Efficient Chemoenzymatic Synthesis of (*RS*)-, (*R*)-, and (*S*)-Bunitrolol

Linga Banoth,[a] Bhukya Chandarrao,[a] Brahmam Pujala,[b] Asit K. Chakraborti,*[b] U. C. Banerjee*[a]

[a] Department of Pharmaceutical Technology (Biotechnology), National Institute of Pharmaceutical Education and Research (NIPER), Sector 67, S. A. S. Nagar-160062, Punjab, India
E-mail: ucbanerjee@niper.ac.in

[b] Department of Medicinal Chemistry, National Institute of Pharmaceutical Education and Research (NIPER), Sector 67, S. A. S. Nagar-160062, Punjab, India
E-mail: akchakraborti@niper.ac.in

Received: 10.09.2013; Accepted after revision: 25.11.2013

Abstract: A new chemical and the first chemoenzymatic synthesis of β-adrenergic receptor blocking agent bunitrolol is reported in racemic (*RS*) and enantioenriched forms (*R* and *S*). The intermediates (*R*)- and (*S*)-1-chloro-3-(2-cyanophenoxy)propan-2-ol intermediates were synthesized from the corresponding racemic alcohol through enzymatic kinetic resolution. The commercial available lipases PS-C and CCL exhibited complementary enantioselectivity during transesterification of the racemic alcohol with vinyl acetate affording the (*R*)-alcohol along with (*S*)-acetate and the (*S*)-alcohol along with (*R*)-acetate, respectively, and represent an example of enzymatic switch for reversal of enantioselectivity. The effects of various reaction parameters, such as temperature, time, substrate and enzyme concentration, and reaction medium, on the activity and enantioselectivity were optimized. The (*R*)- and (*S*)-alcohols were converted into (*S*)-and and (*R*)-bunitrolol, respectively, by treatment with *tert*-butylamine. The (*R*)- and (*S*)-acetates, obtained enzymatically were deacetylated to the corresponding alcohol by chemical hydrolysis and further converted into (*S*)-and and (*R*)-bunitrolol by chemical means. This is the first chemoenzymatic synthesis of both of the enantiomers of the drug. (*RS*)-, (*R*)-, and (*S*)-Bunitrolol were also synthesized following the 'all chemical' routes from (*RS*)-, (*R*)-, and (*S*)-epichlorohydrin via the corresponding (*RS*)-, (*S*)-, and (*R*)-2-cyanoglycidyl ether and the (*RS*)-, (*R*)-, and (*S*)-1-chloro-3-(2-cyanophenoxy)propan-2-ol intermediates with improved overall yields and better enantiomeric excesses compared to the reported processes.

Key words: bunitrolol, biocatalysis, lipase, drug, overall enantioselectivity synthesis

Cardiovascular diseases (CVDs) account for one third (16.6 million) of global deaths in 2001 and they are the leading cause of death worldwide.[1] Effective life-saving medicines for the management of cardiovascular disorders,[2] including hypertension,[3] angina pectoris, cardiac arrhythmias, and other disorders[4] related to the sympathetic nervous system, are β-adrenergic blocking agents that have been in use for more than 25 years. However, the increase in total peripheral resistance by β-adrenergic blocking agents is thought to oppose their antihypertensive effect[5] and this has led to the development of drugs with dual α- and β-adrenergic blocking effects.[6] Bunitrolol (**1**), a vasodilatory drug known for its β-adrenergic blocking properties,[7] is expected to meet new requirements for therapeutic advantage in the acute vasodilatory effect as its α-adrenoreceptor blocking action may contribute to the decrease in total peripheral resistance when hypertensive patients are treated chronically with bunitrolol.[8]

The several approaches to the synthesis of **1** are summarized in Scheme 1.[9] The most common strategy for the synthesis of the 1,2-amino alcohol class of β-adrenergic blocking agents involves the opening of the epoxide ring by an amine.[10] Efforts in this direction in the synthesis of **1** involve the reaction of *tert*-butylamine with the requisite epoxide, 2-cyanophenyl glycidyl ether **2**.[9] However, most of these have one or more drawbacks such as a multistep procedure for the preparation of **2** that requires a costly and toxic osmium catalyst, corrosive reagents, or long reaction times.[9a,c]

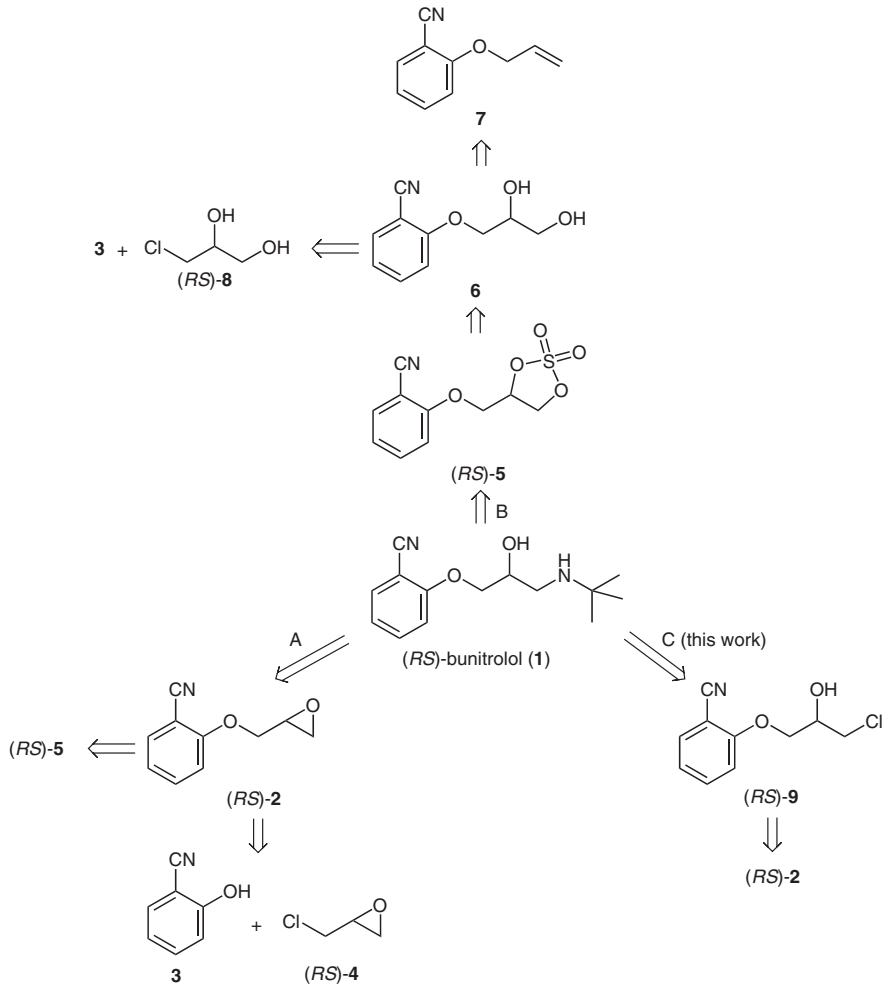
Although currently **1** is marketed in its racemic form, the *S*-enantiomer is the eutomer with 20 times higher potency[11] than that of the distomeric *R*-form, which has undesirable effects,[11,12] and the two enantiomers have different metabolic profiles.[13] This has generated an interest in preparing the enantioenriched/enantiopure forms of **1**.

The reported methodologies (routes A and B) for the synthesis of enantiopure/enantioenriched **1** involves the epoxide ring-opening reaction of the glycidic ethers (*R*)- and (*S*)-**2**, prepared from enantioenriched epichlorohydrin (*R*)-**4**,[9b] multistep reaction of (*R*)- and (*S*)-**6** obtained through spontaneous resolution of the alkylated product of 2-cyanophenol **3** with (*RS*)-**8**,[9c] and a multistep procedure involving toxic osmium tetroxide mediated asymmetric dihydroxylation of **7**.[9a] These reported procedures afford overall 26–37% chemical yields with 60–88% ee.

Herein we describe (route C) concise new chemical and novel chemoenzymatic synthetic routes for (*RS*)-, (*R*)-, and (*S*)-bunitrolols with much improved overall chemical yields (59–61%) with higher (80–94%) enantiomeric excesses.

