Biocatalytic Approach for the Synthesis of Enantiopure Acebutolol as a β_1-Selective Blocker

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ABSTRACT A new chemoenzymatic route is reported to synthesize acebutolol, a selective β_1 adrenergic receptor blocking agent in enantiopure (*R* and *S*) forms. The enzymatic kinetic resolution strategy was used to synthesize enantiopure intermediates (*R*)- and (*S*)-N-(3-acetyl-4-(3-chloro-2-hydroxypropoxy)phenyl)butyramide from the corresponding racemic alcohols. The results showed that out of eleven commercially available lipase preparations, two enzyme preparations (Lipase A, *Candida antarctica*, CLEA [CAL CLEA] and *Candida rugosa* lipase, 62316 [CRL 62316]) act in enantioselective manner. Under optimized conditions the enantiomeric excess of both (*R*)- and (*S*)-N-(3-acetyl-4-(3-chloro-2-hydroxypropoxy)phenyl)butyramide were 99.9 and 96.8%, respectively. N-alkylation of both the (*R*) and (*S*) intermediates with isopropylamine gave enantiomerically pure (*R* and *S*)- acebutolol with a yield 68 and 72%, respectively. This study suggests a high yielding, easy and environmentally green approach to synthesize enantiopure acebutolol. *Chirality 27:382–391, 2015.* © 2015 Wiley Periodicals, Inc.

KEY WORDS: β-blockers; lipase; enantioselectivity; transesterification; enantiopure drug

β-blockers are used in the treatment of several disease conditions such as hypertension,[1] angina pectoris, cardiac arrhythmias,[2] myocardial infarction,[3] migraine,[4] and anxiety disorders.[5] They act as competitive antagonists of catecholamines at adrenoceptors. Propranolol is a noncardioselective β-blocker having membrane-stabilizing properties without intrinsic sympathomimetic activity. However, acebutolol (Ac) is a β_1-selective-blocking agent having both intrinsic sympathomimetic activity and membrane stabilizing properties.[6,7] The interaction of drugs and membranes is necessary for the biological effect of the drugs.[8] Moreover, numerous cardiovascular diseases normally are associated with the modification of membrane lipid composition and structure.[9,10]

Acebutolol is used in the treatment of high blood pressure, abnormal heart rhythms, and sometimes chest pain.[11–17] Similar to other β-blockers, acebutolol is used as a racemic mixture, despite the fact that its β-blocking activity is attributed to the *S*-enantiomer and its major metabolite *S*-diacetolol.[18] Moreover, it has been reported that both the enantiomers of adrenergic β-receptor blockers are not only different from the pharmacodynamic point of view but their pharmacokinetic properties are also very different.[19–21] Chemically, acebutolol is an aryloxypropanol amine derivative bearing one chiral center. Generally, the cardioselectivity and β-blocking activity of *S*-enantiomers of aryloxypropanol amine is 50–500 times more than the *R*-enantiomers. Several strategies for the synthesis of acebutolol have been reported in the literature (Scheme 1).[22–25]

Only a few reports are available for the enantiopure synthesis of this drug molecule. One of the reported strategies (strategy 1) uses chiral halohydrin or glyceryl acetonide to convert phenol **3** to enantiopure halohydrin **5**. This halohydrin is subsequently converted to acebutolol by amination and hydrolysis. In another strategy (strategies 2, 3) the chiral epichlorohydrin was used for converting phenol **3** to epoxide **2**. This epoxide was then converted to acebutolol by ring opening reaction with amine. Finally, the resolution-based approach has been reported to convert the racemic acebutolol to the enantiopure form. This shows that only limited methods are available for the enantiopure synthesis of this important class of drug molecule. Most of the reagents used are costly and the yield and enantiomeric excess is poor. The increase in popularity of biocatalysis-based asymmetric synthesis and our recent work involving the lipase-based kinetic resolution method for the synthesis of enciprazine and bunitrolol led our attention to this problem. We tried to address this issue by devising a new strategy (strategy 4), which involved the kinetic resolution with lipase converting the halohydrin **11** to enantiopure halohydrin, followed by amination to acebutolol.

MATERIALS AND METHODS

Analysis

All the enzymatic reactions were carried out in an incubator (Kuhner, Switzerland) at 200 rpm. Thin-layer chromatography (TLC) plates were obtained from Merck (Germany) and silica gel (60–120 mesh) from (SRL, India) was used in column chromatography. A Finnigan-Mat LCQ instrument (San Jose, CA) with C-18 hypersil ODS (4.6 mm x 15 mm, 5 m) column was used for liquid chromatography, mass spectrometry (LC-MS) analysis. ^{1}H NMR and ^{13}C NMR spectra were obtained with Bruker (Billerica, MA) DPX 400 (^{1}H 400 MHz and ^{13}C 100 MHz), chemical shifts were expressed in δ units relative to the tetramethylsilane (TMS) signal as an internal reference in $CDCl_3$. IR spectra were recorded on Nicolet (Madison, WI) FT-IR impact 400 instruments as neat for liquid samples or KBr pellets for solid samples. Optical rotation was measured in a Rudolph, Autopol IV polarimeter. The enantiomeric excess (ee) was determined by high-performance liquid chromatography

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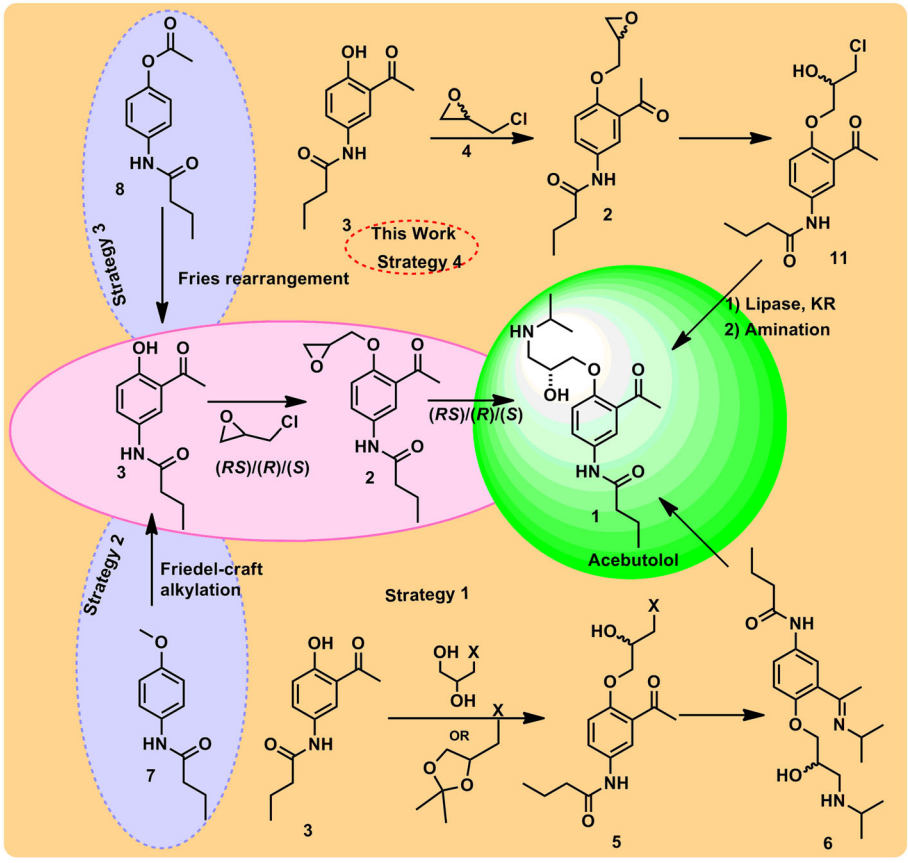


Scheme 1. Strategies for the synthesis of acebutolol.

(HPLC) (Shimadzu, Japan, LC-10AT 'pump, SPD- 10A UV-VIS detector) using a Chiralcel OJ-H column (0.46 mm x 250 mm; 5 µm, Daicel Chemical Industries, Japan) at 254 nm. The conditions were, mobile phase: hexane: 2-propanol (4:1); flow rate: 1 mL/min; column temperature: 25 °C.

