Chemoenzymatic synthesis of (*S*)-Pindolol using lipases

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ABSTRACT

A straightforward chemoenzymatic synthesis of (*S*)-Pindolol has been developed. The key step involved the enzymatic kinetic resolution of *rac*-2-acetoxy-1-(1*H*-indol-4-yloxy)-3-chloropropane with lipase from *Pseudomonas fluorescens* via hydrolytic process to obtain enantiomerically enriched halohydrin *(*2*S*)-1-(1*H*-indol-4-yloxy)-3-chloro-2-propanol (96% *ee*) and (2*R*)-2-acetoxy-1-(1*H*-indol-4-yloxy)-3-chloropropane (97% *ee*). The latter was subjected to a hydrolysis reaction catalyzed by *Candida rugosa* leading to *(*2*R*)-1-(1*H*-indol-4-yloxy)-3-chloro-2-propanol (97% *ee*), followed by a reaction with isopropylamine, producing (*S*)-Pindol (97% *ee*) in quantitative yield.

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1. Introduction

Pindolol, 1-(1*H*-indol-4-yloxy)-3-(isopropylamino)-2-propanol (Fig. 1), is a pharmaceutically active substance, commercially marketed under the brand name Visken (Sandoz) or Barbloc (Alpha) [1]. It is noteworthy that (*S*)-Pindolol (***S*-5**) is 200 times more active than (*R*)-Pindolol [2]. This drug is a nonselective β-blocker, which has a higher intrinsic sympathomimetic activity as compared to any currently available β-blocker, acting effectively as antianginal, antihypertensive, and antiarrhythmic [3]; interestingly, Pindolol is also used in the treatment of glaucoma [4]. It is an effective agent for the treatment of hypertension in pregnancy, since it has no effects on fetal haemodynamics and does not affect fetal cardiac function [5]. Beside these established uses, (*S*)-Pindolol can be used in combination with antidepressants selective inhibitors of serotonin reuptake (SSRI), overcoming the action of these drugs [6,7]. Although an old-fashioned drug, new applications have been reported for the (*S*)-Pindolol, such as in the treatment of fibromyalgia and related fatigue syndromes [8], premenstrual syndrome and premenstrual dysphoric disorder [9], as well as aiding in the treatment of cachexia and sarcopenia [10].

Biocatalysis offers an alternative approach to conventional chemical processes for the production of single-isomer chiral drugs. Lipases are highlighted as being among the most frequently used enzymes for the production of drugs and their intermediates [11-

14]. The use of lipases is mainly due to the characteristics of the regio-, chemo- and enantioselectivity in the resolution process of racemates, without the use of cofactors. Moreover, this class of enzymes has generally excellent stability in the presence of organic solvents, facilitating the solubility of the organic substrate to be modified [14].

The chemoenzymatic synthesis of β-blockers such as propranolol, toliprol, moprolol, alprenolol, penbutenol, atenolol, practolol, oxprenolol, acebutolol, sotalol and nifenalol is a well-documented subject in the literature [15-27].

Many reports regarding the synthesis of (*S*)-Pindolol include the preparation of chiral intermediates via lipase-mediated kinetic resolution as a key step. An example is the hydrolysis of *rac*-1,2-diacetoxy-3-chloropropane, in the presence of lipoprotein lipase, leading to (*S*)-1,2-diacetoxy-3-chloropropane which was converted to (*S*)-3-(4-indolyloxy)-1,2-propanediol (90% *ee* and 20% yield). In this case, the authors stated that this latter chiral intermediate can be used in the synthesis of (*S*)-Pindolol [28]. Another example is the hydrolysis of *rac*-1-acetoxy-2,3-dichloropropane in the presence of pancreatin to give (*S*)-1-acetoxy-2,3-dichloropropane (90% ee). The latter was reacted with 4-hydroxyindole in basic medium, yielding the crude (*S*)-Pindolol in 45% yield. After a benzene treatment, the (*S*)-Pindolol was recovered in 31% yield [$[\alpha]_D^{25}$ = - 2.52 (*c* 1.0, MeOH)]. Finally, after recrystallization from benzene, the (*S*)-Pindolol was obtained in 12% yield [$[\alpha]_D^{25}$ = - 3.4 (*c* 5.9, MeOH)] [29]. Enantiomerically pure (*S*)-Pindolol was prepared from (*R*)-3-chloro-1,2-propanediol in 65% yield [30,31] as well as from (*S*)-glycidyl nosylate in 83% yield [32], but the authors did not report how these intermediates were obtained. Another synthesis of (*S*)-Pindolol (88% yield and 92% *ee*) include a catalytic asymmetric nitroaldol reaction using Lanthanum-Lithium-(*R*)-BINOL, (*R*)-LLB, as chiral catalyst [33].

In general, a major drawback in the use of epichlorohydrin or glycidyl esters as chiral intermediates in the preparation of β-blockers is a competition between substitution at C-1 and ring-opening at C-3 during the nucleophilic attack of the anion aryloxy, leading to a decrease in the optical purity of the final product [1,30,34]. An effective approach to circumvent this problem is the enzymatic kinetic resolution of chlorohydrins, *rac*-1-aryloxy-3-chloro-2-propanol, to obtain the corresponding chiral compound to be used as intermediate. This approach has been applied in the synthesis of a series of (*S*)-β-blockers as propranolol, toliprol, moprolol, alprenolol, penbutenol, atenolol, practolol and oxprenolol. In this case, two complementary methods were used, the hydrolysis of *rac*-2-acetoxy-1-aryloxy-3-chloropropane and acetylation of *rac*-1-aryloxy-3-chloro-2-propanol using vinyl acetate as acyl donor and *tert*-butyl methyl ether (TBME) as solvent, both methods in the presence of lipase from *Pseudomonas* sp. [15]. More recently, CAL-A (CLEA) [25] and CAL-B [27] mediated the kinetic resolution of halohydrins, precursors of the β-blocker Atenolol, with E values > 200. It is noteworthy that various optically pure aromatic chlorohydrins were prepared via dynamic kinetic resolution (DKR) with the use of lipase from *Pseudomonas cepacia* (PS-C "Amano" II) combined with an efficient racemization with Ru complex catalyst [35].

To the best of our knowledge, there are no reports relating to the preparation of chiral halohydrin 1-(1*H*-indol-4-yloxy)-3-chloro-2-propanol from lipase-mediated kinetic resolution to be used as an intermediate for the synthesis of (*S*)-Pindolol.