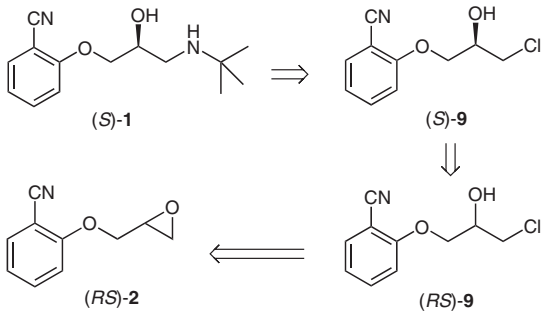
While designing a new synthetic route, we took into consideration the increasing influence of green chemistry tools on medicinal chemistry and chemistry research based organization[14] and the necessity to improve existing transformations to enrich the medicinal chemist's tool box for more general and amenable applications.[15] Integrating biocatalysis in synthesis is an elegant approach to expand the organic toolbox and it adds and additional dimension

SYNTHESIS 2014, 46, 0479–0488
Advanced online publication: 11.12.2013
DOI: 10.1055/s-0033-1340465; Art ID: SS-2013-Z0620-OP



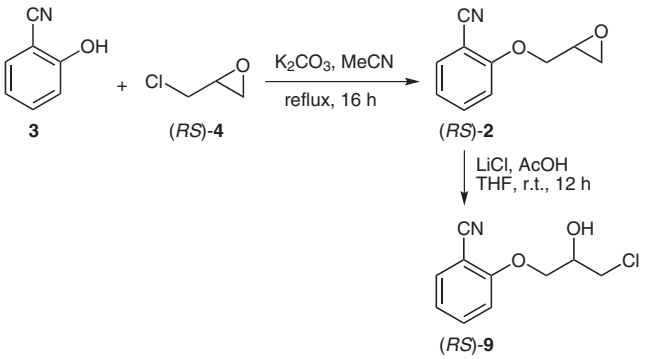
Scheme 1 Various synthetic strategies for **1**

in greener chemistry.[16] In this context, the enzymatic kinetic resolution of a racemic secondary alcohol provides the desired scope[17] and encouraged us to design a new chemoenzymatic route for (*R*)- and (*S*)-bunitrolols (Scheme 2).



Scheme 2 New chemoenzymatic route for (*R*)- and (*S*)-bunitrolols

The starting racemic epoxide (*RS*)-**2** was prepared in 80% yield by the reaction of **3** with (*RS*)-**4** in the presence of potassium carbonate in acetonitrile under reflux following the reported procedure.[10b] The treatment of (*RS*)-**2** with lithium chloride in tetrahydrofuran and acetic acid afforded the desired substrate (*RS*)-**9** required for enzymatic kinetic resolution[18] (Scheme 3).



Scheme 3 Synthesis of (*RS*)-**9**

To assess the catalytic efficiency of the enzyme and to derive the best operative experimental conditions for kinetic resolution, it is necessary to have authentic samples of (*R*)- and (*S*)-**9** and the corresponding O-acylated derivatives (*R*)- and (*S*)-**10**, respectively.

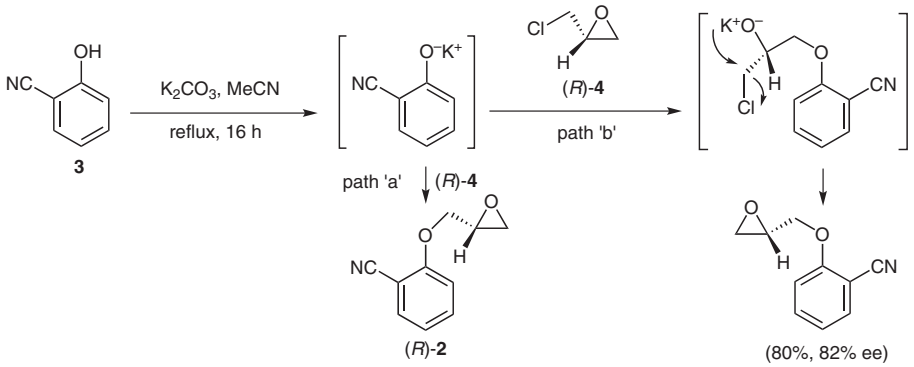
The requisite starting materials (*S*)- and (*R*)-**2** for the authentic samples of (*R*)- and (*S*)-**9** were obtained by reaction of **3** with (*R*)- and (*S*)-**4**, respectively, following the modified procedure.[10b] The enantiomeric excess/optical purity was determined by chiral HPLC and from the optical rotation value. As observed in a previous report,[10b] alkylation using (*R*)-**4** resulted in (*S*)-**2** (80% yield, 82% ee) and (*S*)-**4** afforded (*R*)-**2** (78% yield, 81% ee) (Scheme 4). The formation of (*S*)-**2** from (*R*)-**4** can be attributed due to the nucleophilic ring opening of the epoxide ring at the least substituted carbon atom of the epoxide followed by nucleophilic displacement of the chlorine atom (Scheme 4, path b), rather than direct nucleophilic substitution of the chlorine atom (Scheme 4, path a). This, accounts for the observed inversion of configuration.

Ring opening of (*S*)-**2** by lithium chloride following the same procedure as used for (*RS*)-**2** afforded (*R*)-**9** in 84% yield with 82% ee. Similarly, (*S*)-**9** was obtained from (*R*)-**2** in 82% yield with 81% ee. The treatment of (*RS*)-**9** with acetic anhydride at room temperature under neat conditions in the presence of zirconium(IV) chloride (2 mol%)[19] afforded (*RS*)-**10** in 95% yield. Acetylation of (*R*)- and (*S*)-**9** following a similar procedure resulted in the formation of (*R*)- and (*S*)-**10** in 89% and 87% yields with 82% and 81% ee, respectively.

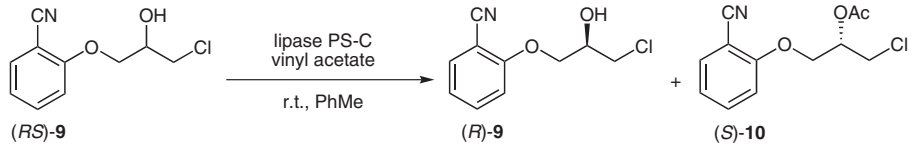
With the substrate (*RS*)-**9** and authentic samples of (*R*)- and (*S*)-**9** and (*R*)- and (*S*)-**10** in hand, it was planned to derive the best operative enzymatic kinetic resolution procedure using various lipases.

Initially, commercially available lipases: *Pseudomonas cepacia* immobilized on sol-gel-Ak (PS-C), immobilized lipozyme *Mucor miehei* (MML), *Candida antarctica* immobilized on acrylic resin (CAL), lipase A *Candida antarctica* (CAL-A), *Candida rugosa* (CRL L8525), *Candida rugosa* (CRL L-1754), *Candida cylindracea* (CCL), *Aspergillus niger*, porcine pancreas lipase, lipase AY 'Amano' 30 were screened for the transesterification of (*RS*)-**9** with vinyl acetate in toluene (Scheme 5).

The lipases PS-C exhibited best activity for conversion of (*RS*)-**9** into (*R*)-**9** and (*S*)-**10**, respectively. The lipases CCL exhibited best activity for conversion of (*RS*)-**9** into (*S*)-**9** and (*R*)-**10**, respectively. Thus, the enzymes PS-C and CCL showed complementary action with respect to enantioselectivity. These enzymes were better in terms of conversion and different enantioselectivity compared to other lipases (Table 1).



Scheme 4 Formation of (*S*)-**2** from (*R*)-**4**



Scheme 5 Enzymatic kinetic resolution of (*RS*)-**9**

Table 1 Lipase-Catalyzed Transesterification of (*RS*)-**9** with Vinyl Acetate[a]

Lipase	Time (h)	C[b] (%)	ee[c] (%) of **10**	ee[c] (%) of **9**	E[b]	Config of **10**
CRL L1754	48	18	55	12	1	*R*
CRL L8525	48	76	11	33	1.61	*R*
CCL	48	32	59	27	2	*R*
PS-C	48	30	79	34	2	*S*
CAL-A	48	2	16	0.3	1	*RS*
MML	48	3	4	0.1	1	*RS*

[a] Conditions: (*RS*)-**9** (1 mmol), toluene (3.5 mL) treated with vinyl acetate (5.4 mmol) at 25 °C in the presence of the enzyme (15 mg/mL).
[b] For the method of calculation see the experimental section.
[c] Enantiomeric excess of the substrate **9** or product **10** was determined by HPLC analysis (Daicel Chiralcel OD-H column, hexane–*i*-PrOH, 80:20, 0.5 mL/min flow rate, 254 nm).