Reagents

N-(3-acetyl-4-hydroxyphenyl)butyramide, (*RS*)-epichlorohydrin, (*R*)-epichlorohydrin, (*S*)-epichlorohydrin, and the lipase preparations from *Candida antarctica* (CAL L4777), *Candida rugosa* (CRL 62316), *Candida rugosa* (CRL 90860), *Candida rugosa* L-1754 (CRL L1754), *Candida rugosa* (CRL 62316), *Aspergillus niger* (ANL 62301), and porcine pancreas lipase (PPL) were purchased from Sigma (St. Louis, MO). Immobilized lipase in sol-gel-Ak from *Pseudomonas cepacia* (PCL 62279), immobilized lipozyme from *Mucor miehei* (MML 62350), lipase A *Candida antarctica* (CAL CLEA) were procured from Fluka (Buchs, Switzerland) and lipase AY "Amano"30 (CRL LY amino) was purchased from Amano Chemical (Elgin, IL). The analytical or commercial grade solvents were procured from various commercial sources. HPLC-grade solvents were obtained from Sigma.

Synthesis of (*RS*)-N-(3-acetyl-4-(3-chloro-2-hydroxypropoxy)phenyl) butyramide(11). A previously reported method was used to synthesize (*RS*)-N-(3-acetyl-4-(3-chloro-2-hydroxypropoxy)phenyl)butyramide(*RS*)-**11** with some modifications.[26] Briefly, (*RS*)-**4** (2.3 mL, 20 mmol, 1.5 eq.) was added to the mixture of **3** (4.4 g, 20 mmol, 1 eq.) and K_2CO_3 (5.5 g, 20 mmol, 2 eq.) in anhydrous MeCN (100 mL) and the reaction mixture was heated for 28 h under reflux. The reaction mixture was then cooled, filtered, and washed with MeCN and the combined organic layer was concentrated using rotavapor. The residue was purified using silica gel (60–120 mesh) column chromatography and eluted with hexane:ethyl acetate (9:1) to afford (*RS*)- **2**.

(*RS*)-N-(3-acetyl-4-(oxiran-2-ylmethoxy)phenyl)butyramide (**2**), a yellow liquid (82% yield, 4.5 g); ^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.00 (t, J = 7.4 Hz, 3H), 1.67-1.76 (m, 2H), 2.33 (t, *J* = 7.28 Hz, 2H), 2.64 (bs, 3H), 2.77 (q, *J* = 2.64 Hz, 1H), 2.91 (t, *J* = 4.48 Hz, 1H), 3.39-3.43 (m, 1H), 3.95 (dd, *J* = 6.48, 11.2 Hz, 1H), 4.47 (dd, *J* = 2.48 H, 11.24 Hz, 1H), 7.10 (d, 1H), 7.75-7.81 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 12.6, 18.9, 30.6, 38.3, 43.6, 49.6, 70.1, 113.1, 121.5, 125.9, 127.9, 132.1, 154.5, 173.1, 199.9; MS (APCI) (*m/z*) 266.43. (*R*)-N-(3-acetyl-4-(oxiran-2-ylmethoxy)phenyl)butyramide (**2**), a yellow liquid (81% yield, 1.1 g); 96% ee. $[\alpha]_D^{20}$ -20.4 (*c* 1.0, MeOH) $[\alpha]_D^{20}$ -20.8 (*c* 0.013, MeOH), op 98%. (*S*)- N-(3-acetyl-4-(oxiran-2-ylmethoxy)phenyl)butyramide (**2**), a yellow liquid (84% yield, 1.2 g); 94% ee. $[\alpha]_D^{20}$ +19.9 (*c* 1.0, MeOH).

Acetyl chloride (2.1 mL, 20 mmol, 1.5 eq) was added to a stirred solution of (*RS*)-**2** (5.5 g, 20 mmol, 1 eq in 100 mL (DCM: water: 1:1). The consequential reaction mixture was stirred at 5 °C for 2 h and on completion of the reaction (TLC), the mixture was extracted with DCM (50 mL) and washed with water. After organic layer separation, Na_2SO_4 was added to it to absorb moisture, then filtered and concentrated under vacuum. The remainder was purified by silica column (60-12 mesh) eluting with ethyl acetate: hexane (15:85) to obtain (*RS*)-**11**. The (*RS*)-**11** was then subjected to chiral HPLC analysis using a Chiralcel OJ-H column and the two enantiomers (*S*)- and (*R*)- **11** were eluted after 18.7 min and 19.8 min (hexane:2-propanol: 4:1), respectively, and were present in a ratio of 49:51. Following a similar procedure, the (*R*)- and (*S*)-**11** were prepared from (*S*)- and (*R*)-**2**, respectively.

(*RS*)-N-(3-acetyl-4-(3-chloro-2-hydroxypropoxy)phenyl)butyramide (**11**), a white solid (92% yield, 5.7 g); ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.02 (t, *J* = 6.76 Hz, 3H) 1.75-1.81 (q, 2H), 2.35 (t, *J* = 7.32 Hz, 2H), 2.62 (s, 3H), 3.33 (bs, 1H), 3.75-3.77 (m, 2H), 4.20-4.24 (m, 3H), 6.97 (d,

J = 8.92 Hz, 1H), 7.20 (bs, 1H), 7.72-7.733 (m, 1H), 7.81-7.84 (m, 1H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 15.6, 20.6, 27.9, 40.5, 48.1, 70.9, 72.5, 118.9, 120.7, 121.3, 130.3, 134.4, 154.9, 168.3, 195.2; MS (APCI) (*m/z*) 302.87.

(*R*)-N-(3-acetyl-4-(3-chloro-2-hydroxypropoxy)phenyl)butyramide (**11**), a white solid, (91% yield, 1.4 g); The product was then subjected to chiral HPLC analysis using chiral OJ-H column, the two enantiomers were eluted at t_S = 17.9 min and t_R = 16.9 min (80:20::hexane: 2-propanol) with peak areas of 3.5 and 96.5%, respectively (93% ee).

(*S*)-N-(3-acetyl-4-(3-chloro-2-hydroxypropoxy)phenyl)butyramide (**11**), a white solid (94% yield, 1.5 g); The product was then subjected to chiral HPLC analysis using chiral OJ-H column, the two enantiomers were eluted at t_S = 17.9 min and t_R = 16.9 min (80:20::hexane: 2-propanol) with peak areas of 97 and 3%, respectively (94% ee).

Synthesis of (*RS*)-1-(2-acetyl-4-butyramidophenoxy)-3-chloropropan-2-yl acetate (10). A well-known method was used to synthesize (*RS*)-**10** with some modifications.[26] Briefly, (*RS*)-10 was synthesized by treating (*RS*)-**11** (0.16 g, 0.5 mmol, 1 eq) with Ac_2O (0.05 g, 0.5 mmol, 1 eq) in the pyridine (0.04 g, 0.5 mmol, 1 eq) at 30 °C in a small RBF with magnetic stirring. After disappearance of (*RS*)-**11** (TLC, 2 h), ice water (50 mL) was added to the reaction mixture and pH 3 was adjusted with 3 M HCl. The final product was extracted with ethyl acetate and brine solution. The organic layer was then separated and concentrated under vacuum to afford (*RS*)-**10**.

(*RS*)-1-(2-acetyl-4-butyramidophenoxy)-3-chloropropan-2-yl acetate (**10**), a yellow liquid (92% yield, 0.17 g); ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.85-2.87 (m, 1H), 2.93-2.96 (m, 1H), 3.39-3.43 (m, 1H), 4.11-4.15 (dd, 1H), 4.36-4.40 (dd, 1H), 7.01-7.06 (m, 2H), 7.51-7.59 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 44.5, 49.8, 69.3, 102.3, 112.68, 116.2, 121.3, 133.8, 134.4, 160.0; MS (APCI) (*m/z*): 344.92

The (*RS*)-**10** was then subjected to chiral HPLC analysis using a chiral OJ-H column, the two enantiomers were eluted at t_R = 51 min and t_S = 54 min (83:17: hexane: 2-propanol) with peak areas of 50.8 and 49.2%, respectively.

(*S*)-1-(2-acetyl-4-butyramidophenoxy)-3-chloropropan-2-yl acetate (**10**), a yellow liquid (93% yield, 0.17 g); The product was then subjected to chiral HPLC analysis using a chiral OJ-H column, the two enantiomers were eluted at t_R = 51 min and t_S = 54 min (83:17::hexane: 2-propanol) with peak areas of 5% and 95%, respectively, (90% ee).

