Available online www.jocpr.com

Journal of Chemical and Pharmaceutical Research, 2015, 7(5):311-322

Review Article

ISSN : 0975-7384
CODEN(USA) : JCPRC5

Lipases-catalyzed enantioselective kinetic resolution of alcohols

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ABSTRACT

The purpose of this brief review is to report overviews of the state of art in the kinetic resolution of racemic alcohols using lipases. This review includes types of lipases, function, and applications in organic synthesis, as well as their mechanism in hydrolysis of esters. Moreover the review highlight success stories about the separation of different alcohols racemates explaining the advantages of the methods employed. A particular attention was drawn to the literature published within the period from 2010 upwards to June 2015.

Key words: lipases, kinetic resolution, chiral alcohols

INTRODUCTION

Optically active compounds as single enantiomers have a considerable pharmacological importance because of their high selectivity in interaction with drug receptors. Consequently, chiral compounds are becoming more important in drug therapy and in academic research [1-3]. Enzymes are considered ideal tools for the preparation of enantiomerically pure compounds and have been widely exploited for the resolution of racemic mixtures. [4]

The first person being able to separate a racemic mixture into the two enantiomers was Louis Pasteur, who as early as in 1849 physically separated tartaric acid crystals of opposite optical activity. Ever since, the separation of a racemate into its two enantiomers has been the most prominent way to separate enantiomers in numerous applications. This resolution is often performed through the addition of an enantiopure (auxiliary) reagent leading to the preferred separation of one diastereoisomer over the other, based on the difference in physical properties. Removal of the auxiliary compound produces the desired enantiopure product. Another commonly used technique is treatment of a racemate with an enzyme, which recognizes one enantiomer and converts it into a different product, while the other enantiomer remains untouched. These kinetic resolutions can be carried out not only in small scale laboratory experiments, but also in large scale industrial processes. [5-7]

Lipase-catalyzed resolution is an effective method to produce optically pure enantiomers[8]. Thus chiral-recognition of alcohols by lipases has become a research hotspot over the last few years. A large number of hydrolytic lipases, have been used successfully for the kinetic resolution of racemic secondary alcohols via enantioselective acylation or hydrolysis of their esters. On the other hand, a very limited number of enzymes are capable to resolve racemate of primary alcohols or their esters. Thus, only lipases from *Pseudomonas* and *Porcine Pancreas* (PPL) are known to efficiently resolve these substrates.[9]

1.1. Advantages of enzymes biocatalysis:

Enzymatic catalysis in reactions of organic synthesis becomes an integral part of modern technological processes. Biocatalytic processes have been intensely studied and have become a useful and green

alternative in stereoselective synthesis due to their multiple advantages among them,

(1) a wide range of possible reactions;
(2) in certain cases, as opposed to the reactions of organic synthesis, a process can be performed in a single stage, and the protection of functional groups is not required;
(3) mild reaction conditions suitable for complex and chemically unstable molecules;
(4) catalysts are highly active at low concentrations;
(5) the reusability of the catalyst, e.g. with cells immobilization;
(6) the safety of the biocatalyst (enzyme) and its complete degradation in the environment;
(7) the possibility to reduce or fully eliminate reaction byproducts.[10, 11]

1.2. Enzymatic reaction in organic solvent

Pros and cons

Enzymatic transformation in organic solvents is an emerging area of research for production of several industrial products. As most organic compounds of commercial interest are very sparingly soluble and are sometimes unstable in aqueous solution, water is a poor solvent for nearly all applications in industrial chemistry. Furthermore, the high boiling point and low vapor pressure of water result in tedious and expensive purification of products from an aqueous based biotransformation system. Side reactions like hydrolysis, racemization, polymerization and decomposition are often accompanying aqueous reaction[12a]. Chemists realized these limitations on the use of enzymes in aqueous media and started to develop enzymatic procedures in organic solvents. Biocatalytic transformations in organic solvents offer the following advantages:

1- The use of low-boiling-point organic solvents facilitates the recovery of the product and leads to better overall yield.
2- Increasing solubility of non-polar substrates in organic solvents resulted in faster rate of conversion.
3- Deactivation of substrate and/or product inhibition is minimized.
4- Unwanted side reactions such as hydrolysis often occur in water are largely suppressed.
5-Microbial contamination is negligible in the case of using living cells in biotransformation
6- Ease of enzyme recovery by simple filtration and reusability so no need for immobilization of enzymes.
7- In organic solvents (except ethanol) enzymes denaturation is minimized.
8- Equilibriums are thermodynamically shifted to synthesis over hydrolysis. [12b]

The use of enzymes in organic solvents, however, has some drawbacks: Their decreased catalytic activities (due to the heterogeneous system), which are generally several orders of magnitude lower than those in aqueous solution. Many enzymatic reactions are prone to substrate or product inhibition, which deactivates the enzymes at higher substrate or product concentration, leading to a decrease in the reaction rate and enantioselectivity.[13, 14]

2. Lipases

Lipases (EC 3.1.1.3) belong to the class of serine hydrolases. The two major classes of hydrolases are esterases and lipases [15, 16]. Lipase is one of the most promising enzymes for broad practical application in organic synthesis.

Theyareobtainedinsatisfactoryyieldsfromanimals,plants,andnaturalorrecombinantmicroorganisms,andhave foundalotofapplicationsinfoodandpharmaceuticalindustriesandtechnologies,assignificantbiocatalyst[17].

