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Biocatalytic reduction of racemic 2-arenoxycycloalkanones by yeasts *P. glucozyma* and *C. glabrata*: one way of achieving chiral 2-arenoxycycloalcohols

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Abstract Chiral β-aryloxy alcohols are interesting building blocks that form part of drugs like β adrenergic antagonists. Acquiring cyclic rigid analogs to obtain more selective drugs is interesting. Thus, we used whole cells of yeast strains *Pichia glucozyma* and *Candida glabrata* to catalyze the reduction of several 2-arenoxycycloalkanones to produce chiral 2-arenoxycycloalcohols with good/excellent enantioselectivity. In both cases, the alcohol configuration that resulted from the carbonyl group reduction was *S*. Yeast *P. glucozyma* allowed the conversion of both enantiomers of the starting material to produce 2-arenoxycycloalcohols with configuration (1*S*, 2*R*) and (1*S*, 2*S*). The reaction with *C. glabrata* nearly always allowed the kinetic resolution of the starting ketone, recovering 2-arenoxycycloalkanone with configuration *S* and (1*S*, 2*R*)-2-arenoxycycloalcohol.

All the four possible stereoisomers of 2-phenoxycyclo hexanol and the two enantiomers of 2-phenoxycyclohexanone were obtained by combining the biocatalyzed reaction with the oxidation/reduction of the chiral compounds with standard reagents. This is a simple approach for the synthesis of the rigid chiral moiety 2-arenoxycycloalcohols contained in putative β-blockers 2-arenoxycycloalkanepropanolamines.

Keywords Biocatalyst · Chiral 2-arenoxycycloalcohols · *C. glabrata* · *P. glucozyma* · Kinetic resolution

Introduction

Using biocatalysts to perform chemical transformations of organic compounds offers considerable advantages when compared with conventional chemical catalysts: their capacity to produce regio- and stereoselective transformations, their high specificity that results in few side products, and, finally, the use of very mild conditions with low waste pollutant production (Crabtree 2009; Heus et al. 2015; Tao and Kazlauskas 2011; Yang and Ding 2014). For all these reasons, biocatalysts have been broadly used in pharmaceutical, agrochemical, and food industries (Breuer et al. 2004; De Gonzalo et al. 2011; Hollmann et al. 2011; Tao et al. 2009).

Using whole cells as biocatalysts entails several advantages over the use of isolated enzymes: reactions can be carried out without the addition of expensive external cofactors needed in redox reactions, because cells have systems for their efficient in situ recycling; the natural environment in the cells increase the enzyme stability thus preventing denaturation and loss of activity; no additional costs are necessary in the isolation and purification of the enzyme; and, finally, cells act as "reactors" containing multiple enzymes that can work in multistep transformations (Kisukuri and Andrade 2015; Robertson and Steer 2004).

Whole cells of different species have been widely employed for a number of asymmetric transformations,

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especially in bioreduction reactions for preparing chiral alcohols. Although *Saccharomyces cerevisiae* has been the most investigated microorganism (Bariotaki et al. 2012; Csuk and Glänzer 1991; Komentani et al. 1996; Moore et al. 2007; Pscheidt and Glieder 2008; Servi 1990; Yamada and Shimizu 1988), other non-conventional less known yeasts have provided interesting results in other studies conducted by us and others (Andreu and del Olmo 2013, 2014; Contente et al. 2015; Delhi and Gotor 2002; Martínez Lagos et al. 2000; Forti et al. 2015; Fragnelli et al. 2012; Huang et al. 2015; Husain et al. 2011; Martinez Lagos et al. 2002; Martínez Lagos et al. 2004).

Propranolol, an aryloxypropanolamine, was developed in the 1960s (Black et al. 1964) and was the first adrenergic antagonist with β selectivity. The eutomer of all these adrenergic β-blockers is the enantiomer with configuration *S*. Preparation of precursors of such compounds by biocatalytic methods has been described in the literature, sometimes with lipases to resolve racemic halohydrins (Bermudez et al. 1996) and sometimes using yeasts and other microorganisms to reduce stereoselectively haloketones (Martínez Lagos et al. 2000; Martinez Lagos et al. 2002; Martínez Lagos et al. 2004).

Substantial efforts have been made to find cardioselective antagonists that do not block vascular or bronchial β2-receptors. Rigidification of structure is a frequently used drug optimization technique to reduce side effects (Fang et al. 2014) and consists in locking the molecule into a more rigid conformation recognized only by the receptor of interest. A usual method to fix conformations, which could be useful for β-blockers aryloxypropanolamines, is the formation of cycles (Fig. 1). In this case, two new stereogenic centers and up to eight possible stereoisomers with different potential activities would be generated.

The aim of this work was to explore the potential of some non-conventional yeast strains as biocatalysts for the stereoselective reduction of 2-arenoxycycloalkanones to produce the chiral moiety 2-arenoxycycloalkanols. This could be the first and most straightforward approach to the more complex putative β-blockers aryloxycycloalkanepropanolamines.

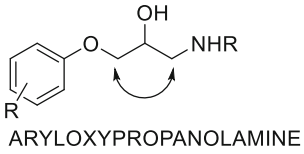

Fig. 1 Fixing conformations by forming cycles

Materials and methods

General

The commercially available reagents were purchased from Sigma-Aldrich. The yeast strains used here were kindly supplied by the Colección Española de Cultivos Tipos (CECT): *Torulospora delbrueckii* (CECT 1015), *Debaryomyces etchellsii* (CECT 11406), *Pichia fermentans* (CECT 1455), *Candida glabrata* (CECT 1448), *Pichia glucozyma* (CECT 11449), *Pichia jadinii* (CECT 1060), and *Kluyveromyces marxianus* (CECT 1018).

Reactions were monitored by thin-layer chromatography (TLC) on Merck silica plates 60 F_{254}, and flash chromatography was performed on Merck silica gel (60 particle size, 0.040–0.063 mm). Melting points were determined in a Cambridge instrument coupled to a Reichter termovar and uncorrected. NMR spectra were recorded with Bruker DRX 300 spectrometers using deuterated chloroform as a solvent. Chemical shifts are reported in parts per million in relation to the residual solvent peak. HRMS were recorded on an AB SCIEX TripleTOF 5600 LC/MS/MS in the infusion positive mode system. Optical rotations were performed on a Perkin Elmer 241 Polarimeter at λ = 589 nm. High-performance liquid chromatography (HPLC) was carried out in a Merck Hitachi Lachrom system. The specific conditions for the determination of enantiomeric excess (a measure of the purity of a chiral substance which indicates the amount of one enantiomer in relation to the other) are described per case and were calculated using the next formula: ee % = 100*([major enantiomer]-[minor enantiomer])/([major enantiomer] + [minor enantiomer]). The semipreparative separations were performed in a LiChrospher 100 RP-18 (10 μm, 250 × 10 mm ID) column, using the appropriate mixture of acetonitrile and water in all cases.