Enantioselective transesterification of (*RS*)-11. In a 5 mL conical flask a mixture of (*RS*)-**11** (20 mM) in 100 μL [BMIM]PF_6, vinyl acetate (5.4 mmol) and toluene (900 μL) were taken. Lipases from different commercial sources[27] (lipase A, *Candida antarctica* CLEA, *Candida rugosa* 90860, *Candida rugosa* 62316, *Candida rugosa* L-1754, *Candida cylindracea*, *Aspergillus niger*, porcine pancreas and AY "Amano"30, immobilized lipase in sol-gel-Ak from *Pseudomonas cepacia*, immobilized lipozyme from *Mucor miehei*, lipase acrylic resin from *Candida antarctica*) were used to carry out the reaction. All the enzyme preparations were individually added, flasks were then capped and placed in a shaker at 30 °C (200 rpm). After the completion of the reaction, the contents were extracted using isopropyl alcohol and subjected to HPLC analysis to determine the conversion and the enantiomeric excess.[28]

Optimization of Transesterification Reaction

Various organic solvents such as diethyl ether, 1,4-dioxane, diisopropyl ether, DCM, toluene, MeCN, benzene, *tert*-butyl methyl ether, heptane, and isooctane were used[29–33] to find their effect on the transesterification of (*RS*)-**11**. The optimum time was determined by carrying out the reaction and collecting the samples at various time intervals. Various aspects of enzymatic acylation such as enantioselectivity, conversion, and greenness[34] have been reported to be influenced by the acyl donors.[35] The enantioselectivity and rate of conversion of enzyme-catalyzed kinetic resolution was therefore studied using various acyl donors. The reaction temperature also influences the rate and enantioselectivity of the transesterification reaction.[36,37] Various enzyme concentrations (15, 30, 45, 60, and 90 mg/mL) were used with a fixed substrate concentration (20 mM). Substrate concentrations were also varied (10, 20, 30, 40, and 50 mM) to achieve optimum substrate concentration for the reaction. Samples were analyzed for conversion and enantioselectivity of the enzymes used.

Preparative-Scale Transesterification Reaction

The resolution of (*RS*)-**11** was performed by subjecting 50 mL (20 mmol substrate, 0.31 g) reaction mixture to resolution by CAL CLEA and CRL 62316 lipases at 37 and 30 °C using vinyl acetate as acyl donor in 1,4-dioxane and toluene, respectively. The reaction mixture was filtered and the solvent was evaporated in rotavapor and the resulting dried residue was subjected to silica column chromatography using hexane:ethyl acetate (17:3) as a mobile phase. The isolated yield of (*S*)-**11** was 48%, 0.15 g with ee 99.7%, (Chiralcel OJ-H) and that of (*R*)-**10** was 46%, 0.16 g with ee 98%, (Chiralcel OJ-H) with CAL CLEA enzyme. It was observed that with CRL 62316 the isolated yield of (*R*)-**11** was 45%, 0.14 g, with ee 98% (Chiralcel OJ-H) and that of (*S*)-**10** was 48%, 0.17 g with ee 95% (Chiralcel OJ-H).

Deacylation of (*RS*)/(*R*)/(*S*)-10. A solution of K_2CO_3 (0.27 g, 2 mmol) in deionized water (1 mL) was added to a solution of **10** (0.35 g, 1 mmol) in methanol (5 mLL) and the resultant reaction mixture was allowed to stir for 2 h at room temperature (30 °C). After completion, the reaction mixture was extracted with EtOAc (3 x 15 mL) and water (10 mL).[38] The combined organic extracts were dried over Na_2SO_4 and concentrated under vacuum to obtain the crude which was purified by silica gel column chromatography (100–200 mesh) to obtain the corresponding alcohol.

(*RS*)-**11**: a light yellow liquid (97% yield, 0.30 g). (*R*)-**11**: a light yellow liquid, (94% yield, 0.29 g); The product was then subjected to chiral HPLC analysis using Chiralcel OJ-H column, the two enantiomers were eluted at t_S = 18.7 min and t_R = 19.8 min (80:20::hexane: 2-propanol) with peak areas of 1.0 and 99%, respectively, (98% ee). (*S*)-**11**: a light yellow liquid, (97% yield, 0.30 g); The product was then subjected to chiral HPLC analysis using Chiralcel OJ-H column, the two enantiomers were eluted at t_S = 18.7 min and t_R = 19.8 min (80:20::hexane: 2-propanol) with peak areas of 99.2 and 0.8%, respectively (99% ee).

Synthesis of (*R*)/ (*S*)- 1. The (*R*)/(*S*)-**11** (0.31 g, 1.0 mmol) was treated with isopropylamine (0.081 mL, 1.0 mmol) in methanol (10 mL) and Et_3N (0.14 mL, 1.0 mmol) for 12 h under reflux conditions. Upon reaction completion, the mixture was diluted with ethyl acetate (15 mL) and washed with water.[39] The organic layer was separated and dried on Na_2SO_4 and concentrated under vacuum. The residue was purified silica gel (60-12 mesh) column chromatography eluted with hexane:ethyl acetate (17:3) to obtain (*R*)/(*S*)- **1**. (*R*)-acebutolol (**1**), a white solid (93% yield, 0.31 g); ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.98 (t, J = 7.36, 3H), 1.08-1.10 (m, 6H), 1.65-1.74 (m, 2H), 2.29 (t, J = 7.4, 2H), 2.66-2.70 (m, 1H), 2.79-2.87 (m, 2H), 4.03-4.11 (m, 3H), 7.08 (d, J = 8.92, 1H), 7.73-7.79 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 12.6, 18.9, 21.1, 21.2, 38.3, 48.5, 49.6, 68.34, 71.6, 113.2, 121.6, 125.9, 127.8, 131.8, 154.8, 173.1, 200.2; MS (APCI) (*m/z*): 325.44. (*S*)-acebutolol (**1**), a white solid (90% yield, 0.30 g); 82% ee. $[\alpha]_D^{20}$ +15.0 (*c* 1.0, EtOH)

Resolution of enzymatically prepared (*R*)/(*S*)-11. (*R*)-N-(4-(3-chloro-2-hydroxypropoxy)-3-methoxyphenyl)butyramide (**11**), a white solid (44% yield, 0.66 g); The product was then subjected to chiral HPLC analysis using chiral OJ-H column, the two enantiomers were eluted at t_S = 18.7 min and t_R = 19.9 min (80:20::hexane: 2-propanol) with peak areas of 1.7 and 98%, respectively (97% ee). (*S*)-1-(4-butanoylaminophenoxy)-3-chloropropan-2-yl acetate (**10**), a yellow liquid (45.3% yield, 0.31 g); The product was then subjected to chiral HPLC analysis using the chiral OJ-H column, the two enantiomers were eluted at t_R = 42.5 min and t_S = 38.7 min (hexane: 2-propanol::4:1) with peak areas of 0.600 and 99.4%, respectively (98.8% ee).

RESULTS AND DISCUSSION

Biocatalytic routes for the synthesis of enantiopure acebutolol derivatives are preferred over chemical routes

due to the green nature of the procedure. Here, chemoenzymatic routes to prepare (*R*) and (*S*)-acebutolol is proposed using lipase mediated kinetic resolution (Scheme 2). Preparation of chemical entities using ionic liquid as reaction medium and the use of mild biocatalysts such as lipase makes these routes preferable over the use of harsh chemicals and solvents.

Synthesis of (RS)-N-(3-acetyl-4-(3-chloro-2-hydroxypropoxy)phenyl)butyramide (11)

N-(3-acetyl-4-hydroxyphenyl)butyramide (**3**) was reacted with 2-(chloromethyl)oxirane (*RS*)-**4** in the presence of K_2CO_3 in MeCN under reflux and afforded N-(3-acetyl-4-(oxiran-2-ylmethoxy)phenyl)butyramide (*RS*)-**2** with 82% yield. Further, the desired substrate for the lipase catalyzed kinetic resolution, (*RS*)-**11** (92% yield) was obtained by the reaction of (*RS*)-**2** with acetyl chloride in DCM and water (Scheme 3).