Herein, we wish to report the chemoenzymatic synthesis of (*S*)-Pindolol (***S*-5**) in which the key step was the preparation of (2*R*)-1-(1*H*-indol-4-yloxy)-3-chloro-2-propanol (***R*-3**) (Fig. 1) as intermediate, in the presence of lipases. We have focused especially on the optimization of the reactional conditions.

2. Experimental

2.1. Enzymes

(i) Immobilized lipases: *C. antarctica* lipase type B immobilized on acrylic resin (CAL-B, Novozym 435, 7,300.0 U/g) and *R. miehei* lipase immobilized on anionic resin (RML, 150.0 U/g) were purchased from Novozymes®. *T. lanuginosus* lipase immobilized on immobead-150 (TLL, 250.0 U/g), *R. oryzae* lipase immobilized on immobead-150 (ROL, 340.0 U/g) and Amano lipase PS from *B. cepacia* immobilized on diatomaceous earth (PS-IM, ≥ 500 U/g) were acquired from Sigma-Aldrich® (ii) Crude lipase preparations: *P. fluorescens* lipase (AK, 22,100.0 U/g), *P. camemberti* lipase (G, 50.0 U/g), *A. melleus* lipase (Acylase I, 200.0 U/g), Amano lipase PS from *B. cepacia* (PS, ≥ 30,000U/g), lipase from *R. niveus* (RNL, ≥ 1.5 U/mg), Amano lipase from *M. javanicous* (MJL, ≥ 10,000 U/g) and lipase from *A. niger* (ANL, 200 U/g) were acquired from Sigma-Aldrich®. Porcine pancreas lipase (PPL, 46.0 U/g solid), and *C. rugosa* lipase (CRL, 1.4 U/g) were obtained from Sigma®.

2.2. Chemical materials

Chemical reagents were purchased from different commercial sources and used without further purification. Acetone, hexane and dichloromethane were acquired from Synth®. Toluene was obtained from Dinamica® and dioxane from Carlo Erba®. Isopropanol (IPA), acetonitrile and hexane, HPLC grade, were purchased from TEDIA®. Tetrahydrofuran (THF), heptane, cyclohexane and *tert*-butylmethylether (TBME) were acquired from Sigma-Aldrich®. Solvents used in the reaction of biocatalysis were distilled over an adequate desiccant under nitrogen. Analytical TLC analyses were performed on aluminum sheets pre-coated with silica gel 60 F254 (0.2

mm thick) from Merck®. Flash chromatographies were performed using silica gel 60 (230-240 mesh).

2.3. Analysis

Melting points of *rac*-2-acetoxy-1-(1*H*-indol-4-yloxy)-3-chloropropane (***rac*-4**) and (*S*)-Pindolol (***S*-5**) were determined in open capillary tube Mettler Toledo model FP62 and are uncorrected. IR spectra were recorded on a Perkin - Elmer 1720-X F7 using NaCl plates or KBr in pellets. ^{1}H and ^{13}C NMR were obtained using Spectrometer Bruker model Avance DRX-500, operating at frequency of 500 MHz for hydrogen and frequency of 125 MHz for carbon. The chemical shifts are given in delta (δ) values and the absolute values of coupling constants (*J*) in Hertz (Hz). Measurement of the optical rotation was done in a Perkin-Elmer 241 polarimeter. High performance liquid chromatography (HPLC) analyses were carried out in a Shimadzu chromatograph model LC solution 20A using a chiral column Chiracel® OD-H (150 m x 4.6 mm I.D.) with oven set at 35 °C . For the following of the reaction time courses: Flow was 1.0 mL/min using hexane:IPA (80:20) as eluent and UV detector at 265 nm. Retention times were: (*S*)-acetate (***S*-4**) 7.8 min; (*R*)-acetate (***R*-4**) 8.8 min; (*S*)-alcohol (***S*-3**) 10.9 min; (*R*)-alcohol (***R*-3**) 25.1 min.

2.4 Calculation of enantiomeric excess, conversion and enantioselectivity

The efficiency of the kinetic resolution was evaluated based on the optical purity of the compounds, expressed in terms of enantiomeric excess of the substrate ($e.e._s$) and product ($e.e._p$), using the following Eq. (1) and (2):

$$e.e._s = \frac{X - Y}{X + Y} \qquad (1)$$

$$e.e._{p} = \frac{X - Y}{X + Y} \quad (2)$$

X denote the major enantiomer and Y denote the minor enantiomer represented by their chromatographic peak areas.

The conversion (*c*) is calculated using Eq. (3):

$$c = \frac{e.e._{s}}{e.e._{s} + e.e._{p}} \quad (3)$$

Enantioselectivity was expressed as enantiomeric ratio (E) and calculated by Eq. (4):

$$\mathrm{E} = \frac{\ln[1 - c(1 + e.e._{p})]}{\ln[1 - c(1 - e.e._{p})]} \quad (4)$$

The results of the $e.e._{s}$ and $e.e._{p}$ are expressed in percentage using the Eq. (5) and (6):

$$e.e._{s} = \frac{X - Y}{X + Y} \text{ x } 100 \quad (5)$$

$$e.e._{p} = \frac{X - Y}{X + Y} \text{ x } 100 \quad (6)$$

Additionally, the conversion (*c*) is also calculated in percentage, using (5) and (6).

*2.5. Synthesis of rac-1-(1*H*-indol-4-yloxy)-3-chloro-2-propanol (**rac-3**)*

To a suspension of 500 mg (3.75 mmol) of 4-hydroxyindole (**1**) in 5 mL of aqueous solution of sodium hydroxide (NaOH 0.75 M), 2.5 mL (32 mmol) of (±)-epichlorohydrin in 2 mL of dioxane was added. The reaction was stirred for 6 h at room temperature under N_2. The progress of the reaction was monitored by thin layer chromatography using dichloromethane as eluent. After this time, the solvent was

evaporated under reduced pressure. The crude product was purified by flash chromatography on silica gel using dichloromethane as eluent, yielding *rac*-1-(1*H*-indol-4-yloxy)-3-chloro-2-propanol (***rac*-3**) (66% yield) and (1*H*-indol-4-yloxy)(2-oxiranyl)methane (***rac*-2**) (22% yield). This latter was converted to *rac*-1-(1*H*-indol-4-yloxy)-3-chloro-2-propanol (***rac*-3**) as follows: to a solution of 152.4 mg (0.81 mmol) of (1*H*-indol-4-yloxy)(2-oxiranyl)methane (***rac*-2**) in 5 mL of dichloromethane, 5 mL of concentrated HCl was added. The reaction mixture was stirred for 1 hour. Then, the product was extracted with dichloromethane (3 x 5 mL) and dried with anhydrous Na_2SO_4. After filtration, the solvent was evaporated under reduced pressure. The crude product was purified by chromatography using silica gel and dichloromethane as eluent. Finally, the *rac*-1-(1*H*-indol-4-yloxy)-3-chloro-2-propanol (***rac*-3**) was obtained with an overall yield of 85% (718.8 mg).