To evaluate the effect of the solvent on the enantioselectivity of the enzymatic reaction,[21] various other organic solvents with varying log *P* values, such *tert*-butyl methyl ether, isooctane, chloroform, dichloromethane etc, were investigated for the transesterification of (*RS*)-**9** with vinyl acetate in the presence of PS-C and CCL (Table 2). The maximum enantioselectivity was observed in *tert*-butyl methyl ether and isooctane for the PS-C-catalyzed reactions. On the other hand, for reactions using CCL, maximum enantioselectivity was observed in toluene. It has been reported[22] that in polar solvents (log $P < 2$), the rate of biocatalytic reactions is lower compared to that in nonpolar solvents (log $P > 4$). Moderate reactions rates are observed in organic solvents with log *P* value between 2 and 4.[23a] It has been observed that hydrophobic solvents are unable to strip away the water molecules associated with the enzymes and in this process enzymes retain the required degree of hydration to remain catalytically active, whereas hydrophilic solvents, due to their water loving nature, strip away water molecules from the enzyme complex, which leads to catalytic deactivation.[23b,c] In the case of isooctane, a hydrophobic solvent, a positive correlation between the activity of lipase and increased log *P* value of the solvent could be seen. Thus isooctane and *tert*-butyl methyl ether were used for subsequent enzymatic reactions with PS-C and toluene was used for CCL.

A time dependent PS-C-catalyzed transesterification reaction of (*RS*)-**9** was performed with vinyl acetate separately in *tert*-butyl methyl ether and isooctane and in toluene for CCL-catalyzed reactions. The conversion and enantiomeric excess were determined using chiral HPLC. In the case of *tert*-butyl methyl ether the conversion increased with time. Maximum conversion (C = 25.3%) was achieved after 96 hours and thereafter no significant change in the rate of conversion was observed. On the other hand, the enantiomeric excess and E values of the product decreased with time (E = 47.1 at 96 h to E = 25.5 at 168 h and ee_P = 94.3% at 96 h to 89.4% at 168 h) (Figure 1).

Table 2 The Effect of Organic Solvent on Enantioselectivity in the Resolution of (*RS*)-**9** with Lipase PS-C[a]

Solvent	Log *P*	C[b] (%)	ee[c] (%) of **10**	ee[c] (%) of **9**	E[b]
***S*-Selective with PS-C**					
MeCN	–0.33	2	2	4	1
1,4-dioxane	–1.1	5	70	4	1
t-BuOMe	1.35	18	92	20	2
i-Pr_2O	1.9	7	37	3	1
benzene	2.0	4	90	4	1
heptane	4.0	17	92	19	1
toluene	2.5	30	79	34	1
isooctane	4.5	43	95	72	11
***R*-Selective with CCL**					
benzene	2.0	23	73	22	2
CH_2Cl_2	1.25	3	68	2	1
i-Pr_2O	1.9	59	56	81	–
Et_2O	0.85	41	70	49	4
1,4-dioxane	–1.1	3	55	1	1
toluene	2.5	46	86	74	13
hexane		66	45	88	–
isooctane	4.5	42	46	33	2

[a] Conditions: (*RS*)-**9** (1 mmol), toluene (3.5 mL) treated with vinyl acetate (5.4 mmol) at 25 °C in the presence of the lipase (15 mg/mL) for 48 h.
[b] For the method of calculation see the experimental section.
[c] Enantiomeric excess of the substrate **9** or product **10** was determined by HPLC analysis (Daicel Chiralcel OD-H column, hexane–*i*-PrOH, 80:20, 0.5 mL/min flow rate, 254 nm).

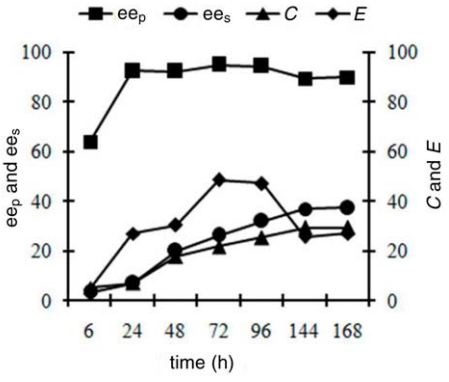


Figure 1 Course of PS-C-catalyzed transesterification of (*RS*)-**9** in *tert*-butyl methyl ether

Maximum conversion (C = 46.2%) was achieved after 48 hours of reaction and thereafter no significant change in the rate of conversion was observed when the reaction medium was isooctane. On the other hand, the enantiomeric ratio of the product decreased with time (E = 84.5 at 48 h to E = 63.67 at 168 h) (Figure 2). Thus, 96 and 48 hours were taken as the optimum time for further study in *tert*-butyl methyl ether and isooctane, respectively.

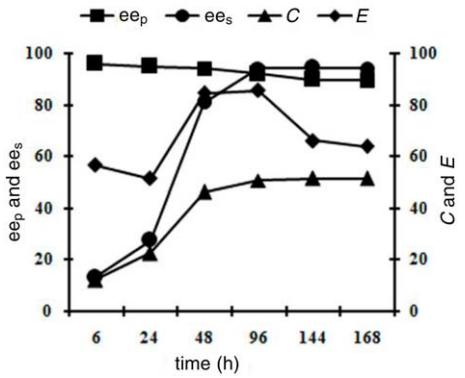


Figure 2 Course of PS-C-catalyzed transesterification of (*RS*)-**9** in isooctane

For the CCL-catalyzed reaction, maximum conversion (C = 48%) was achieved after 36 hours and there after no significant change in the rate of conversion was observed when the reaction medium was toluene. The enantiomeric ratio of the product increased with time (Figure 3). Thus, 36 hours was taken as an optimum time for further study in toluene.

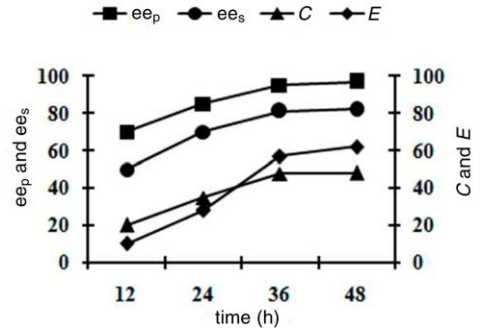


Figure 3 Course of CCL-catalyzed transesterification of (*RS*)-**9** in toluene

The acyl donor has significance influence on enzymatic acylation[24] on various aspects such as conversion, enantioselectivity, and greenness of the process.[24a] Therefore, the effect of the acyl donor on the rate of conversion and enantioselectivity of the PS-C-catalyzed kinetic resolution of (*RS*)-**9** was studied separately in *tert*-butyl methyl ether (Table 3) and isooctane (Table 4) and the best results were obtained in using vinyl acetate and poor results were obtained with other acyl donors. The superiority of vinyl acetate as the acyl donor for enzymatic reactions has been ascribed to the ability of the in situ generated vinyl alcohol to tautomerize to acetaldehyde, there by not acting as a competitive substrate and shifting the equilibrium to the product.[24b]

Table 3 Effect of Acyl Donors on the PS-C-Catalyzed Transesterification of (*RS*)-**9** in *tert*-Butyl Methyl Ether[a]

Acyl donors[a]	C[b] (%)	ee[c] (%) of **10**	ee[c] (%) of **9**	E[b]
BnOAc	–	–	–	–
EtOAc	4	87	3	1
butanoic anhydride	4	86	4	1
isobutanoic anhydride	3	79	3	1
vinyl acetate	26	96	33	2

[a] Conditions: (*RS*)-**9** (1 mmol) in *t*-BuOMe (3.5 mL) treated with acyl donor (5.4 mmol) at 25 °C in the presence of the lipase PS-C (15 mg/mL) for 96 h.
[b] For the method of calculation see the experimental section.
[c] Enantiomeric excess of (*R*)-**9** or (*S*)-**10** determined by HPLC analysis (Daicel Chiralcel OD-H column, hexane–*i*-PrOH, 80:20, 0.5 mL/min flow rate, 254 nm).

