INTRODUCTION

The synthesis of optically active compounds has been a challenging area of organic synthesis with a long history. In 1848, it was for the legendary Louis Pasteur to carry out the first resolution of a racemate into enantiomers and to recognize subsequently, that "optical activity is a consequence of molecular asymmetry". He also developed the two important methods, diastereomer crystallization and fermentation which are still used for the industrial-scale resolution of racemates.

In recent years, interest in the synthesis of optically active compounds in homochiral form has gained new impetus as a consequence of ever increasing awareness about the importance of optical purity in the context of biological activity. Several biologically active molecules, such as pharmaceuticals, food additives, agrochemicals, etc., are often chiral molecules.

Several chiral drug molecules exhibit different biological activity within their enantiomer pairs. For example, the (S)-enantiomer of the drug propranolol (1), which is used to treat hypertension, has 100 times of β-adrenergic activity of the (R)-enantiomer. Similarly, the (R)-enantiomer of thalidomide is a drug used for morning sickness while the (S)-enantiomer 2 is teratogenic. Such a phenomenon of property differentiation within enantiomer pairs is also exhibited by food additives and agrochemicals. For
example both enantiomers of sucrose are equally sweet, but only the naturally occurring D-enantiomer is metabolized, making the synthetic L-enantiomer a potential dietary sweetener. Similarly, the (2R,3R)-enantiomer of paclobutrazol (3) is a fungicide while the (2S,3S)-enantiomer acts as a plant growth regulator.

This emergence of chirality as one of the key issues in pharmaceutical\(^4\) and agrochemical\(^5\) research has led to a flurry of activity among synthetic organic chemists. Consequently, asymmetric synthesis,\(^7\) once thought to be a rather esoteric subject, has become a major field of activity in both academic and industrial laboratories all over the world. Research over the last twenty years has resulted in great advances in developing efficient and economic methods for the synthesis of a variety of optically active compounds. The arsenal of synthetic organic chemists has now become very rich in chiral building blocks and methods for their preparation and elaboration.
The biocatalytic approach, in particular, has recently emerged as a major tool for the synthesis of homochiral compounds with enzymes being increasingly recognized as potential chiral catalysts. Consequently, biocatalysis in organic synthesis has become a well-defined area of research. The field is served by a steady stream of monographs, reviews and specialist conferences.

Enzymes have become highly attractive catalysts mainly due to their high stereospecificity, regioselectivity, broad substrate specificity and ability to work under mild conditions. Moreover, enzymes are versatile and catalyze a broad spectrum of reactions. There is an enzyme catalyzed equivalent for most types of organic reactions with few exceptions such as Diels-Alder reaction and Cope rearrangement.

The native enzymes can be modified to suit the requirements via immobilization, chemical modification of active site and site-directed mutagenesis that may result in increased operational stability and change in stereoselectivity. With the advent of monoclonal antibodies, it has become possible to synthesize artificial enzymes, the abzymes or catalytic antibodies, that have been shown to catalyze required reactions in stereoselective manner.

Applications of enzymes for synthesis of optically active molecules and other organic transformations have been of current interest with rich literature. As it is not possible to review
all the bibliographic material, some selective examples, that represent fundamental contributions and describe current status of the field, were chosen to illustrate the importance of enzymes in organic synthesis.

Enzymes are conventionally classified into six groups based on the type of reaction they catalyze: Oxidoreductases, transferases, hydrolases, lyases, isomerases and ligases. Oxidoreductases and hydrolases are the most widely used enzymes for the preparation of optically active molecules. Esterhydrolases are of particular interest since they do not require expensive cofactors. Recent work of Klibanov on the utility of these enzymes in organic media has made esterhydrolases more popular among organic chemists. These esterhydrolases have been employed for synthesis of enantiomerically enriched molecules via

(1) enantioselective hydrolysis of prochiral (or meso diesters) and esters of racemic carboxylic acids and acylated racemic alcohols.

(2) enantioselective esterification of racemic carboxylic acids and acylation of racemic alcohols.

(3) enantioselective transesterification of prochiral (or meso) diols, racemic alcohols and esters.