The chemical synthesis of racemic 2-arenoxycyclo alkanones and their full spectroscopic characterization, including the assignation of absolute configuration, is described in the Supplementary Material. Figure S1 shows the spectra and HPLC chromatogram corresponding to (+/−)-**1a**. Synthesis corresponding to all four stereoisomers of 2-arenoxycycloalkanols **2** by chemical reduction of racemic ketones **1** is also included in the Supplementary Material. Figure S2 shows, as example, full characterization of all stereoisomers of **2a**.

Biocatalytic reduction of racemic 2-arenoxycycloalkanones 1: general procedure

For this work, the seven different yeast strains described above were screened.

Cells were grown overnight in a flask containing 200 mL of YPD medium (1 % (*w*/*v*) yeast extract, 2 % (*w*/*v*) bactopeptone, 2 % (*w*/*v*) glucose) to an OD_{600} of

approximately 10 by incubation in an orbital shaker (170 rpm) at 30 °C. Then, a volume corresponding to 500 or 1000 OD_{600} units was taken for each experiment. After centrifugation, the pellet was resuspended by pipetting up and down in 25 or 50 mL of sterile water that contained 2 % (*w*/*v*) glucose. This solution was used to guarantee the metabolic activity of the cells and the cofactor NADH regeneration. The mixture was incubated in a flask for 30 min at 30 °C with orbital shaking. Then the racemic ketone (25 or 50 mg, final concentration of 1 mg/mL) was added, and the reaction was maintained under the above conditions for the time indicated in each case (previously determined by analyses of various aliquots taken at different times). Next, the reaction mixture was stopped by centrifuging cells, and the aqueous supernatant was extracted with ethyl acetate (3 × 40 mL). Combined organic phases were dried over sodium sulfate and the solvent was vacuum-evaporated. To determine the percentage of transformation, the crude material was analyzed by ^{1}HNMR and chiral HPLC. Finally, it was purified by silica gel column chromatography or by semipreparative RP HPLC. The specific conditions for the separation of the remaining ketone and the diastereomeric alcohols in each case are described in Supplementary Material.

Characterization of chiral 2-arenoxycycloalkanols

(−)-(*1S*, *2R*)-*2-Phenoxycyclohexanol* ((*1S*, *2R*)-2a) (Bai et al. 2010) (Fig. S3): mp 42–43 °C lit 43–44 °C.

^{1}H-RMN ($CDCl_3$, 300 MHz) δ 1.28–1.30 (m, 2H, C$\underline{H}_2$), 1.53–1.62 (m, 4H, C$\underline{H}_2$), 1.78–1.96 (m, 2H, C$\underline{H}_2$), 2.12 (m, 1H, O$\underline{H}$), 3.86–3.87 (m, 1H, C$\underline{H}$–O), 4.29–4.33 (m, 1H, C$\underline{H}$–O), 6.87 (m, 3H, C$\underline{H}$ Ar), 7.28 (m, 2H C$\underline{H}$ Ar) ppm.

^{13}C-RMN ($CDCl_3$, 75 MHz): 21.4, 21.8, 26.5, 30.4, 69.2, 77.3, 116.3, 121.2, 129.5, 157.4 ppm.

ODH Chiralcel column, hexane from 0 to 30 min, Hex/*i*prOH 99/1 from 35 min to the end; flow = 1 mL/min; wavelength = 214 nm: 57.0 min.

$[\alpha]_D^{20}$ = −36 (*c* 0.8, $CHCl_3$), 92 % ee.

(+)-(*1S*, *2S*)-*2-Phenoxycyclohexanol* ((*1S*, *2S*)-2a) (Basavaiah et al. 1995; Töke et al. 2006) (Fig. S3): white solid, mp 86–88 °C, lit 82–83 °C.

^{1}H-RMN ($CDCl_3$, 300 MHz) δ 1.25–1.28 (m, 4H, C$\underline{H}_2$), 1.53–1.68 (m, 2H, C$\underline{H}_2$), 2.02–2.07 (m, 2H, C$\underline{H}_2$), 2.54 (m, 1H, O$\underline{H}$), 3.61–3.69 (m, 1H, C$\underline{H}$–O), 3.89–3.94 (m, 1H, C$\underline{H}$–O), 6.87 (m, 3H, C$\underline{H}$ Ar), 7.1 (m, 2H C$\underline{H}$ Ar) ppm.

^{13}C-RMN ($CDCl_3$, 75 MHz): 23.9, 24.0, 29.1, 32.0, 69.2, 82.2, 116.3, 121.2, 129.5, 157.8 ppm.

ODH Chiralcel column, hexane from 0 to 30 min, Hex/*i*prOH 99/1 from 35 min to the end; flow = 1 mL/min; wavelength = 214 nm: 51.6 min.

$[\alpha]_D^{20}$ = +77 (*c* 0.7, $CHCl_3$), 100 % ee.

(−)-(*1S*, *2R*)-*2-(4-Methoxyphenoxy)cyclohexanol* ((*1S*, *2R*)-2b) (Bai et al. 2010):

^{1}H-RMN ($CDCl_3$, 300 MHz) δ 1.26–1.29 (m, 2H, C$\underline{H}_2$), 1.56–1.62 (m, 4H, C$\underline{H}_2$), 1.79–1.90 (m, 2H, C$\underline{H}_2$), 2.18 (m, 1H, O$\underline{H}$), 3.77 (s, 3H, O–C$\underline{H}_3$), 3.84–3.86 (m, 1H, C$\underline{H}$–O), 4.14–4.18 (m, 1H, C$\underline{H}$–O), 6.74–6.77 (m, 2H, C$\underline{H}$ Ar), 6.81–6.85 (m, 2H C$\underline{H}$ Ar) ppm.

^{13}C-RMN ($CDCl_3$, 75 MHz): 21.7, 26.6, 30.4, 55.6, 69.2, 78.6, 114.6, 118.1, 151.2, 154.3 ppm.

ODH Chiralcel column, Hex/*i*prOH 99/1; flow = 1 mL/min; wavelength = 214 nm: 33.7 min.

$[\alpha]_D^{20}$ = −29.6 (*c* 0.1, $CHCl_3$), 83 % ee.

(+)-(*1S*, *2S*)-*2-(4-Methoxyphenoxy)cyclohexanol* ((*1S*, *2S*)-2b) (Matsunaga et al. 2000)**:** solid, mp 84–86 °C, lit 84–86 °C.

