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Sachin Mulik†, Saptarshi Ghosh†, Jayeeta Bhaumik, Uttam C. Banerjee*

Biocatalytic synthesis of (*S*)-Practolol, a selective β-blocker

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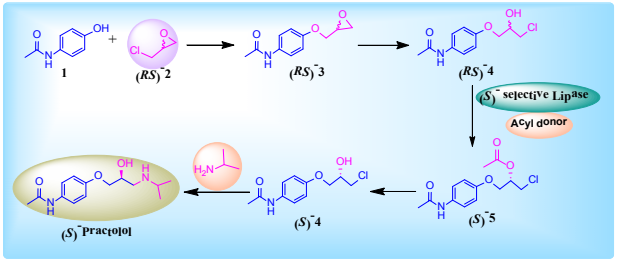
Abstract: The present study describes an efficient chemoenzymatic synthesis of enantiopure (*S*)-Practolol, a selective β-adrenergic receptor blocker. Prior to the synthesis of the target, a synthetic protocol for (*RS*)-*N*-4-(3-chloro-2-hydroxypropoxy)phenylacetamide, an essential precursor, was developed. Various commercial lipases were screened for the kinetic resolution of (*RS*)-*N*-4-(3-chloro-2-hydroxypropoxy)phenylacetamide using toluene as solvent and vinyl acetate as an acyl donor. Among various lipases screened, *Pseudomonas cepacia* sol-gel AK showed the highest enantioselectivity (96% enantiomeric excess with 50% conversion), affording (*S*)-1-(4-acetamidophenoxy)-3-chloropropan-2-yl acetate. Optimization of the reaction parameters was carried out in order to find the best-suited conditions for the biocatalysis. Furthermore, the enantiopure intermediate was hydrolyzed and the resulting product was reacted with isopropylamine to afford (*S*)-Practolol. This biocatalytic procedure depicts a green technology for the synthesis of (*S*)-Practolol with better yield and enantiomeric excess.



Keywords: enantiopure, lipase, chemoenzymatic, enantioselectivity, biocatalysis

***Corresponding author: Uttam C. Banerjee,** Department of Pharmaceutical Technology (Biotechnology), National Institute of Pharmaceutical Education and Research, Sector-67,,S. A. S. Nagar-160062, Punjab, E-mail: ucbanerjee@niper.ac.in
Sachin Mulik, Saptarshi Ghosh, Jayeeta Bhaumik, Department of Pharmaceutical Technology (Biotechnology), National Institute of Pharmaceutical Education and Research, Sector-67,,S. A. S. Nagar-160062, Punjab
† Equal authorship

1 Introduction

Optically active aryloxypropanolamines have been widely employed as starting materials for the synthesis of β-blockers, which are used in the management of sympathetic nervous system-associated disorders such as hypertension, angina pectoris, cardiac arrhythmias, depression, loss of appetite, asthma, migraine, glaucoma, treatment of inflammatory disorders, and pain [1-4]. All β-blockers have at least one chiral center in their structure and thus have two or more enantiomers [5]. For practolol, the *(S)*-enantiomer is the eutomer component of racemic drugs and the *(R)*-enantiomer is the distomer, displaying other (often undesirable) activity [6] with different rates of metabolic oxidation [7]. As a β-blocker, *(S)*-Practolol is known to be 60 times more potent than *(R)*-practolol [8]. Several approaches to obtain optically active aryloxypropanolamine have been reported [9,10]. The compound may be synthesized by the asymmetric dihydroxylation of aryl-allyl ethers through a OsO_4-catalyzed reaction [11]. In practice, however, this process is relatively inefficient and requires an expensive chiral chemical catalyst [11]. Enantiopure aryloxypropanolamine can also be synthesized by a crystallization approach [12,13]. One of the most commonly used methods for the synthesis of β-amino alcohols is the direct aminolysis of epoxides using different promoters or catalysts in the presence of conventional solvents. These include calcium trifluoromethanesulfonate [14], ionic liquids [15], bismuth(III) triflate, polymer-supported chiral Co(salen) complexes, copper(II) compounds, microwave irradiation, and others. The epoxide was synthesized from a phenol and epichlorohydrin using different catalysts, including cesium fluoride [16], sodium hydride, cesium carbonate, and β-cyclodextrin, as reported in the literature [17].

However, the majority of these approaches have one or more drawbacks, including the use of expensive reagents (e.g., chiral transition metal complexes), insufficient catalyst selectivity, and sensitivity in asymmetric hydrogenation and the crystallization process [11-13]. Therefore, to obtain enantiopure aryloxypropanolamine

in sufficient/bulk quantity, it is desirable to develop an efficient, simple, and green synthesis method. Nowadays, enzymatic methodologies constitute an important alternative for existing chemical syntheses. Lipases are a very attractive group of enzymes for synthetic purposes because of their stability, selectivity, and ability to perform without cofactors [18]. The lipase-mediated synthesis of chiral secondary alcohols for the enantiopure synthesis of β-blockers has been previously reported [19,20]. Synthesis of the enantiopure intermediate of Practolol using lipase from *Pseudomonas sp.* with 48% conversion and 92% enantiomeric excess has also been described [21]. Furthermore, the enantiopure intermediate has been used for the synthesis of (*S*)-Practolol in 30% yield [21].

Here, we report a chemoenzymatic method for lipase-mediated kinetic resolution of (*RS*)-N-(4-(3-chloro-2-hydroxypropoxy)phenylacetamide for the synthesis of enantiopure (*S*)-N-(4-(3-chloro-2-hydroxypropoxy) phenylacetamide. The enantiopure phenylacetamide derivative was then used as a precursor for the synthesis of enantiopure (*S*)-Practolol. The effect of different reaction parameters on the kinetic resolution of the racemic alcohol using commercial lipases was also studied.