A large number of reports describing the applications of esterhydrolases to organic synthesis have appeared in literature. Since the thesis deals with the synthesis of optically active
molecules via hydrolysis of racemic esters, emphasis has been made for hydrolytic reactions catalyzed by esterhydrolases.

Several commercially available esterases and lipases have been employed for this purpose. Some of the more frequently used among those are:

- Pig liver esterase (PLE)
- Porcine pancreas lipase (PPL)
- Horse liver esterase (HLE)
- Candida cylindracea lipase (CCL)
- Bacillus subtilis lipase
- Asperigillus niger lipase (ANL)
- Pseudomonas sp. lipase (PSL)
- Pseudomonas cepacia lipase (PCL)
- Pseudomonas AK lipase (Lipase AK)
- Pseudomonas sp. SAM II lipase

Among these pig liver esterase\textsuperscript{28,29} is certainly the most widely used esterase for the synthesis of enantiomerically enriched compounds. PLE was employed as a catalyst for the kinetic resolution of mandelic acid ester by Dakin as early as in 1903.\textsuperscript{30}

Enantioselective hydrolysis of prochiral diesters and diacyl prochiral diols:

**Prochiral diesters:**

Several esterases and lipases such as PLE, PPL, PFL, etc., are capable of catalyzing enantiotopically specific hydrolysis of prochiral diesters. One of the oldest reaction of this type is due to Cohen et al.\textsuperscript{31} They showed that $\alpha$-chymotrypsin (CHT) catalyzes the hydrolysis of diethyl $\beta$-acetamidoglutarate ($4a$) in enantiospecific manner resulting in the production of (R)-ethyl-
hydrogen $\beta$-acetamidoglutamate (4b) in optically pure form (Eq. 1).

\[
\begin{array}{c}
\text{NHAc} \\
\text{EtOOC} & \text{COOEt} \\
\end{array}
\stackrel{\text{CHT}}{\longrightarrow}
\begin{array}{c}
\text{HOOC} \\
\text{COOEt} \\
\end{array}
\]

Later in 1975, Sih and coworkers\textsuperscript{32} reported PLE catalyzed enantiospecific hydrolysis of dimethyl $\beta$-hydroxy-$\beta$-methylglutarate (5a) to produce (S)-hydrogen-methyl $\beta$-hydroxy-$\beta$-methylglutarate (5b) in 99% enantiomeric excess, which was subsequently transformed into either enantiomer of mevalonolactone (6) (Scheme 1).

SCHEME 1:

Ohno and coworkers\textsuperscript{33} employed PLE for asymmetric hydrolysis of dimethyl $\beta$-acylamino glutarate (7a) which gave (S)-half ester 7b in 93% enantiomeric excess. The (S)-7b was subsequently converted into the azetidinone 8, a versatile precursor for carbapenem antibiotics (Scheme 2).
In 1984, Schneider et al.\textsuperscript{34} reported the enantioselective synthesis of monoalkyl malonates (9b) with 8-86% enantiomeric purity via PLE-catalyzed hydrolysis of corresponding diesters (9a). Later, Norin et al.\textsuperscript{35} studied the effect of size of substituent on the enantioselectivity of PLE-catalyzed hydrolysis of dialkylated propanedioic acid esters (9a). They have also synthesized optically pure (S)-α-methylphenylalanine (10a, R' = benzyl, R'' = Me), (S)-α-methyltyrosine (10b, R' = 4-hydroxybenzyl, R'' = Me) and (S)-α-methyl-3,4-dihydroxyphenylalanine (10c, R' = 3,4-dihydroxybenzyl, R'' = Me) employing 9b as chiral synthons (Scheme 3).\textsuperscript{36}

**SCHEME 3:**

\[ R' = \text{alkyl or aryl}, \ R'' = \text{ethyl or methyl} \]

Jones et al.\textsuperscript{37} studied the effect of reaction conditions on stereoselectivity of PLE catalyzed hydrolysis of dimethyl β-methylglutarate (11a) in detail (Eq. 2). Later, Ohno et al.\textsuperscript{38} reported
a similar study on the effect of size of acyl group of \( \beta \)-acylaminoglutarate (7a) on stereoselectivity of PLE catalyzed hydrolysis.

\[
\begin{align*}
\text{MeOOC} & \quad \text{COOMe} & \quad \text{PLE} & \quad \text{MeOOC} & \quad \text{COOMe} \\
11a & \quad & & 11b
\end{align*}
\]

At \( +20^\circ\text{C} \), pH = 7.0, MeOH = 0\%, 79\% e.e.
At \( -10^\circ\text{C} \), pH = 7.0, MeOH = 20\%, 97\% e.e.