Enantioselectivity of lipases easily solves the problem of producing optically pure isomers, which is sometimes difficult to achieve by organic synthesis methods. Commercial lipase preparations that are currently produced by industry can be used as biocatalysts for the reproduction of such processes in synthetic organic chemistry.[18]

In recent years, lipases have been widely used as an ecofriendly biocatalyst for the synthesis of pharmaceutical active intermediates and chemicals because of their stability, broad range of substrate scope and easy availability from bacteria and fungi. Kinetic resolution of various racemates using lipases is considered to be a green method for the separation of enantiomers as it works at mild reaction conditions.[19, 20]

lipase mediated kinetic resolutions have found broad applications in the synthesis of various optically active intermediates such as secondary alcohols, amines, amino alcohols, epoxides, amino acids and carboxylic acids. Lipases possess the same enantiomeric preference in both acylation and alcoholysis/hydrolysis process and this constitutes a viable access to the both enantiomeric forms of the chiral products.[3, 21, 22]

2..Typess and function of lipases

Lipases are highly stable enzymes, which remain active even under unfavorable conditions as elevated temperatures, *etc*. They have essential roles in the digestion, transport and processing of dietary lipids (triglycerides, fats, oils). Lipases exert their natural function on the hydrolysis/synthesis of carboxyl ester bonds of long-chain triacylglycerols[17, 23], **Figure1**. A number of lipases are unable to hydrolyze ester bonds at secondary positions, as most of microbial lipases do, while another group of these enzymes hydrolyzes both primary and secondary esters. A third group of lipases exhibits fatty acid selectivity, and cleaves ester bonds of particular types of fatty acids.

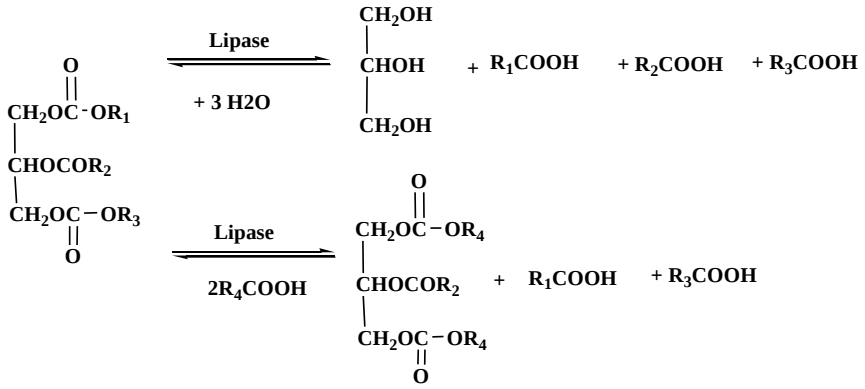


Figure1.Examples of lipase-catalyzed reactions of triglycerides

2. 2. Applications of Lipase

Microbial lipases are also more stable than their corresponding plant and animal enzymes and their production is more convenient, safer and can be obtained in bulk at low cost. Microbial lipases are widely diversified in their enzymatic properties and substrate specificity, which make them very attractive for industrial applications. They have many applications in variable fields of industry including:

(a) The food industry (modification of fats to develop organoleptic and nutritional qualities)
(b) The paper (removal of pitch from paper pulp), textile and leather industries
(c) Additives in detergents,
(d) Synthesis of biopolymers,
(e) Biodiesel production,
(f) Synthesis of optically pure compounds and fine chemicals of interest in the pharmaceutical (antibiotics, anti-inflammatory drugs),
(g) Cosmetic (flavor and fragrance compounds)
(h) Agrochemical (herbicides, insecticides) industries
(i) Waste treatment.[19, 24-26]

2.3. Potential of Lipases in Synthetic Organic Chemistry

The potential of lipases in organic chemistry is fully exploited by replacing the aqueous medium with an organic one. The direction of the reaction is dependent upon the adequate solvent medium used, e.g., aqueous or organic solvents.**Figure**2 shows some reactions catalyzed by lipase depending on the reaction solvent. The first lipase-catalyzed reaction performed in organic medium was reported in 1984 by Zaks and Klibanov[27, 28]. From the mechanistic point of view, in organic solvents the acyl-enzyme intermediate (R_1CO-E) typical to lipase catalysis (**Figure**3) may be subjected to a nucleophilic attack by different nucleophiles (NuH), such as by alcohols (ROH), thiols (RSH), amines (RNH_2), ammonia (NH_3), hydrogen peroxide (H_2O_2), and carboxylic acids (RCO_2H). Thus, reactions like alcoholysis, thiolysis, aminolysis, ammonolysis, peracid formation and acidolysisare enabled. Interesterification with another ester ($R_1CO_2R_3$) is a special type of alcoholysis in which the nucleophile (R_3OH) is generated *in-situ*. Alcoholysis, thiolysis and interesterification are actually transesterifications, consisting of the formation of a new ester (R_1CONu) from a substrate ester ($R_1CO_2R_2$). Exhaustive reviews are available concerning practical applications of lipase-catalyzed transesterification. [22, 29]