Table 4 Effect of Acyl Donors on the PS-C-Catalyzed Transesterification of (*RS*)-**9** in Isooctane[a]

Acyl donors[a]	C[b] (%)	ee[c] (%) of **10**	ee[c] (%) of **9**	E[b]
BnOAc	–	–	–	–
EtOAc	4	51	2	1
butanoic anhydride	4	64	3	1
isobutanoic anhydride	4	37	2	1
vinyl acetate	43	95	72	11

[a] Conditions: (*RS*)-**9** (1 mmol) in isooctane (3.5 mL) treated with acyl donor (5.4 mmol) at 25 °C in the presence of the lipase PS-C (15 mg/mL) for 48 h.
[b] For the method of calculation see the experimental section.
[c] Enantiomeric excess of (*R*)-**9** or (*S*)-**10** determined by HPLC analysis (Daicel Chiralcel OD-H column, hexane–*i*-PrOH, 80:20, 0.5 mL/min flow rate, 254 nm).

The influence of temperature on activity and enantioselectivity of the PS-C-catalyzed kinetic resolution of (*RS*)-**9** using vinyl acetate as the acyl donor in *tert*-butyl methyl ether (Figure 4) and isooctane (Figure 5) was also determined. The resolution was carried out at 15, 25, 30, 45, and 60 °C and the conversion and the enantiomeric excess were determined using chiral HPLC after 96 hours in *tert*-butyl methyl ether (Figure 4) and after 48 hours in isooctane (Figure 5). It was found that in *tert*-butyl methyl ether the conversion had increased from 5% at 15 °C to 46% at 45 °C and then decreased to 25% at 60 °C. On the other hand, the enantiomeric excess of the product decreased from 94% at 25 °C to 90% at 60 °C [i.e. enantiomeric ratio (E) decreased from E = 47 to E = 26] (Figure 4). In the

case of isooctane, it was observed that the conversion increased from 22% at 15 °C to 52% at 45 °C and then decreased to 35% at 60 °C. On the other hand, the enantiomeric ratio (*E*) decreased from *E* = 85 to *E* = 48) (Figure 5). Thus, 25 °C was used as the optimum temperature for PS-C-catalyzed kinetic resolution to achieve good enantiomeric ratio and enantiomeric excess both in *tert*-butyl methyl ether and isooctane.

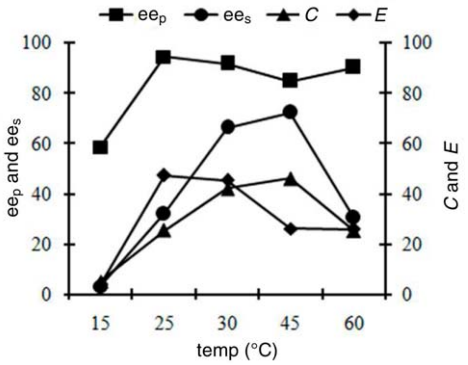


Figure 4 The effect of temperature on the PS-C-catalyzed transesterification of (*RS*)-**9** with vinyl acetate in *tert*-butyl methyl ether

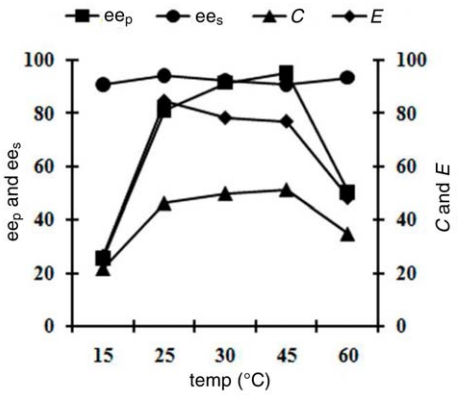


Figure 5 The effect of temperature on the PS-C-catalyzed transesterification of (*RS*)-**9** with vinyl acetate in isooctane

As the enzyme concentration often exhibits a significant effect on the rate of conversion as well as the enantiomeric excess,[25] the reaction of (*RS*)-**9** with vinyl acetate was carried out using different concentrations of PS-C (10, 15, 20, 23, 25, 30, 60, and 120 mg/mL) separately in *tert*-butyl methyl ether and isooctane. It was observed that with the increase in enzyme concentration, the conversion increased up to a certain level after which there was no significant change in the conversion. Although the conversion obtained at 20 mg/mL (46%) was slightly less than at 25 mg/mL (50%) of the enzyme, the enantiomeric excess and *E* values were better at 20 mg/mL (Figure 6). In the case of isooctane, the conversion (46%) obtained at 20 mg/mL enzyme concentration was slightly less (48%) than that of at 25 mg/mL but the enantiomeric excess and *E* values were better at 20 mg/mL enzyme level (Figure 7). Thus, for all the subsequent experiments, an enzyme concentration of 20 mg/mL was used.

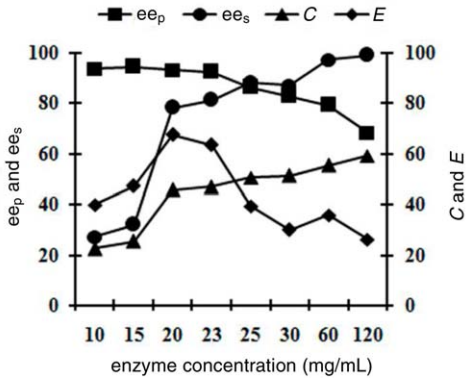


Figure 6 Effect of enzyme concentration on the PS-C-catalyzed transesterification of (*RS*)-**9** with vinyl acetate in *tert*-butyl methyl ether

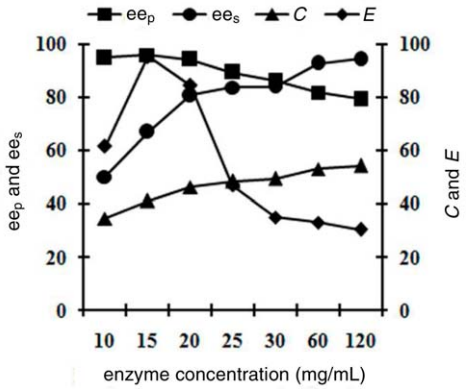


Figure 7 Effect of enzyme concentration on the PS-C-catalyzed transesterification of (*RS*)-**9** with vinyl acetate in isooctane

As the enzyme concentration often exhibits a significant effect on the rate of conversion as well as the enantiomeric excess, the reaction of (*RS*)-**9** with vinyl acetate was carried out using different concentrations of CCL (10, 50, 75, and 150 mg/mL) in toluene. The optimum enzyme concentration was 50 mg/mL with conversion (46%), ee_S = 82%, ee_p = 95%, and *E* = 100 (Figure 8).

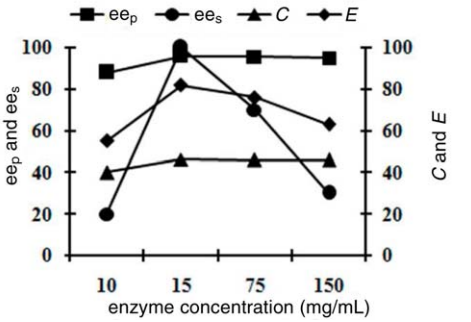
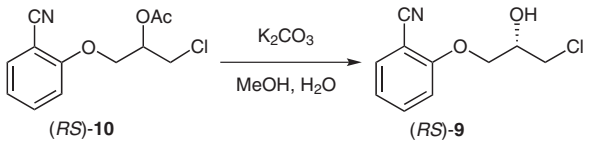


Figure 8 Effect of enzyme concentration on the CCL-catalyzed transesterification of (*RS*)-**9** with vinyl acetate in toluene

The liberation of the parent alcohol from the acetylated intermediate (*RS*)-, (*R*)-, and (*S*)-**10** was achieved by deacetylation in aqueous potassium carbonate at room temperature for two hours[26] (Scheme 6).