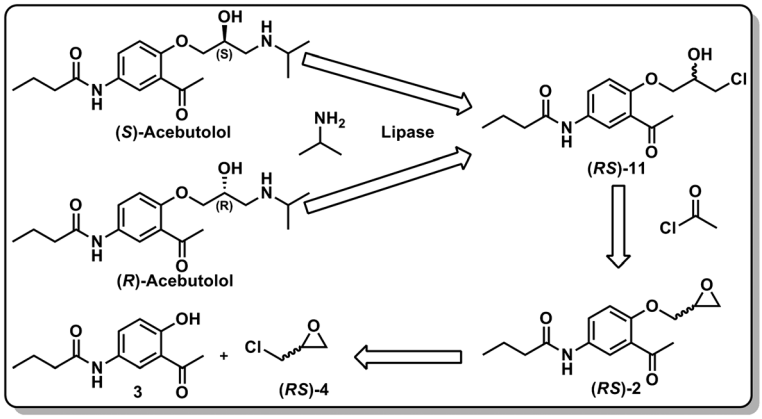
Synthesis of Standard Compounds Through the Chemical Route

The standard samples of (*R*)- or (*S*)-**11** and their O-acetylated derivatives (*R*)- or (*S*)-**10** were synthesized to optimize the enzymatic kinetic resolution conditions. For this purpose, first (*R*)-**2** [81% yield, 92% ee and optical rotation [α] +4.22 (*c* 1.0, $CHCl_3$)] and (*S*)-**2** [84% yield, 94% ee and optical rotation [α] +4.28 (*c* 1.0, $CHCl_3$)] were synthesized by the reaction between compound **3** and (*S*)- and (*R*)-**4**, respectively (Scheme 4). Chiral HPLC and optical rotation analysis was used to determine the optical purity of synthesized compounds. Here, the nucleophilic ring opening of oxirane at the least substituted carbon atom followed by nucleophilic displacement of chlorine atom through S_N^2(concerted) mechanism (Scheme 4) may have been responsible for the inversion of configuration.[26,40] Following the same mechanism, (*R*)-**11** can be prepared from (*S*)-**4**.

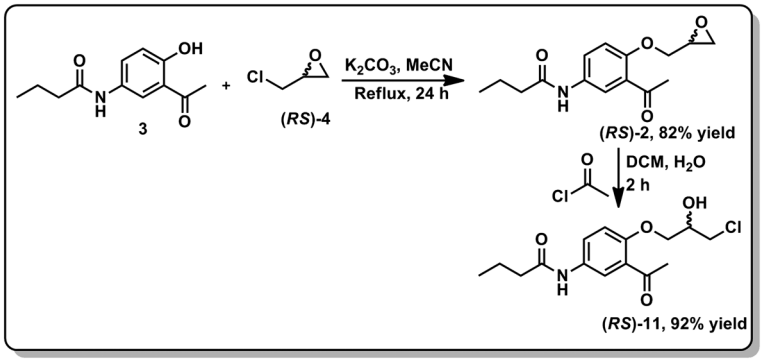
(*R*)-**11** (91% yield and 96% ee) and (*S*)-**11** (94% yield and 95% ee) were afforded by the ring opening of (*S*)- and (R)-**2**, respectively, using acetyl chloride. In the pyridine, the treatment of (*RS*)-**11** with Ac_2O at 4 °C gave the (*RS*)-**10** in 92% yield. Using similar procedure acetylation of (*R*)-**11** and (*S*)-**11** afforded, (*R*)-**10** and (*S*)-**10** (91% yield and 94% ee and 93% yield and 93% ee, respectively) (Scheme 5).

Screening of Lipases for Kinetic Resolution of (RS)-11

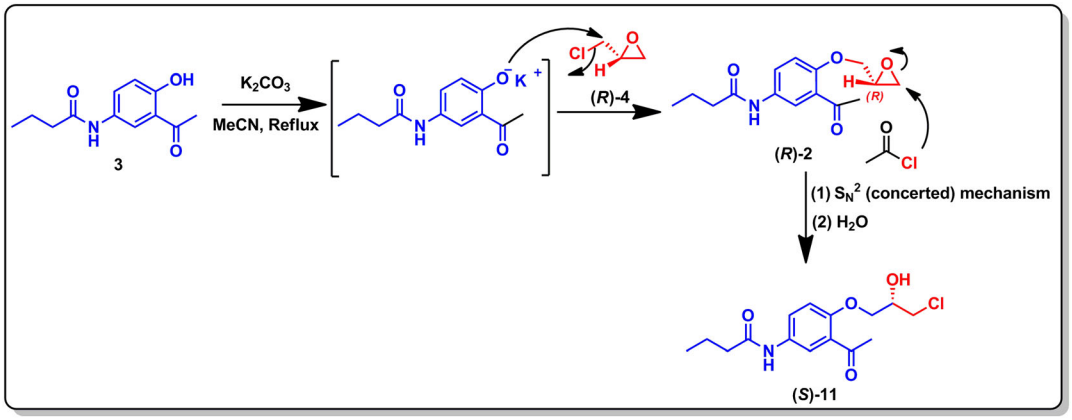
The substrates (*RS*)-**11** and the standard samples of (*R*)/(*S*)-**11** and (*R*)/(*S*)-**10** were used to determine the efficient method for the enzymatic kinetic resolution. Commercial lipases from different sources were screened using vinyl acetate as acyl donor for the transesterification of (*RS*)-**11** in toluene (Scheme 6).



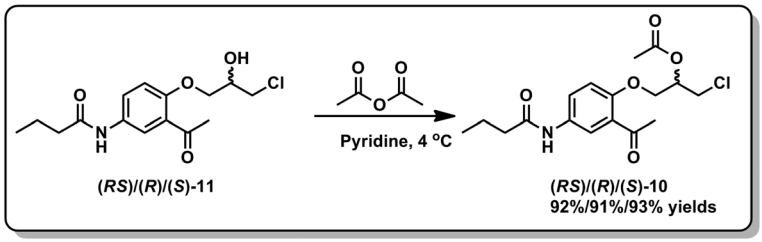
Scheme 2. Retrosynthetic pathway towards the synthesis of enantiopure acebutolol.



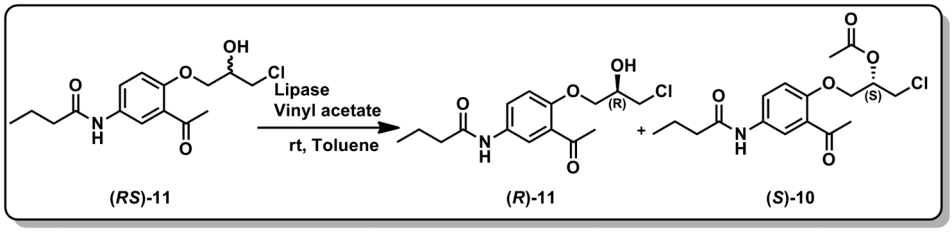
Scheme 3. Synthesis of (*RS*)-**11**.



Scheme 4. Mechanism of formation of (*S*)-**11** from (*R*)-**4**.



Scheme 5. Synthesis of (*RS*)/(*R*)/(*S*)-**10**.



Scheme 6. Enzymatic kinetic resolution of (*RS*)-**11**.

Among the various enzymes used, only two of them (CAL CLEA and CRL 62316) were found to be better in terms of conversion and enantioselectivity. For the conversion of (*RS*)-**11** to (*R*)-**11** and (*S*)-**10** the CAL CLEA showed better activity and the conversion of (*RS*)-**11** to (*S*)-**11** and (*R*)-**10** was most efficiently done by CRL 62316. It is to be noted that the two enzymes showed opposite enantioselectivity (Table 1).

Screening of Organic Solvents

The solvent effect on the enantioselectivity of enzymatic reactions are well known.[29–32] Lipases are maximally used by the chemists because of their high stability in organic solvents.[33] In this study a number of solvents with different log *P* values were investigated for the resolution of (*RS*)- **11** (Table 2). It has been reported in the literature[42] they demonstrate that the rates of biocatalytic reactions are less with polar solvents (log $P < 2$) as compared to apolar solvents (log $P > 4$). (*RS*)-**11** is not soluble in vinyl acetate. A detailed solubility study of (*RS*)-**11** was carried out using eleven various organic solvents to screen and optimize resolution conditions (Supplementary Material Table 1). It was found that (*RS*)-**11** is insoluble in most of the organic solvents except 1,4-dioxane, acetonitrile, and DCM; however, in order to screen various other organic solvents to carry out the lipase catalyzed reactions in interphase, it is mandatory to make (*RS*)-**11** soluble in some solvents. Further ionic liquids were used to make (*RS*)-**11** soluble in the presence of various organic solvents and its miscibility with isopropyl alcohol were tested out. Miscibility of ionic liquids is important for extraction of **11** from the reaction mixture using isopropyl alcohol as the extracting solvent (Supplementary Material Table 2). It was found that (*RS*)-**11** is soluble in [BMIM]BF_4 and [BMIM]PF_6 but insoluble in [EMIM]BF_4. Also, it was found that [BMIM]PF_6 was