^{1}H-RMN ($CDCl_3$, 300 MHz) δ 1.25–1.37 (m, 4H, C$\underline{H}_2$), 1.70–1.74 (m, 2H, C$\underline{H}_2$), 2.07–2.11(m, 2H, C$\underline{H}_2$), 2.60 (m, 1H, O$\underline{H}$), 3.64–3.70 (m, 1H, C$\underline{H}$–O), 3.77 (s, 3H, O–C$\underline{H}_3$), 3.80–3.88(m, 1H, C$\underline{H}$–O), 6.80–6.83 (m, 2H, C$\underline{H}$ Ar), 6.86–6.91 (m, 2H C$\underline{H}$ Ar) ppm.

^{13}C-RMN ($CDCl_3$, 75 MHz): 23.9, 24.0, 29.3, 32.0, 55.6, 73.3, 83.6, 114.2, 118.3, 151.4, 154.2 ppm.

ODH Chiralcel column, Hex/*i*prOH 99/1; flow = 1 mL/min; wavelength = 214 nm: 26.2 min.

$[\alpha]_D^{20}$ = +55.0 (*c* 0.8, $CHCl_3$), 100 % ee.

(−)-(*1S*, *2R*)-*2-(4-Bromophenoxy)cyclohexanol* ((*1S*, *2R*)-2c) (Bai et al. 2010)**:** white solid, mp 65–67, lit 66–67 °C.

^{1}H-RMN ($CDCl_3$, 300 MHz) δ 1.23–1.36 (m, 2H, C$\underline{H}_2$), 1.36–1.64 (m, 4H, C$\underline{H}_2$), 1.64–1.76 (m, 2H, C$\underline{H}_2$), 2.18 (m, 1H, O$\underline{H}$), 3.84–3.86 (m, 1H, C$\underline{H}$–O), 4.24–4.28 (m, 1H, C$\underline{H}$–O), 6.76 (m, 2H, C$\underline{H}$ Ar), 7.30 (m, 2H C$\underline{H}$ Ar) ppm.

^{13}C-RMN ($CDCl_3$, 75 MHz): 21.8, 26.8, 30.8, 69.7, 78.2, 83.0, 113.8, 118.6, 132.8, 157.0 ppm.

ODH Chiralcel column, Hex/*i*prOH 97/3; flow = 0.5 mL/min; wavelength = 214 nm: 27.4 min.

$[\alpha]_D^{20}$ = −22.0 (*c* 0.1, $CHCl_3$), 90 % ee.

(+)-(*1S*, *2S*)-*2-(4-Bromophenoxy)*cyclohexanol ((*1S*, *2S*)-2c) (Basavaiah et al. 1995): white solid, mp 87–89 °C, lit 88–89 °C.

^{1}H-RMN ($CDCl_3$, 300 MHz) δ 1.25–1.36 (m, 2H, C$\underline{H}_2$), 1.52–1.76 (m, 4H, C$\underline{H}_2$), 2.07–2.12(m, 2H, C$\underline{H}_2$), 2.60 (m, 1H, O$\underline{H}$), 3.65–3.75 (m, 1H, C$\underline{H}$–O), 3.88–3.99(m, 1H, C$\underline{H}$–O), 6.83 (m, 2H, C$\underline{H}$ Ar), 7.36 (m, 2H C$\underline{H}$ Ar) ppm.

^{13}C-RMN ($CDCl_3$, 75 MHz): 21.9, 24.2, 29.4, 32.5, 73.7, 82.9, 113.8, 118.6, 132.8, 132.9, 156.9 ppm.

ODH Chiralcel column, Hex/*i*prOH 97/3; flow = 0.5 mL/min; wavelength = 214 nm: 22.4 min.

$[\alpha]_D^{20}$ = +23.0 (*c* 0.2, $CHCl_3$), 100 % ee.

(−)-(*1S*, *2R*)-*2-(Naphthalene-2-yloxy)cyclohexanol*((*1S*, *2R*)-2d) (Bai et al. 2010) (Fig. S8): white solid, mp 71–72 °C, lit 70–72 °C.

^{1}H-RMN ($CDCl_3$, 300 MHz) δ 1.27–1.43 (m, 2H, C$\underline{H}_2$), 1.47–1.69 (m, 4H, C$\underline{H}_2$), 1.61–2.0 (m, 2H, C$\underline{H}_2$), 2.10 (m, 1H, O$\underline{H}$), 3.92–3.98 (m, 1H, C$\underline{H}$–O), 4.47–4.49 (m, 1H, C$\underline{H}$–O), 7.10–7.14 (m, 2H, C$\underline{H}$ Ar), 7.27 (m, 1H C$\underline{H}$ Ar), 7.37 (m, 1H C$\underline{H}$ Ar), 7.67 (m, 3H C$\underline{H}$ Ar) ppm.

^{13}C-RMN ($CDCl_3$, 75 MHz): 21.7, 24.0, 29.0, 30.5, 69.3, 77.3, 109.5, 119.6, 123.8, 126.5, 126.7, 127.6, 129.1, 129.7, 134.4, 155.0 ppm.

ODH Chiralcel column, Hex/*i*prOH 90/10 flow = 0.5 mL/min; wavelength = 214 nm: 44.1 min.

$[\alpha]_D^{20}$ = −51 (*c* 0.3, $CHCl_3$), 100 % ee.

(+)-(*1S*, *2S*)-*2*-(*Naphthalene-2-yloxy*)*cyclohexanol* ((*1S*, *2S*)-2d) (Fig. S8)**:** white solid, mp 139–141 °C.

^{1}H-RMN ($CDCl_3$, 300 MHz) δ 1.25–1.52 (m, 4H, C$\underline{H}_2$), 1.70–1.74 (m, 2H, C$\underline{H}_2$), 2.04–2.21(m, 2H, C$\underline{H}_2$), 2.60 (m, 1H, O$\underline{H}$), 3.63–3.74 (m, 1H, C$\underline{H}$–O), 4.05–4.12(m, 1H, C$\underline{H}$–O), 7.07–7.12 (m, 1H, C$\underline{H}$ Ar), 7.15–7.20 (m, 1H, C$\underline{H}$ Ar), 7.24–7.29 (m, 1H, C$\underline{H}$ Ar), 7.33–7.38 (m, 1H, C$\underline{H}$ Ar), 7.63–7.70 (m, 3H, C$\underline{H}$ Ar) ppm.

