-performance liquid chromatography (HPLC) analysis was carried out in an Agilent® Technologies 1200 series system, using a 15 cm × 2.1 mm × 5 μm Supelco Astec cellulose DMP column obtained from Sigma-Aldrich®, with a UV-Vis detector. Thin layer chromatography (TLC) analysis was performed using aluminum plates coated with F254 nm silica gel. The *C. albicans* CCT 0776 yeast strain was purchased from the André Tosello Research Foundation (Campinas, São Paulo State, Brazil) and stored at ultra-low temperature (–80 °C) in 20% glycerol.

Growth conditions of *C. albicans*, cell immobilization, synthesis, characterization of the racemic alcohols (±)-**1a**-**i** and isolated products (*R*)-**1a**-**i** obtained via biocatalysis were reported in a previous study.[14]

Screening of ketoreductases: reduction of acetophenone (**2a**)

The ketoreductases obtained from Codexis, as a lyophilized pounder with 40-70% of the enzyme, were screened in accordance with the producer's protocol. Approximately 10 mg of KREDs 1-5 (Table 1, entries 1-5) were weighed out into separate labelled vials, which were added with 1 mL of a fresh KRED Recycle Mix N solution (containing 50 mmol L^{-1} of **2a**, 250 mmol L^{-1} of potassium phosphate, 2 mmol L^{-1} of magnesium sulfate, 1.1 mmol L^{-1} of NAD^+, 1.1 mmol L^{-1} of $NADP^+$, 80 mmol L^{-1} of D-glucose and 10 U mL^{-1} of GDH (glucose dehydrogenase), pH 7.0). The reaction was mixed at 30 °C and 180 rpm for 19 h. An aliquot of 250 μL of the reaction mixture was extracted with 500 μL of ethyl acetate and dried with sodium sulfate. Conversion and enantiomeric excess were measured by GC-mass spectrometry (MS) and GC-FID. For ADH-PL (Table 1, entry 25), 15 μL of enzyme suspension was used, while for KREDs 6-24 (Table 1, entries 6-24), 10 mg of each enzyme was weighed out into separate labelled vials, which were added with

0.9 mL of KRED Recycle Mix P (containing 125 mmol L^{-1} of potassium phosphate, 1.25 mmol L^{-1} magnesium sulfate, 1 mmol L^{-1} of $NADP^+$, pH 7.0) and 0.1 mL of 250 mmol L^{-1} **2a** solution in 2-propanol. The reaction was mixed at 30 °C and 180 rpm for 24 h. A 250 µL aliquot of the reaction mixture was extracted with 500 µL of ethyl acetate and dried with sodium sulfate. Conversion and enantiomeric excess (ee) were measured by GC-MS and GC-FID.

General procedure for deracemization by stereoinversion using immobilized *C. albicans* cells in calcium alginate beads and a ketoreductase

It was added 0.5 mmol of substrate dissolved in 0.150 mL of acetone as co-solvent to a 125 mL Erlenmeyer flask containing 11 g of *C. albicans* immobilized in calcium alginate beads suspended in approximately 10 mL of distilled water. The flask was incubated at 30 °C and 180 rpm in an orbital shaker, until complete oxidative kinetic resolution (OKR) was achieved, which was monitored by GC-MS (conversion) and GC-FID or HPLC (ee). Next, 1 mL of the reaction was collected, filtered through a 0.2 µm regenerated cellulose membrane filter, and transferred to a 2 mL vial containing 50 mg of dry Recycle Mix N or 25 mg of dry Recycle Mix P, added with 100 µL of 2-propanol and 4 mg of an *anti*-Prelog KRED or 15 µL of ADH-PL. A procedure using 2 mg of KRED instead of 4 mg exhibited no loss of efficiency. The reaction was mixed at 30 °C and 180 rpm for 24 h. A 250 µL aliquot of the reaction mixture was extracted with 500 µL of ethyl acetate and dried with sodium sulfate. Conversion and ee were determined by GC-MS and GC-FID or HPLC. The process was scaled up using the total reaction volume from the OKR step (10 mL); 100 µL of ADH-PL was added with 1 mL of 2-propanol and 250 mg of dry Recycle Mix P. The reaction was mixed at 30 °C and 180 rpm for 5 h. The product was extracted with 10 mL ethyl acetate (3 times), dried with sodium sulfate and the solvent removed in vacuum, producing a colorless liquid, (*R*)-**1h**, 70% yield (0.35 mmol). MS (*m/z*, %): 152 (M^+, 47), 137 (44), 135 (20), 109 (100), 94 (30), 77 (33), 43 (17); ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.48 (d, *J* 6.4 Hz, 3H), 1.99 (s, 1H), 3.81 (s, 3H), 4.86 (q, *J* 6.4 Hz, 1H), 6.81 (m, 1H), 6.94 (m, 2H), 7.25 (t, *J* 8.0 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 25.15, 55.23, 70.34, 110.92, 112.90, 117.70, 129.55, 147.62, 159.79; $[\alpha]_D^{20}$ +28 (*c* 1.0; $CHCl_3$) 91% ee (*R*). Lit.[46] $[\alpha]_D^{20}$ +29 (*c* 1.0; $CHCl_3$), 54% ee for the (*R*)-enantiomer. The ee was determined by GC-FID, with an oven temperature of 100 °C for 3 min, then increased to 180 °C at 1.5 °C min^{-1} and maintained constant for 10 min; retention time: t_R = 22.95 min [(*R*)-enantiomer]; t_S = 23.60 min [(*S*)-enantiomer].