2 Materials and Methods

2.1 Reagents

N-(4-Hydroxyphenyl)acetamide, (*RS*)-epichlorohydrin, *(R)*-epichlorohydrin, acetic anhydride, acetyl chloride, vinyl acetate, and pyridine were purchased from Sigma-Aldrich (USA). Sodium sulphate and potassium carbonate was purchased from SDFCL (India) and Merck (India), respectively. Commercial lipases from different sources were purchased from Sigma-Aldrich (USA), including the following: *Candida antarctica*, *Candida rugosa* 62316, *Candida rugosa* L-1754, *Candida rugosa* 90860, *Candida cylindracea*, *Aspergillus niger*, *Pseudomonas cepacia*, *Mucor meihei* and porcine pancreas lipase. Analytical- or commercial-grade solvents for synthesis and extraction were acquired from commercial sources. HPLC-grade solvents, such as hexane, isopropanol, and others, were purchased from J.T. Baker and Merck Ltd.

2.2 Analytical methods

^{1}H NMR and ^{13}C NMR spectra were obtained on a Bruker DPX 400 spectrometer (ν ^{1}H = 400 MHz and ν ^{13}C = 100 MHz), chemical shifts are expressed in δ units relative to the tetramethylsilane (TMS) signal as an internal reference in $CDCl_3$. IR spectra (wavenumber in cm^{-1}) were recorded on Nicolet FT-IR impact 400 spectrometers (neat for liquids and KBr pellets for solids). Analytical TLC of all reactions was performed on Merck prepared plates, and silica gel (60–120 mesh) was used for column chromatography. The enantiomeric excess (*ee*) was determined by HPLC analysis performed on a Shimadzu LC-10AT instrument using a Chiralcel OD-H column (0.46 mm × 250 mm; 5 µm, Daicel Chemical Industries, Japan) under the following conditions: mobile phase, 8:2 hexane/2-propanol; flow rate, 1 mL min^{-1}; and absorbance, 254 nm.

2.3 Synthesis of (*RS*)-N-(4-(oxiran-2-yl-methoxy)phenylacetamide (3)

To a stirred solution of 4-acetamidophenol (3.02 g, 20.0 mmol) and K_2CO_3 (2.76 g, 20.0 mmol) in anhydrous acetonitrile (20 mL), *(RS)*-epichlorohydrin (2.5 mL, 20 mmol) was added and the reaction was continued at 60°C for 24 h. The reaction mixture was then cooled and filtered. The filtrate was then concentrated, and ethyl acetate and water was added to the residue. The organic layer was separated and concentrated on a Rotavapor to afford the desired product, *(RS)*-N-(4-(oxiran-2-ylmethoxy) phenylacetamide (**3**): light yellow liquid (97% yield). GC-MS: *m/z* 207.

2.4 Synthesis (*RS*)-N-(4-(3-chloro-2-hydroxypropoxy)phenylacetamide (4)

To a stirred solution of **3** (2.07 g, 10.0 mmol) in 20 mL dichloromethane (DCM), water (10 mL) was added. After 5 min, acetyl chloride (0.67 mL, 10 mmol) was added dropwise. The resultant reaction mixture was stirred at room temperature for 3 h. On completion, the reaction mixture was diluted with DCM and water, and aqueous $NaHCO_3$ was added. Then, the DCM layer was separated, dried on Na_2SO_4, and concentrated under vacuum. The residue was purified by column chromatography (silica, 60–120 mesh; 9:1 hexane/ethylacetate) to afford pure (*RS*)-N-(4-(3-chloro-2-hydroxypropoxy)-phenylacetamide (**4**).

The product (*RS*)-**4** was then analyzed by chiral HPLC using a Chiralcel OD-H column, and the two enantiomers were eluted at 28.8 and 33.7 min, respectively (8:2 hexane/2-propanol, 1 mL min^{-1}, 254 nm; see supporting information).

(*RS*)-*N*-4-(3-Chloro-2-hydroxypropoxy) phenylacetamide (**4**): White solid (96% yield). ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.09 (s, 1H), 2.12–2.17 (s, 3H), 2.6 (s, 1H), 3.7–3.8 (m, 2H), 4.03–4.09 (m, 1H), 4.18–4.23 (m, 1H) 6.86–6.88 (d, *J* = 8 Hz, 2H), 7.12 (br s, 1H), 7.38–7.41 (d, *J* =

12 Hz, 2H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 24.4, 45.9, 68.8, 69.8, 114.9, 121.9, 155.1, 168.3. GC-MS: *m/z* 242.94.

2.5 Synthesis of (*RS*)-1-(4-acetamidophenoxy)-3-chloropropan-2-yl-acetate (5)

To a stirred solution of *(RS)*-**4** (0.12 g, 0.50 mmol) in acetic anhydride (2 mL), 100 µL pyridine was added as base. The reaction was continued at 4°C for ~6 h. On completion, the reaction mixture was added to ice cold water (50 mL), and the resulting mixture was acidified with 3 M HCl to adjust the pH to 1.0–2.0. The mixture was then extracted three times with ethyl acetate, and the combined organic layers were concentrated on a Rotavapor.

(*RS*)-1-(4-Acetamidophenoxy)-3-chloropropan-2-yl-acetate: off-white solid (90% yield). ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.96–2.2 (br s, 6H), 3.66–3.75 (m, 2H), 4.14–4.15 (dd, J = 4 Hz, 2H), 5.31 (br s, 1H), 6.85–6.87 (d, *J* = 8 Hz, 2H), 7.26 (s, 1H), 7.34–7.39 (m, 2H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 20.9, 24.5, 29.7, 30.9, 42.5, 66.4, 71.1, 115, 121.9, 131.7, 155, 168.3, 170.2. GC-MS: *m/z* 285.