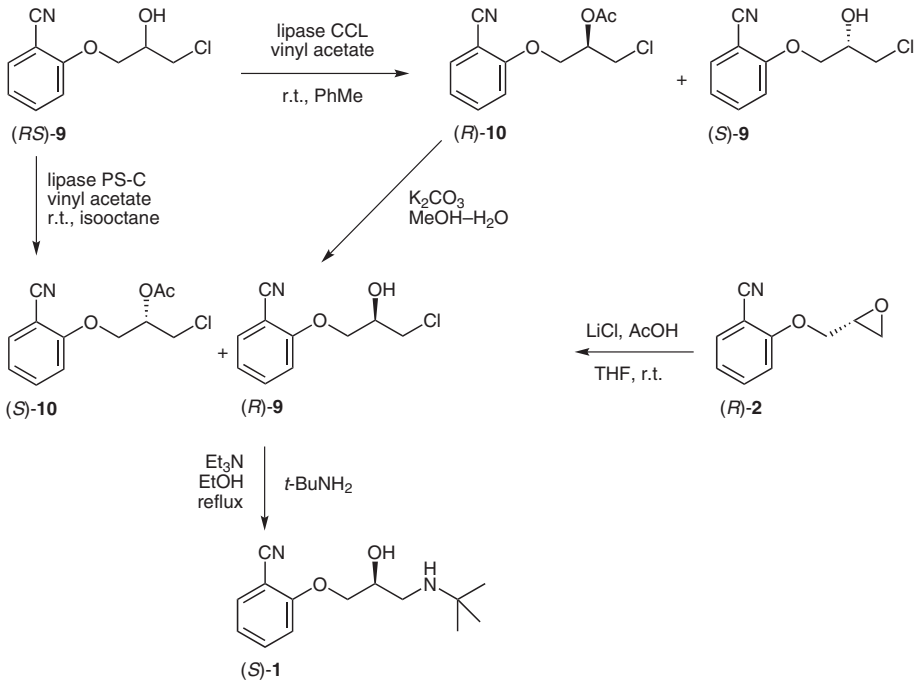
Scheme 6 Synthesis of (*RS*)-, (*R*)-, and (*S*)-**9**

The treatment of (*R*)-**9** [obtained from (i) the enzymatic kinetic resolution of (*RS*)-**9** with vinyl acetate by lipase PS-C, (ii) the enzymatic kinetic resolution of (*RS*)-**9** with vinyl acetate by lipase CCL to form (*R*)-**10** followed by deacetylation, and (iii) epoxide ring opening of (*S*)-**2** with lithium chloride] with *tert*-butylamine in ethanol under reflux for overnight[27] afforded (*S*)-bunitrolol [(*S*)-**1**] (Scheme 7).

Similarly, the treatment of (*S*)-**9** [obtained from (i) the enzymatic kinetic resolution of (*RS*)-**9** with vinyl acetate by lipase CCL, (ii) the enzymatic kinetic resolution of (*RS*)-**9** with vinyl acetate by lipase PS-C to form (*S*)-**10** followed by deacetylation, and (iii) epoxide ring opening of (*R*)-**2** with lithium chloride] with *tert*-butylamine in ethanol under reflux for overnight[27] afforded (*R*)-bunitrolol [(*R*)-**1**].

The present study describes an efficient chemical synthesis, and the first chemoenzymatic synthesis, of the enantiomerically enriched cardiovascular drug bunitrolol in improved overall yields (59–61% as compared to reported yields of 26–37%) and higher enantiomeric access (80–94% as compared to the reported of 60–88% ee). This commercially available lipases PS-C and CCL offered complementary activity during the transesterification of (*RS*)-**9** with vinyl acetate to afford the key intermediates (*R*)- and (*S*)-**9** for (*R*)- and (*S*)-**10** required for the synthesis of enantioenriched (*R*)- and (*S*)-bunitrolol. This offers a handle for an enzymatic switch towards the production of bunitrolol in an enantiodivergent fashion. These enantiomeric intermediates are also used for the synthesis of other chiral drugs such as epanolol and bucindolol. An 'all-chemical' synthetic route was also achieved for efficient synthesis of (*RS*)-, (*R*)-, and (*S*)-bunitrolol.

Enzymatic reactions were carried out on a 'stackable Kuhner-shaker' at 200 rpm. ^{1}H and ^{13}C NMR spectra were obtained with a Bruker DPX 400 (^{1}H 400 MHz and ^{13}C 100 MHz) relative to TMS as an internal reference in $CDCl_3$. IR spectra were recorded on Nicolet FT-IR impact 400 instrument as either neat for liquid or KBr pellets for solid samples. Analytical TLC of all the reactions was performed on Merck prepared plates. Column chromatography was performed using SRL silica gel (60–120 mesh). MS analysis was carried out on Finninganmat LCQ instrument (USA). Optical rotation was measurement in a Rudolph, Autopol IV polarimeter. Enantiomeric excesses were determined by HPLC performed on Shimadzu LC-10AT pump, SPD-10A UV-VIS detector using a Chiralcel OD-H column (0.46 mm × 250 mm; 5 μm, Daicel Chemical Industries, Japan) under the following conditions: mobile



Scheme 7 Chemical and chemoenzymatic synthesis of (*S*)-bunitrolol [(*S*)-**1**]

phase, hexane–*i*-PrOH, 80:20; flow rate, 0.5 mL/min; column temperature, 25 °C at 254 nm.

(*RS*)-Epichlorohydrin, (*R*)-epichlorohydrin, 2-hydroxybenzonitrile, *Candida antarctica* (5.72 U/mg), *Candida rugosa* L8525 (13060 U/mg), *Candida rugosa* L-1754 (742 U/mg), *Candida cylindracea* (≥2 U/mg), *Aspergillus niger* (184 U/g), and porcine pancreas lipase (54 U/mg) and chemicals were purchased from Sigma. Solvents required for the synthesis and extraction were acquired from commercial sources and they were either of analytical or commercial grades. HPLC grade solvents used for HPLC analysis were obtained from J. T. Baker, Rankem, and Merck Ltd. immobilized lipase in sol-gel-Ak from *Pseudomonas cepacia* (186 U/g), immobilized lipozyme from *Mucor miehei* (140 U/g), lipase A, *Candida antarctica* lipase (5.72 U/mg) were purchased from Fluka and lipase AY 'Amano'30 was purchased from Amano Chem Ltd. They were used without any further treatment. Although the definition of enzyme activity depends on enzyme preparation, in most of the cases 1 unit of enzyme corresponds to the amount of the enzyme that frees 1 μmol fatty acid per min at the specified pH and temperature with the corresponding glyceryl esters as the substrates. The strains 5b1, 5b2, 5a1, 1b1 (N), 5d1, 1b1 used were previously isolated from soil in our laboratory for the resolution of (*RS*)-3-[5-(4-fluorophenyl)-5-hydroxypentanoyl]-4-phenyloxazolidin-2-one, an intermediate for ezitimibe synthesis. These isolates were maintained on selective media at 4 °C.

Conversions were calculated from the enantiomeric excess using the formula: Conversion (C) = $ee_S/(ee_S + ee_P)$[20a–e] [for Tables 3 and 4 (*R*)-**9** (substrate S) and (*S*)-**10** (product P)]; E values were calculated using the formula: $E = [\ln (1 - C (1 + ee_P)]/[\ln (1 - C (1 - ee_P)]$.[20e,f]

(*RS*)-, (*R*)-, and (*S*)-2-(Oxiranylmethoxy)benzonitrile [(*RS*)-2, (*R*)-2, and (*S*)-2]

To a mixture of **3** (2.38 g, 20 mmol) and K_2CO_3 (11.04 g, 40 mmol) in anhyd MeCN (100 mL) was added (*RS*)-**4** (3.5 mL, 30 mmol), and the mixture was heated under reflux for 16 h.[16] The mixture was cooled to r.t., filtered, and washed with MeCN, and the combined organic layers were concentrated under vacuum. The residue was purified by column chromatography (silica gel, 60–120 mesh, EtOAc–hexane, 15:85) to afford (*RS*)-**2** (2.8 g, 80%) as a white solid.

(*R*)-2

White solid; yield: 680 mg (78%); 81% ee; $[\alpha]_D^{20}$ –14.8 (*c* 1.0, EtOH) [Lit.[13] $[\alpha]_D^{20}$ –16.9 (*c* 1.0, EtOH), 94% ee].