The substrate concentration study with (±)-**1a** (35-200 mmol L^{-1}) was performed with 11 g of *C. albicans* immobilized in calcium alginate beads suspended in approximately 10 mL of distilled water for 1 h. Next, 1 mL of the reaction was collected, filtered through a 0.2 µm regenerated cellulose membrane filter, and transferred to a 2 mL vial containing 4 mg of the ketoreductase KRED-P1-A04, and 25 mg of dry Recycle Mix P. The amount of co-substrates were proportional to initial amount of **1a**, 4 equiv. of acetone in the first step, and 37 equiv. of 2-propanol in the second step.

Synthesis of (*S*)-1-(3-methoxyphenyl)ethylamine ((*S*)-**3h**)

Triphenylphosphine (1.2 equiv.) and phthalimide (1 equiv.) were added to a solution of (*R*)-**1h** (0.23 mmol) in 3 mL of dry tetrahydrofuran (THF), under nitrogen atmosphere and magnetic stirring. The solution was cooled to 0 °C and diisopropyl azodicarboxylate (DIAD, 1.2 equiv.) was added. The mixture was heated to 25 °C and stirred for approximately 3 h. The solvent was removed by vacuum and the product was purified by flash chromatography using a hexane/ethyl acetate gradient as the mobile phase and a Biotage Isolera Spektra One system. The protected amine was dissolved in THF (3 mL) and ethanol (1 mL), and hydrazine monohydrate (50 µL) was added. The reaction was stirred at 66 °C for 2 h. The white suspension formed was filtered and washed with THF, and the organic solvent was evaporated in vacuum, producing a light-yellow oil, (*S*)-**3h**, with 60% yield (0.14 mmol). MS (*m/z*, %): 151 (M^+, 9), 136 (100), 109 (16), 94 (10), 77 (8), 44 (12); ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.39 (d, 3H, *J* 6.8 Hz), 1.86 (br s, 2H), 3.82 (s, 3H), 4.10 (q, 1H, *J* 6.8 Hz), 6.78 (m, 1H), 6.93 (m, 2H), 7.26 (m, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 25.5, 51.3, 55.2, 111.4, 112.2, 118.1, 129.5, 149.4, 159.9; $[\alpha]_D^{20}$ –26.0 (*c* 1.0; $CHCl_3$) 91% ee (*S*), Lit.[47] $[\alpha]_D^{20}$ –26.8 (*c* 1.15; $CHCl_3$) ≥ 96% ee for the (*S*)-enantiomer.[47] In order to determine the enantiomeric excess, the enantioenriched amine (*S*)-**3h** was acetylated and its GC chromatogram compared to that obtained for the acetylated racemic compound. Thus, chemical acetylation was carried out using approximately 1 mg of the amine (*S*)-**3h** dissolved in ethyl acetate (1 mL), added with K_2CO_3 (10 mg) and acetic anhydride (250 µL). The mixture was shaken at 30 °C and 900 rpm for 1 h and an aqueous 10 mol L^{-1} NaOH solution (200 µL) was added. The organic layer was separated, dried with anhydrous sodium sulfate and transferred to a GC glass vial for analysis.[48] Enantiomeric excess was determined by GC-FID at temperatures of 120-160 °C (0.4 °C min^{-1}) and 160-180 °C (5 °C min^{-1}) and