2.6 Enantioselective transesterification of (*RS*)-4

In a 5 mL conical flask, (*RS*)-**4** (20 mM), toluene (0.9 mL), and vinyl acetate (10 mM, 100 µL) were combined. Lipases from different sources were investigated: immobilized lipase in sol-gel-Ak from *Pseudomonas cepacia*, immobilized lipozyme from *Mucor meihei*, lipase acrylic resin from *Candida Antarctica*, *Candida antarctica* lipase A CLEA, *Candida rugosa* (90860), *Candida rugosa* (L-1754); *Candida rugosa* (62316), *Candida cylindracea*, *Aspergillus niger*, porcine pancreas lipase, and lipase AY "Amano" 30. The flasks were capped and placed in an incubator shaker set to 30°C and 200 rpm. Samples (300 µL) were taken from each reaction mixture and analyzed by HPLC. Conversion and enantiomeric excess (*ee*) were calculated from the acquired HPLC chromatogram.

2.7 Optimization of transesterification reaction conditions

The effect of different organic solvents (*i.e.*, acetonitrile, 1,4 dioxane, *tert*-butyl methyl ether, diethyl ether, DCM, benzene, and toluene) on the transesterification of (*RS*)-**4** was studied. To determine the optimum temperature, reactions were carried out at temperatures from 20 to 40°C. Different acyl donors such as ethyl acetate, isopropenyl acetate, acetic anhydride, and vinyl acetate (10 mM) were used to determine the effect of acyl donor on the conversion and enantiomeric excess. To study the effect of incubation time, reactions were carried out at different time intervals (3, 6, 9 and 12 h). Reactions were carried out in increasing substrate concentrations (10, 20, 30, 40 and 50 mM) to determine the effect of substrate concentration on the conversion and enantiomeric excess. Finally, in order to optimize the enzyme concentration with respect to constant substrate concentration (10 mM), various enzyme concentrations (15, 30, 45, 60 and 90 mg mL^{-1}) were tested.

2.8 Preparative-scale transesterification reaction

The resolution of *(RS)*-**4** was carried out on a preparative scale under the optimized conditions. The reaction was performed with 10 mM substrate on a 50 mL scale using *Pseudomonas cepacia* lipase (PCL) at 35°C for 12 h. Vinyl acetate was used as an acyl donor and *tert*-butyl methyl ether acted as solvent. After 12 h, the reaction mixture was filtered and the filtrate was concentrated under vacuum. The resulting dried product was subjected to column chromatography (silica, 60–120 mesh) using hexane/ethyl acetate (9:1) as the mobile phase. The product was analyzed by HPLC (Chiralcel-ODH column, 9:1 hexane/2-propanol; see supporting information).

(*R*)-N-(4-(3-Chloro-2-hydroxypropoxy) phenylacetamide: (49% yield). ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.09 (s, 1H), 2.12–2.17 (s, 3H), 2.6 (br s, 1H), 3.7–3.8 (m, 2H), 4.03–4.09 (m, 1H), 4.18–4.23 (t, *J* = 2 Hz, 1H) 6.86–6.88 (d, *J* = 8 Hz, 2H), 7.12 (br s, 1H), 7.38–7.41 (d, *J* = 12 Hz, 2H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 24.4, 45.9, 68.8, 69.8, 114.9, 121.9, 155.1, 168.3. GC-MS: *m/z* 242.94.

(*S*)-1-(4-Acetamidophenoxy)-3-chloropropan-2-yl-acetate: off-white solid (48% yield). ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.96–2.2 (br s, 6H), 3.66–3.75 (m, 2H), 4.14–4.15 (dd, *J* = 4 Hz, 2H), 5.31 (br s, 1H), 6.85–6.87 (d, *J* = 8 Hz, 2H), 7.26 (s, 1H), 7.34–7.39 (m, 2H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 20.9, 24.5, 29.7, 30.9, 42.5, 66.4, 71.1, 115, 121.9, 131.7, 155, 168.3, 170.2. GC-MS: *m/z* 285.

2.9 Enantioselective transesterification of (*RS*)-4 followed by the deacylation of (*S*)-5

After the successful enantioselective acylation of (*RS*)-**4**, the acylated product (*S*)-**5** was purified by column chromatography (silica, 60–120 mesh, 9:1 hexane/ethyl acetate) and concentrated in a Rotavapor. Following a literature method with modification, the acetylated intermediate (*S*)-**5** (0.57 g, 2.0 mmol) was hydrolyzed with aqueous K_2CO_3 (0.41 g, 3.0 mmol) at room temperature

(30°C) for 2 h to liberate the parent alcohol (*S*)-**4** [22]. The product was isolated by extraction with ethyl acetate and characterized by NMR, GC-MS, and HPLC (see Supporting Information).

(*S*)-N-(4-(3-Chloro-2-hydroxypropoxy) phenylacetamide (**4**): (92% yield). ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.09 (s, 1H), 2.12–2.17 (s, 3H), 2.6 (s, 1H), 3.7–3.8 (m, 2H), 4.03–4.09 (m, 1H), 4.18–4.23 (t, *J* = 2 Hz, 1H), 6.86-6.88 (d, *J* = 8 Hz, 2H), 7.12 (br s, 1H), 7.38–7.41 (d, *J* = 12 Hz, 2H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 24.4, 45.9, 68.8, 69.8, 114.9, 121.9, 155.1, 168.3. GC-MS: *m/z* 242.94.

2.10 Synthesis of (*S*)-Practolol

(*S*)-1-Chloro-3-(2-cyanophenoxy)propan-2-ol (**4**) was treated with isopropylamine in the presence of potassium carbonate in acetonitrile at 70°C for 12 h. On completion, the reaction mixture was extracted with ethyl acetate. The ethyl acetate layer was separated and concentrated under vacuum. The residue was purified by column chromatography using silica (60–120 mesh) and eluting with hexane/ethyl acetate (9:1) to obtain (*S*)-Practolol.

(*S*)-N-{4-[2-Hydroxy-3-(propan-2-yl-amino)propoxy] phenylacetamide: white solid (75% yield). GC-MS: *m/z* 267.33. Mass spectra: *m/z* 267.23.

3 Results and Discussions

Several reports related to the synthesis of enantiopure Practolol were available. However, the longer reaction times (3–7 days) and lower enantiomeric excesses were major drawbacks of these methods [23,24]. A chemoenzymatic method with improved enantiomeric excess and reduced reaction time is reported here.