(*S*)-2

White solid; yield: 700 mg (80%); 82% ee; $[\alpha]_D^{20}$ +15.0 (*c* 1.0, EtOH) [Lit.[13] $[\alpha]_D^{20}$ +10.7 (*c* 1.0, EtOH), 59% ee].

^{1}H NMR (400 MHz, $CDCl_3$): δ = 2.85–2.87 (m, 1 H), 2.93–2.96 (m, 1 H), 3.39–3.43 (m, 1 H), 4.13 (dd, *J* = 5.3, 11.4 Hz, 1 H), 4.38 (dd, *J* = 2.9, 11 Hz, 1 H), 7.01–7.06 (m, 2 H), 7.51–7.59 (m, 2 H).

^{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 (+ESI): *m*/*z* = 176.34 (identical with an authentic sample[12]).

(*RS*)-, (*R*)-, and (*S*)-1-Chloro-3-(2-cyanophenoxy)propan-2-ol [(*RS*)-9, (*R*)-9, and (*S*)-9]

To a stirred solution of (*RS*)-**2** (175 mg, 1 mmol) in THF (10 mL) containing AcOH (0.2 mL) was added LiCl (85 mg, 2 mmol).[17] The resultant mixture was stirred at r.t. for 12 h and on completion of the reaction (TLC), the mixture was diluted with EtOAc (15 mL) and washed with aq $NaHCO_3$ to remove excess AcOH. The organic layer was separated, dried (Na_2SO_4), and concentrated under vacuum. The residue was purified by column chromatography (silica gel, 60–12 mesh, EtOAc–hexane, 15:85) to obtain (*RS*)-**9** which was then subjected to chiral HPLC analysis (Chiralcel OD-H column, hexane–*i*-PrOH, 80:20): t_R = 21.45 (*S*), 23.6 min (*R*), ratio 49:51.

(*RS*)-9

White solid; yield: 173 mg (82%).

IR (neat): 3466 cm^{-1} (OH).

^{1}H NMR (400 MHz, $CDCl_3$): δ = 2.85 (s, 1 H, OH), 3.79–3.87 (m, 2 H), 4.18–4.25 (m, 2 H), 4.28–4.36 (m, 1 H), 7.0–7.08 (m, 2 H), 7.53–7.58 (m, 2 H).

^{13}C NMR (100 MHz, $CDCl_3$): δ = 42.0, 45.6, 69.4, 102.2, 112.60, 116.2, 121.6, 133.7, 134.5, 159.81.

MS (+ESI): *m*/*z* = 212.18.

Following a similar procedure (*R*)-**9** and (*S*)-**9** were prepared from (*S*)-**2** and (*R*)-**2**, respectively.

(*R*)-9

White solid; yield: 177 mg (84%); 82% ee; $[\alpha]_D^{20}$ –3.0 (*c* 1.0, $CHCl_3$). Enantiomeric excess was determined by chiral HPLC analysis (chiral OD-H column, hexane–*i*-PrOH, 80:20): t_R = 21.45 (*S*), 23.6 min (*R*), peak areas of 9% and 91%, respectively.

(*S*)-9

White solid; yield: 173 mg (82%); 81% ee; $[\alpha]_D^{20}$ +2.9 (*c* 1.0, $CHCl_3$). Enantiomeric excess was determined by chiral HPLC analysis (chiral OD-H column, hexane–*i*-PrOH, 80:20): t_R = 21.45 (*S*), 23.6 min (*R*), peak areas of 90.5% and 9.5% respectively.

(*RS*)-, (*R*)-, and (*S*)-1-Chloro-3-(2-cyanophenoxy)propan-2-yl Acetate [(*RS*)-10, (*R*)-10, and (*S*)-10]

Compound (*RS*)-**10** was synthesized chemically through the treatment of (*RS*)-**9** (106 mg, 0.5 mmol) with Ac_2O (0.065 mL, 0.6 mmol) in the presence of $ZrCl_4$ (5 mg, 2 mol%) in MeCN at r.t. under magnetic stirring. After disappearance of (*RS*)-**9** (TLC, 2 h), H_2O was added to the mixture and it was washed with $NaHCO_3$. The organic layer was then separated and concentrated under vacuum to afford (*RS*)-**10** (115 mg, 91%) as a white solid. (*RS*)-**10** was subjected to chiral HPLC analysis (Chiralcel OD-H column, hexane–*i*-PrOH, 80:20): t_R = 26.2 (*R*), 28.0 min (*S*), ratio 49.9:50.1.

IR (KBr): 1745 cm^{-1} (ester).

^{1}H NMR (400 MHz, $CDCl_3$): δ = 2.12–2.16 (s, 3 H), 3.84–3.95 (m, 2 H), 4.32 (d, *J* = 4.9 Hz, 2 H), 5.34–5.4 (m, 1 H), 6.99–7.09 (m, 2 H), 7.53–7.58 (m, 2 H).

^{13}C NMR (100 MHz, $CDCl_3$): δ = 42.02, 66.6, 70.5, 77.0, 102.58, 112.49, 115.8, 121.69, 133.8, 134.3, 159.7.

MS (+ESI): *m*/*z* = 253.92.

Following a similar procedure (*R*)-**10** and (*S*)-**10** were prepared from (*R*)-**9** and (*S*)-**9**, respectively.

(*R*)-10

White solid; yield: 113 mg (89%); 82% ee; $[\alpha]_D^{20}$ –26.02 (*c* 1.0, $CHCl_3$). Enantiomeric excess was determined by chiral HPLC analysis (chiral OD-H column, hexane–*i*-PrOH, 80:20): t_R = 26.2 (*R*), 28 min (*S*), peak areas of 91% and 9%, respectively.

(*S*)-10

White solid; yield: 110 mg (87%); 81% ee; $[\alpha]_D^{20}$ +25.89 (*c* 1.0, $CHCl_3$). Enantiomeric excess was determined by chiral HPLC analysis (chiral OD-H column, hexane–*i*-PrOH, 80:20): t_R = 26.2 (*R*), 28 min (*S*), peak areas of 9.5% and 90.5%, respectively.

Enantioselective Transesterification of (*RS*)-9

To a round-bottomed flask (10 mL) containing a magnetic bead were added a mixture of (*RS*)-**9** (1 mmol) in *t*-BuOMe (3.5 mL) and vinyl acetate (5.40 mmol). Lipases from different sources [commercial lipase from lipase A, *Candida Antarctica*, *Candida rugosa* L8525, *Candida rugosa* L-1754, *Candida cylindracea*, *Aspergillus niger*, porcine pancreas, and AY 'Amano'30] and crude lipase [from strains 5b1, 5b2, 5a1, 1b1 (N), 5d1, 1b1 laboratory strains] were used to carry out the reaction. The round-bottomed flask was capped and placed on a magnetic stirrer, which was maintained at

r.t. Immobilized lipase in sol-gel-Ak from *Pseudomonas cepacia*, immobilized lipozyme from *Mucor miehei*, lipase acrylic resin from *Candida antarctica*, were individually taken into a separate 10-mL conical flask, the flasks were capped and placed in shaker which was maintained at 25 °C (200 rpm). Samples (300 μL) were withdrawn from mixture and conversion and the enantiomeric excess of the reaction were monitored by HPLC.

Optimization of Transesterification Reaction

The effect of different organic solvents (MeCN, 1,4-dioxane, *t*-BuOMe, *i*-Pr_2O, Et_2O, CH_2Cl_2, benzene, heptanes, isooctane, and toluene) was determined on the transesterification of *rac*-CCPP. The optimum temperature was determined by carrying out reaction at different temperatures in the range 15–60 °C. To find out the effect of different acyl donors, different acyl donors such as EtOAc, BnOAc, butanoic anhydride, and isobutanoic anhydride (5.40 mmol) were used. Finally in order to optimize the enzyme concentration with respect to constant substrate concentration (1 mmol), various enzyme concentrations (10, 15, 20, 23, 25, 30, 60, and 120 mg/mL) were used. The samples were taken at regular time intervals and analyzed for enantioselectivity of transesterification reaction.