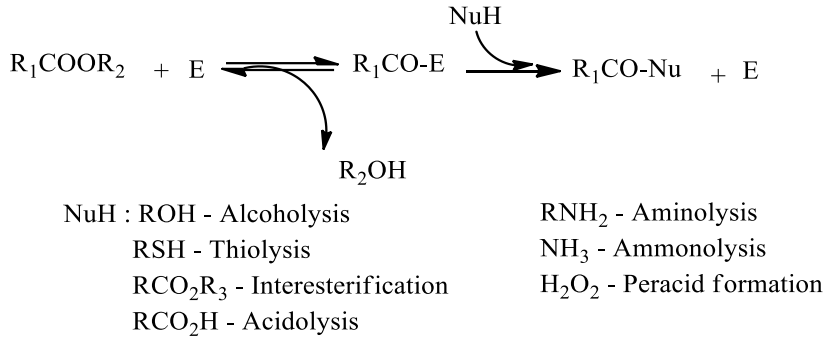


Figure 2.Synthetic potential of lipases in organic solvents

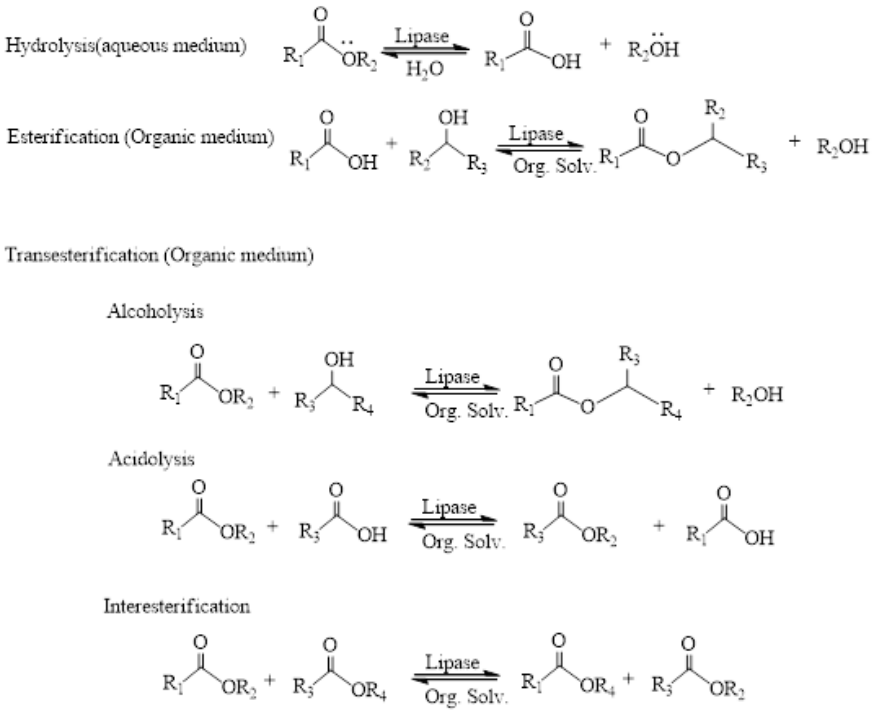


Figure 3: Some reactions catalyzed by lipases

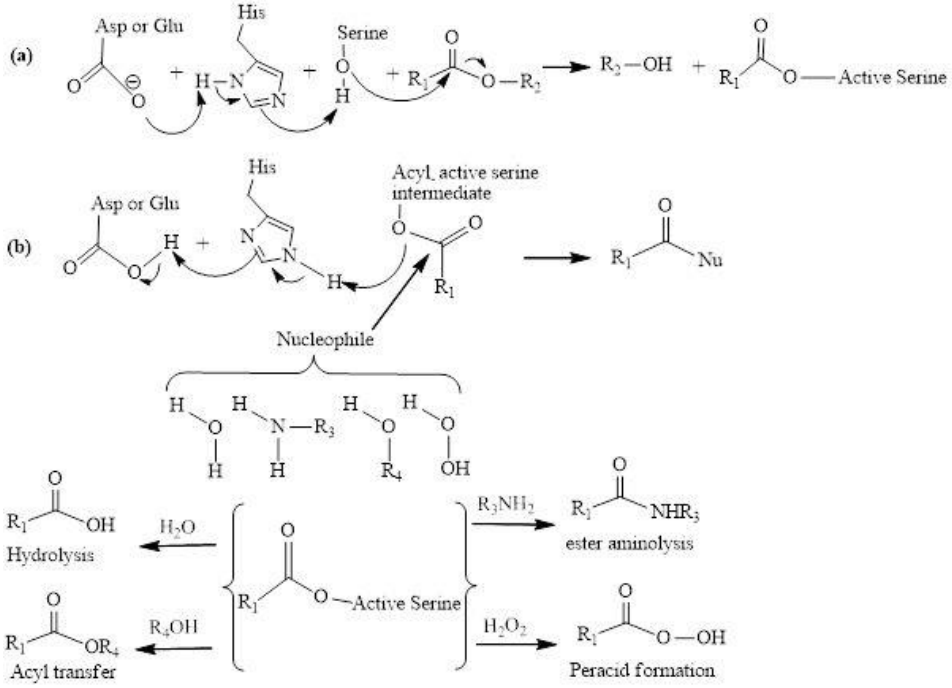


Figure4: Mechanism of lipase-catalyzed ester bond hydrolysis.

2.4. Mechanism of lipase-catalyzed ester bond hydrolysis

Lipases perform catalysis via a motif comprising three residues (serine, histidine and aspartate or glutamate) (also referred as "catalytic triad"). In the catalytic site of serine hydrolases, the reaction occurs through a *bi-bi* ping-pong mechanism and a nucleophilic attack on the carbonyl group promoted by a serine, a histidine and an aspartate or glutamate residue, **Figure 4a**. The resulting "acyl enzyme" intermediate can, in turn, react with a nucleophile, such as water, alcohols or amines, regenerating the enzyme, **Figure 4b** [30, 31]. Different reaction mechanisms describe lipase-catalyzed hydrolysis, esterification, and transesterification reactions, depending also on the specific used medium; thus, mostly non-Michaelis–Menten kinetic models have been suggested, which are applied in non-isotropic media and comprise steps leading to lipase activation and the formation of the corresponding enzyme–substrate complexes.