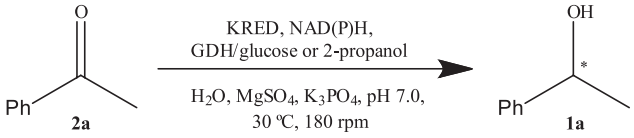
kept constant for 5 min; t_S = 91.86 min [(*S*)-enantiomer]; t_R 93.08 min [(*R*)-enantiomer].

Results and Discussion

Twenty-five commercial KREDs were screened for acetophenone (**2a**) reduction to determine enantioselectivity and conversion to 1-phenylethanol (**1a**). As shown in Table 1, all KREDs exhibited high conversion (90-98%) in reducing **2a** after 4-24 h. Twelve KREDs yielded the enantiomerically enriched alcohol **1a** with > 80% ee, while five KREDs showed Prelog selectivity and produced (*S*)-**1a** with 96-99% ee (Table 1, entries 2-4, 22 and 24), and seven KREDs displayed *anti*-Prelog selectivity producing (*R*)-**1a** with 87-99% ee (Table 1, entries 5, 6, 13, 20, 21 and 25). The reactions were monitored for 4-24 h; based on Codexis' screening protocol, however, in some reactions > 90% conversion was detected after 1 h or, in certain cases, 15 min.

Of the seven KREDs that displayed *anti*-Prelog activity, NADH-dependent KRED-NADH-110 converted 96% of acetophenone (**2a**) to (*R*)-**1a** with 87% ee (Table 1, entry 5). It was selected to couple with resting cells of *C. albicans*, a yeast capable of oxidizing alcohols,[14,29,44] and developing a deracemization process of (±)-**1a** by stereoinversion. Initially, we attempted to combine *C. albicans* and the KRED in a one-pot process; however, this was ineffective, likely due to competition between enzymes with opposite stereoselectivity for the substrate. The yeast was immobilized in calcium alginate beads to ensure easier removal of the biocatalyst from the media

Table 1. Screening of commercial ketoreductases for reduction of acetophenone (**2a**)



entry	KRED	Cofactor	Recycling system	time / h	**1a**	
					Conversion[d] / %	ee[e] / %
1[a]	KRED-101	NADPH	GDH/glucose	19	93	4 (*S*)
2[a]	KRED-119	NADPH	GDH/glucose	19	93	98 (*S*)
3[a]	KRED-130	NADPH	GDH/glucose	19	98	> 99 (*S*)
4[a]	KRED-NADH-101	NADH	GDH/glucose	19	96	> 99 (*S*)
5[a]	KRED-NADH-110	NADH	GDH/glucose	19	96	87 (*R*)
6[b]	KRED-P1-A04	NADPH	2-propanol	4	96	> 99 (*R*)
7[b]	KRED-P1-B02	NADPH	2-propanol	4	98	3 (*S*)
8[b]	KRED-P1-B05	NADPH	2-propanol	4	96	28 (*S*)
9[b]	KRED-P1-B10	NADPH	2-propanol	4	98	4 (*S*)
10[b]	KRED-P1-B12	NADPH	2-propanol	4	98	1 (*R*)
11[b]	KRED-P1-C01	NADPH	2-propanol	4	98	4 (*S*)
12[b]	KRED-P1-H08	NADPH	2-propanol	4	97	9 (*S*)
13[b]	KRED-P1-H10	NADPH	2-propanol	4	96	93 (*R*)
14[b]	KRED-P2-B02	NADPH	2-propanol	4	98	5 (*S*)
15[b]	KRED-P2-C02	NADPH	2-propanol	4	98	7 (*S*)
16[b]	KRED-P2-B02	NADPH	2-propanol	4	96	90 (*R*)
17[b]	KRED-P2-DO3	NADPH	2-propanol	4	98	4 (*S*)
18[b]	KRED-P2-D11	NADPH	2-propanol	4	97	53 (*S*)
19[b]	KRED-P2-D12	NADPH	2-propanol	4	97	8 (*R*)
20[b]	KRED-P2-G03	NADPH	2-propanol	4	96	89 (*R*)
21[b]	KRED-P2-H07	NADPH	2-propanol	4	96	> 99 (*R*)
22[b]	KRED-P3-B03	NADPH	2-propanol	4	95	> 99 (*S*)
23[b]	KRED-P3-G09	NADPH	2-propanol	4	94	46 (*S*)
24[b]	KRED-P3-H12	NADPH	2-propanol	4	96	96 (*S*)
25[c]	ADH-PL	NADPH	2-propanol	24	90	> 99 (*R*)