^{13}C-RMN ($CDCl_3$, 75 MHz): 21.5, 23.9, 29.7, 32.2, 73.5, 82.1, 109.5, 119.6, 123.8, 126.5, 126.7, 127.6, 129.1, 129.7, 134.4, 155.0 ppm.

HRMS (ESI) Calcd for $[M + H, C_{16}H_{18}O_2]^+$: 243.1380; Found: 243.1369.

ODH Chiralcel column, Hex/*i*prOH 90/10; flow = 0.5 mL/min; wavelength = 214 nm: 31.7 min.

$[\alpha]_D^{20}$ = +49.0 (*c* 0.2, $CHCl_3$), 100 % ee.

(+)-(*1S*, *2R*)-*2*-(*Pyridin-2-yloxy*)*cyclohexanol* ((*1S*, *2R*)-2e) (Bai et al. 2010)**:** white solid, mp 56–58 °C, lit 54–56 °C.

^{1}H-RMN ($CDCl_3$, 300 MHz) δ 1.35–1.47 (m, 2H, C$\underline{H}_2$), 1.63–1.75 (m, 4H, C$\underline{H}_2$), 1.79–2.0 (m, 2H, C$\underline{H}_2$), 3.3 (m, 1H, O$\underline{H}$), 3.94–3.99 (m, 1H, C$\underline{H}$–O), 5.17–5.21 (m, 1H, C$\underline{H}$–O), 6.75–6.78 (m, 2H, C$\underline{H}$ Ar), 6.85–6.88 (m, 1H C$\underline{H}$ Ar), 6.85–6.88 (m, 1H C$\underline{H}$ Ar), 7.54–7.60 (m, 1H C$\underline{H}$ Ar), 8.08–8.11 (m, 1H C$\underline{H}$ Ar) ppm.

^{13}C-RMN ($CDCl_3$, 75 MHz): 21.8, 21.9, 27.8, 30.4, 70.1, 75.4, 111.9, 116.9, 138.9, 146.5, 163.6 ppm.

IC Chiralpak column, Hex/*i*prOH 98/2 flow = 1 mL/min; wavelength = 214 nm: 22.5 min.

$[\alpha]_D^{20}$ = +4.3 (*c* 0.45, $CHCl_3$), 71 % ee.

(−)-(*1S*, *2S*)-*2*-(*Pyridin-2-yloxy*)*cyclohexanol* ((*1S*, *2S*)-2e): white solid, mp 110–112 °C.

^{1}H-RMN ($CDCl_3$, 300 MHz) δ 1.12–1.47 (m, 4H, C$\underline{H}_2$), 1.62–1.75 (m, 2H, C$\underline{H}_2$), 2.02–2.11 (m, 2H, C$\underline{H}_2$), 3.64–3.75 (m, 1H, C$\underline{H}$–O), 4.25 (m, 1H, O$\underline{H}$), 4.69–4.77 (m, 1H, C$\underline{H}$–O), 6.75–6.78 (m, 1H, C$\underline{H}$ Ar), 6.85–6.89 (m, 1H C$\underline{H}$ Ar), 7.55–7.61 (m, 1H C$\underline{H}$ Ar),8.02–8.10 (m, 1H C$\underline{H}$ Ar) ppm.

^{13}C-RMN ($CDCl_3$, 75 MHz): 23.5, 24.3, 30.5, 33.3, 74.1, 80.6, 111.9, 116.8, 139.4, 146.0, 164.3 ppm.

HRMS (ESI) Calcd for $[M + H, C_{11}H_{15}NO_2]^+$: 194.1176; Found: 194.1168.

IA Chiralpak column, Hex/*i*prOH 98/2 flow = 1 mL/min; wavelength = 214 nm: 18.2 min.

$[\alpha]_D^{20}$ = −6.6(*c* 0.40, $CHCl_3$), 100 % ee.

(−)-(*1S*, *2R*)-*2*-*Phenoxycyclopentanol* ((*1S*, *2R*)-2f) (Bai et al. 2010): colorless oil.

^{1}H-RMN ($CDCl_3$, 300 MHz) δ 1.49–1.52 (m, 2H, C$\underline{H}_2$), 1.72–1.97 (m, 4H, C$\underline{H}_2$), 2.35 (m, 1H, O$\underline{H}$), 4.16–4.21 (m, 1H, C$\underline{H}$–O), 4.45–4.50 (m, 1H, C$\underline{H}$–O), 6.86–6.90 (m, 3H, C$\underline{H}$ Ar), 7.19–7.25 (m, 2H C$\underline{H}$ Ar) ppm.

^{13}C-RMN ($CDCl_3$, 75 MHz): 19.7, 28.2, 31.1, 73.3, 79.6, 115.7, 121.2, 129.5, 157.5 ppm.

IA Chiralpak column, hexane from 0 to 30 min, Hex/*i*prOH 99/1 from 35 min to the end; flow = 1 mL/min; wavelength = 214 nm: 52.3 min.

$[\alpha]_D^{20}$ = −68 (*c* 0.6, $CHCl_3$), 90 % ee.

(+)-(*1S*, *2S*)-*2*-*Phenoxycyclopentanol* ((*1S*, *2S*)-2f) (Seemayer and Schneider 1991): colorless oil.

^{1}H-RMN ($CDCl_3$, 300 MHz) δ 1.61–1.86 (m, 4H, C$\underline{H}_2$), 1.75 (m, 1H, O$\underline{H}$), 2.01–2.21 (m, 2H, C$\underline{H}_2$), 4.23–4.27 (m, 1H, C$\underline{H}$–O), 4.23–4.27 (m, 1H, C$\underline{H}$–O), 4.43–4.47 (m, 1H, C$\underline{H}$–O), 6.83–6.86 (m, 3H, C$\underline{H}$ Ar), 7.18–7.23 (m, 2H C$\underline{H}$ Ar) ppm.

^{13}C-RMN ($CDCl_3$, 75 MHz): 21.2, 29.8, 32.6, 77.3, 84.3, 115.5, 120.6, 129.4, 157.9 ppm.

IA Chiralpak column, hexane from 0 to 30 min, Hex/*i*prOH 99/1 from 35 min to the end; flow = 1 mL/min; wavelength = 214 nm: 59.7 min.

$[\alpha]_D^{20}$ = +57 (*c* 0.6, $CHCl_3$), 100 % ee.

(−)-(*1S*, *2R*)-*2*-(*4-Methoxyphenoxy*)*cyclopentanol* ((*1S*, *2R*)-2 g): mp 61–63 °C.