TABLE 1. Lipase-catalyzed transesterification of (*RS*)-11 with vinyl acetate[a]

Type of lipase[b]	Time (h)	*C* (%)[c]	ee_s (%)[d]	ee_p (%)[e]	*E*[f]	Configuration of 10
CRL AMANO	48	17	15	71	6.8	*R*
CAL	48	44	35	44	3.5	*S*
CCL	48	26	21	59	4.7	*R*
CAL CLEA	48	48.7	89.3	94.1	99.6	*S*
CRL 62316	48	48	85	91	61	*R*
CRL L1754	48	27	18	49	3.4	*R*

[a]Conditions: (*RS*)-**11** (20 mM) in toluene (1 mL) was treated with of vinyl acetate (5.4 mmol) at 30 °C in the presence of the enzyme (15 mg/mL).
[b]CRL AMANO, CAL, CCL CAL CLEA, CRL 62316, & CRL L1754 lipase,
[c]Conversions were calculated from the enantiomeric excess (ee) of (*R*)-**11** (substrate S) and (*S*)-**10** (product P) using the formula: Conversion (C) = $ee_S/(ee_S + ee_P)$.
[d]Enantiomeric excess of (*R*)/(*S*)-**11** was determined by HPLC analysis (Daicel Chiralcel OD-H column) 80:20; hexane: 2-propanol, 1 mL/min flow rate at 254 nm.
[e]Enantiomeric excess of (*R*)/(*S*)-**10** was determined by HPLC analysis (Daicel Chiralcel OD-H column) 80:20; hexane: 2-propanol, 1 mL/min flow rate at 254 nm.
[f]*E* values were calculated using the formula: $E = \ln[(ee_p(1 - ee_s)/(ee_p + ee_s)]/\ln[(ee_p(1 + ees)/(ee_P + ee_s)]$.[28,41]

immiscible with isopropyl alcohol which is important for the extraction of (*R*)/(*S*)-11 and (*R*)/(*S*)-10 from the reaction mixture. The solvent effect of CAL CLEA and CRL 62316 for the kinetic resolution of (*RS*)-**11** was studied using vinyl acetate as the acyl donor at 30 °C. The 1,4-dioxane for CAL CLEA and toluene for CRL 62316 enzyme preparations were suggested for the maximum enantioselectivity and enantiomeric excess of substrate and product as compared to the other solvents.

Effect of Reaction Time

CAL CLEA and CRL 62316 catalyzed transesterification reaction of (*RS*)-**11** were carried out separately in 1,4-dioxane and toluene, respectively. The chiral HPLC was used to determine conversion and enantiomeric excess. In the 1,4-dioxane

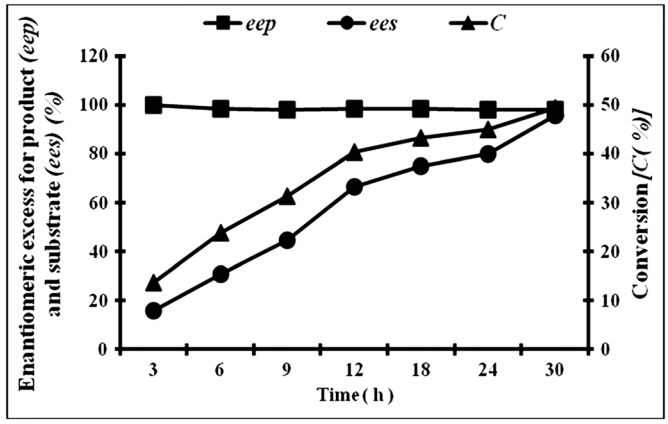


Fig. 1. Course of CAL CLEA catalyzed transesterification of (*RS*)-**11** in 1,4-dioxane.

solvent, it was found that mmaximum conversion (*C* = 49%), enantiomeric excess of substrate (96%), and enantiomeric excess of the product (98%) were achieved at 30 h. The enantiomeric ratio and enantiomeric excess of the product improved up to 30 h (*E* = 381) (Fig. 1). In toluene, the conversion and enantiomeric excess of substrate increased with the reaction time. Maximum conversion (49%), enantiomeric excess of substrate (88%) and product (91%) were achieved after 30 h of reaction. The enantiomeric ratio increased up to 30 h (*E* = 60) (Fig. 2). Slower reacting enantiomers have the disadvantage of being converted to the undesired isomer if the reaction time is prolonged, resulting in a less satisfactory enantiomeric excess. Thus, both solvents (toluene and 1,4-dioxane) were chosen and the optimum reaction time (30 h) was used to carry out further studies.

Effect of Acyl Donors

The enantioselectivity and rate of conversion of CAL CLEA and CRL 62316-catalyzed kinetic resolution of (*RS*)-**11** was studied in toluene and 1,4-dioxane, respectively, using

TABLE 2. Effect of organic solvent on the enantioselectivity in the resolution of (*RS*)-11 with lipase[a].

		S-selectivity with CAL CLEA				*R*-selectivity with CRL 62316			
Solvent	Log *P*	*C*[b] (%)	ee_s[c] (%)	ee_p[d] (%)	*E*[e]	*C*[b] (%)	ee_s[c] (%)	ee_p[d] (%)	*E*[e]
Acetonitrile	-0.33	55.2	99.9	80.9	66.8	23	2.1	6.9	1.2
1,4-dioxane	-1.1	49.6	93.8	95.4	149	5.8	2.6	42	2.5
t-butyl methyl ether	1.35	81	82	19	3.2	32	26	56	4.5
Diethyl ether	0.85	87	53	8.0	1.7	30	21	49	3.6
Dichloromethane	1.25	77	26	7.9	1.4	18	8.8	39	2.5
Benzene	2	78	80	22	3.3	30	33	76	10
Heptane	4	84	66	13	2.2	59	74	52	6.8
Toluene	2.5	48.8	90.4	94.9	122	50	86	87	40
Cyclohexane	3.41	77	71	21	2.9	47	66	73	13
Hexane	3.5	72	36	14	1.8	54	80	67	12
Isooctane	4.5	87	85	13	2.7	54	73	62	9.0

[a]Conditions: (*RS*)-**11** (20 mM) in organic solvent (1 mL) was treated with vinyl acetate (5.40 mmol) in the presence of lipase (15 mg/mL).
[b]Conversions were calculated from the enantiomeric excess (*ee*) of (*R*)-**11** (substrate S) and (*S*)-**10** (product P) for CAL CLEA and enantiomeric excess (*ee*) of (*S*)-**11** (substrate S) and (*R*)-10 (product P) for CRL using the formula: Conversion (C) = $ee_S/(ee_S + ee_P)$.
[c]Enantiomeric excess of (*R*)-**11** (substrate S) for CAL CLEA and (*S*)-**11** (substrate S) for CRL 62316 determined by HPLC analysis (Daicel Chiralcel OJ-H column) 80:20; hexane/2-propanol, 1 mL/min flow rate at 254 nm.
[d]Enantiomeric excess of (*R*)-**10** (product P) for CAL CLEA and (*S*)-**10** (product P) for CRL determined by HPLC analysis (Daicel Chiralcel OJ-H column) 80:20; hexane/2-propanol, 1 mL/min flow rate at 254 nm.
[e]*E* values were calculated using the formula: $E = \ln[(ee_p(1 - ee_s)/(ee_p + ee_s)]/\ln[(ee_p(1 + ees)/(ee_P + ee_s)]$.[28,41]

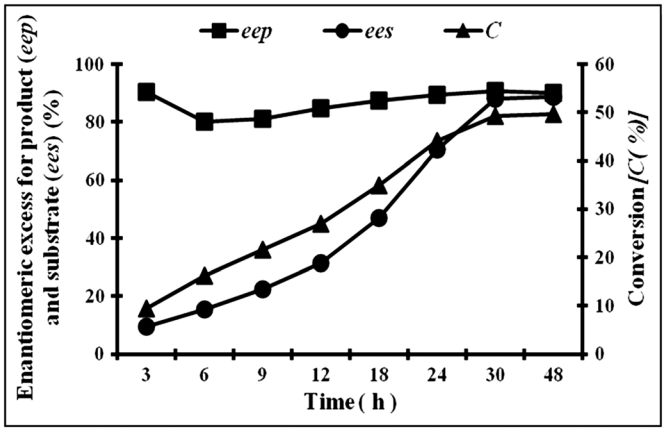


Fig. 2. Course of CRL 62316 catalyzed transesterification of (*RS*)-**11** in toluene.