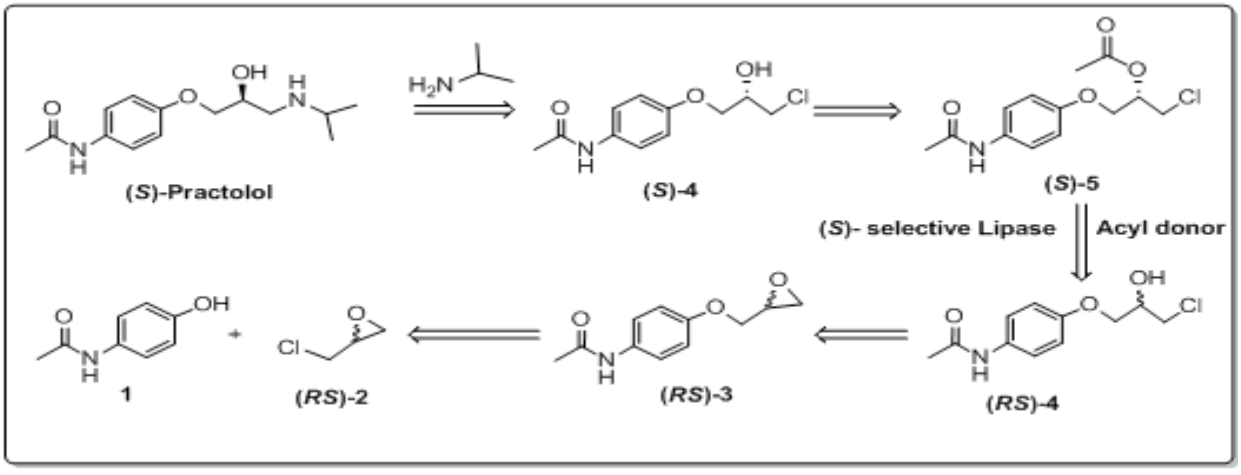
For the preparation of enantiopure (*S*)-Practolol, the retrosynthetic pathway shown in Scheme 1 can be followed. First, reacting 4-acetamidophenol with *(RS)*-epichlorohydrin (**2**) produces the racemic intermediate (*RS*)-**4**. The racemic alcohol (*RS*)-**4** in the presence of lipase as a biocatalyst and acyl donor affords (*S*)-**5** as a precursor for (*S*)-Practolol.

3.1 Synthesis of (*RS*)-N-4-(3-chloro-2-hydroxypropoxy)phenylacetamide (4)

Racemic epoxide N-4-(oxiran-2-yl-methoxy) phenylacetamide (**3**) was prepared by the reaction of 4-acetamidophenol (**1**) with *(RS)*-epichlorohydrin (**2**) in the presence of K_2CO_3 in MeCN at 60°C. Upon completion, the reaction mixture was filtered and the filtrate was extracted with ethyl acetate. The resulting crude epoxide was then purified by column chromatography (silica, 60–120 mesh; 9:1 hexane/ethyl acetate). The desired intermediate (*RS*)-**3** was isolated as a light yellow liquid in 97% yield. Ring opening of the epoxide in (*RS*)-**3** by acetyl chloride in water afforded the desired product, (*RS*)-*N*-4-(3-chloro-2-hydroxypropoxy) phenylacetamide (**4**), in 96% yield (Scheme 2).

3.2 Synthesis of (*RS*)-1-(4-acetamidophenoxy)-3-chloropropan-2-yl-acetate (5)

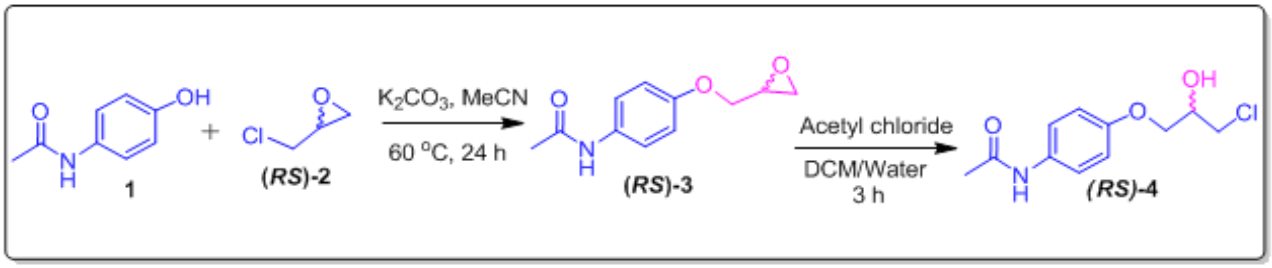
To isolate the standard acylated product, (*RS*)-**4** was reacted with acetic anhydride and pyridine at 4°C to afford (*RS*)-**5** (Scheme 3). The product obtained was subjected to chiral HPLC analysis using a Chiralcel OD-H column, and the two enantiomers (*R*)-**5** and (S)-**5** were eluted at 21.18 and 42.4 min (8:2 hexane/2-propanol, 1 mL min^{-1}, 254 nm), respectively.



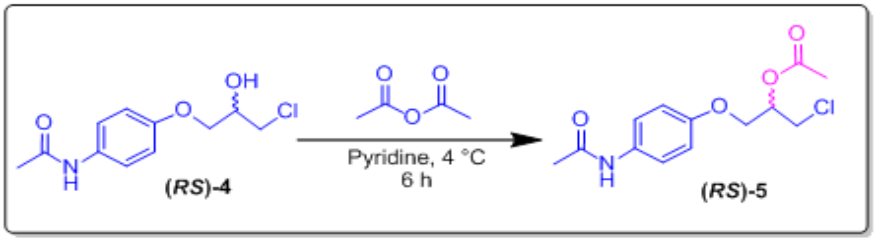
Scheme 1. Retrosynthetic pathway for the synthesis of (*S*)-Practolol.