*2.6. Synthesis of rac-2-acetoxy-1-(1*H*-indol-4-yloxy)-3-chloropropane (**rac-4**)*

DMAP (22 mg, 0.19 mmol) and acetic anhydride (360 μL, 3.8 mmol) were added to a *rac*-1-(1*H*-indol-4-yloxy)-3-chloro-2-propanol (***rac*-3**) (50 mg, 0.38 mmol) solution in dichloromethane (4.0 mL). The reaction was stirred at room temperature during 1 h and after that time, the solvent was evaporated under reduced pressure. The resulting crude product was purified by flash chromatography on silica gel , using dichloromethane as eluent, to afford the desired *rac*-2-acetoxy-1-(1*H*-indol-4-yloxy)-3-chloropropane (***rac*-4**) as a yellow liquid in 93% yield (94.5 mg).

*2.7. Synthesis of (2R)-1-(1*H*-indol-4-yloxy)-3-chloro-2-propanol (**R-3**) via hydrolysis*

A suspension of *rac*-2-acetoxy-1-(1*H*-indol-4-yloxy)-3-chloropropane (***rac*-4**) (5 mg, 0.019 mmol) and 10 mg of lipase from *Pseudomonas fluorescens* in a mixture of 152 μL of phosphate buffer 100 mM (pH 7.0) and 38 μL of THF (80/20 v/v) was shaken

at 40 °C and 250 rpm for 25 h. After this time, the products were extracted with EtOAc (3 x 1.0 mL) and the organic phases were combined and dried with anhydrous Na_2SO_4, filtered and the solvent evaporated under reduced pressure. The crude product was purified by flash chromatography on silica gel using dichloromethane as eluent, yielding 2 mg of (2*R*)-2-acetoxy-1-(1*H*-indol-4-yloxy)-3-chloropropane (***R*-4**) (40.5% % yield and 97% *ee*) and 2 mg of (2*S*)-1-(1*H*-indol-4-yloxy)-3-chloro-2-propanol (***S*-3**) (46.7% yield and 96% *ee*). Then, a suspension of (2*R*)-2-acetoxy-1-(1*H*-indol-4-yloxy)-3-chloropropane (***R*-4**) (5 mg, 0.019 mmol) and 5 mg of lipase from *Candida rugosa* (ratio 1:1 in weight respect to the ***R*-4**) in a mixture of 152 µL of phosphate buffer 100 mM (pH 7.0) and 38 µL of THF (80/20 v/v) was shaken at 40 °C, 250 rpm for 12 h. After this time, the reaction mixture was centrifuged, the supernatant was removed and the product was extracted with EtOAc (3 x 1.0 mL). The organic phases were combined and dried with anhydrous Na_2SO_4, filtered and the solvent evaporated under reduced pressure, yielding 4.3 mg of *(*2*R*)-1-(1*H*-indol-4-yloxy)-3-chloro-2-propanol (***R*-3**) (100% yield, $[\alpha]_D^{20}$ = + 2.19 (*c* 0.9, $CHCl_3$) for 97% *e.e.* of the (*R*)-enantiomer).

2.8. Synthesis of (*S*)-*Pindolol (**S*-5**)*

A solution of (2*R*)-1-(1*H*-indol-4-yloxy)-3-chloro-2-propanol (***R*-3**) (100 mg, 0.44 mmol) in excess of isopropylamine (4 mL) was stirred under reflux for 12 h. After this time, the excess of amine was evaporated under reduced pressure and the product was extracted with EtOAc (3 x 1.0 mL). The solvent was evaporated under reduced pressure, yielding 109.5 mg of (*S*)-Pindolol (***S*-5**) as a white solid in quantitative yield, ($[\alpha]_D^{20}$ = - 4.1 (*c* 0.62, MeOH) for 97% *e.e.* of the (*S*)-enantiomer. Lit $[\alpha]_D^{20}$ = - 3.8 (*c* 0.62, MeOH) for 92% *e.e.* of the (*S*)-enantiomer) [33].

*2.9. General procedure for the lipase-catalyzed hydrolysis of rac-2-acetoxy-1-(1*H*-indol-4-yloxy)-3-chloropropane (**rac-4**) (screening) Table 1*

A suspension of *rac*-2-acetoxy-1-(1*H*-indol-4-yloxy)-3-chloropropane ***rac*-4** (5 mg, 0.019 mmol), 5 mg of lipase (ratio 1:1 in weight respect to the ***rac*-4**) in a mixture of 152 μL of phosphate buffer 100 mM (pH 7.0) and 38 μL of THF (80/20 v/v) was shaken at 30 °C, 250 rpm for varied times (ranging from 12 to 36 h). After each period, the products were extracted with EtOAc (3 x 1.0 mL) and the organic phases were combined and dried with anhydrous Na_2SO_4, filtered and the solvent evaporated under reduced pressure. The crude product was purified by flash chromatography on silica gel using dichloromethane as eluent, yielding (2*R*)-2-acetoxy-1-(1*H*-indol-4-yloxy)-3-chloropropane (***R*-4**) and (2*S*)-1-(1*H*-indol-4-yloxy)-3-chloro-2-propanol (***S*-3**). Their enantiomeric excess were determined by HPLC.