Nevertheless, it should be emphasized that any lipase-catalyzed process (including synthesis) is influenced by the lipase stability, selectivity, mass transfer and other factors. A variety of lipase-forms can be used as a biocatalyst, i.e. as:

(a) Whole-cell catalysis (lipases kept inside the host cell), in either a free or immobilized form, concomitantly taking into account the cost of side reactions,
(b)Liquid formulated lipases (lipases dissolved in aqueous solutions)
(c) Immobilized lipases (lipases immobilized in solid matrices) either by crosslinking, or encapsulation, or adsorbing and/or covalent linking onto a matrix. In addition it should be taken into account that lipases should be able to retain water, since these enzymes may need the interface to work.[32]

The most widely used lipase-catalyzed methods include three main types of reactions that yield enantiopure compounds **Figure5**. These are:

1. Kinetic resolution (KR)[5, 33]
2. Dynamic kinetic resolution(DKR) [34, 35]
3. Desymmetrization.[34, 36, 37]

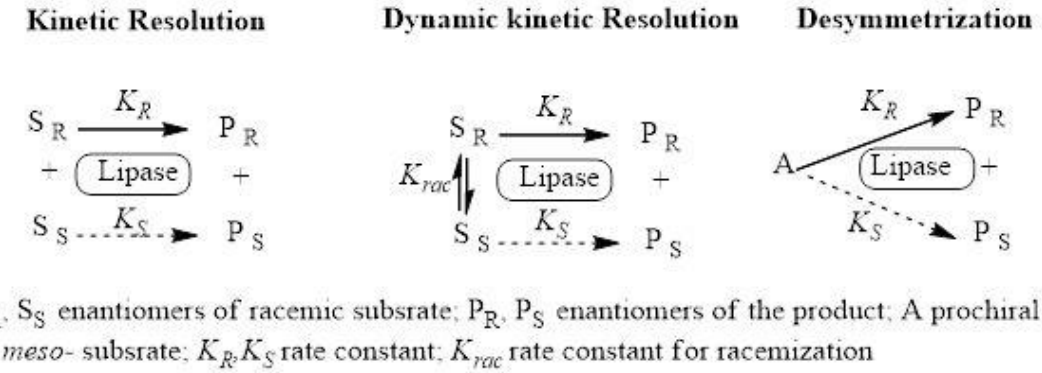


Figure5: Lipase-catalyzed methods for the preparation of enantiopure compounds

Enzymatic kinetic resolution is based on the difference between the reaction rates (k_R, k_S) of the enantiomers (*SR, SS)* of a racemate in the presence of an enzyme as a chiral catalyst. In the optimal case of a kinetic resolution, the transformation of one of the enantiomers into the product takes place while the other enantiomer stays unreacted. Kinetic resolution then has an advantage to provide the both enantiomers of a racemate with excellent enantiopurity in the presence of a highly enantioselective enzyme. This is considered as an advantage, especially for drug development where strict regulations require the characterization of biological activity for all possible stereoisomers of a certain drug. When only one enantiomer of a racemate is needed, kinetic resolution offers a relatively low yield, the maximum yield being 50% for one enantiomer.

Dynamic kinetic resolution combines kinetic resolution with the *in-situ* racemization of the unreacted enantiomer. Racemization can take place chemically or enzymatically. DKR was thoroughly reviewed by Pellisier. The racemization of the unreacted enantiomers in DKR is more often performed chemically, because the group of racemases is relatively small. The need for racemization in Nature, governed by stereospecific interactions, is rare. Schnell *et al*[38]have reviewed few racemases applicable for biotransformation

Desymmetrization of *meso* and prochiral compounds has the potential to fulfill theoretical yields of 100%. This method is not that often used as enzymatic kinetic resolution because the substrate *meso* or prochiral has to be available.

2.5. Mechanism of enantioselectivity toward chiral primary alcohol by lipase from *Pseudomonas cepacia*

Chiral alcohols, including secondary and primary alcohols, are a common type of optically active chemicals. As chiral building blocks or chiral sources, secondary and primary alcohols play important roles in medicine, cosmetic production, and food chemistry.[39]

Kazlauskas *et al*.[40]investigated the resolution of 94-pairs of enantiomers of chiral alcohols catalyzed by lipases including PCL *(Pseudomonas cepacia lipase*), and proposed an empirical law of enantiopreference. According to this rule, the discrimination of the enantiomers is based on the size of the substituents (medium-size substituent-M, large-size substituent-L), **Figure6**, attached to the asymmetric center, which bind to different hydrophobic pockets at the active site of the enzyme. For a secondary alcohol with the alcohol group pointing backwards, the enantiomer with the large group to the left and the medium group to the right will react fastest. The fast-reacting enantiomer is the (*R*)-enantiomer, assuming that the larger group has the higher priority. This groundbreaking law was considered to be the origin of enantioselectivity by lipases.[41]

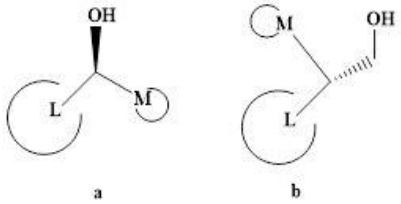


Figure 6.Empirical rules summarize the enantiopreference of PCL toward chiral alcohols. (a) Shape of the favored enantiomer of secondary alcohols. M represents a medium-sized substituent, e.g., CH_3, and L represents a large substituent, e.g., Ph. (b) Shape of the favored enantiomer of primary alcohols. This rule for primary alcohols is reliable only when the stereocenter lacks an oxygen atom (as in case of M=CH_3 while L= PhO. Note that the OH of secondary alcohols points toward the reader, while the CH_2OH of primary alcohols points away from the reader

Based on this law and using various genetic approaches, a number of studies on improving, manually altering lipase's enantioselectivity and even reversing the enantio-preference were reported. Moreover, researchers were able to quantitatively predict with reasonable precision the enantioselectivity ratio of particular lipase-catalyzed resolution of chiral compounds using modern computational tools such as molecular docking and QSAR (quantitative structure–activity relationship) calculation.[42]