[a]10 mg of KRED, 1 mL of KRED Recycle Mix N with 50 mmol L^{-1} of **2a**; [b]10 mg of KRED or [c]15 µL of enzyme suspension, 0.9 mL of KRED Recycle Mix P, 0.1 mL of 2-propanol, 25 mmol L^{-1} of **2a**; [d]conversion measured by gas chromatography-mass spectrometry (GC-MS); [e]enantiomeric excess (ee) determined by gas chromatography-flame ionization detector (GC-FID). KRED: ketoreductase; NADH: nicotinamide adenine dinucleotide; ADH-PL: alcohol dehydrogenases from *Parvibaculum lavamentivorans*; NADPH: nicotinamide adenine dinucleotide phosphate; GDH: glucose dehydrogenase.

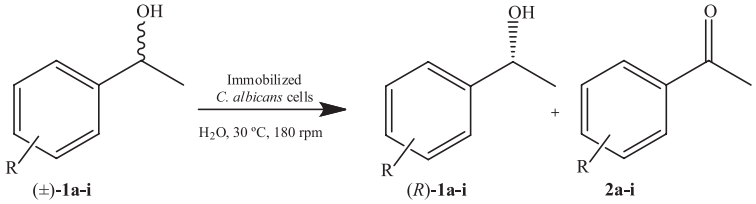
after oxidation (first step). Besides there is the advantage to reuse the beads during four oxidative kinetic resolution (OKR) cycles of (±)-1-phenylethanol (**1a**) without activity loss in each cycle.

Oxidative kinetic resolution (OKR) of (±)-1-arylethanols was performed using immobilized cells in Ca-alginate beads, with reaction times between 40 min and 16 h. For each substrate (**1a**-**i**) the OKR time was optimized in order to obtain the best result possible. The (*R*)-**1a**-**i** were obtained with conversion of 49-79% and 75-99% ee, as well as the corresponding ketone with conversion of 21-51% (Table 2). For (±)-**1a** (Table 2, entry 1), OKR was reached after 40 min and *C. albicans* immobilized in Ca-alginate beads oxidized the (*S*)-enantiomer to ketone acetophenone (**2a**) with 51% conversion, obtaining 49% of (*R*)-**1a** with 94% ee. OKR of (±)-1-(4-chlorophenyl)ethanol ((±)-**1b**) and (±)-1-(4-bromophenyl)ethanol (±)-**1d** yielded the (*R*)-alcohols after a reaction of 4 and 6 h, with 79 and 75% ee, respectively, whereas the reaction was finalized in 1 h for (±)-1-(4-fluorophenyl)ethanol ((±)-**1c**), which also exhibited a halogen in position 4 of the aromatic ring, and (*R*)-1-(4-fluorophenyl)ethanol ((*R*)-**1c**) was obtained with 57% conversion and 90% ee (entry 2-4, Table 2). The presence of a hydroxyl group in position 3 or 4 of the aromatic ring (entry 7 and 9, Table 2) promoted a significant increase in ORK time, from 40 min to 16 h.

A sequential process was proposed for deracemization via stereoinversion: efficient OKR of (±)-**1a** (0.5 mmol) was observed after 40 min with *C. albicans* cells immobilized in calcium alginate beads: 51% acetophenone (**2a**) and 49% (*R*)-1-phenylethanol ((*R*)-**1a**), with 94% ee. Subsequently, the beads were removed and KRED-NADH-110, $NAD(P)^+$, D-glucose, and GDH were dispersed in 1 mL of the reaction mixture. After 15 min of reduction, 90% of (*R*)-**1a** with 91% ee was obtained, resulting in successful deracemization in less than 1 h (Figure 2). The *anti*-Prelog ketoreductases KRED-P1-H10, KRED-P2-H07, KRED-P1-A04, KRED-P2-C11 and ADH-PL, which are dependent of NADPH (nicotinamide adenine dinucleotide phosphate) cofactor, were also coupled with the immobilized yeast cells. In this process, 2-propanol was used to regenerate the NADPH cofactor, enabling efficient deracemization of (±)-1-arylethanols and yielding (*R*)-enantiomers (Table 3).