^{1}H-RMN ($CDCl_3$, 300 MHz) δ 1.53–1.64 (m, 2H, C$\underline{H}_2$), 1.77–2.01 (m, 4H, C$\underline{H}_2$), 2.43 (m, 1H, O$\underline{H}$), 3.77 (s, 3H), 4.20–4.25 (m, 1H, C$\underline{H}$–O), 4.41–4.46 (m, 1H, C$\underline{H}$–O), 6.81–6.89 (m, 4H, C$\underline{H}$ Ar) ppm.

^{13}C-RMN ($CDCl_3$, 75 MHz): 19.7, 28.3, 31.1, 55.6, 73.3, 85.2, 114.7, 117.1, 151.6, 154.2 ppm.

HRMS (ESI) Calcd for $[M + H, C_{12}H_{16}O_3]^+$: 209.1172; Found: 209.1166.

ODH Chiralcel column, Hex/*i*prOH 92/8; flow = 0.5 mL/min; wavelength = 214 nm: 26.8 min.

$[\alpha]_D^{20}$ = −29 (*c* 0.4, $CHCl_3$), 100 % ee.

(+)-(*1S*, *2S*)-*2*-(*4-Methoxyphenoxy*)*cyclopentanol* ((*1S*, *2S*)-2 g) (Matsunaga et al. 2000): white solid, mp 46–49 °C.

^{1}H-RMN ($CDCl_3$, 300 MHz) δ 1.56–1.85 (m, 4H, C$\underline{H}_2$), 2.02–2.16 (m, 2H, C$\underline{H}_2$), 3.76 (s, 3H), 4.27–4.31 (m, 1H, C$\underline{H}$–O), 4.41–4.45 (m, 1H, C$\underline{H}$–O), 6.80–6.86 (m, 4H, C$\underline{H}$ Ar) ppm.

^{13}C-RMN ($CDCl_3$, 75 MHz): 21.1, 29.8, 32.6, 55.6, 77.7, 86.5, 114.7, 117.1, 151.6, 154.2 ppm.

ODH Chiralcel column, Hex/*i*prOH 92/8; flow = 0.5 mL/min; wavelength = 214 nm: 18.3 min.

$[\alpha]_D^{20}$ = +39 (*c* 0.6, $CHCl_3$), 100 % ee.

Obtaining and characterizing (1*R*, 2*S*)-2a and (1*R*, 2*R*)-2a (Fig. S6)

(*S*)-**1a** and (*R*)-**1a** were reduced with sodium borohydride in methanol. The mixture of stereoisomers obtained in each case was separated by chromatography in a silica gel column (hexane to hexane/ethyl acetate 99/1) and characterized.

(+)-(*1R, 2S*)-*2-Phenoxycyclohexanol*: ODH Chiralcel column, hexane from 0 to 30 min, Hex/*i*prOH 99/1 from 35 min to the end; flow = 1 mL/min; wavelength = 214 nm: 48.6 min.

$[\alpha]_D^{20}$ = +36 (*c* 0.6, $CHCl_3$), 95 % ee.

(−)-(*1R, 2R*)-*2-Phenoxycyclohexanol* (Basavaiah et al. 1995): ODH Chiralcel column, hexane from 0 to 30 min, Hex/*i*prOH 99/1 from 35 min to the end; flow = 1 mL/min; wavelength = 214 nm: 50.2 min.

$[\alpha]_D^{20}$ = −67 (*c* 0.5, $CHCl_3$), 91 % ee.

Results

Yeast screening and methodology optimization

Racemic 2-phenoxycyclohexanone **1a** was chosen as a model substrate of arenoxycycloalkanone. Reduction of the ketone group allows access to four stereoisomers of 2-phenoxycyclohexanol **2a** (Fig. 2): the *cis* pair as the result of adding hydride to the *re* face of the carbonyl group, while the *trans* pair results from adding to the *si* one.

The goal of this work was to obtain each stereoisomer with the highest enantiomeric purity, and for this purpose, seven different yeast strains were screened as biocatalysts. The chiral HPLC analysis of the results indicated that the enantiomer obtained in excess in each pair was the same with almost all the strains (Table 1). According to the obtained data, *P. glucozyma* and *C. glabrata* can be considered the most promising microorganisms to carry out transformation and were hence chosen for the next experiments. In the first case, all the starting ketone was transformed into *cis* and *trans*-2-phenoxycyclohexanols **2a** with very good and excellent enantiomeric excess, respectively. In the second case, the microorganism carried out a kinetic resolution of the starting ketone. The recovery of one of the enantiomers of **1a** was possible, and also a good yield in enantiomerically enriched 2-

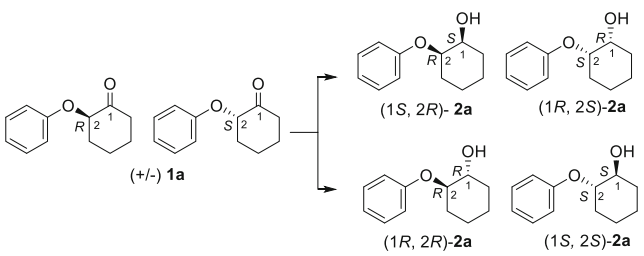

Fig. 2 All the possible stereoisomers of 2-phenoxycyclohexanol

Table 1 Results of the stereoselective reduction of *rac*-**1a** with different yeast strains

Yeast/time (h)	1a[1]−/ee[2]	*cis*-2a[1]/ee[2]	*trans*-2a[1]/ee[2]
T. delbrueckii/48	75/12	7/100	18/100
D. etchellsii/24	32/40	48/0	20/99
P. fermentans/48	78/0	11/100	11/100
C. glabrata/32	40/99	52/92	8/100
P. glucozyma/24	-	57/73	43/100
P. jadinii/48	14/55	62/14	24/0
K. marxianus/70	40/44	10/100	50/16

Conditions: biocatalyst (500 OD_{600} units) in 25 mL of sterile water that contained 2 % glucose, substrate (25 mg), 30 °C, 190 rpm

[1] Conversion (%): percentage of conversion was determined by ^{1}HNMR. Assignation of signals for diastereomeric alcohols was done in accordance with the literature data (Bai et al. 2010; Basavaiah et al. 1995; Töke et al. 2006)

[2] Enantiomeric excess (%): determined by chiral HPLC (Materials and Methods)

phenoxycyclohexanol *cis*. *P. fermentans* and *T. delbrueckii* offered very good results from an enantiomeric viewpoint, but the conversion was too poor in both cases.

The reaction was run again in a higher volume using *P. glucozyma* and *C. glabrata* as biocatalysts (Table 2 and Fig. 3), and the products were purified. The absolute configuration of the enantiomer in excess in each pair was determined.