Nonetheless, the success of these studies was only based on secondary alcohols, whose chiral center is the hydroxyl α-C of the alcohol ($\mathbf{C^{\alpha}_{alc}}$). Research, either genetic or computational, on stereo-recognition of primary alcohols, whose chiral center is not the $\mathbf{C^{\alpha}_{alc}}$ has been limited. These alcohols could only be resolved by a limited number of lipases including PCL. Although by these lipases, only a small number of primary alcohols could be recognized, and even toward this small number of alcohols, the lipases enantiopreference did not obey Kazlauskas' Rule.[41] studied mechanism behind the deviant behavior of stereo-recognition of these substrates (whose chiral center is not the $\mathbf{C^{\alpha}_{alc}}$) uncovered a new mechanism for enantioselectivity of lipase is proposed. PCL stereo-recognition of chiral primary alcohols (and their esters) is mostly decided by the possibility and extent of the formation of a hydrogen bond between the $O^{non\text{-}\alpha}$ of the substrates and the Tyr^{29}-OH of the lipase. Moreover, a larger acyl moiety was found to push the $O^{non\text{-}\alpha}$ further away from Tyr^{29}-OH, thereby reducing the likelihood of this key hydrogen bond, and thus decreasing the enantioselectivity. This mechanism rather satisfactorily clarifies the lipase's deviant behavior of stereo-recognition of chiral primary alcohols which does not follow the former empirical rules and laws for secondary alcohols.[43]

2.6. Towards Enantiopure Alcohols by Lipase-catalyzed Kinetic Resolution

Several strategies were invented for the preparation of enantiopure alcohols using conventional methods such as those based on reduction of ketones:

(i) an asymmetric transfer hydrogenation of ketones using mixture of formic acid–triethylamine, sodium formateas a hydrogen source,

(ii) an organocatalytic reduction of ketones,

(iii)reduction of ketone using silane as a hydrogen source with metal,

(iv) reduction of ketones by using molecular hydrogen.

Other reduction methods were also investigated as by oxidative kinetic resolution (OKR) of alcohol and selective hydrolysis of epoxides.

These methodologies gave good yield and enantiomeric excess of compounds, but suffers from several drawbacks such as use of molecular hydrogen that requires high pressure autoclave, low enantiomeric excess, longer reaction time, use of expensive metal precursor, phosphine based ligands, additives, multistep synthesized chiral ligands and no catalyst recovery, which limits their practical catalytic applications.[44] Thus, the synthesis of enantiomerically pure alcohol and its acetate derivative is a challenging task, which can be achieved simply via greener biocatalytic pathway.

2.6.1. Lipase-catalyzed Kinetic resolution of primary alcohol

While lipase-catalyzed enantioselective access to enantiomerically pure secondary alcohols is a very efficient tool in organic synthesis, the kinetic resolution of racemates of primary alcohols by the same method is more difficult to achieve. This is due to the lower enantioselectivity of lipases towards chiral primary alcohols. To enhance the enantioselectivity of lipases towards primary alcohols, Sakai *et al*. reported a low-temperature method.

- A systematic study on the effects of the remote stereogeniccentre on the enantioselectivity and conversion through lipase-catalyzed kinetic resolutions of (±)-1, (±)-2 and (±)-3, **Figure7**, in a continuous-flow system under the optimized conditions were reported by Schönstein *et al*.[45]

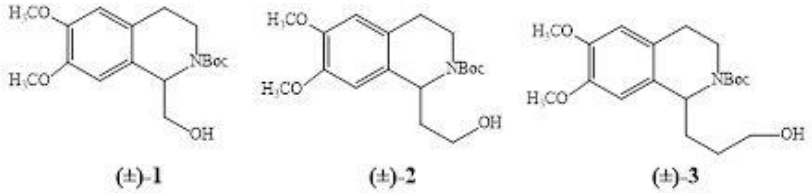
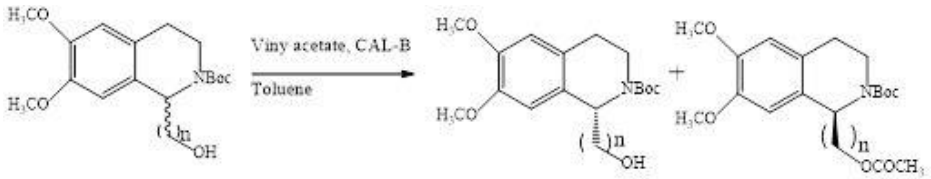


Figure7: Amino alcohols with remote stereogeniccentre.

The preparative-scale resolution of (±)-3 was performed with high enantioselectivity (*E*>200). The resulting amino alcohol (*S*)-3 and amino ester (*R*)-4, obtained with high enantiomeric excess (*ee*= 99%), were transformed into the desired calycotomine (*S*)-5 and (*R*)-5 (*ee* = 99%), **Figure** 8.[45]



(±) 1 n=1 *(S)*-1 *(R)*-4 (n=1)
(±) 2 n=2 *(S)*-2 *(R)*-5 (n=2)
(±) 3 n=3 *(R)*-3 *(S)*-6 (n=3)

Figure 8: Preparation of calycotomine enantiomers

Burkholderia cepacia lipase (BCL) showed high enantioselectivity toward chiral primary alcohols, but this enantioselectivity is often unpredictable, especially for substrates that contain an oxygen at the stereocenter. For example, BCL resolves β-substituted-γ-acetyloxymethyl-γ-butyrolactones (acetates of a chiral primary alcohol) by hydrolysis of the acetate, but the enantioselectivity varies with the nature and orientation of the β-alkyl substituent, **Figure** 9. BCL favors the (*R*)-primary alcohol when the β-alkyl substituent is hydrogen (*E*=30) or *trans* methyl (*E*=38), on the other hand, the (*S*)-primary alcohol was strongly favored (*E*=145) when R is *cis* methyl[46].