*2.10. General procedure for the lipase-catalyzed acetylation of rac-1-(1*H*-indol-4-yloxy)-3-chloro-2-propanol (**rac-3**) Table 3*

To a suspension of the *rac*-1-(1*H*-indol-4-yloxy)-3-chloro-2-propanol (***rac*-3**) (4 mg, 0.018 mmol) and lipase (4 mg) in dry organic solvent (0.18 μL) under nitrogen atmosphere, vinyl acetate (7.8 μL, 0.085 mmol) was added, and the reaction was shaken (250 rpm) at temperatures ranging from 30 °C to 40 °C. Aliquots were regularly analyzed by HPLC and after the adequate time, the reaction was stopped and the enzyme filtered off and washed with the respective solvent (3 mL). The solvent was evaporated under reduced pressure and the crude products purified by flash chromatography on silica gel using dichloromethane as eluent. This procedure yielded (2*S*)-2-acetoxy-1-(1*H*-indol-4-yloxy)-3-chloropropane (***S*-4**) and (2*R*)-1-(1*H*-indol-4-

yloxy)-3-chloro-2-propanol (***R*-3**), being their enantiomeric excess determined by HPLC.

rac-1-(1*H*-indol-4-yloxy)-3-chloro-2-propanol (***rac*-3**): Oil. R_f (100% CH_2Cl_2): 0.30. IR υ_{max} (cm^1): 3403, 2924, 1616, 1585, 1502, 1440, 1241, 1087 and 736 cm^{-1}. ^1H NMR (500 MHz, CD_3OD) δ (ppm): 2.66 (br s, 1H), 3.8 (dd, 1H, *J*=11.2 Hz and 5.6 Hz), 3.9 (dd, 1H, *J*= 11.2 Hz and 5.0 Hz), 4.2-4.3 (m, 3H), 6.6 (d, 1H, *J*= 7.6 Hz), 6.7 (m, 1H), 7.1-7.2 (m, 3H) and 8.2 (br s, 1H).^{13}C NMR (125 MHz, CD_3OD) δ (ppm): 46.4 (CH_2), 68.9 (CH_2), 70.2 (CH), 99.9 (CH), 101.2 (CH), 105.4 (CH), 118.9(C), 122.9 (CH), 123.2 (CH), 137.6 (C) and 152.1 (C).

rac-(1*H*-indol-4-yloxy)(2-oxiranyl)methane (***rac*-2**): Oil. R_f (100% CH_2Cl_2): 0.57. IR υ_{max} (cm^{-1}): 3401, 1361, 1240 and 1086 cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$) δ (ppm): 2.8 (dd, 1H, *J*= 5.0 Hz and 2.6 Hz), 2.9 (m, 1H), 3.4-3.5 (m, 1H), 4.2 (dd, 1H, *J*= 11.1 Hz and 5.5 Hz), 4.4 (dd, 1H, *J*= 11.1 Hz and 3.2 Hz), 6.5 (d, 1H, *J*= 7.6 Hz), 6.7 (m, 1H), 7.0-7.1 (m, 3H) and 8.2 (br s, 1H). ^{13}C NMR (125 MHz, $CDCl_3$) δ (ppm): 45.1 (CH_2), 50.6 (CH), 69.0 (CH_2), 100.2 (CH), 101.1 (CH), 105.2 (CH), 119.0 (C), 122.9 (CH), 123.0 (CH), 137.6 (C) and 152.4 (C).

rac-2-acetoxy-1-(1*H*-indol-4-yloxy)-3-chloropropane (***rac*-4**): Solid. Mp: 101-106 °C. R_f (100% CH_2Cl_2): 0.65. IR υ_{max} (cm^{-1}): 3409, 1736, 1224, and 1047 cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$) δ (ppm): 2.1 (s, 3H), 3.8 (dd, 1H, *J*= 11.7 Hz and 5.5 Hz), 3.9 (dd, 1H, *J*= 11.7 Hz and 4.8 Hz), 4.3 (m, 2H), 5.4-5.5 (m, 1H), 6.5 (d, 1H, *J*= 7.6 Hz), 6.7 (m, 1H), 7.0-7.1 (m, 3H), and 8.2 (br s, 1H). ^{13}C NMR (125 MHz, $CDCl_3$) δ (ppm): 21.1 (CH_3), 43.0 (CH_2), 66.5 (CH_2), 71.5 (CH), 100.0 (CH), 101.2 (CH), 105.4 (CH), 119.0 (C), 122.9 (CH), 123.1 (CH), 137.6 (C), 152.1 (C) and 170.5 (C).

(*S*)-Pindolol (***S*-5**): Solid. Mp: 168-170 °C. IR υ_{max} (cm^{-1}): 3303, 2964, 1242, 1086, and 879 cm^{-1}. ^{1}H NMR (500 MHz, CD_3OD) δ (ppm): 1.12 (d, 3H, *J*= 5.4 Hz), 1.13 (d, 3H, *J*= 5.2 Hz), 2.7-2.8 (m, 1H), 2.9-3.0 (m, 2H), 4.0-4.2 (m, 3H), 6.5 (dd, 1H, *J*= 6.7 Hz and 1.4 Hz), 6.53-6.54 (m, 1H), 7.0-7.1 (m, 3H). ^{13}C NMR (125 MHz, CD_3OD) δ (ppm): 22.3 (CH_3), 22.4 (CH_3), 50.1 (CH), 51.1 (CH_2), 69.8 (CH), 72.0 (CH_2), 99.7 (CH), 101.3 (CH), 106.3 (CH), 120.2 (C), 123.1 (CH), 124.2 (CH), 139.3 (C) and 153.6 (C). $[\alpha]_D^{20}$ = - 4.1 (*c* 0.62, MeOH) for 97% *e.e.* of the (*S*)-enantiomer. Lit $[\alpha]_D^{20}$ = - 3.8 (*c* 0.62, MeOH) for 92% *e.e.* of the (*S*)-enantiomer [33].

3. Results and Discussion

The chemoenzymatic route for the production of (*S*)-Pindolol (***S*-5**) is depicted in Fig. 1. The key step involved the preparation of the enantiomerically enriched halohydrin *(*2*R*)-1-(1*H*-indol-4-yloxy)-3-chloro-2-propanol (***R*-3**) via kinetic resolution in the presence of a lipase.