The deracemization process was efficient and produced (*R*)-1-arylethanols ((*R*)-**1a**-**i**) with excellent conversion (up to 99%) and high ee (up to > 99%) in less than 20 h (oxidative and reductive steps). However, for **1f** and **1g**, successful OKR with *C. albicans* cells immobilized in Ca-alginate beads (Table 2, entries 6 and 7) was observed in the first step, but no or low selective reduction of the respective ketones (**2f** and **2g**) was detected (second step) with the KREDs studied after 24 h (Table 3, entries 15 and 16). The entire deracemization process of

Table 2. Oxidative kinetic resolution (OKR) of 0.50 mmol of 1-arylethanol with *C. albicans* cells immobilized in calcium alginate beads



entry[a]	Substrate	R	time / h	Alcohol	
				Conversion[b] / %	ee[c] / %
1	(±)-**1a**	H	0.67	49	94 (*R*)[c]
2	(±)-**1b**	4-Cl	4	73	79 (*R*)[c]
3	(±)-**1c**	4-F	1	57	90 (*R*)[c]
4	(±)-**1d**	4-Br	6	79	75 (*R*)[c]
5	(±)-**1e**	4-Me	1	55	93 (*R*)[c]
6	(±)-**1f**	4-OMe	2	49	> 99 (*R*)[c]
7	(±)-**1g**	4-OH	16	49	> 99 (*R*)[c]
8	(±)-**1h**	3-OMe	2	57	93 (*R*)[c]
9	(±)-**1i**	3-OH	16	53	96 (*R*)[d]

[a]0.5 mmol of **1a**-**i**, 0.150 mL of acetone, 11 g of *C. albicans* immobilized in calcium alginate beads, 10 mL of H_2O. The reactions were mixed at 30 °C and 180 rpm; [b]conversion monitored by gas chromatography-mass spectrometry (GC-MS); [c]enantiomeric excess (ee) determined by gas chromatography-flame ionization detector (GC-FID); [d]ee was determined by HPLC.

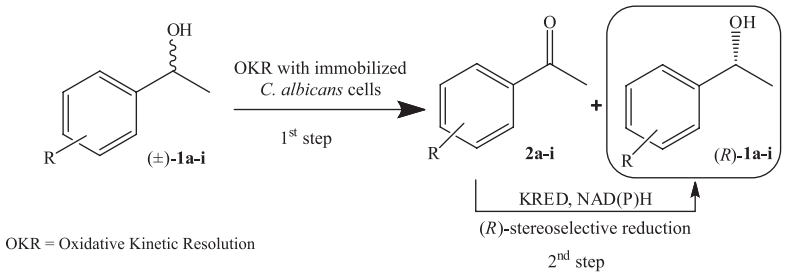


Figure 2. Deracemization by stereoinversion of (±)-1-arylethanols using immobilized *C. albicans* cells in calcium alginate beads and an *anti*-Prelog KRED.

Table 3. Deracemization of (±)-1-arylethanols by stereoinversion using immobilized *C. albicans* cells in calcium alginate beads and a commercial KRED