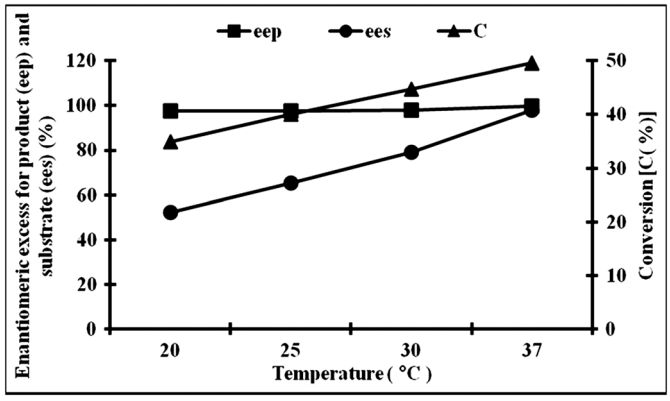


Fig. 3. Effect of temperature on the CAL CLEA catalyzed transesterification of (*RS*)-**11** with vinyl acetate in 1,4-dioxane.

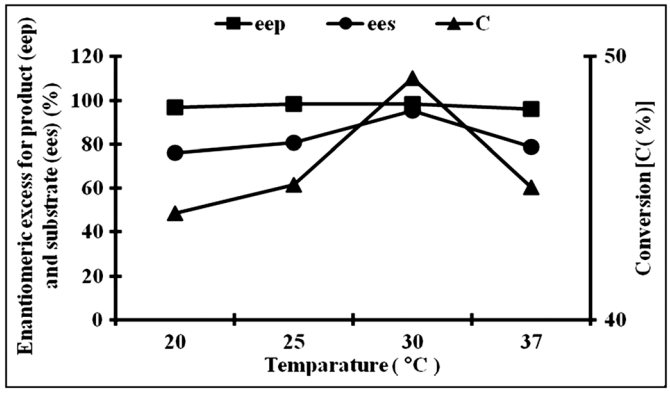


Fig. 4. Effect of temperature on the CRL catalyzed transesterification of (*RS*)-**11** with vinyl acetate in toluene.

various acyl donors (Table 3). Out of the six acyl donors screened, vinyl acetate showed the best results; whereas poor results were obtained with other acyl donors. Vinyl acetate has the tautomerization ability of the in situ generated vinyl alcohol to acetaldehyde. It shifts the equilibrium to the product without acting as a competitive substrate.

Effect of Temperature

The activity and enantioselectivity of the CAL CLEA and CRL 62316 catalyzed kinetic resolution of (*RS*)-**11** using vinyl acetate as the acyl donor in 1,4-dioxane and toluene, respectively, was determined at various temperatures. The conversion and the enantiomeric excess were determined after the resolution at 20, 25, 30, and 37 °C in 1,4-dioxane (Fig. 3) and in toluene (Fig. 4), respectively. It was found that the conversion (50%), the enantiomeric excess of the product (99.6%) and substrate (98%), and enantiomeric ratio (*E*, 2641) were highest in 1,4-dioxane at 37 °C (Fig. 3), while in the case of toluene, the maximum conversion (49%), enantiomeric excess of the product (98%) and substrate (95%), and enantiomeric ratio (*E*, 447) were observed at 30 °C (Fig. 4).

Effect of Enzyme Concentration

Resolution was carried out using diverse concentrations of CAL CLEA and CRL 62316 preparations (15, 30, 45, 60, and 90 mg/mL) in 1,4-dioxane and toluene, respectively. These experiments were conducted to understand the effect of enzyme concentration on the conversion, enantiomeric excess, and enantiomeric ratio of the product. In 1,4-dioxane, the maximum enantiomeric ratio (3606), enantiomeric excess of the product (99.7) and substrate (99) with 50% conversion was achieved with 15 mg/mL CAL CLEA enzyme preparation (Fig. 5). In toluene, the conversion increased with the increase in enzyme concentration up to a certain level, after which the enzyme became nonselective. The maximum

TABLE 3. Effect of acyl donors on the transesterification of (*RS*)-11[a]

	CAL CLEA catalyzed in 1,4-dioxane[a]				CRL 62316 catalyzed in toluene[a]			
Acyl donors	*C* (%)[b]	ee_s (%)[c]	ee_p(%)[d]	*E*[e]	*C* (%)[b]	ee_s (%)[c]	ee_p(%)[d]	*E*[e]
Acetic anhydride	0.3	0.0	6.6	1.1	4.7	0.47	9.4	1.2
Benzyl acetate	4.6	3.8	78	8.5	—	99	—	—
Ethyl acetate	2.9	1.8	61	4.3	—	0.44	—	—
Isopropenyl acetate	33	43	89	27	27	27	72	7.8
Phenylethyl acetate	3.5	1.7	47	2.8	—	0.82	—	—
Vinyl acetate	49.9	99	99	1057	50	89	90	55

[a]Conditions: (*RS*)-**11** (20 mM) in 1 mL solvent was treated with the acyl donor (5.4 mmol) at 30 °C in the presence of the lipase preparations (15 mg/mL).
[b]Conversions were calculated from the enantiomeric excess (ee) of (*R*)-**11** (substrate S) and (*S*)-**10** (product P) using the formula: Conversion (C) = $ee_S/(ee_S + ee_P)$.
[c]Enantiomeric excess of (*S*)-**10** determined by HPLC analysis (Daicel Chiralcel OJ-H column) 80:20; hexane/2-propanol, 1 mL/min flow rate at 254 nm.
[d]Enantiomeric excess of (*R*)-**11** determined by HPLC analysis (Daicel Chiralcel OJ-H column) 80:20; hexane/2-propanol, 1 mL/min flow rate at 254 nm
[e]*E* values were calculated using the formula: $E = \ln[(ee_p\ (1 - ee_s)/(ee_p + ee_s)]/\ln[(ee_p(1 + ees)/(ee_P + ee_s)]$.[28,41]

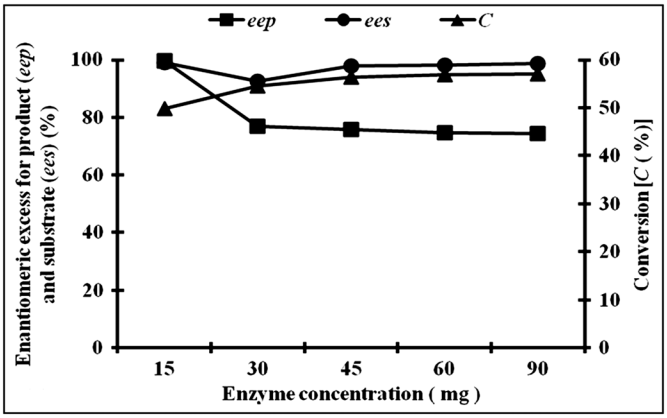


Fig. 5. Effect of enzyme concentration on CAL CLEA catalyzed transesterification of (*RS*)-**11** in 1,4-dioxane.

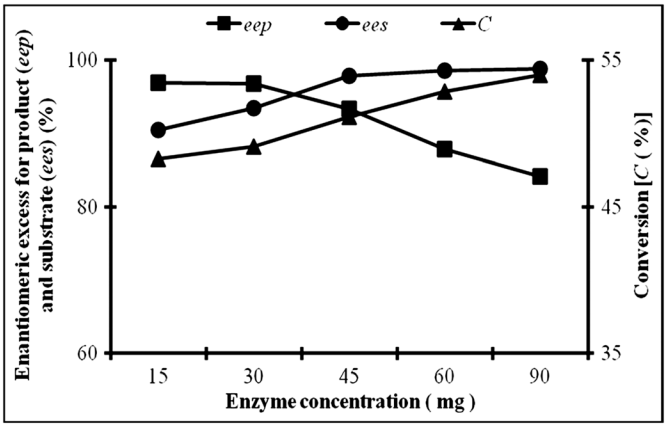


Fig. 6. Effect of enzyme concentration on CRL 62316 catalyzed transesterification of (*RS*)-**11** in toluene.

enantiomeric ratio (214), enantiomeric excess of the product (97) and substrate (93) with a conversion of 49% was obtained with 30 mg/mL CRL 62316 enzyme preparation in toluene reaction medium (Fig. 6). For all the subsequent experiments, an enzyme concentration of 15 and 30 mg/mL of CAL CLEA and CRL in 1,4-dioxane and toluene were used, respectively. Enantiomeric ratio (*E*) was calculated from the values of ee_p and conversion using the equation as described in footnote of Table 3.