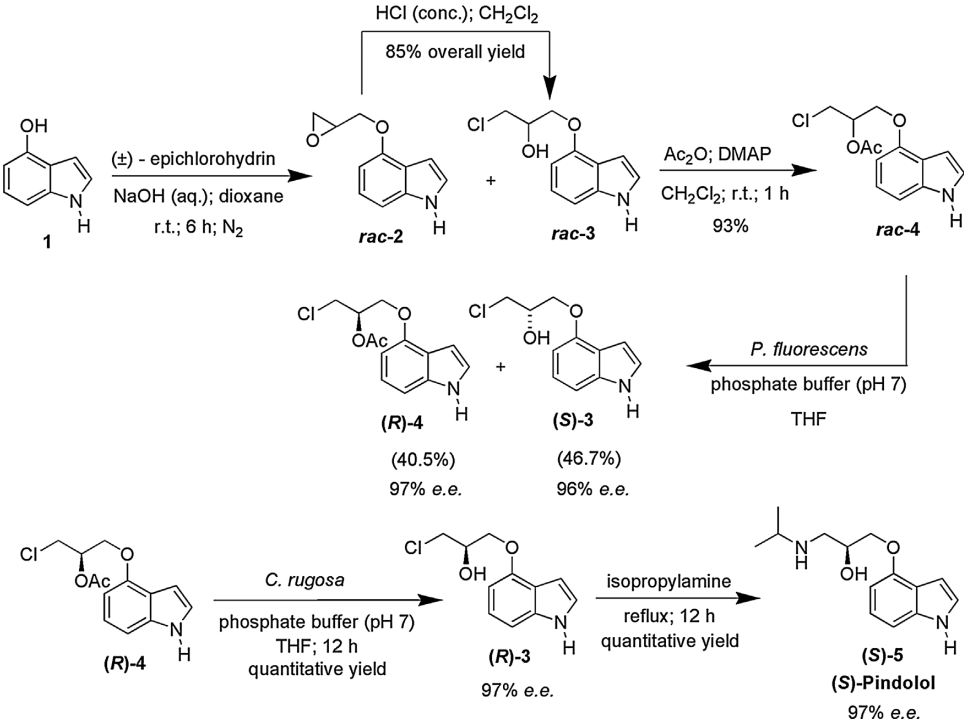

Fig. 1. Chemoenzymatic synthesis of (*S*)-Pindolol.

*3.1. Synthesis of halohydrin rac-1-(1*H*-indol-4-yloxy)-3-chloro-2-propanol (**rac-3**) and its acetate (**rac-4**)*

Initially, the halohydrin *rac*-1-(1*H*-indol-4-yloxy)-3-chloro-2-propanol (***rac*-3**) was obtained from the reaction between the commercially available reagents 4-hydroxyindole (**1**) and epichlorohydrin. In this case, beyond the expected halohydrin, *rac*-1-(1*H*-indol-4-yloxy)-3-chloro-2-propanol (***rac*-3**) (66% yield), the epoxide *rac*-(1*H*-indol-4-yloxy)(2-oxiranyl)methane (***rac*-2**) (22% yield) was obtained. Then, the epoxide was converted to the desired halohydrin after treatment with concentrated HCl. Thus, it was possible to obtain the *rac*-1-(1*H*-indol-4-yloxy)-3-chloro-2-propanol (***rac*-3**) with global yield of 85%. Subsequently, by the use of acetic anhydride and 4-dimethylaminopyridine (DMAP), the corresponding acetate *rac*-2-acetoxy-1-(1*H*-indol-4-yloxy)-3-chloropropane (***rac*-4**) was prepared with 93% yield. Both compounds, ***rac*-3** and ***rac*-4**, were analyzed by chiral HPLC to find the adequate conditions for the separation of their enantiomers (Fig. 2).

INSERT FIGURE 2 A and 2 B HERE

Fig. 2. A) HPLC chromatograms of *rac*-2-acetoxy-1-(1*H*-indol-4-yloxy)-3-chloropropane (***rac*-4**: (*S*) t_R 19.1 min.; (*R*) t_R 20.9 min.)

Fig. 2 B) *rac*-1-(1*H*-indol-4-yloxy)-3-chloro-2-propanol (***rac*-3**: (*S*) t_R 27.7 min.; (*R*) t_R 34.0 min.).

*3.2. Kinetic resolution of rac-2-acetoxy-1-(1*H*-indol-4-yloxy)-3-chloropropane (**rac-4**) via hydrolysis reaction*

Initially, thirteen commercially available lipases were evaluated in the kinetic resolution of *rac*-2-acetoxy-1-(1*H*-indol-4-yloxy)-3-chloropropane (***rac*-4**) via hydrolysis reaction,

Fig. 3 (Path a). The reactions were conducted at 30 °C, using 1:1 ratio (w:w) of substrate/lipase, phosphate buffer (pH 7.0) and THF as a co-solvent (buffer/co-solvent 4:1, v:v). The reactions were monitored by chiral HPLC and stopped within 12-36 h, when the conversions reached values close to 50%. The experimental data are summarized in Table 1.

INSERT FIGURE 3 HERE

Fig. 3. (Path a) Enzymatic kinetic resolution of ***rac*-4** via hydrolysis reaction. (Path b) Enzymatic kinetic resolution of ***rac*-3** via acetylation reaction.

Table 1. Kinetic resolution via hydrolysis of *rac*-2-acetoxy-1-(1*H*-indol-4-yloxy)-3-chloropropane (***rac*-4**) using lipases.[a]

Entry	Lipase	Reaction time (h)	*e.e.*$_s$ (%)[b]	*e.e.*$_p$ (%)[b]	*c* (%)[c]	E[d]
1	*P. camemberti*	24	-	-	0	-
2	*R. oryzae* immobilized on immobead-150	24	-	-	0	-
3	Porcine pancreas	24	-	-	0	-
4	*R. miehei* immobilized on anionic resin	24	-	-	0	-
5	*R. niveus*	24	-	-	0	-
6	Amano lipase from *M. javanicous*	24	-	-	0	-
7	*P. fluorescens*	36	81	87	48	36

8[e]	*P. fluorescens*	23	90	94	49	100
9[f]	*P. fluorescens*	25	97	96	50	207
10	*T. lanuginosus* immobilized on immobead-150	36	80	94	46	79
11[e]	*T. lanuginosus* immobilized on immobead-150	33	87	93	48	78
12[f]	*T. lanuginosus* immobilized on immobead-150	24	81	89	48	43
13	Novozym 435	12	91	81	53	30
14	*C. rugosa*	20	37	54	41	5
15	Amano lipase PS from *B. cepacia* immobilized on diatomaceous earth	12	63	78	45	15
16	Amano lipase PS from *B. cepacia*	12	55	63	47	8
17	*A. niger* [g]	24	54	64	46	8

[a] Conditions: 30 ºC, lipase:***rac*-4** (1:1) at 250 r.p.m.

[b] Determined by HPLC.