Preparative-Scale Transesterification Reaction

The resolution of (*RS*)-**9** was carried out on a preparative scale under optimized conditions. The reaction was performed by subjecting the substrate mixture (45 mL, 1 mmol) to resolution by PS-C and CCL lipase at 25 °C and 30 C using vinyl acetate as the acyl donor in isooctane and with toluene as the solvent. When the transformation was ca. 50% (48 h, 46.2% conversion and 36 h, 46.32% conversion) the mixture was filtered off and enzyme preparation was washed with solvent. The solvent was evaporated under reduced pressure and the resulting dried residue was subjected to flash chromatography (EtOAc–hexane, 15:85). Using PS-C after 48 h were isolated (*R*)-**9**; yield: 146 mg (46%); 81% ee (Chiralcel OD-H), and (*S*)-**10**; yield: 139 mg (37%); 94% ee (Chiralcel OD-H). Using CCL after 36 h were isolated (*S*)-**9**; yield: 148 mg (47%); 82% ee (Chiralcel OD-H), and (*R*)-**10**; yield: 170 mg (45%); 95% ee (Chiralcel OD-H).

PS-C-Catalyzed Reactions

(*R*)-9

White solid; yield: 146 mg (46%); $[\alpha]_D^{20}$ –3.0 (*c* 1.0, $CHCl_3$).

IR (neat): 3466 cm^{-1} (OH).

^1H NMR (400 MHz, $CDCl_3$): δ = 2.85 (s, 1 H, OH), 3.79–3.87 (m, 2 H), 4.18–4.25 (m, 2 H), 4.28–4.36 (m, 1 H), 7.0–7.08 (m, 2 H), 7.53–7.58 (m, 2 H).

^{13}C NMR (100 MHz, $CDCl_3$): δ = 42.0, 45.6, 69.4, 102.2, 112.60, 116.2, 121.6, 133.7, 134.5, 159.8.

(*S*)-10

White solid; yield: 139 mg (37%); $[\alpha]_D^{20}$ +30.36 (*c* 1.0, $CHCl_3$).

IR (KBr): 1745 cm^{-1} (ester).

^1H NMR (300 MHz, $CDCl_3$): δ = 2.12–2.16 (s, 3 H), 3.84–3.95 (m, 2 H), 4.32 (d, *J* = 5 Hz, 2 H), 5.34–5.4 (m, 1 H), 6.99–7.09 (m, 2 H), 7.53–7.58 (m, 2 H).

^{13}C NMR (100 MHz, $CDCl_3$): δ = 42.02, 66.6, 70.5, 77.0, 102.58, 112.49, 115.8, 121.69, 133.8, 134.3, 159.7.

CCL-Catalyzed Reactions

(*S*)-9

White solid; yield: 148 mg (47%); $[\alpha]_D^{20}$ +3.0 (*c* 1.0, CH_2Cl_2).

(*R*)-10

White solid; yield: 1.70 g (45%); $[\alpha]_D^{20}$ –30.14 (*c* 1.0, CH_2Cl_2).

Deacylation of (*RS*)-, (*R*)-, and (*S*)-10

A solution of K_2CO_3 (270 mg, 2 mmol) in distilled H_2O (1 mL) was added to a solution of **10** (250 mg, 1 mmol) in MeOH (5 mL) and the resultant mixture was allowed to stir at r.t. for 2 h. After completion of the reaction, the mixture was extracted with EtOAc (3 × 15 mL) and H_2O (10 mL). The combined organic extracts were dried (Na_2SO_4) and concentrated under vacuum to obtain the crude product, which was purified by column chromatography (silica gel, 100–200 mesh) to obtain the corresponding alcohol.

(*RS*)-9

White solid; yield: 170 mg (82%).

(*R*)-9

White solid; yield: 170 mg (81%); 95% ee; $[\alpha]_D^{20}$ –3.46 (*c* 1.0, $CHCl_3$).

(*S*)-9

White solid; yield: 173 mg (82%); 94% ee; $[\alpha]_D^{20}$ +3.43 (*c* 1.0, $CHCl_3$).

Synthesis of (*RS*)-1

Compound (*RS*)-**9** (100 mg, 1 mmol) was treated with *t*-$BuNH_2$ (110 mg, 0.16 mL, 1 mmol) in EtOH (10 mL) in the presence of Et_3N (100 mg, 0.35 mL, 1 mmol) for 10 h. On completion of the reaction (TLC), the mixture was diluted with EtOAc (15 mL) and washed with H_2O. The organic layer was separated and dried (Na_2SO_4), and concentrated under vacuum. The residue was purified by column chromatography (silica gel, 60–12 mesh, EtOAc–hexane, 15:85) to give (*RS*)-**1** (114 mg, 92%) as a white solid.

^1H NMR (400 MHz, $CDCl_3$): δ = 1.14 (s, 9 H), 2.76–2.81 (m, 2 H), 3.64–3.68 (m, 3 H), 4.11 (d, *J* = 4.8 Hz, 1 H), 6.99–7.03 (m, 2 H), 7.50–7.56 (m, 2 H).

^{13}C NMR (100 MHz, $CDCl_3$): δ = 28.9, 44.3, 50.6, 67.8, 72.7, 102.1, 112.5, 121.0, 133.6, 134.3, 160.5. MS (+ESI): *m*/*z* = 248.15.

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

Compound (*R*)- and (*S*)-**9** (100 mg, 1 mmol), obtained either by chemical synthesis from (*R*)- and (*S*)-**4** or kinetic resolution of (*RS*)-**9** by the enzyme PS-C/CCL, was treated with *t*-$BuNH_2$ (110 mg, 0.16 mL, 1 mmol) in EtOH (10 mL) in the presence of Et_3N (100 mg, 0.35 mL, 1 mmol) for 10 h. On completion of the reaction (TLC), the mixture was diluted with EtOAc and washed with H_2O. The EtOAc layer was separated and dried (Na_2SO_4), and it was concentrated under vacuum. The residue was purified by column chromatography (silica gel, 60–12 mesh, EtOAc–hexane, 15:85) to obtain (*S*)- and (*R*)-**1**. In all cases optical rotation can be compared to Lit.[9a] $[\alpha]_D^{20}$ –10.0 (*c* 1.4, H_2O), 60% ee.

For Chemoenzymatic Routes

Using Enzymatically Prepared (*R*)- and (*S*)-9

(*S*)-1

Yield: 114 mg (92%); 80% ee; $[\alpha]_D^{20}$ –13.4 (*c* 1.4, H_2O).

(*R*)-**1**

Yield: 115 mg (93%); 81% ee; $[\alpha]_D^{20}$ +13.5 (*c* 1.4, H_2O).

By Deacylation of Enzymatically Prepared (*R*)- and (*S*)-10

(*S*)-1

Yield: 114 mg (92%); 94% ee; $[\alpha]_D^{20}$ –15.7 (*c* 1.4, H_2O).

(*R*)-1

Yield: 112 mg (91%); 94% ee; $[\alpha]_D^{20}$ +15.58 (*c* 1.4, H_2O).

For All-Chemical Route

(*S*)-1

Yield: 112 mg (91%); 80% ee; $[\alpha]_D^{20}$ –13.4 (*c* 1.4, H_2O).

(*R*)-1
Yield: 114 mg (92%); 80% ee; $[\alpha]_D^{20}$ +13.3 (*c* 1.4, H_2O).

Supporting Information for this article is available online at http://www.thieme-connect.com/ejournals/toc/synthesis.