Effect of Substrate Concentration

It is necessary to study the effect of substrate concentration on the activity and enantiomeric excess of any enzyme-catalyzed chiral reaction. Various substrate concentrations (10, 20, 30, 40, and 50 mM) were used in the reaction mixture for the resolution of (*RS*)-**11** with CAL CLEA and CRL 62316. In the case of CAL CLEA, it was found that maximum conversion was obtained with 20 mM substrate (C = 49%, ee_p = 100% and ee_s = 98%) (Fig. 7). In case of CRL 62316, it was found that maximum conversion was obtained with 10 mM substrate (C = 49%, ee_p = 99% and ee_s = 95%) (Fig. 8).

Deacylation of (RS) /(R)/(S)-10

In aqueous K_2CO_3 at room temperature, enantiopure, and racemic halohydrin **11** was obtained from deacylation of **10** after 2 h reaction (Scheme 7).

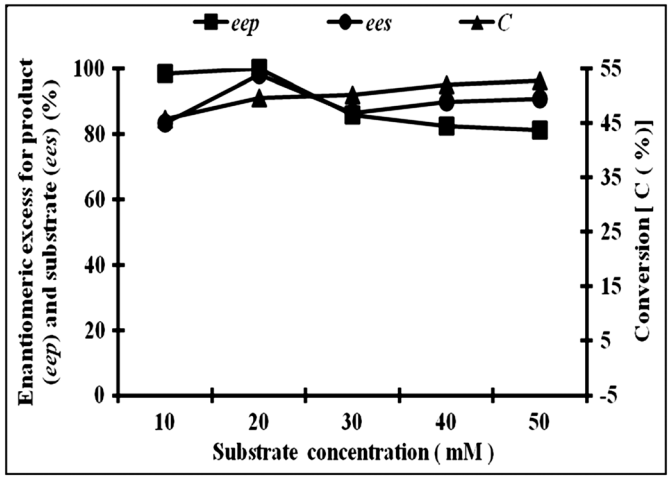


Fig. 7. Effect of substrate concentration on CAL CLEA catalyzed transesterification of (*RS*)-**11** in 1,4-dioxane.

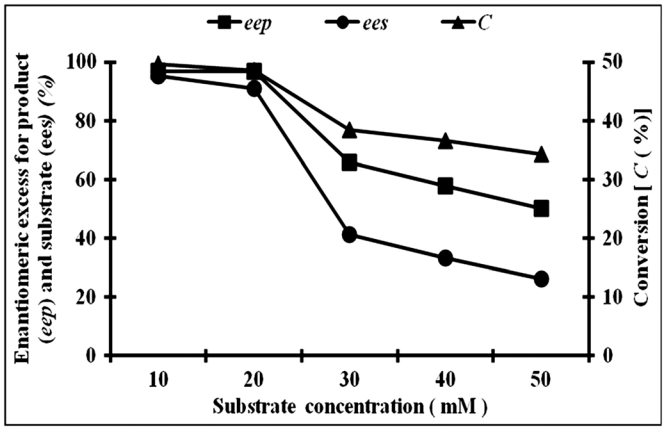
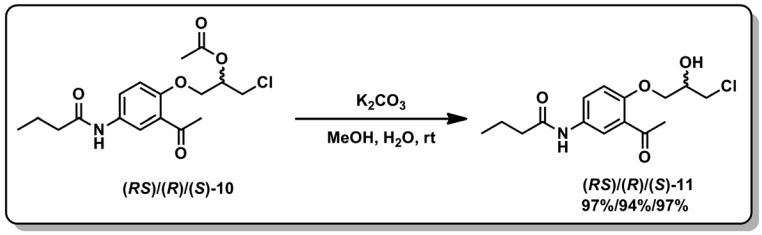


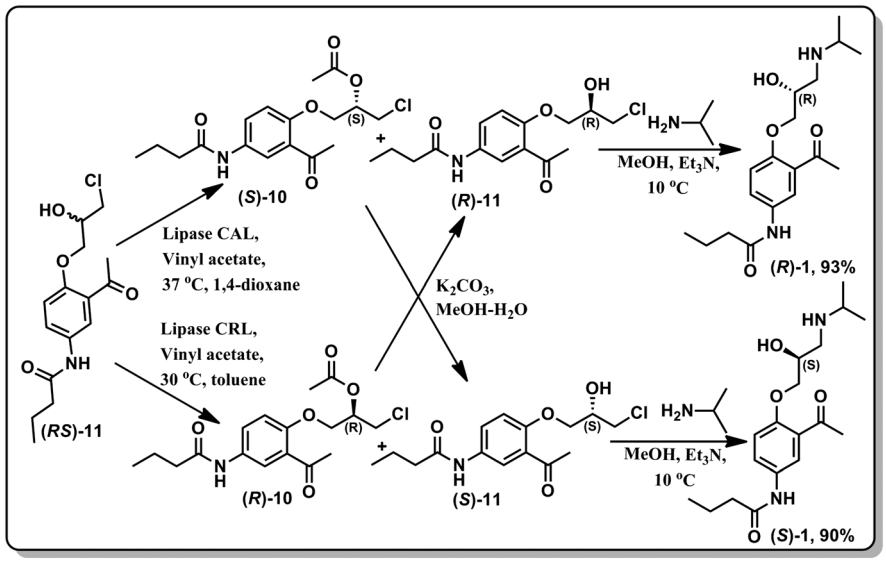
Fig. 8. Effect of substrate concentration on CRL catalyzed transesterification of (*RS*)-**11** in toluene.

Synthesis of (R) and (S)-Acebutolol (1)

From the enzymatic kinetic resolution of (*RS*)-**11** with vinyl acetate and CAL CLEA lipase enantiopure (*R*)-**11** and (*S*)-**10** was obtained. Optimum conditions for this reaction were: organic solvent: 1,4-dioxane; time: 30 h; temperature: 37 °C; enzyme concentration: 15 mg/mL and 20 mM substrate to achieve C = 49.59%, E = 11325, ee_p = 99.91% and ee_s = 98.30%. (*S*)-**10** was further hydrolyzed with K_2CO_3 to afford (*S*)-**11**. Enantiopure compound (*R*)-**11** was further reacted with isopropylamine in MeOH in the presence of Et_3N under reflux condition to afford (*R*)-acebutolol **1** (Yield 93%, Scheme 8). Similarly, from the enzymatic kinetic resolution of (*RS*)-**11** with vinyl acetate and CRL 62316, lipase enantiopure (*S*)-**11** and (*R*)-**10** was obtained. Optimum conditions for this reaction were; organic solvent: toluene; time: 30 h; temperature: 30 °C; enzyme concentration: 30 mg/mL and 10 mM substrate to achieve C = 49.59%, E = 239, ee_p = 96.8%, and ee_s = 95.3%. Enantiopure compound (*S*)-**11** was further reacted with isopropylamine in MeOH in presence of Et_3N under reflux condition to afford (*S*)- acebutolol **1** (yield 90%, Scheme 8). Additionally, (*R*) and (*S*)-**11** was chemically synthesized by the reaction of epichlorohydrin derivative (*S*) and (*R*)-**2**, respectively, with acetyl chloride as a standard compound.



Scheme 7. Synthesis of (*RS*) /(*R*)/(*S*)-**11**.



Scheme 8. Chemo-enzymatic synthesis of (*R*) and (*S*)-acebutolol (**1**).

CONCLUSIONS

Efficient chemical and chemoenzymatic synthesis of the enantiomerically pure cardiovascular drug acebutolol is reported in this study with improved overall yield (68–72%) and higher enantiomeric excess (95–99%). (*R*)-Acebutolol was obtained from (*RS*)-**11** via (*R*)-**10** enantiopure intermediate using CAL CLEA lipase with optimized reaction parameters (solvent, acyl donor, time, temperature, and enzyme concentration of the substrate). Similarly, (*S*)-acebutolol was obtained from (*RS*)-**11** via the (*S*)-**10** enantiopure intermediate using CRL 62316 lipase with optimized reaction parameters. This simple and high-yielding biocatalytic route to synthesize enantiopure acebutolol is an excellent example of green chemistry. This biocatalytic can be applied for the synthesis of various enantiopure drugs and drug intermediates.

ACKNOWLEDGMENTS

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SUPPORTING INFORMATION

Additional supporting information may be found in the online version of this article at the publisher's web-site.