3.3 Screening of lipases for the kinetic resolution of (RS)-N-4-(3-chloro-2-hydroxypropoxy) phenylacetamide (4)

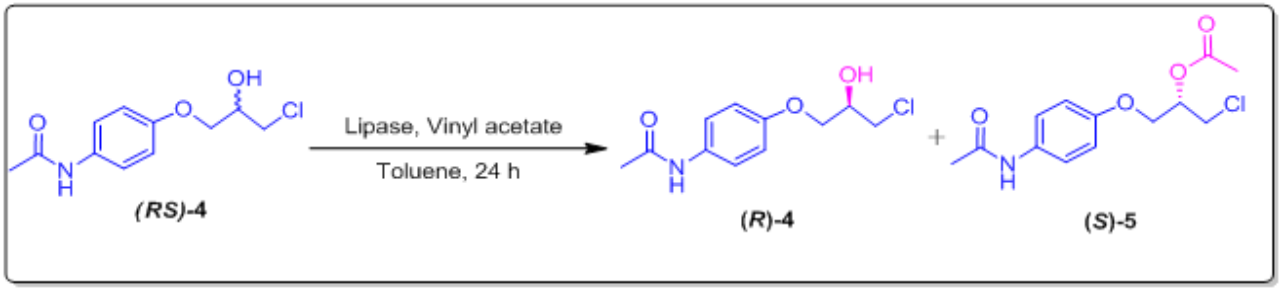
Commercially available lipases were screened for the kinetic resolution of (*RS*)**-4** with vinyl acetate in toluene (Scheme 4). Considerable conversion was achieved using lipozyme immobilized from *Mucor meihei*, Lipase A, *Candida antarctica* (CLEA), lipase immobilized in sol-gel-AK from *P. cepacia* (PCL), and lipase from *Candida rugosa* (62316) (Table 1). Among these, lipase from PCL provided the best conversion and highest enantiomeric excess and was therefore selected for all subsequent studies.



Scheme 2. Synthesis of (*RS*)-N-4-(3-chloro-2-hydroxypropoxy)phenylacetamide (**4**).



Scheme 3. Synthesis of (*RS*)-1-(4-acetamidophenoxy)-3-chloropropan-2-yl-acetate (**5**).



Scheme 4. Lipase mediated kinetic resolution of *(RS)*-4.

Table 1. Screening of various lipases for the kinetic resolution of (*RS*)-4.[a]

Name of Lipase	ee$^{b}_{s}$ (%)	ee$^{b}_{p}$ (%)	Conversion[c] (%)	E[d]
Lipase acrylic resin from *Candida antarctica*	19.2	> 99	16.1	24.8
Lipase from *Candida rugosa* (62316)	22.8	74.2	23.4	8.4
Lipase from *Candida rugosa* (90860)	4.8	> 99	4.6	2.8
Lipase from *Candida cylindracea*	34	79.5	29.9	12.2
Lipozyme immobilized from *Mucor meihei*	14.2	> 99	12.4	16.3
Lipase A from *Candida antarctica* (CLEA)	83.9	95.5	46.8	116.7
Lipase from *Pseudomonas cepacia*, sol-gel AK	96.2	95.3	48.3	157.2
Lipase AY "Amano" 30	10.6	85.5	11	14.2
Porcine pancreas lipase	18.3	89.6	17	22

[a]Conditions: 20 mM (*RS*)-4, 0.9 mL toluene (as solvent), 15 mg mL^{-1} lipase, and 10 mM vinyl acetate at 30°C for 24 h.

[b]Enantiomeric excesses of substrate and product determined by HPLC analysis: Daicel Chiralcel OD-H column; 80:20 hexane/2-propanol; 1 mL min^{-1} flow rate at 254 nm.

[c]Conversions calculated from the enantiomeric excess of substrate (*ee*) and product (*ee*): conversion (C) = *ee*/(*ee*+ *ee*).

[d] *E* values were calculated from *E* = [ln (1 – C (1 + *ee*)]/ [ln (1 –C (1 - *ee*)]

3.4 Effect of organic solvent

Organic solvents play a significant role in lipase-mediated kinetic resolution of racemic alcohols [25-28]. The effect of various solvents on the activity and enantioselectivity of PCL for the kinetic resolution of (*RS*)-**4** was studied using vinyl acetate as an acyl donor at 37°C. Both the reaction rate and enantioselectivity were greatly affected by the choice of solvent (Table 2). The rate of biocatalysis in organic solvents is low in polar solvents with log P < 2, is moderate in solvents having a log P between 2 and 4, and is higher in apolar solvents having log P > 4 [29]. Lipases are also known to carry out transesterification reactions in polar solvents [30-33]. *Tert*-butyl methyl ether (log P = 1.35), hexane (log P = 3.5), toluene (log P = 2.5), acetonitrile (log P = -0.33), dichloromethane (log P = 1.25) and 1,4-dioxane (log P = -1.1) were investigated for the resolution of (*RS*)-**4**. Among these organic solvents, *tert*-butyl methyl ether facilitated the highest enantioselectivity.

3.5 Effect of reaction time

In order to optimize the reaction time, samples were collected periodically from the reaction mixture. The enzyme was then removed by filtration and the conversion and enantiomeric excess were determined using chiral HPLC. Figure 1 provides evidence that the conversion rate increased as the reaction time increased, and the maximum conversion (50% with 100% *ee*) was achieved at 12 h; thereafter, no significant change in the rate of conversion was observed. Extending the reaction time might cause the faster-reacting enantiomer to reach its state of equilibrium more rapidly. However, a longer reaction time might also accelerate conversion into the slower-reacting enantiomer, resulting in a less satisfactory enantiomeric excess of the desired product. Therefore, 12 h was selected as the optimum reaction time for further studies. This time frame is significantly shorter compared to the previously reported methods, which required 3–7 days [23,24].