References

(1) WHO Report 2003 on Global strategy on diet, physical activity, and health.
(2) Connolly, M. E.; Kersting, F.; Dollery, C. T. *Prog. Cardiovasc. Dis.* **1976**, *19*, 203.
(3) (a) Shanks, R. G.; Wood, T. M.; Dornhorst, A. C.; Clark, M. L. *Nature (London)* **1966**, *212*, 88. (b) Triggle, D. J. In *Burger's Medicinal Chemistry*, 4th ed.; Wolff, M. S., Ed.; Wiley-Interscience: New York, **1981**, 225. (c) Cree, J. D.; Geukens, H.; Leempoels, J.; Verhaegen, H. *Drug Dev. Res.* **1986**, *8*, 109.
(4) (a) Owen, D. A. L.; Marsden, C. D. *Lancet* **1965**, *286*, 1259. (b) Granville-Grossman, K. L.; Turner, P. *Lancet* **1966**, *287*, 788. (c) Hadden, D. R.; Montgomery, D. A.; Shanks, R. G.; Weaver, J. A. *Lancet* **1968**, *292*, 852. (d) Gas, D.; Kregar, M. *Ann. Intern. Med.* **1970**, *70*, 985. (e) Grosz, H. J. *Lancet* **1972**, *300*, 564. (f) Weber, R. B.; Reinmuth, O. M. *Neurology* **1972**, *22*, 366. (g) Morelli, H. F. *Ann. Intern. Med.* **1973**, *78*, 913. (h) Young, R. R.; Growdon, J. H.; Shahani, B. T. *N. Engl. J. Med.* **1975**, *293*, 950.
(5) (a) Ulrych, M.; Frohlich, E. D.; Dustan, H. P.; Page, I. H. *Circulation* **1968**, *37*, 411. (b) Tarazi, R. C.; Dustan, H. P. *Am. J. Cardiol.* **1972**, *29*, 633.
(6) (a) Farmer, J. B.; Kennedy, I.; Levy, G. P.; Marshall, R. J. *Br. J. Pharmacol.* **1972**, *45*, 660. (b) Prichard, B. N. C.; Thompson, F. O.; Boakes, A. J.; Joekes, A. M. *Clin. Sci. Mol. Med., Suppl.* **1975**, *48*, 97s. (c) Mehta, J.; Cohn, J. N. *Circulation* **1977**, *55*, 370.
(7) (a) Engelhardt, A.; Traunecker, W. *Naunyn-Schmiedeberg's Arch. Pharmacol.* **1969**, *263*, 203. (b) Mylecharane, E. J.; Raper, C. *Eur. J. Pharmacol.* **1971**, *16*, 14. (c) Cheymol, G.; Honnorat, C.; Schmitt, H. *Eur. J. Pharmacol.* **1972**, *17*, 341. (d) Nayler, W. G.; Tay, J. *J. Pharmacol. Exp. Ther.* **1972**, *180*, 302.
(8) Satoh, K.; Nunoki, K.; Goto, T.; Taira, N. *J. Cardiovasc. Pharmacol.* **1985**, *7*, 508.
(9) Route A: (a) Sayyed, I. A.; Thakur, V. V.; Nikalje, M. D.; Dewakar, G. K.; Kotkar, S. P.; Sudalai, A. *Tetrahedron* **2005**, *61*, 2831. (b) Bredikhin, A. A.; Bredikhina, Z. A.; Zakharychev, D. V.; Akhatova, F. S.; Krivolapov, D. B.; Litvinov, I. A. *Mendeleev Commun.* **2006**, *16*, 245. Route B: (c) Bredikhina, Z. A.; Akhatova, F. S.; Zakharychev, D. V.; Bredikhin, A. A. *Tetrahedron: Asymmetry* **2008**, *19*, 1430.
(10) (a) Pujala, B.; Rana, S.; Chakraborti, A. K. *J. Org. Chem.* **2011**, *76*, 8768. (b) Shivani Pujala, B.; Chakraborti, A. K. *J. Org. Chem.* **2007**, *72*, 3713. (c) Chakraborti, A. K.; Kondaskar, A.; Rudrawar, S. *Tetrahedron* **2004**, *60*, 9085. (d) Chakraborti, A. K.; Rudrawar, S.; Kondaskar, A. *Eur. J. Org. Chem.* **2004**, 3597. (e) Chakraborti, A. K.; Rudrawar, S.; Kondaskar, A. *Org. Biomol. Chem.* **2004**, *2*, 1277. (f) Chakraborti, A. K.; Kondaskar, A. *Tetrahedron Lett.* **2003**, *44*, 8315. (g) For a review: Schneider, C. *Synthesis* **2006**, 3919.
(11) Narimatsu, S.; Kato, R.; Horie, T.; Ono, S.; Tsutsui, M.; Yabusaki, Y.; Ohmori, S.; Kitada, M.; Ichioka, T.; Shimada, N. *Chirality* **1999**, *11*, 1.
(12) (a) Mehvar, R.; Brocks, D. R. *J. Pharm. Pharm. Sci.* **2001**, *4*, 185. (b) Nathanson, J. A. *J. Pharmacol. Exp. Ther.* **1988**, *245*, 94. (c) Narimatsu, S.; Mizukami, T.; Huang, Y.; Masubuchi, Y.; Suzuki, T. *J. Pharm. Pharmacol.* **1996**, *48*, 1185.
(13) Masuda, K.; Tamagake, K.; Katsu, T.; Torigoe, F.; Saito, K.; Hanioka, N.; Yamano, S.; Yamamoto, S.; Narimatsu, S. *Chirality* **2006**, *18*, 167.
(14) Alfonsi, K.; Colberg, J.; Dunn, P. J.; Fevig, T.; Jennings, S.; Johnson, T. A.; Kleine, H. P.; Knight, C.; Nagy, M. A.; Perry, A.; Stefaniak, M. *Green Chem.* **2008**, *10*, 31.
(15) Roughley, S. D.; Jordan, A. M. *J. Med. Chem.* **2011**, *54*, 3451.
(16) Clouthier, C. M.; Pelletier, J. N. *Chem. Soc. Rev.* **2012**, *41*, 1585.
(17) Kaler, A.; Meena, V. S.; Singh, M.; Pujala, B.; Chakraborti, A. K.; Banerjee, U. C. *Tetrahedron Lett.* **2011**, *52*, 5355.
(18) Bajwa, J. S.; Anderson, R. C. *Tetrahedron Lett.* **1991**, *32*, 3021.
(19) Chakraborti, A. K.; Gulhane, R. *Synlett* **2004**, 627.
(20) (a) Ding, W.; Li, M.; Dai, R.; Deng, Y. *Tetrahedron: Asymmetry* **2012**, *23*, 1376. (b) Bottalla, A.-L.; Queyroy, S.; Azzi-Schue, N.; Vanthuyne, N.; Gastaldi, S.; Bertrand, M. P.; Gil, C. *Tetrahedron: Asymmetry* **2009**, *20*, 2823. (c) Okudomi, M.; Nogawa, M.; Chihara, N.; Kaneko, M.; Matsumoto, K. *Tetrahedron Lett.* **2008**, *49*, 6642. (d) Barnier, J. P.; Rayssac, V.; Morisson, V.; Blanco, L. *Tetrahedron Lett.* **1997**, *38*, 8503. (e) Chen, C.-S.; Fujimoto, Y.; Girdaukas, G.; Sih, C. J. *J. Am. Chem. Soc.* **1982**, *104*, 7294. (f) Straathof, A. J. J.; Jongejan, J. A. *Enzyme Microb. Technol.* **1997**, *21*, 559.
(21) (a) Khmelnitsky, Y. L.; Rich, J. O. *Curr. Opin. Chem. Biol.* **1999**, *3*, 47. (b) Hudson, E. P.; Eppler, R. K.; Clark, D. S. *Curr. Opin. Biotechnol.* **2005**, *16*, 637.
(22) Dordick, J. S. *Enzyme Microb. Technol.* **1989**, *11*, 194.
(23) (a) Laane, C.; Boeren, S.; Vos, K.; Veeger, C. *Biotechnol. Bioeng.* **1987**, *30*, 81. (b) Klibanov, A. M. *Nature (London)* **2001**, *409*, 241. (c) Parida, S.; Dordick, J. S. *J. Am. Chem. Soc.* **1991**, *113*, 2253.
(24) (a) Paravidino, M.; Hanefeld, U. *Green Chem.* **2011**, *13*, 2651. (b) Egri, G.; Baitz-Gacs, E.; Poppe, L. *Tetrahedron: Asymmetry* **1996**, *7*, 1437.
(25) (a) Banoth, L.; Singh, M.; Tekewe, A.; Banerjee, U. C. *Biocatal. Biotransform.* **2009**, *27*, 263. (b) Banoth, L.; Narayan, T. K.; Banerjee, U. C. *Tetrahedron: Asymmetry* **2012**, *23*, 1272. (c) Banoth, L.; Narayan, T. K.; Pujala, B.; Chakraborti, A. K.; Banerjee, U. C. *Tetrahedron: Asymmetry* **2012**, *23*, 1564.
(26) Khan, F. A.; Dash, J.; Jain, D.; Prabhudas, B. *J. Chem. Soc., Perkin Trans. 1* **2001**, 3132.
(27) Zaidlewicz, M.; Tafelska-Kaczmarek, A.; Prewysz-Kwinto, A. *Tetrahedron: Asymmetry* **2005**, *16*, 3205.