[c] Conversion, $c = e.e._s/(e.e._s+e.e._p)$.

[d] Enantiomeric ratio, $E = \ln[1-c(1+e.e._p)]/\ln[1-c(1-e.e._p)]$

[e] Conditions: 40 ºC, lipase:***rac*-4** (1:1) at 250 r.p.m.

[f] Conditions: 40 ºC, lipase:***rac*-4** (2:1) at 250 r.p.m.

[g] Inverse configurations

Lipases from *P. camemberti*, *R. oryzae* immobilized on immobead-150, porcine pancreas, *R. miehei* immobilized on anionic resin, *R. niveus* and Amano lipase from *M. javanicous* (Table 1, entries 1-6) were inactive in kinetic resolution of *rac*-2-acetoxy-1-(1*H*-indol-4-yloxy)-3-chloropropane (***rac*-4**), under the above conditions. Novozym 435, Amano PS from *B. cepacia* immobilized on diatomaceous earth and Amano PS from *B. cepacia* were the most active among the evaluated lipases, leading to conversion values near 50% in only 12 h of reaction, however with low-to-medium E-values, ranging from 8 to 30 (Table 1, entries 13, 15-16). *C. rugosa* showed an intermediate activity, leading to 41% of conversion in 20 h, with low enantioselectivity (Table 1, entry 14). The best enantioselectivity (E = 79) was presented by lipase from *T. lanuginosus*, with 46% conversion after 36 h (Table 1, entry 10). In order to decrease the reaction time, the temperature was increased from 30 to 40 °C, showing only a slight acceleration from 36 h to 33 h, keeping the enantioselectivity basically unchanged (Table 1, entry 11). A further attempt to decrease the reaction time was performed by increasing the enzyme-substrate ratio from 1:1 to 2:1. Actually, the reaction time decreased to 24 h, however followed by a decrease in enantioselectivity with E value of 43 (Table 1, entry 12). Lipase from *P. fluorescens* has provided a conversion value close to 50% with moderate enantioselectivity (E= 36), but in a very long reaction time, 36 h, (Table 1, entry 7). In order to reduce the reaction time, the temperature was increased from 30 to 40 °C (Table 1, entry 8). In this case, besides decreasing the reaction time from 36 to 23 h, there was an increase in selectivity of kinetic resolution with E values ranging from 36 to 100 (Table 1, entry 8). It is generally assumed that enantioselectivity in enzyme-catalyzed reactions decreases with increasing temperature. However, there are several examples in the literature of enzymatic reactions in which

enantioselectivity increases with increasing temperature [36-44]. In these situations, the degree of flexibility in the active site of the enzyme is more evident than the stereostatic and electrostatic effects among the components of the reaction, leading to a preference of the enantiomer favored by entropy [37,39,44].

This latter result prompted us to verify if the reaction time would be decreased with a greater amount of enzyme. Thus, the ratio enzyme/substrate was increased from 1:1 to 2:1 (Table 1, entry 9). However, we noticed a slight increase in the reaction time from 23 to 25 h, but with significant increment in the enantioselectivity (E from 100 to 207), Table 1, entries 8 and 9. Recently, Wahab et al. reported an increase in enantioselectivity in the esterification of (*R*,*S*)-ibuprofen in the presence of lipase from the thermophilic *Geobacillus zalihae* with increasing enzyme loading [45]. Concerning the enantiopreference, all the evaluated lipases showed a marked preference for leading to halohydrin ***S*-3**, with the notable exception of lipase from *A. niger*, which produces the halohydrin ***R*-3** albeit with low enantioselectivity (E value of 8; Table 1, entry 17).

Based on the optimized conditions for systems previously studied in our research group [46,47], hydrolysis reactions of the *rac*-2-acetoxy-1-(1*H*-indol-4-yloxy)-3-chloropropane (***rac*-4**) were performed in the presence of THF as co-solvent. The enantioselectivity of the reaction underwent a dramatic increase with increasing temperature and enzyme:substrate ratio. However, at this stage of our investigation it was not clear the influence of the THF as co-solvent on the activity and selectivity of lipase from *P. fluorescens*. Therefore, we decided to investigate the effect of other co-solvents on the efficiency of the enzymatic kinetic resolution of *rac*-4. In addition, the kinetic resolution of ***rac*-4** was performed in the absence of co-solvents.

*3.3. Co-solvent effect on the enzymatic kinetic resolution of rac-2-acetoxy-1-(1*H*-indol-4-yloxy)-3-chloropropane (**rac-4**) via hydrolytic process*

The study of the effect of co-solvent in the hydrolysis of *rac*-2-acetoxy-1-(1*H*-indol-4-yloxy)-3-chloropropane (***rac*-4)** was carried out in the presence of lipase from *P. fluorescens*, as it presented the best results of conversion and enantioselectivity (Table 1, entry 9). Apart from THF, which was evaluated in three different ratio with respect to the buffering medium, hydrolysis reactions were carried out in the presence of acetone, acetonitrile, dioxane, isopropanol, ethanol, *tert*-butyl methyl ether (TBME) and hexane as co-solvents (buffer/co-solvent 4:1, v:v), keeping the temperature at 40 °C, ratio lipase/substrate 2:1 (w:w) and 25 h of reaction time. It should be noted that the aforementioned reaction was also performed in the absence of co-solvent. All the experimental data have been summarized in Table 2.

Table 2. Co-solvent effect on the lipase from *P. fluorescens*-mediated enzymatic kinetic resolution of *rac*-2-acetoxy-1-(1*H*-indol-4-yloxy)-3-chloropropane (***rac*-4**)[a]

Entry	Co-solvent	log*P*	*e.e.*$_s$ (%)[b]	*e.e.*$_p$(%)[b]	*c*(%)[c]	E[d]
1	None	-	55	73	43	11
2	Acetone	-0.24	85	84	50	31
3	Acetonitrile	-0.34	75	89	46	39
4	Dioxane	-0.27	62	88	41	29
5	Isopropanol	-0.16	50	68	42	8
6	Ethanol	-0.10	9	68	12	6
7	THF[e]	0.46	97	96	50	207
8	THF[f]	0.46	80	72	53	15
9	THF[g]	0.46	98	95	51	180

10	TBME	0.94	83	82	50	26
11	Hexane	3.90	2	12	14	2

[a] Conditions: 40 ºC, 25 h and lipase from *P. fluorescens*:***rac*-4** (2:1) at 250 r.p.m.