2.6.2. Lipase-catalyzed Kinetic resolution of secondary alcohol

Secondary alcohols are frequently used as excellent targets in lipase-catalyzed kinetic resolutions.This is due to their utility in organic synthesis and their higher enantioselectivity in resolutions, compared to those in primary and tertiary alcohols, which are difficult to achieve.

S-1-(2-Furyl) ethanol serves as an important chiral building block for the preparation of various natural products, fine chemicals, and is widely used in the chemical and pharmaceutical industries. Lipase-catalyzed kinetic resolution of (*R/S*)-1-(2-furyl) ethanol using different acyl donors was

investigated. Vinyl acetate was found to be the best acyl donor. Different immobilized lipases such as *Rhizomu cormiehei* lipase, *Thermomyceslanuginosus* lipase, and *Candida antarctica* lipase B were evaluated for this reaction, *C. antarctica* lipase B, immobilized on acrylic resin (Novozym 435), was found to be the best catalyst in n-heptane as solvent, **Figure10**.

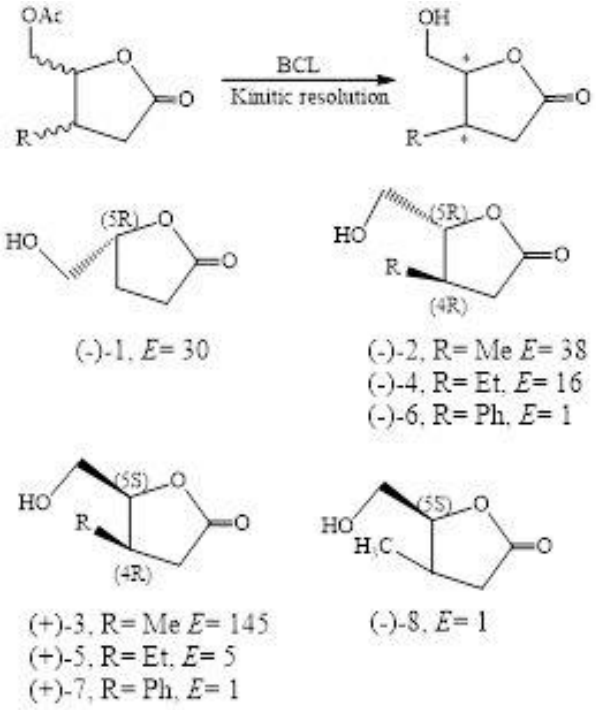


Figure9.Enantiopreference of the BCL-catalyzed kinetic resolution of γ-acetyloxymethyl-γ-butyrolactones by hydrolysis of the acetate and the *cis* vs. *trans* orientation of the β- (1–8) substituent (*R*) influences the enantioselectivity

The effect of various parameters was studied in a systematic manner. Maximum conversion of 47% and enantiomeric excess of the substrate (*ee*) of 89% were obtained in 2 h. This process is more economical, green, and easily scalable than the chemical processes.[47]

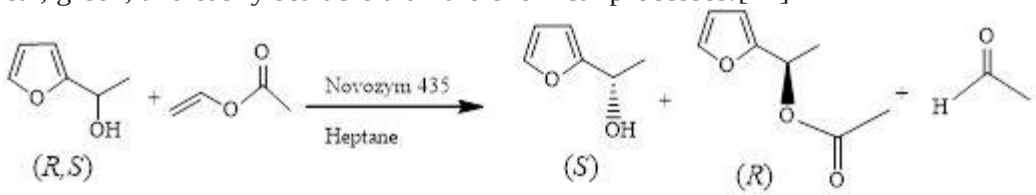
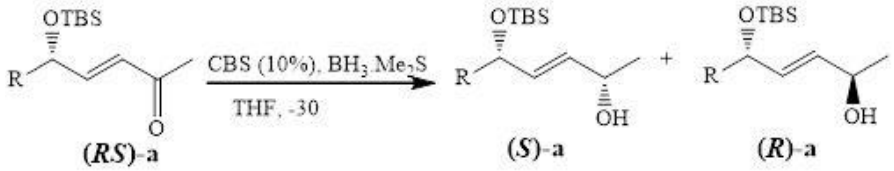


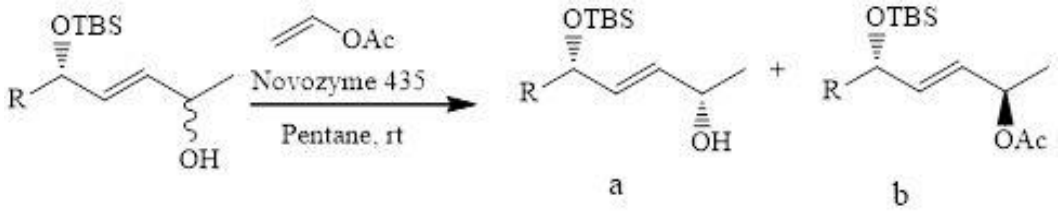
Figure10:Kinetic resolution of chiral 1-(2-Furyl) ethanol

PiotrSzczesniak *et al*., focused on the stereoselective transformation of enone into alcohols *S* and *R*. Disappointingly, Corey−Bakshi−Shibata reduction of enone **a** with $BH_3{\cdot}Me_2S$ in the presence of chiral oxazaborolidine(CBS) yielded a hardly separable mixture of alcohols (*R*) and (*S*) in a 4:1 ratio, **Figure11**. Lowering the reaction temperature from −30 to −78 °C did not increase the reaction selectivity. After that they turned to enzymatic kinetic resolution of diastereomeric alcohols. For this purpose, enone **a** was subjected to Luche reduction ($NaBH_4$ and $CeCl_3$) to afford a mixture of enantiomeric alcohols, **Figure12**. Enzymatic kinetic resolution of a with vinyl acetate in the presence of lipase Novozyme 435 provided alcohol (*S*)-**a**(*ee*> 95%,) along with acetate(*R*)-**b**. Basic hydrolysis of the latter gave alcohol **6a** in 88% yield (*ee*> 95%).[45, 48]