Table 2. The effect of organic solvent on the enantioselectivity in the resolution of (RS)-4 with immobilized lipase in sol-gel-AK from P. cepacia (PCL).[a]

Solvent					Log *P*	ee_s[b] (%)	ee_p[b] (%)	*C*[c] (%)
1,4-Dioxane					-1.1	10.7	> 99	9.7
Acetonotrile	-0.33	60.3	> 99	37.6				
Diethyl ether					0.85	60.4	> 99	37.6
Dichloromethane					1.25	7.1	> 99	6.6
t-Butyl methyl ether					1.35	99.8	> 99	49.9
Benzene					2.0	90.4	> 99	47.5
Toluene					2.5	96.2	> 99	49.5
Hexane					3.5	25.9	25.2	50.7

[a]Conditions: 20 mM *(RS)*-4, 1 mL solvent, lipase (15 mg mL^{-1}), 10 mM vinyl acetate at 37°C for 24 h. PCL = immobilized lipase in sol-gel-AK from *Pseudomonas cepacia*.

[b]Enantiomeric excesses of substrate and product determined by HPLC (Daicel Chiralcel OD-H column) 80:20 hexane/2-propanol, 1 mL min^{-1} flow rate at 254 nm.

[c]Conversions were calculated from enantiomeric excess of substrate (ee_s) and product (ee_p): conversion (C) = $ee_s / (ee_{s+} ee_p)$

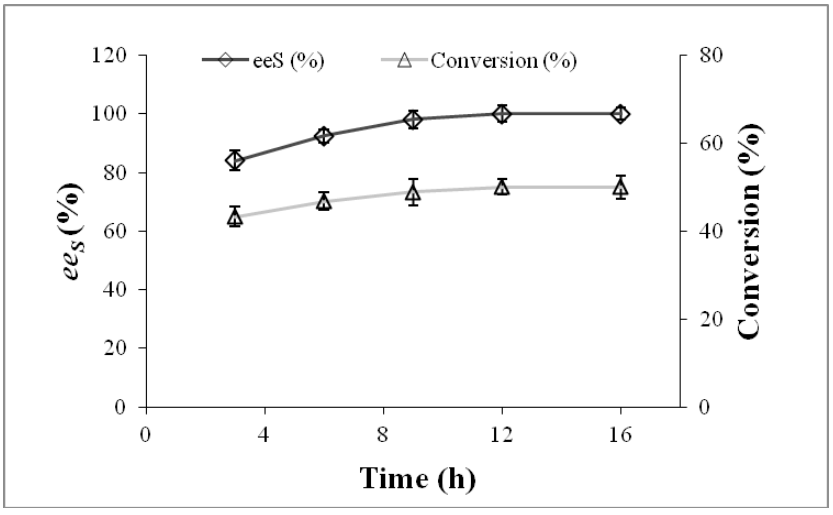


Figure 1. Course of reaction of immobilized PCL sol-gel AK catalyzed transesterification of (*RS*)-**4**.

3.6 Effect of temperature

The effect of temperature on activity and enantioselectivity of PCL for the resolution of (*RS*)-**4** was determined (Figure 2). The resolution was carried out at 20, 25, 30, 35, and 40°C, and the conversion and enantiomeric excess were determined using chiral HPLC after 12 h. The conversion was found to increase from 44.7% at 20°C to 50.0% at 35°C, and then it decreased to 39.8% at 40°C. On the other hand, the enantiomeric excess of the substrate (*ee*) had decreased from 100% *ee* at 35°C to 66% *ee* at 40°C. Therefore, 35°C was selected as the optimum temperature for the PCL-catalyzed resolution of (*RS*)-**4** to achieve good conversion and enantiomeric excess.

3.7 Effect of substrate concentration

The effect of substrate concentration on lipase-mediated kinetic resolution was studied with increasing substrate concentrations (10–50 mM). With increasing concentration of substrate, the conversion decreased from 49.8% at 10 mM to 30.8% at 50 mM (Figure 3). Similarly, the enantiomeric excess decreased from 100% at 10 mM to 64.5% at 50 mM. This might be due to the substrate inhibition of lipase activity at higher substrate concentration. These values are slightly improved from those obtained from the previously reported method, which used 10 mM substrate to give 48% conversion and 92% *ee* [21].

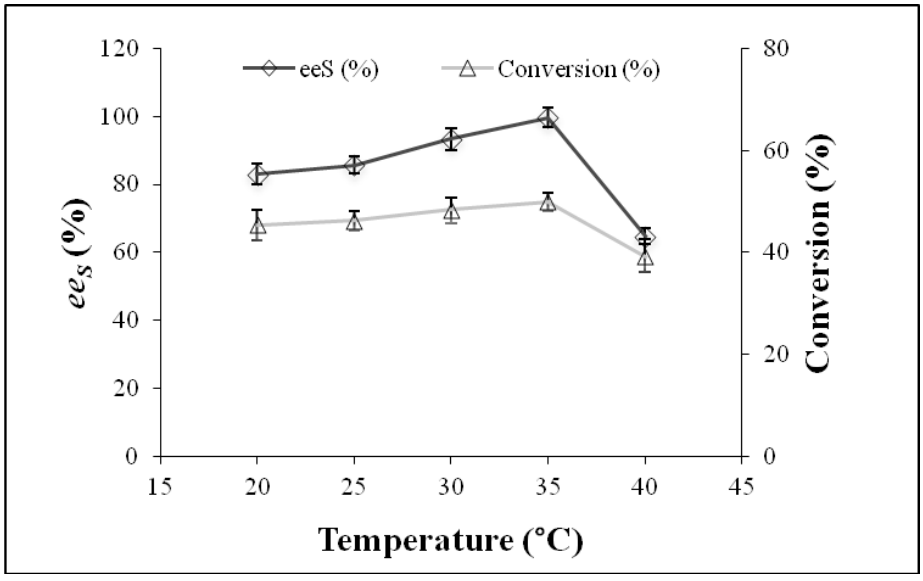


Figure 2. Effect of temperature on the kinetic resolution of (*RS*)-**4** by immobilized lipase in sol-gel-Ak from *P. cepacia*.