[b] Determined by HPLC.

[c] Conversion, $c = e.e._s/(e.e._s+e.e._p)$.

[d] Enantiomeric ratio, $E = \ln[1-c(1+e.e._p)]/\ln[1-c(1-e.e._p)]$

[e] (buffer/THF 8:2, v:v)

[f] (buffer/THF 9:1, v:v)

[g] (buffer/THF 7:3, v:v)

In the absence of co-solvent, the enantioselectivity of lipase from *P. fluorescens* was very low, with E-value of 11 (Table 2, entry 1). All evaluated co-solvents with negative log *P* values showed E-values range from low to moderate, 6-39 (Table 2, entries 2-6). It should be noted that protic solvents such as isopropanol and ethanol (Table 2, entries 5 and 6) were the ones that led to the lowest values of enantioselectivity (E). Probably, these solvents interfere in the active enzymatic site through hydrogen bonds. On the other hand, when less hydrophilic THF was used as co-solvent (buffer/co-solvent 8:2, v:v), the enantioselectivity was greatly increased up to an E-value of 207 (Table 2, entry 7). It should be noted that the addition of THF in proportions above or below relative to the aforementioned led to lower enantioselectivity (Table 2, entries 8-9). In contrast, solvents with increasing log *P* values such as TBME and hexane (Table 2, entries 10 and 11), did not offer high enantioselectivity, most probably because of a partial loss of efficiency in dissolving the substrate. As far as we know, there is no general rule-relating enhancement of enantioselectivity to the nature of the co-solvent in enzymatic hydrolysis reactions in monophasic aqueous systems containing a minor fraction of an

organic solvent. A possible explanation for obtaining a high enantioselectivity value in the kinetic resolution of ***rac*-4** can be performed by evoking the concept of induce-fit motions of the enzyme [48]. It is known that the two enantiomers of acetates from secondary alcohols have two different modes of attachment to the active site of a lipase. The large group (indole moiety) of the most reactive enantiomer **(*S*)-4** points out from the active site (larger pocket) while the middle group (chlorine atom) is oriented toward the stereoselective pocket (smaller pocket), constituting a favorable mode. For the less reactive enantiomer **(*R*)-4**, the large group is positioned in the stereoselective pocket, while the medium group is oriented out of the active site (unfavorable mode).These different modes of binding the enantiomers in lipase result in the enantioselectivity [49,50]. It is evident that the high value of enantioselectivity obtained in the kinetic resolution of ***rac*-4** in the presence of *P. fluorescens* is due to a combination of effects, which includes increasing the reaction temperature and adding an ideal amount of THF as the co-solvent. The addition of THF should strip off an amount of water from enzyme, modifying the microenvironment in the active site of the lipase. Probably, the new microenvironment favors a better accommodation of the indole moiety of the **(*S*)-4** enantiomer. The increase in temperature should be responsible for an increase in the conformational flexibility of the lipase and this added to the effect of the addition of THF should promote a more effective fitness for indole moiety of **(*S*)-4** with the large binding pocket of the enzyme. These combined effects lead to better accommodation of the **(*S*)-4** enantiomer at the active site and a greater stabilization of the complex between the substrate and enzyme. Therefore, the favorable mode should become even more favorable, resulting in an increase in enantioselectivity in the kinetic resolution of ***rac*-4**.

Once that the kinetic resolution of *rac*-2-acetoxy-1-(1*H*-indol-4-yloxy)-3-chloropropane (***rac*-4**) was optimized for the production of enantiomerically enriched (2*R*)-2-acetoxy-

1-(1*H*-indol-4-yloxy)-3-chloropropane (***R*-4**) (97% *ee*), we decided to move forward and explore the enzymatic acetylation of *rac*-1-(1*H*-indol-4-yloxy)-3-chloro-2-propanol (***rac*-3**) mediated by *P. fluorescens*, in the presence of organic solvents.

*3.4. Kinetic resolution of rac-1-(1*H*-indol-4-yloxy)-3-chloro-2-propanol* (***rac*-3**) *via acetylation reaction*

Due to the high values of conversion and enantioselectivity obtained in the hydrolysis of *rac*-2-acetoxy-1-(1*H*-indol-4-yloxy)-3-chloropropane (***rac*-4**) (Table 1, entry 7), catalysed by lipase from *P. fluorescens*, we selected this enzyme to mediate the kinetic resolution of *rac*-1-(1*H*-indol-4-yloxy)-3-chloro-2-propanol (***rac*-3**) via acetylation (Fig 3, Path b). Based on previously reported studies, we choose the vinyl acetate as acyl donor [46,47]. Various solvents were evaluated at temperatures ranging between 30 and 40 °C, (Table 3).

Table 3. Kinetic resolution of *rac*-1-(1*H*-indol-4-yloxy)-3-chloro-2-propanol (***rac*-3**) via acetylation using lipase from *P. fluorescens*.[a]

Entry	Solvent	Reaction time (h)	Temperature (°C)	$e.e._s$ (%)[b]	$e.e._p$ (%)[b]	c (%)[c]	E[d]
1	THF	24	30	2	>99	2	>200
2	THF	24	40	4	91	4	22
3	Heptane	24	30	13	88	13	18
4	Toluene	24	30	15	75	17	8
5	Cyclohexane	24	30	20	53	27	4
6	Hexane	24	30	35	79	31	12
7	TBME	24	30	21	91	19	26
8	TBME	24	40	72	69	51	11
9	TBME[e]	24	30	24	84	22	14
10	TBME[e]	68	30	47	80	37	14

11	MeCN	24	30	9	94	9	35
12	MeCN	68	30	23	94	20	40
13	MeCN	24	35	11	>99	10	>200
14	MeCN	48	35	13	>99	11	>200
15	MeCN	72	35	14	>99	12	>200
16	MeCN	120	35	14	>99	12	>200
17	MeCN	24	40	15	>99	13	>200
18	MeCN	48	40	26	94	22	42

[a]Conditions: lipase from *P. fluorescens*:***rac*-3** (1:1), vinyl acetate as acyl donor (5 eq.) in organic solvent, at 250 r.p.m.

[b] Determined by HPLC.

[c] Conversion, $c = e.e._s/(e.e._s+e.e._p)$.