Two of the four possible stereoisomers were obtained with very good enantioselectivity with both microorganisms. *P. glucozyma* reduced the carbonyl group of both enantiomers of the starting ketone to generate a new stereocenter with configuration *S*, thus obtaining (−)-(1*S*, 2*R*)-**2a** and (+)-(1*S*, 2*S*)-**2a** as products. *C. glabrata* showed more affinity for reacting with one of the enantiomers of the starting material, isolating the alcohol (−)-(1*S*, 2*R*)-**2a** and untransformed

Table 2 Reduction of (±)-**1a** with *C. glabrata* and *P. glucozyma*

Yeast	1a[1]/yield[2]/ee[3]	*cis*-2a1/yield[2]/ee[3]	*trans*-2a[1]/yield[2]/ee[3]
C. glabrata	*S*/30/99	1*S*, 2*R*/32/92	1*S*, 2*S*/8/100
P. glucozyma		1*S*, 2*R*/42/73	1*S*, 2*S*/35/100

Conditions: biocatalyst (1000 OD_{600} units) in 50 mL of sterile water that contained 2 % glucose, substrate (50 mg), 30 °C, 190 rpm, time (h): *C. glabrata*, 32; *P. glucozyma*, 24

[1] The absolute configurations of 2-phenoxycyclohexanols were assigned by measuring the specific rotation and in accordance with the data described in the literature (Bai et al. 2010; Basavaiah et al. 1995; Töke et al. 2006). The absolute configuration of the remaining ketone in the reaction catalyzed by *C. glabrata* was unknown and was determined as described in the main text

[2] Isolated yields (%)

[3] Enantiomeric excess (%) was determined by chiral HPLC following the conditions described in Materials and Methods

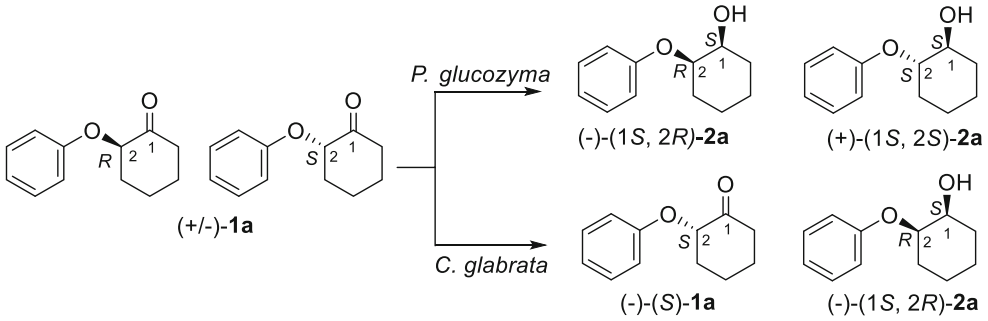

Fig. 3 Main products of the reaction of **1a** with yeasts *P. glucozyma* and *C. glabrata*

levorotatory enantiomer of **1a** as main products, whose absolute configuration (not described in the literature) was then assigned as *S*. This was also confirmed by a comparison with the ketones obtained by oxidation with the pyridinium chlorochromate of the aforementioned alcohols (−)-(1*S*, 2*R*)-**2a** and (+)-(1*S*, 2S)-**2a**, which produced ketone (+)-(*R*)-**1a** and (−)-(*S*)-**1a**, respectively (Figs. S4 and S5).

In order to obtain all the possible stereoisomers of 2-phenoxycyclohexanol **2a** with the highest optical purity, we carried out the reduction of the enantiomerically pure ketone (−)-(*S*)-**1a** recovered from the reaction catalyzed by *C. glabrata* with sodium borohydride. Thus, even when minor racemization was observed under the chemical reduction conditions (less than 5 %), (+)-(1*R*, 2*S*)-**2a** was obtained in very good yield and excellent enantiomeric excess. The (+)-(*R*)-**1a** obtained by the chemical oxidation of (−)-(1*S*, 2*R*)-**2a** was also reduced to provide (−)-(1*R*, 2*R*)-**2a** (Fig. 4 and Fig. S6).

Exploring the scope of the methodology

The reactions with both yeast strains under standard conditions were carried out using several 2-arenoxycycloalkanones with different ketone and aromatic moieties as substrates (Fig. 5 and Tables 3 and 4). The assignation of the absolute configurations of the 2-arenoxycycloalkanols obtained was made in almost all cases by comparing their specific rotation with the data described in the literature (see Materials and methods). The configuration of alcohols (+)-*trans*-**2d**, (−)-*trans*-**2e**, and (−)-*cis*-**2 g** from the biocatalyzed reactions were previously unknown, and according to the results obtained with the other substrates, we assigned the configuration (1*S*, 2*S*) for (+)-*trans*-**2d** and (−)-*trans*-**2e** and (1*S*, 2*R*) for (−)-*cis*-**2g**. In order to confirm this, enantiomerically pure alcohols (−)-c*is*-**2d**, for which configuration (1*S*, 2*R*) was assigned according to the literature data, and (+)-*trans*-**2d** were oxidized with PCC. The comparison of the obtained ketones allowed the unequivocal assignation of the absolute configuration (1*S*, 2*S*) to (+)-*trans*-**2d** (Figs. S7, S8, and S9)**.** The same procedure was carried out with enantiomerically pure alcohols (−)-c*is*-**2g** and (+)-*trans*-**2g** (whose configuration according to the literature was (1*S*, 2*S*)), which allowed the unequivocal assignation of configuration (1*S*, 2*R*) to (−)-*cis*-**2 g**.

As in substrate **1a**, *P. glucozyma* reacted with both enantiomers of the starting material to yield mainly (1*S*, 2*R*) and (1*S*, 2*S*)-2-arenoxycycloalkanols (Table 3). Enantioselectivity was excellent in the *trans* alcohol (100 %) and, almost in all cases, good to excellent in the *cis* one (60–100 %), depending particularly on the arenoxy component. The best results were obtained for the substrates with the phenoxy or 4-methoxyphenoxy moiety (**1a** in Table 2, **1b**, **1f**, and **1g**) and