entry[a]	Substrate	R	Reduction catalyst	time / h	**1a-i**	
					Conversion[e] / %	ee / %
1[b]	**1a**	H	KRED-P1-A04	2	90	97 (*R*)[f]
2[b]			KRED-P1-H10	2	98	92 (*R*)[f]
3[d]			ADH-PL	9	92	95 (*R*)[f]
4[b]	**1b**	4-Cl	KRED-P2-C11	5	> 99	82 (*R*)[f]
5[b]			KRED-P2-H07	5	> 99	83 (*R*)[f]
6[c]			ADH-PL	12	> 99	84 (*R*)[f]
7[b]	**1c**	4-F	KRED-P1-A04	3	90	94 (*R*)[f]
8[b]			KRED-P2-C11	3	92	95 (*R*)[f]
9[c]			ADH-PL	7	91	95 (*R*)[f]
10[b]	**1d**	4-Br	KRED-P1-A04	19	> 99	89 (*R*)[f]
11[b]			KRED-P2-C11	7	> 99	84 (*R*)[f]
12[c]			ADH-PL	14	> 99	79 (*R*)[f]
13[b]	**1e**	4-CH_3	KRED-P2-C11	2	96	96 (*R*)[f]
14[b]			KRED-P2-H07	2	97	95 (*R*)[f]
15[b]	**1f**	4-OCH_3	KRED-P1-A04	24	50	> 99 (*R*)[f]
16[c]	**1g**	4-OH	ADH-PL	40	52	87 (*R*)[f]
17[b]	**1h**	3-OMe	KRED-P1-A04	19	95	93 (*R*)[f]
18[b]			KRED-P2-H07	3	99	93 (*R*)[f]
19[d]			ADH-PL	10	96	91 (*R*)[f]
20[b]	**1i**	3-OH	KRED-P1-A04	17	94	96 (*R*)[g]
21[b]			KRED-P2-H07	17	94	96 (*R*)[g]

[a]1st step: 0.5 mmol of **1a-i**, 0.150 mL of acetone, 11 g of immobilized *C. albicans*, 10 mL of H_2O; 2nd step: 1 mL of the reaction, 25 mg of dry Recycle Mix P, 0.100 mL of 2-propanol; [b]4 mg of KRED or [c]15 μL of ADH-PL; [d]10 mL of oxidative kinetic resolution (OKR) (first step), 0.100 mL of ADH-PL, 1 mL of 2-propanol and 250 mg of dry Recycle Mix P; [e]conversion monitored by gas chromatography-mass spectrometry (GC-MS); [f]enantiomeric excess (ee) determined by gas chromatography-flame ionization detector (GC-FID); [g]ee determined by HPLC. KRED: ketoreductase; ADH-PL: alcohol dehydrogenases from *Parvibaculum lavamentivorans*.

(±)-1-phenylethanol ((±)-**1a**) lasted about 2 h, including 40 min of OKR using immobilized *C. albicans* cells and 1 h of reduction with KRED-P1-A04 or KRED-P1-H10 (entries 1 and 2, Table 3), yielding (*R*)-1-phenylethanol ((*R*)-**1a**) with 90-98% conversion and 92-97% ee. Reduction of acetophenone (**2a**) with ADH-PL was slower, lasting 8 h (entry 3, Table 3). In this case, the complete procedure took 9 h and provided 92% (*R*)-**1a** with 95% ee.

The process was scaled up (10 times) with (±)-**1a** and (±)-**1h** using the ADH-PL. Reproducibility of the results was maintained (entries 3 and 19, Table 3), leading to (*R*)-**1a** and (*R*)-**1h** with excellent conversion (above 90%), 91-95% ee and 70% yield of (*R*)-**1h** starting from 0.5 mmol of racemate (±)-**1h**.

Substrate concentration was evaluated from 35-200 mmol L^{-1} of (±)-**1a**, using *C. albicans* cells immobilized in Ca-alginate beads and 4 mg of KRED-P1-A04 after 2 h (Table 4). The process demonstrated to be efficient for concentration until 110 mmol L^{-1}, furnishing (*R*)-**1a** (91-94%, 90-94% ee, entries 1-4,

Table 4. Study of the substrate concentration in deracemization of (±)-**1a**, using *C. albicans* cells immobilized in Ca-alginate beads and 4 mg of KRED-P1-A04 after 2 h

entry[a]	Substrate concentration ((±)-**1a**) / (mmol L^{-1})	**1a**	
		Conversion[b] / %	ee[c] / %
1	35	94	93 (*R*)
2	55	92	94 (*R*)
3	82	92	93 (*R*)
4	110	91	90 (*R*)
5	150	92	79 (*R*)
6	200	75	40 (*R*)

[a]1st step: (±)-**1a** 35-200 mmol L^{-1}, 4 equiv. of acetone, 11 g of immobilized *C. albicans*, 10 mL of H_2O, 1 h; 2nd step: 1 mL of the reaction, 25 mg of dry Recycle Mix P, 37 equiv. of 2-propanol, 4 mg of KRED-P1-A04, 1 h; [b]conversion monitored by gas chromatography-mass spectrometry (GC-MS); [c]enantiomeric excess (ee) determined by gas chromatography-flame ionization detector (GC-FID).