Recently, \textit{Pseudomonas} sp. lipase (PSL) has been successfully employed for asymmetric hydrolysis of diester 12a to produce the \( \text{(R)} \)-half ester 12b in \( >98\% \) enantiomeric excess which was subsequently transformed into either enantiomer of a potent LTD\(_4\) antagonist 13 (Scheme 4).\(^{39}\)

**SCHEME 4:**
Diacyl prochiral diols:

Asymmetric hydrolysis of diacyl prochiral diols catalyzed by esterhydrolases has been a well known method to provide the corresponding optically active monoacyl diols. Schneider et al.\textsuperscript{40} hydrolyzed the diacetate $\text{14a}$ with lipoprotein lipase to produce $(R)$-$\text{14b}$ in 91\% enantiomeric excess (Eq. 3).

\[ \text{OCH}_2\text{Ph} \quad \text{Lipoprotein lipase} \quad \text{OCH}_2\text{Ph} \]

14a \quad \text{14b}

Sakai et al.\textsuperscript{41} reported PFL catalyzed hydrolysis of 2-methyl-1,3-propanediol diacetate ($\text{15}$) ($R$=Me) to produce $(R)$-monoacetyl diol $\text{16}$ in >99\% enantiomeric excess. Guanti et al.\textsuperscript{42} reported PPL catalyzed asymmetric hydrolysis of several 2-aryl-1,3-propanediol diacetates ($\text{15}$) ($R$=aryl) which gave the (S)-monoacetyl diols $\text{17}$ in 90-96\% enantiomeric excess (Scheme 5).

Scheme 5:
Mori et al.\textsuperscript{43} employed lipase P for asymmetric hydrolysis of diacetate of 2-vinyl-1,3-propanediol (18a) to provide (R)-18b in 90\% enantiomeric excess which is an important synthon for antibiotic 1233A (Scheme 6).

\textbf{SCHEME 6:}

\begin{center}
\begin{tikzpicture}
  \node[below] at (0,0) {18a};
  \node[below] at (1.5,0) {18b};
  \node at (0,0.75) {AcO};
  \node at (1.5,0.75) {OAc};
  \node at (0,1.5) {HO};
  \node at (0,2.25) {Lipase P};
  \node at (2,0) {Antibiotic 1233A};
  \draw[->] (0,0.25) -- (0,1.25);
  \draw[->] (1.5,0.25) -- (1.5,1.25);
\end{tikzpicture}
\end{center}

Guanti et al.\textsuperscript{44} during their studies to prepare new chiral building block, asymmetrized tris(hydroxymethyl)methane(THYM) (20) investigated the asymmetric hydrolysis of a variety of 2-(E)-alkenyl-1,3-propanediol diacetates (19a) catalyzed by PPL to obtain corresponding (E)-alkenyl derivatives 19b with high optical purities (>95\%). (Scheme 7). The corresponding 2-(Z)-alkenyl derivatives gave less satisfactory results.

\textbf{SCHEME 7:}

\begin{center}
\begin{tikzpicture}
  \node[below] at (0,0) {19a};
  \node[below] at (1.5,0) {19b};
  \node[below] at (0,1.5) {AcO};
  \node[below] at (1.5,1.5) {OAc};
  \node[below] at (0,3.0) {R = n-hexyl, isopropyl, cyclohexyl.};
  \node[below] at (2.25,0) {PPL};
  \node at (2.75,0) {R' = H};
  \node at (2.75,1) {R'' = SiPh\textsubscript{2}Bu\textsuperscript{t}};
  \node at (2.75,2.25) {R'' = CH\textsubscript{2}OCH\textsubscript{2}Ph};
\end{tikzpicture}
\end{center}
Enantioselective hydrolysis of meso diesters and diacyl meso diols:

Enzymes are now widely recognized as potential catalysts for asymmetric synthesis with their abilities to asymmetrize meso compounds via enantiotopic group discrimination. PLE has been the most widely used enzyme for this purpose. Other enzymes that have been employed with considerable success are PPL, PCL, SAM II, etc.