Enone (R)	Catalyst	Ratio(*R*:*S*)	Yeild %
Me	(*S*)-Me-CBS	4:1	78
Me	(*R*)-Me-CBS	1:4	80
Ph	(*S*)-Me-CBS	81:19	92
Ph	(*R*)-Me-CBS	13:87	71

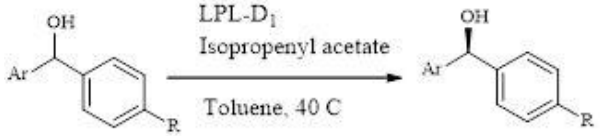
Figure 11. Resolution of enone using $BH_3 \cdot Me_2S$ in the presence of chiral oxazaborolidine (CBS)



Enol	a (Yield %)	b(Yield %)
R= Me	**94**	**96**
R=Ph	**96**	**98**
R= Bn	**92**	**84**

Figure12: kinetic resolution of enone by lipase in using vinyl acetate as acyl donner

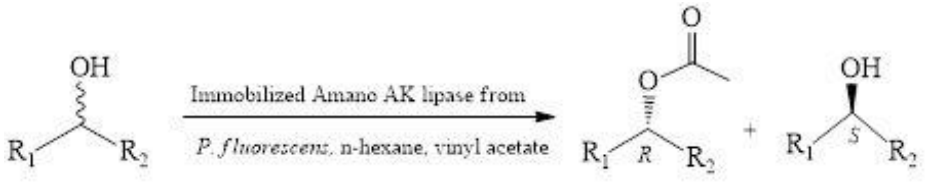
Dynamic kinetic resolution (DKR) provides a powerful methodology for the complete transformations of racemates to single enantiomers. The DKR of diarylmethanols including aryl heteroarylmethanols, **Figure13,** was recently reported. Enantiomerically enriched diarylmethanols are useful as the precursors or building blocks for the synthesis of pharmaceutically important compounds such as antihistaminic, antiarrhythmic, and anticholinergic agents. Several chemical procedures have been explored to provide the routes to their synthesis. Two common approaches include the enantioselective additions of aryl nucleophiles to aromatic aldehydes and the asymmetric hydrogenations of diaryl ketones. The enzymatic methods are available as well. They include the asymmetric reduction of ketones employing ketoreductases and the lipase-catalyzed kinetic resolution of racemic alcohols. All of these chemical and enzymatic methods have advantages and disadvantages. In particular, the enzymatic kinetic resolution has a serious limitation that the theoretical maximum yield is 50% for the wanted enantiomer. The DKR procedure described provides higher yields.[49, 50]



Ar	(*E*) R=isopropyl	R=isobutyl
2-Furyl	93	145
3-Furyl	78	146
2-Thiophenyl	81	141
3-Pyridyl	79	>200
Ph	45	114
Ph-*m*-F	40	76
Ph-*p*-F	28	81
4-Pyridyl	57	>200

Figure 13. Selected example of kinetic resolved diarylalcohols

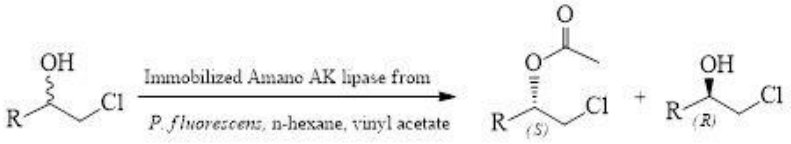
Ferreira, et al,[51] reported the use of enzyme-catalysedtransesterifications of secondary alcohols by KR using different compounds to produce enantiomerically pure or enriched alcohols and acetates. An efficient protocol was declared for the KR of aliphatic secondary alcohols using mild conditions to produce enantiopure compounds (*ee*> 99) by lipase CAL-B[52], **Figure14.**



R_1	R_2	(S)- alcohol (Yield %)	(*R*)-acetate (Yield %)
i-Bu	Me	40	45
i-Pent	Me	32	31
n-Hex	Me	40	35
n-Bu	Et	35	32
n-Pent	Vinyl	31	50

Figure 14.Lipase-catalyzed resolution of aliphatic secondary alcohol

Ferreira, et al, succeed to found the best conditions for kinetic resolution of aromatic, allylic, and aliphatic compounds, containing a chlorohydrin group mediated by immobilized Amano AK lipase from *Pseudomonas fluorescens*. Thus, the enantioselectivity of the process was sufficient for the production of the desired alcohol and acetates in good yields and high enantiomeric purities. It is a simple, cheap, and practical protocol for enantioselective synthesis of chlorohydrins which are a motif for many bioactive compounds, **Figure15**.[53]



R	*ee (%)* (*S*)	(*R*)	*E*
p-F-phenyl	99	82	510
p-CH_3-phenyl	99	43	304
p-NO_2-phenyl	99	47	317
3,4-CH_3O-phenyl	99	15	230
Phenylethenyl	99	43	304
n-hexanyl	39	99	292

Figure15.Lipase-catalyzed kinetic resolution of racemic chlorohydrin bytransesterification with vinyl acetate