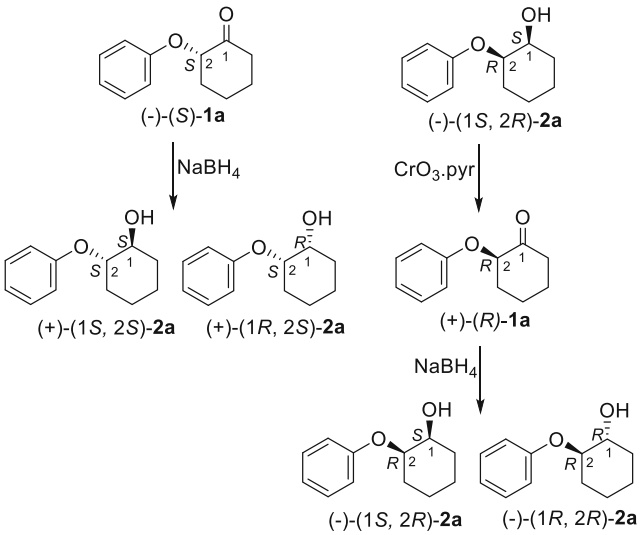

Fig. 4 Strategy to obtain all the possible stereoisomers of **1a** and **2a**

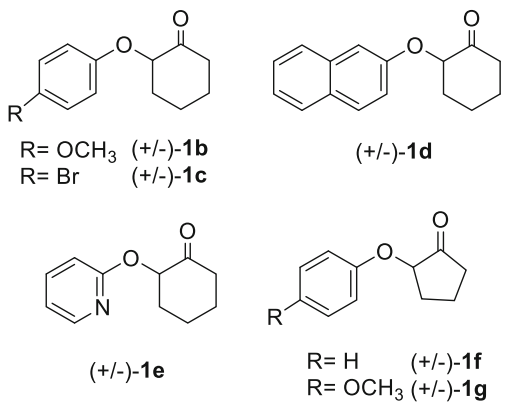

Fig. 5 2-Aryloxycycloalkanones used as substrates for the biocatalytic stereoselective reduction

Table 3 Reactions catalyzed by *P. glucozyma*

(±)-1	1/c-y[2]/ee[3]	*cis*-2[1]/c-y[2]/ee[3]	*trans*-2[1]/c-y[2]/ee[3]
1b	–	(1*S*, 2*R*)-**2b**/55–37/83	(1*S*, 2*S*)-**2b**/45–38/100
1c	**1c**/10–4/nd	(1*S*, 2*R*)-**2c**/50–32/60	(1*S*, 2*S*)-**2c**/40–29/100
1d	**1d**/39–20/0	(1*S*, 2*R*)-**2d**/40–29/53	(1*S*, 2*S*)-**2d**/21–18/100
1e	**1e**/17–14/65	(1*S*, 2*R*)-2**e**/43–40/66	(1*S*, 2*S*)-**2e**/40–37/100
1f	–	(1*S*, 2*R*)-**2f**/52–32/90	(1*S*, 2*S*)-**2f**/48–29/100
1g	**1 g**/10–5/nd	(1*S*, 2*R*)-**2 g**/45–28/100	(1*S*, 2*S*)-**2 g**/45–30/100

Conditions: biocatalyst (1000 OD_{600} units) in 50 mL of sterile water that contained 2 % glucose, substrate (50 mg), 30 °C, 190 rpm, time (h): **1b**, 24; **1c**, 15; **1d**, 72; **1e**, 52; **1f**, 28; **1 g**, 24

[1] Absolute configurations of the 2-aryloxycycloalkanols were assigned by the measurement of the specific rotation and in accordance with data described in the literature (Materials and Methods)

[2] *c* conversion determined by [1] HNMR (%); *y* isolated yield (%)

[3] The enantiomeric excess (%) was determined by chiral HPLC

the worst for the larger and more lipophilic naphthalene-2-yloxy **1d** and 4-bromophenoxy **1c**. Changes in the cycloalkanone component were not that significant and similar results were obtained with cyclopentanone and cyclohexanone (compare **1a–1f** and **1b–1g**).

The reactions catalyzed by *C. glabrata* (Table 4) produced mainly the *cis* 2-arenoxycycloalkanol with (1*S*, 2*R*) configuration in almost all cases, remaining unreacted one enantiomer of the starting ketone. According to this, *S* configuration was assigned to it. Kinetic resolution occurred with good to excellent enantioselectivity for **1a** (Table 2), **1b**, **1e**, **1f**, and **1g** (ee between 73 and 100 %), but it was not possible in those substrates with a more lipophilic or larger arenoxy component (**1c** and **1d**). However, very good enantioselectivity was obtained for these substrates in almost all the *cis* alcohols (70–100 %) and in all cases with the *trans* (100 %) ones.

Discussion

Chiral 2-arenoxycycloalkanols are interesting building blocks in the synthesis of cardioselective adrenergic β1-blockers. Rigidification of structure is a drug optimization strategy that enhances the activity of a drug or cuts its secondary effects (Fang et al. 2014). Flexible molecules can adopt numerous conformations that go unrecognized by the receptor of interest and can hence interact with other receptors to produce other responses or side effects. The formation of cycles is a usual way of locking the drug into its active conformation. However, there are some disadvantages as a result of this technique, for example, the generation of new stereogenic centers and multiple diastereomers, many of which have non-optimal activity. For this reason, the development of simple economic strategies is necessary to synthesize all the stereoisomers to obtain a more active and selective structure.

Reducing the carbonyl group of racemic 2-arenoxycyclo alkanones **1** yields the four possible stereoisomers of 2-arenoxycycloalkanols **2** (Fig. 2). In this work, an easy methodology to synthesize them all with a high degree of enantiomeric purity is described by combining biocatalysis with whole cells of yeasts and conventional chemical reagents (Fig. 6).

The screening of several yeasts strains to catalyze the reduction of 2-phenoxycyclohexanone (*rac*-**1a**), used as a model substrate, indicated that *P. glucozyma* and *C. glabrata* were the most interesting to carry out the biocatalytic reaction, as both microorganisms displayed a complementary behavior (Fig. 3 and Tables 1 and 2). Thus, the reaction with *P. glucozyma* provided two of the four possible stereoisomers, (−)-(1*S*, 2*R*)-**2a** and (+)-(1*S*, 2*S*)-**2a**, with very good enantioselectivity, while the reaction with *C. glabrata* produced *cis*-alcohol (−)-(1*S*, 2*R*)-**2a** and the unreacted enantiomer of **1a** with *S* configuration as the main products. These chiral products were used as starting material to obtain the other two stereoisomer alcohols. Reduction of (−)-*S*-**1a** with sodium borohydride yielded, besides (+)-(1*S*, 2*S*)-**2a**, the *cis* alcohol (+)-(1*R*, 2*S*)-**2a** with excellent stereoselectivity. Oxidation of chiral alcohol (−)-(1*S*, 2*R*)-**2a** from the biocatalyzed reaction with pyridinium chlorochromate gave the enantiomerically pure ketone (+)-*R*-**1a**. Reduction of the