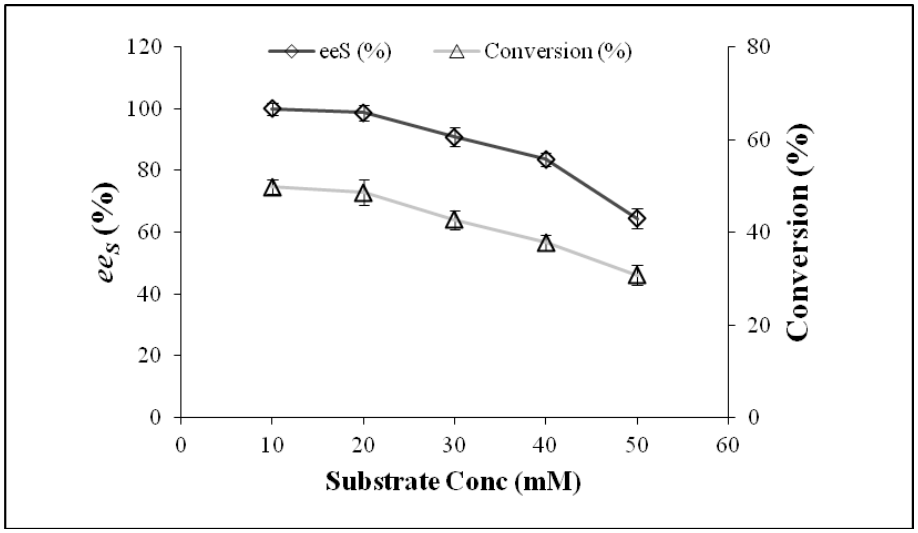


Figure 3. Effect of substrate concentration on the kinetic resolution of (*RS*)-**4** by immobilized PCL in sol-gel-AK.

3.8 Effect of enzyme concentration

To study the effect of enzyme concentration on the conversion and enantiomeric excess, reactions were carried out with increasing concentrations of PCL (15, 30, 45, 60 and 90 mg mL^{-1}). It is evident from Figure 4 that the conversion increased from 41.7% with a 15 mg mL^{-1} enzyme concentration to 50% with 30 mg mL^{-1}. Further increasing the enzyme concentration (up to 90 mg mL^{-1}) did not result in a significant change in the conversion (Figure 4). The enantiomeric excess of the substrate increased from 84.9% with 15 mg mL^{-1} enzyme to 100% with double the enzyme concentration (30 mg mL^{-1}); thereafter, there was no significant change in the *ee* value with increasing concentration. Therefore, an enzyme concentration of 30 mg mL^{-1} was selected for all the subsequent experiments.

3.9 Effect of acyl donors

Effect of acyl donor on the conversion and enantioselectivity of the PCL-catalyzed resolution of (*RS*)-**4** was studied using various acyl donors such as ethyl acetate, vinyl acetate, acetic anhydride, and isopropyl acetate. Vinyl acetate gave the best conversion (49.6%) with 99.4% *ee* (Table 3). The reversibility of the enzymatic process with the use of esters as acyl donors is reported in the literature [33]. In order to render the process irreversible, various activated esters (e.g., vinyl acetate) have been used. A major advantage of using vinyl acetate is the tautomerization of the elusive vinyl alcohol to acetaldehyde, which shifts the equilibrium to the required product [33].

3.10 Preparative scale transesterification of (*RS*)-4

The transesterification of (*RS*)-**4** was carried out on a preparative scale under the optimized reaction conditions, as discussed in section 2.8. Following the reaction, the product was purified by flash chromatography (silica, 60–120 mesh) using hexane/ethyl acetate (9:1) as the mobile phase. After 12 h, the isolated yield of (*S*)-1-(4-acetamidophenoxy)-3-chloropropan-2-yl-acetate was 48% with 99% *ee*. (Chiralcel-ODH) and that of (*R*)-N-(4-(3-chloro-2-hydroxypropoxy)phenylacetamide was 49% with 98.8% *ee* (Chiralcel-ODH).

Table 3. Effect of acyl donors on the kinetic resolution of (*RS*)-4 by immobilized PCL in sol-gel-AK.

Acyl donors[a]	ee_s[b] (%)	C[c] (%)
Ethyl acetate	97	49.2
Isopropenyl acetate	47.5	32.2
Acetic anhydride	47.9	32.4
Vinyl acetate	99.4	49.6

[a]Conditions: 10 mM (*RS*)-4, 1 mL *t*-butyl methyl ether, lipase (30 mg/mL), 10 mM acyl donor at 35°C for 12 h. PCL= *P. cepacia* lipase immobilized in sol-gel-AK

[b]Enantiomeric excesses determined by HPLC (Daicel Chiralcel OD-H column) 80:20 hexane/2-propanol, 1 mL/min flow rate at 254 nm.

[c]Conversions calculated from enantiomeric excess of substrate (*ee*) and product (*ee*): conversion (C) = *ee*/(*ee*+ *ee*).