Table 4). Similar processes were proposed to deracemize secondary alcohols such as 2-octanol, sulcatol, 2-decanol and 1-phenylethanol, with 30-80 mmol L^{-1} substrate concentration.[1,36] Compared to the procedure developed here, we could successfully deracemize 110 mmol L^{-1} of (±)-**1a** and other (±)-1-arylethanols into the (*R*)-enantiomer with high conversion and ee, using commercially available ketoreductases and immobilized yeast.

As an example of the applicability of this procedure, it was obtained (*R*)-**1h** from (±)-1-(3-methoxyphenyl)ethanol ((±)-**1h**) with 70% yield and 91% ee, which was used to prepare (*S*)-1-(3-methoxyphenyl)ethylamine ((*S*)-**3h**), a key intermediate of (*S*)-rivastigmine synthesis that is used to treat Parkinson's and Alzheimer's diseases.[49] Synthesis of (*S*)-**3h** consisted of two steps: a Mitsunobu reaction, with substitution of the hydroxyl group for phthalimide and configuration inversion, followed by phthalimide hydrolysis with hydrazine, yielding 60% (*S*)-**3h** with 91% ee. The preparation of (*S*)-**3h** from (±)-**1h** is shown in Figure 3.

Methodologies with a chemoenzymatic or biocatalytic step have been reported in the preparation of (*S*)-**3h** due to the importance of this compound in organic synthesis;[6,48,50,51] however, none applied deracemization via stereoinversion step with substrate concentration above 100 mmol L^{-1}.

Conclusions

This study demonstrated the significant oxidative potential of *C. albicans* CCT 0776 as an enantioselective catalyst for the oxidation of secondary alcohols, especially (±)-1-arylethanols, and its application in oxidation-reduction sequences for deracemization via stereoinversion of those compounds coupled with commercially available ketoreductases, yielding (*R*)-1-arylethanols, (*R*)-**1a-e** and (*R*)-**1h-i**, with 90-99% conversion and 79-99% ee, after 2-19 h. As an example of the applicability of this procedure, we obtained (*R*)-**1h** from (±)-1-(3-methoxyphenyl)ethanol ((±)-**1h**) with 70% yield and 91% ee, which was used to prepare the (*S*)-rivastigmine precursor (*S*)-1-(3-methoxyphenyl)-ethylamine ((*S*)-**3h**), with 60% yield and 91% ee. These results represent a useful asymmetric green methodology applicable in organic synthesis, since the immobilization of biocatalyst provided the reuse of the catalyst besides the possibility to increase the substrate concentration above 100 mmol L^{-1}.

Supplementary Information

Supplementary data are available free of charge at http://jbcs.sbq.org.br as PDF file.

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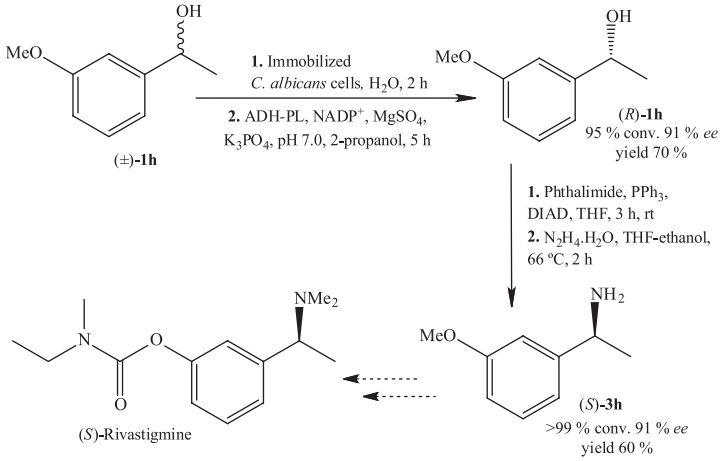

Figure 3. Synthesis of (*S*)-1-(3-methoxyphenyl)ethylamine ((*S*)-**3h**) from (±)-1-(3-methoxyphenyl)ethanol ((±)-**1h**).

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