Table 4 Reactions catalyzed by *C. glabrata*

(±)-1	1/c-y[2]/ee[3]	*cis*-2[1]/c-y[2]/ee[3]	*trans*-2[1]/c-y[2]/ee[3]
1b	(*S*)-**1b**/46–32/73	(1*S*, 2*R*)-**2b**/49–35/78	(1*S*, 2*S*)-**2b**/5–3/100
1c	(*S*)-**1c**/42–30/33	(1*S*, 2*R*)-**2c**/38–28/90	(1*S*, 2*S*)-**2c**/20–12/100
1d	(*S*)-**1d**/46–25/0	(1*S*, 2*R*)-**2d**/27–20/100	(1*S*, 2*S*)-**2d**/27–20/100
1e	(*S*)-**1e**/40–38/77	(1*S*, 2*R*)-**2e**/53–37/71	(1*S*, 2*S*)-**2e**/7–5/100
1f	(*S*)-**1f**/40–28/100	(1*S*, 2*R*)-**2f**/57–42/74	(1*S*, 2*S*)-**2f**/3-nd/nd
1 g	(*S*)-**1 g**/38–25/100	(1*S*, 2*R*)-**2 g**/62–40/60	-

Conditions: biocatalyst (1000 OD_{600} units) in 50 mL of sterile water that contained 2 % glucose, substrate (50 mg), 30 °C, 190 rpm, time (h): **1b**, 45; **1c**, 14; **1d**, 72; **1e**, 52; **1f**, 15; **1 g**, 24

[1,2,3] As in Table 3

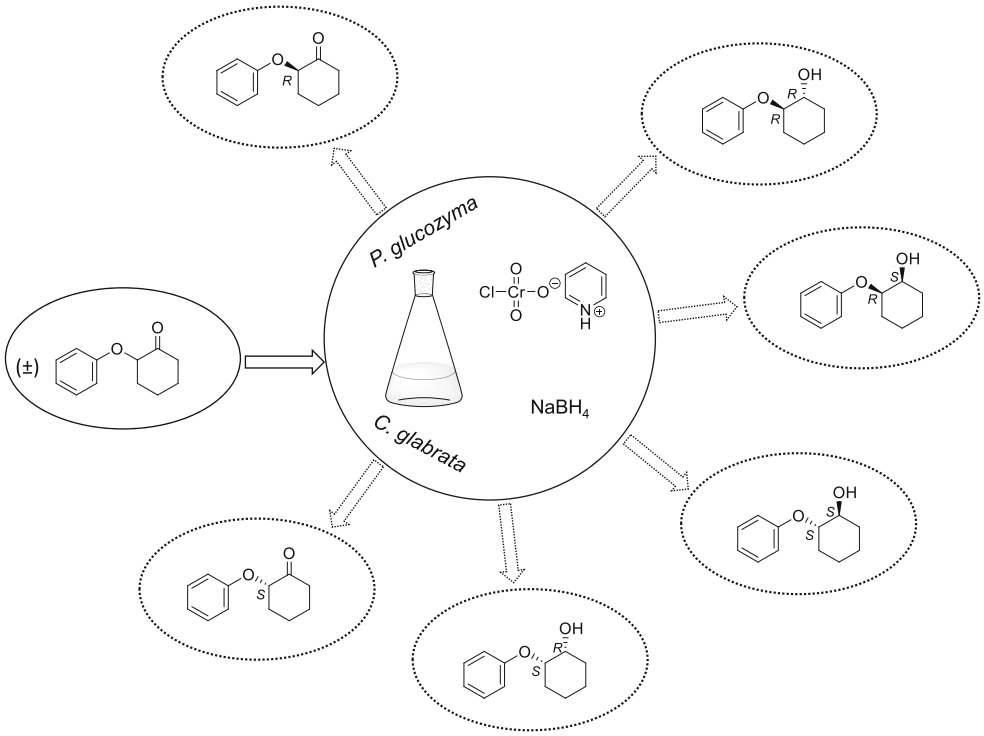

Fig. 6 Methodology to synthesize all the possible stereoisomers of 2-phenoxycyclohexanol with a high degree of enantiomeric purity by combining biocatalysis with whole cells of yeasts and conventional chemical reagents

latter with sodium borohydride allowed us to also obtain, besides (−)-(1*S*, 2*R*)-**2a**, *trans* alcohol (−)-(1*R*, 2*R*)-**2a** (Fig. 4).

In order to investigate the scope of the methodology, several racemic 2-arenoxycycloalkanones with different substituents in the aromatic moiety and ring size were used as substrates for the biocatalytic reaction under the previously optimized conditions (Fig. 5, and Tables 3 and 4). Both yeasts reduced the carbonyl group to produce alcohol with an *S* configuration, but once again, the reaction for *C. glabrata* was faster with the enantiomer of 2-arenoxycycloalkanone with an *R* configuration, and the kinetic resolution of the starting material was possible in nearly all cases.

No influence of cycloalkanones size was found, and similar results were observed in both microorganisms for substrates with a cyclopentanone or cyclohexanone ring. However, the lipophilic nature of the aromatic component seems important in the stereoselectivity of the reaction, as the best results were yielded with those substrates with less lipophilic substituents. As in substrate (+/−)-**1a**, the reaction with *P. glucozyma* yielded *trans*-(1*S*, 2*S*)-2-arenoxycycloalkanols with excellent enantioselectivity and *cis*-(1*S*, 2*R*)-2-arenoxyalkanols with good to excellent stereoselectivity, depending on the arenoxy component. The reactions catalyzed by *C. glabrata* produced mainly *cis*-(1*S*, 2*R*)-2-arenoxycycloalkanol in almost all cases, while the enantiomer of the starting ketone with the *S* configuration remained unreacted. Once again, enantioselectivity was dependent on the arenoxy moiety, and the worst results were obtained with the most lipophilic substrates (**1c** and **1d**) for which the kinetic resolution of the starting ketone was not possible. However, both *cis* and *trans* alcohols were obtained with very good enantioselectivity.

Recent reports have described the usefulness of the microorganism *P. glucozyma* and *C. glabrata* for the stereoselective reduction of several substrates (Contente et al. 2015; Forti et al. 2015; Fragnelli et al. 2012; Huang et al. 2015; Husain et al. 2011). Here, we show a new biotechnological application of whole cells of these non-conventional yeasts to produce in combination with standard chemical reagents all four possible stereoisomers of 2-aryloxycycloalkanols (Fig. 6). These compounds constitute a first approach to synthesize more complex rigid aryloxycycloalkanepropanolamines with putative activity as β-adrenergic blockers.

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Compliance with ethical standards This article does not contain any studies with human participants or animals performed by any of the authors.

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Conflict of interest The authors declare that they have no competing interests.

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