Atenolol is an aryloxypropanolamine derivative bearing one chiral centre. In general β-blocking activity of S-enantiomer of aryloxypropanol amine derivative is 50-500 times higher than the (*R*)-enantiomer[54]. Only few reports are available for the enantiopure synthesis of atenolol. Various catalysts used in the synthesis include sulfated tungstate, CsF, $Sm(OTf)_3$, Jacobson catalyst [(*R,R*) (salen Co(III)OAc)], bimetallic chiralcobaltsalen-type complex and microwave dielectric heating. This demonstrates that only limited methods are available for the enantiopure synthesis of this important drug molecule. Most of the reported chemical reagents are costly, not environment-friendly; require harsh condition and also the yield and enantiomericexcess is poor.[55]

A new green and efficient route was reported [56]recently to prepare of *S*-enantiomer of aryloxypropanol amine derivative. This strategy is a biocatalytic process involved the kinetic resolution with lipase converting the racemic halohydrin to enantiopure form followed by amination to produce enantiopure atenolol, **Figure16**. Out of the commercially available lipases screened, *Candida antarctica* lipase-A (CLEA) showed maximum enantioselectivity in the transesterification of racemic alcohol using vinyl acetate as the acyl donor. The reactions afforded the (*S*)-alcohol along with (*R*)-acetate, with 48.9% conversion (*E* = 210, *ee*= 96.9% and *ee* = 91.1%), **Figure16**.

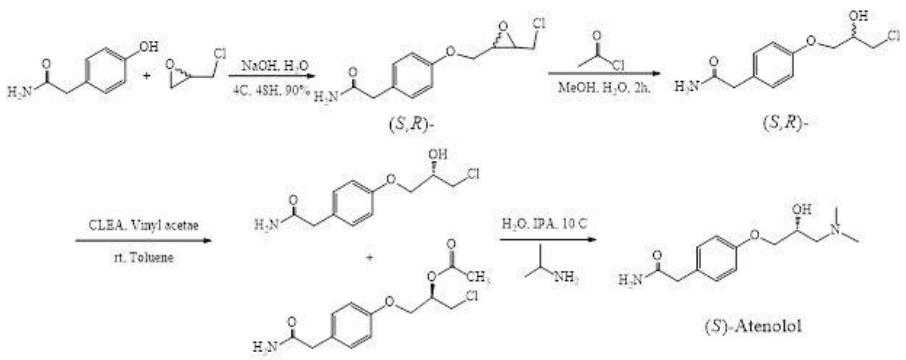
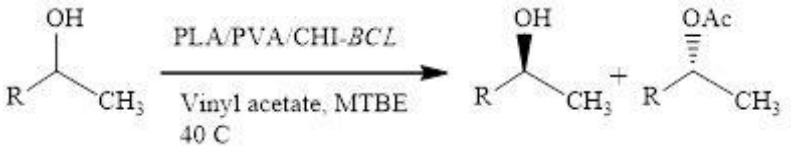


Figure16: Synthesis of (*S*)-Atenolol

Recently, More, V. G. *et al*, reported a highly efficient and biocatalytic heterogeneous protocol for KR of racemic secondary alcohols with vinyl acetate as an acyl donor, using the immobilized biocatalyst *Burkholderia cepacia lipase* (BCL) in methyl *tert*-butyl ether (MTBE). The KR reaction with various substituted aromatic, heterocyclic racemic secondary alcohols gave enantiomerically pure alcohol and its enantioenriched acetate derivatives with high conversion (45–50%) and excellent enantiomeric excess (up to 99% *ee*) at optimized reaction conditions. The reaction works under mild conditions using simple and inexpensive starting materials such as racemic alcohols, vinyl acetate and a biocatalyst. The given protocol provides excellent recyclability with good yield, **Figure17**. After KR, these enantiopure alcohols can be converted into biologically significant compounds, such as Ezetimibe, Prozac, Emend and Sotalol, **Figure18**.[56]



R	Alcohol(*ee*)	Acetate [conversion %, (*ee*)]
Ph	(98)	50 (99)
p-CH_3-Ph	(77)	40 (99)
p-CH_3O-Ph	(99)	51(57)
p-F-Ph	(99)	50 (99)
p-Cl-Ph	(85)	49 (96)
p-Br-Ph	(83)	47 (89)
Naphthyl	(74)	43 (98)
3-pyridyl	(85)	47 (96)

Figure 17.Examples of lipase catalyzed resolution of secondary alcohols

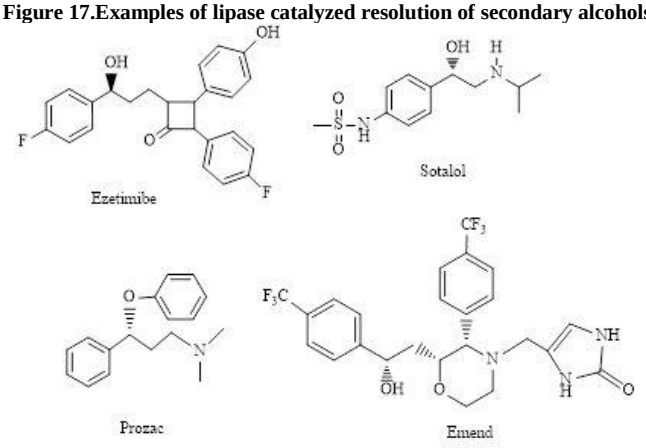


Figure18. Enantiopure alcohol motifs in bioactive compounds

2.6.3. Lipase-catalyzed Kinetic resolution of Tertiary alcohols

The application of lipases for the kinetic resolution of primary and secondary alcohols is widely used. However, the application for tertiary alcohols represents a bottleneck because most of the commercially available enzymes do not accept tertiary alcohols as substrates. Until recently, most studies on the esterase/lipase-catalyzed synthesis of tertiary alcohols had been restricted to very limited substrate spectra with poor enantioselectivity of most resolutions.[57, 58]

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