[d] Enantiomeric ratio, $E = \ln[1-c(1+e.e._p)]/\ln[1-c(1-e.e._p)]$

[e] (10 eq. of vinyl acetate)

As THF was successfully used as co-solvent in the hydrolysis of *rac*-2-acetoxy-1-(1*H*-indol-4-yloxy)-3-chloropropane (***rac*-4**), it was the first solvent evaluated in acetylation of *rac*-1-(1*H*-indol-4-yloxy)-3-chloro-2-propanol (***rac*-3**), in the presence of lipase from *P. fluorescens*. However, THF decreases the enzymatic activity leading to a conversion of only 2%, while the selectivity remained high, E-value > 200 (Table 3, entry 1). With increasing temperature of 30 to 40 °C, the enzyme activity showed only a slight increase to 4% of conversion, with a decrease of E-value to 22, (Table 3, entry 2). In more hydrophobic solvents (i.e., heptane, toluene, cyclohexane and hexane), conversion values range from low to moderate (13-31%), but with quite low enantioselectivity (Table 3, entries 3-6). When the acetylation was carried out in the presence of TBME as solvent, the corresponding acetate (***S*-4**) was obtained with *e.e.* of 91%, but the conversion value was only 19% (Table 3, entry 7). This result encouraged us to provide

conditions to achieve a conversion close to 50% with still high enantioselectivity. Firstly, temperature was increased from 30 to 40 °C, leading a desired conversion value (51%). However, the selectivity decreased dramatically, with E-value of only 11 (Table 3, entry 8). More attempts to optimize reaction conditions in the presence of TBME were performed; the amount of vinyl acetate was doubled from 5 to 10 equivalents and the reaction time prolonged from 24 to 68 h, but the selectivity of the reaction remained low, with E-value of only 14, (Table 3, entries 9-10). In acetonitrile and reaction time of 24 h, the acetylated product (***S*-4**) was obtained with a high value *e.e.* (94%), but low conversion value, only 9% (Table 3, entry 11). By increasing the reaction time from 24 to 68 h, conversion increased from 9 to 20% while maintaining high *e.e.* (94%) of the product, (Table 3, entry 12). To decrease reaction time, we decided to increase the temperature from 30 to 35 °C. Thus, in 24 h, the acetylated product (***S*-4**) was obtained with high selectivity (99% *e.e.*) and E-value > 200, but with conversion of 10% (Table 3, entry 13). In order to achieve 50% conversion, reaction was monitored up to 120 h, but the conversion remained practically unaltered (Table 3, entries 14-16). An increase of 5 °C in the temperature (40 °C) led to only a slight increase in the conversion of 10 to 13%, while maintaining the high *e.e.* (>99%) to (***S*-4**) (Table 3, entry 17). A higher reaction time (48 h) afforded a higher conversion value (22%), but with a decrease in *e.e.* (94%) to (***S*-4**) (Table 3, entry 18).

These results were not so promising when compared with the hydrolytic process. This prompted us to move our attention to further optimize the performance of the hydrolysis process. In the kinetic resolution of *rac*-2-acetoxy-1-(1*H*-indol-4-yloxy)-3-chloropropane (***rac*-4**), the compound with the desired configuration is the remaining substrate (2*R*)-2-acetoxy-1-(1*H*-indol-4-yloxy)-3-chloropropane (***R*-4**). Then, the latter should be subjected to a hydrolysis reaction to get the key intermediate in the synthesis

of (*S*)-Pindolol (***S*-5**), the haloydrin (2*R*)-1-(1*H*-indol-4-yloxy)-3-chloro-2-propanol (***R*-3**). To catalyze this hydrolysis, we choose the lipase from *C. rugosa*, which proved to be active, but not selective (Table 1, entry 14).

*3.5. Synthesis of the key intermediate haloydrin (2R)-1-(1*H*-indol-4-yloxy)-3-chloro-2-propanol (**R-3**)*

Once obtained the (2*R*)-2-acetoxy-1-(1*H*-indol-4-yloxy)-3-chloropropane (***R*-4**) enantiomerically enriched (97% *e.e.*), we turn our attention to the preparation of the halohydrin (2*R*)-1-(1*H*-indol-4-yloxy)-3-chloro-2-propanol (***R*-3**), the key intermediate in the synthesis of (*S*)-Pindolol (***S*-5**). The hydrolysis of (2*R*)-2-acetoxy-1-(1*H*-indol-4-yloxy)-3-chloropropane (***R*-4**) (97% *e.e.*), mediated by lipase from *C. rugosa* in phosphate buffer (pH 7)/THF (4:1, v:v) at 40 °C for 12 h, led to (2*R*)-1-(1*H*-indol-4-yloxy)-3-chloro-2-propanol (***R*-3**) (97% *e.e.*) in quantitative yield.

3.6. Synthesis of (S)-Pindolol

Finally, the haloydrin (2*R*)-1-(1*H*-indol-4-yloxy)-3-chloro-2-propanol (***R*-3**) was subjected to reaction with excess of isopropylamine, under reflux, leading to (*S*)-Pindolol (***S*-5**) (97% *e.e.*), in a quantitative yield.

4. Conclusion

In summary, we have prepared the (*S*)-Pindolol based on a chemoenzymatic process using lipases. Lipase from *P. fluorescens* proved to be a robust catalyst in the kinetic resolution of *rac*-2-acetoxy-1-(1*H*-indol-4-yloxy)-3-chloropropane (***rac*-4**), via hydrolysis reaction, leading to the intermediate (2*R*)-2-acetoxy-1-(1*H*-indol-4-yloxy)-3-chloropropane (***R*-4**) with 97% *ee*. It is noteworthy that the kinetic resolution through the hydrolytic process was efficient when the THF was used as co-solvent and when the reaction temperature was increased from 30 to 40 °C, resulting in high enantioselectivity (E >200). A particular behavior of the high enantioselectivity induced by a higher reaction temperature and the addition of an ideal amount of THF as a co-solvent can be explained by a model in which stabilization of the association between the correctly binding enantiomer and the lipase active site was increased by the great conformational flexibility of the lipase. Therefore, parameters such as temperature and addition of an appropriate co-solvent are ways of tuning the enantioselectivity of an enzyme. Lipase from *C. rugosa* was efficient in the hydrolysis of the chiral acetate yielding as key intermediate the halohydrin (2*R*)-1-(1*H*-indol-4-yloxy)-3-chloro-2-propanol (***R*-3**) with 97% *ee* in quantitative yield. The kinetic resolution was more efficient in the hydrolytic process rather than acylation process in organic solvent.

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