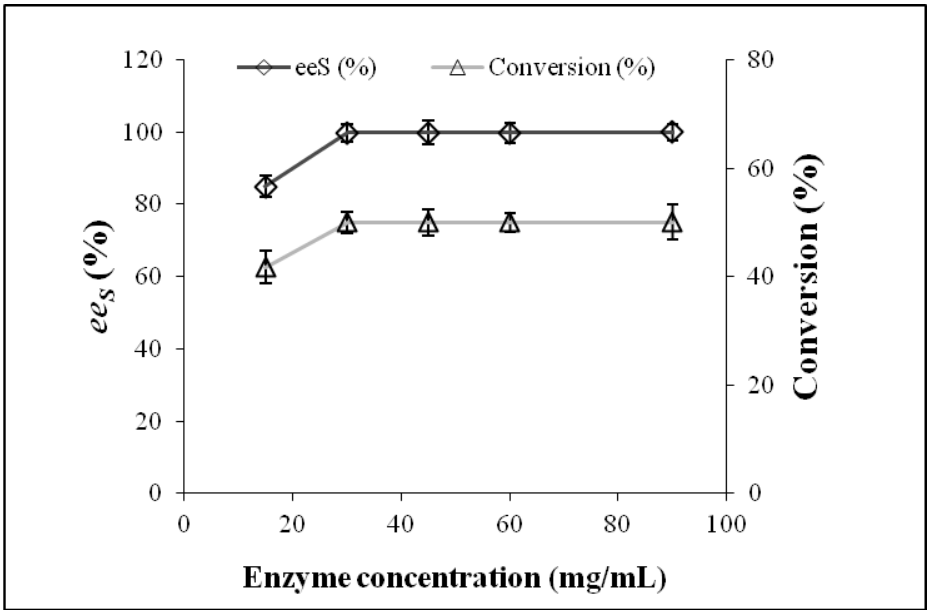


Figure 4. Effect of enzyme concentration on kinetic resolution of (*RS*)-**4** by immobilized PCL in sol-gel-AK.

3.11 Deacylation of (*S*)-5

Deacylation of **(*S*)-5** was necessary to afford the enantiopure alcohol **(*S*)-4** as a precursor to *(S)*-Practolol. Therefore, compound **(*S*)-5** was treated with aqueous K_2CO_3 and the reaction was continued for 2 h at room temperature (30°C) to afford the desired alcohol **(*S*)-4** (Scheme 5). The parent alcohol was extracted from the reaction mixture, purified, and used for the synthesis of (*R*)-Practolol. The product was subjected to chiral HPLC analysis and showed a retention time of 29.9 min, which is similar to that of the standard enantiopure alcohol (Supporting Information).

3.12 Synthesis of (*S*)-Practolol

Enatiopure alcohol (*S*)-N-(4-(3-chloro-2-hydroxypropoxy) phenylacetamide (**4**) was reacted with isopropylamine in the presence of a base (K_2CO_3) using acetonitrile as a solvent to produce enantiopure (*S*)-Practolol in 75% yield (Scheme 6).

4 Conclusions

In this study, we demonstrated a chemoenzymatic synthesis of enantiomerically pure (*S*)-Practolol. Initially, an important precursor, (*RS*)-N-(4-(3-chloro-2-hydroxypropoxy)phenyl-acetamide (**4**), was chemically synthesized in high yield (96%). Later, lipases from various sources were screened for the kinetic resolution of (*RS*)-**4** to afford the enantiopure intermediate. Immobilized lipase in sol-gel-Ak from *Pseudomonas cepacia* (PCL) was chosen over other lipases for its superior selectivity. The effect of varying the reaction parameters (*i.e.*, solvent, reaction time, temperature, substrate, enzyme concentration, *etc*.) on the activity and enantioselectivity of PCL-catalyzed kinetic resolution of (*RS*)-**4** was studied. Under the optimized conditions with 10 mM substrate, 30 mg mL^{-1} lipase, *tert*-butyl methyl ether as solvent, vinyl acetate as acyl donor, and a reaction time of 12 h at 35°C, a conversion of 50% and 100% *ee* was obtained for (*S*)-**5**. Acetylated derivative (*S*)-**5** was then synthesized on a preparative scale and further deacylated to yield the enantiopure acetamide derivative (*S*)-**4**. The reaction of acetamide (*S*)-**4** with isopropylamine afforded the desired enantiopure drug (*S*)-Practolol (**6**) in good yield. The biocatalytic method of (*S*)-Practolol synthesis described here can serve as a substantial platform for the preparation of other potential β-blockers.

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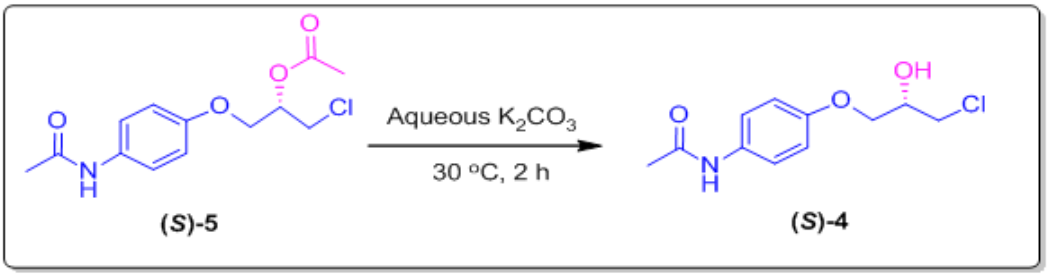
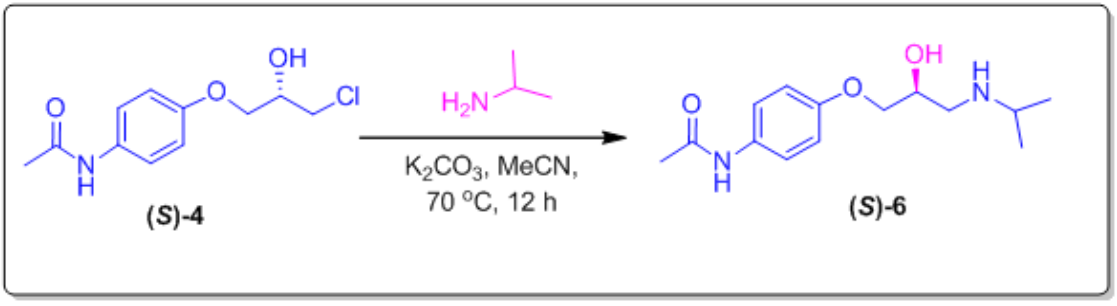
Supporting Information: Additional information (GCMS, NMR, IR spectra of selected compounds, and HPLC chromatograms) may be found in the online version of this article on the publisher's website.



Scheme 5. Deacylation of (*S*)-**5**.



Scheme 6. Synthesis of (*S*)-Practolol (**6**).

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