LITERATURE CITED

1. Lund-Johansen P. Essential hypertension: hemodynamic and therapeutic changes over 20 years. J Cardiovasc Pharmacol 1991; 18: S1–S7.
2. Ricks WB, Harrison DC, Bell PA, Winkle RA. Treatment of drug-resistant life-threatening ventricular arrhythmias with acebutolol. Am J Cardiol 1976; 37: 165.
3. Boissel JP, Leizorovicz A, Picolet H, Peyrieux JC. Secondary prevention after high-risk acute myocardial infarction with low-dose acebutolol. Am J Cardiol 1990; 66: 251–260.
4. Sándor PS, Áfra J, Ambrosini A, Schoenen J. Prophylactic treatment of migraine with β-blockers and riboflavin: differential effects on the intensity dependence of auditory evoked cortical potentials. Headache 2000; 40: 30–35.
5. Hayes PE, Schulz SC. Beta-blockers in anxiety disorders. J Affect Disord 1987; 13: 119–130.
6. Pereira-Leite C, Carneiro C, Soares JX, Afonso C, Nunes C, Lúcio M, Reis S. Biophysical characterization of the drug–membrane interactions: the case of propranolol and acebutolol. Eur J Pharmaceut Biopharmaceut 2013; 84: 183–191.
7. Giacomini JC, Thoden WR. Ancillary pharmacologic properties of acebutolol: Cardioselectivity, partial agonist activity, and membrane-stabilizing activity. Am Heart J 1985; 109: 1137–1144.
8. Seydel JK, Wiese M. Drug-Membrane Interactions: Analysis, Drug Distribution, Modeling. Wiley-VC: Weinheim; 2002.
9. Escribá PV. Membrane-lipid therapy: a new approach in molecular medicine. Trends Mol Med 2006; 12: 34–43.
10. Escribá PV, Sánchez-Dominguez JM, Alemany R, Perona JS, Ruiz-Gutiérrez V. Alteration of lipids, G proteins, and PKC in cell membranes of elderly hypertensives. Hypertension 2003; 41: 176–182.

11. Chatterji AN. A randomized crossover comparison of acebutolol and methyldopa in the treatment of mild to moderate essential hypertension. Curr Med Res Opin 1978; 5: 675–681.
12. Singh BN, Thoden WR, Ward A. A review of its pharmacological properties and therapeutic efficacy in hypertension, angina pectoris and arrhythmia. Drugs 1985; 29: 531–569.
13. Gradman AH, Winkle RA, Fitzgerald JW, Meffin PJ, Stoner J, Bell PA, Harrison DC. Suppression of premature ventricular contractions by acebutolol. Circulation 1977; 55: 785–791.
14. Hansson L, Berglund G, Andersson O, Holm M. Controlled trial of acebutolol in hypertension. Eur J Clin Pharmacol 1977; 12: 89–92.
15. Hanson RN, Holman BL, Davis MA. Synthesis and biologic distribution of radioiodinated. beta-adrenergic antagonists. J Med Chem 1978; 21: 830–833.
16. Basil B, Clark JR, Coffee ECJ, Jordan R, Loveless AH, Pain DL, Wooldridge KRH. New series of cardioselective adrenergic-β-receptor blocking compounds: 1-(2-Acyl-4-acylaminophenoxy)-3-isopropylaminopropan-2-ols. J Med Chem 1976; 19: 399–402.
17. Flouvat B, Roux A, Chau NP, Viallet M, Andre-Fouet X, Woehrle R, Gregoire J. Pharmacokinetics and bioavailability of diacetolol, the main metabolite of acebutolol. Eur J Clin Pharmacol 1981; 19: 287–292.
18. Stoschitzky K, Klein W, Lindner W. Stereoselective release of (S)-atenolol from adrenergic nerve endings at exercise. Lancet 1992; 340: 696–697.
19. Piquette-Miller M, Foster RT, Kappagoda CT, Jamali F. Pharmacokinetics of acebutolol enantiomers in humans. J Pharm Sci 1991; 80: 313–316.
20. Lee EJ, Williams KM. Chirality: clinical pharmacokinetic and pharmacodynamic considerations. Clin Pharmacokinet 1990; 18: 339–345.
21. Olanoff LS, Walle T, Walle UK, Coward TD, Gaffney TE. Stereo-selective clearance and distribution of intravenous propranolol. Clin Pharmacol Ther 1984; 35: 755–761.
22. Hietaniemi LA, Nupponen HE. 1-(4'-Alkylamido)-2'{1[n-(al- kyl)imino]-ethyl}-phenoxy-3-alkylamino-2-propanols and use thereof. U. S. Patent 4579970 1986.
23. Guebitz G, Pierer B, Wendelin W. Resolution of the enantiomers of drugs containing amino alcohol structure after derivatization with bromoacetic acid. Chirality 1992; 4: 333–337.
24. Smith LH. Beta-adrenergic blocking agents (3-Amino-2-hydroxypropoxy) benzamides. J Med Chem 1976; 19: 1119–1123.
25. Wang N, Tang X. An efficient chiral synthesis of (R)-N-[3-acetyl-4-(2-hydroxy-3-isopropylamino-propoxy) phenyl]-butanamide with high enantio-selectivity. Sci China Ser B 2009; 52: 1216–1219.
26. Banoth L, Chandarrao B, Pujala B, Chakraborti AK, Banerjee UC. Efficient chemoenzymatic synthesis of (RS)-, (R)-, and (S)-bunitrolol. Synthesis 2014; 46: 0479–0488.
27. Singh A, Goel Y, Rai AK, Banerjee UC. Lipase catalyzed kinetic resolution for the production of (S)-3-[5-(4-fluoro-phenyl)-5-hydroxy-pentanoyl]-4-phenyl-oxazolidin-2-one: an intermediate for the synthesis of ezetimibe. J Mol Catal B: Enzymatic 2012; 85: 99–104.
28. Straathof AJJ, Jongejan JA. The enantiomeric ratio: origin, determination and prediction. Enzyme Microbial Technol 1997; 21: 559–571.
29. Khmelnitsky YL, Rich JO. Biocatalysis in nonaqueous solvents. Curr Opin Chem Biol 1999; 3: 47–53.
30. Hudson EP, Eppler RK, Clark DS. Biocatalysis in semi-aqueous and nearly anhydrous conditions. Curr Opin Biotechnol 2005; 16: 637–643.
31. Gupta MN. Enzyme function in organic solvents. Eur J Biochem 1992; 203: 25–32.
32. Gupta MN, Roy I. Enzymes in organic media. Eur J Biochem 2004; 271: 2575–2583.
33. Dordick JS. Enzymatic catalysis in monophasic organic solvents. Enzyme Microbial Technol 1989; 11: 194–211.
34. Paravidino M, Hanefeld U. Enzymatic acylation: assessing the greenness of different acyl donors. Green Chem 2011; 13: 2651–2657.
35. Egri G, Baitz-Gács E, Poppe L. Kinetic resolution of 2-acylated-1, 2-diols by lipase-catalyzed enantiomer selective acylation. Tetrahedron: Asymmetr 1996; 7: 1437–1448.
36. Lou WY, Wang W, Li RF, Zong MH. Efficient enantioselective reduction of 4′-methoxyacetophenone with immobilized Rhodotorula sp. AS2. 2241 cells in a hydrophilic ionic liquid-containing co-solvent system. J Biotech 2009; 143: 190–197.
37. Wang P, Ma G, Gao F, Liao L. Enabling multienzyme bioactive systems using a multiscale approach. China Particuology 2005; 3: 304–309.
38. Madivada LR. An efficient synthesis of 1-(2-methoxyphenoxy)-2, 3-epoxypropane: key intermediate of β-adrenoblockers. Org Process Res Dev 2012; 16: 1660–1664.
39. Gandolfi CA. N-Acyl-2-substituted-1,3-thiazolidines, a new class of non-narcotic antitussive agents: studies leading to the discovery of ethyl 2-[(2-methoxyphenoxy)methyl]-β-oxothiazolidine-3-propanoate. J Med Chem 1995; 38: 508–525.
40. Dwivedee BP, Ghosh S, Bhaumik J, Banoth L, Banerjee UC. Lipase catalysed green synthesis of enantiopure atenolol. RSC Adv 2015. DOI:10.1039/C4RA16365F.
41. Faber K. Biotransformations in organic chemistry. Springer: Berlin, Heidelberg; 2011. p 31–63.
42. Laane C, Boeren S, Vos K, Veeger C. Rules for optimization of biocatalysis in organic solvents. Biotechnol Bioeng 1987; 30: 81–87.