Meso diesters:

In 1981, Sih and coworkers\textsuperscript{45} employed PLE for the first time to asymmetrize a meso diester. They asymmetrized dimethyl cis-2,4-dimethylglutarate (21a) via enantiotopically specific hydrolysis catalyzed by PLE and Gliocladium roseum which produced the half esters 21b and 21c in 64\% and 987. enantiomeric excess respectively (Scheme 8).

\textbf{SCHEME 8:}

\begin{center}
\begin{tikzpicture}
\node at (0,0) (a) {HOOC};
\node at (1,0) (b) {COOMe};
\node at (2,0) (c) {MeOOC};
\node at (3,0) (d) {COOMe};
\node at (4,0) (e) {COOH};
\node at (2,1) (f) {21b};
\node at (3,1) (g) {21a};
\node at (4,1) (h) {21c};
\draw[->] (a) -- (b);
\draw[->] (b) -- (c);
\draw[->] (c) -- (d);
\draw[->] (d) -- (e);
\end{tikzpicture}
\end{center}

Ohno \textit{et al.}\textsuperscript{46} hydrolyzed the tricyclic meso diesters 22a and 24a using PLE as catalyst to produce the corresponding optically active mono-acid-esters 22b and 24b in 77\% enantiomeric excess. The half esters 22b and 24b were subsequently converted into nucleosides showdomycin (23) and cordycepin (25) respectively (Scheme 9).
PLE catalyzed asymmetric hydrolysis of cyclic meso diesters 26a-31a to produce the corresponding optically active half esters 26b-31b in 9-100% enantiomeric excess was concurrently reported by Tamm et al., Schneider et al. and Jones et al.
Gais and coworkers\textsuperscript{50} have demonstrated the high utility of PLE in large-scale synthesis of homochiral compounds by preparing the half ester 31b in optically pure form in a mole-scale. The half ester 31b is used as a chiron in the synthesis of several naturally occurring and biologically active compounds (Scheme 10).

SCHEME 10:

Zemlicka et al.\textsuperscript{51} obtained the half ester 32b in 95\% enantiomeric excess from PLE catalyzed hydrolysis of meso diester 32a. Bloch and coworkers\textsuperscript{52} reported PLE catalyzed asymmetric hydrolysis of the bicyclic mesodiesters 33a-35a which gave the corresponding half esters 33b-35b in >97\% enantiomeric excess.

\text{a)} R = \text{Methyl, b)} R = \text{H}
Diacyl meso diols:

Sih and coworkers\textsuperscript{53} described asymmetric hydrolysis of meso diacetates 36a and 37a catalyzed by PLE to produce the corresponding mono acetates 36b and 37b in about 80\% enantiomeric excess. Later, Schneider et al.\textsuperscript{54} showed PPL to be ideal enzyme for the asymmetric hydrolysis of 37a, after screening several enzymes. The mono acetate 37b is very valuable chiron for the synthesis of prostaglandins (Scheme 11).

SCHEME 11:

\[
\begin{align*}
&36 \\
&37 \\
a) & R = \text{Ac}, \quad b) & R = \text{H}
\end{align*}
\]

Wang and Sih\textsuperscript{55} synthesized (+)-biotin in homochiral form starting from the optically active monoacetate 38b, which in turn was obtained from PLE catalyzed enantioselective hydrolysis of corresponding diacetate 38a (Scheme 12).

SCHEME 12:
Vasella et al.\textsuperscript{56} successfully asymmetrized the meso dipropionate 39a via PLE catalyzed enantioselective hydrolysis which produced the optically active monopropionate 39b in 95\% enantiomeric excess (Eq. 4).

\begin{equation}
\text{PLE} \quad \begin{array}{c}
\text{39a} \\
\text{EtCOO} \\
\text{O} \\
\text{O} \\
\text{HO} \\
\text{EtCOO} \\
\text{O} \\
\text{O} \\
\end{array} \quad \begin{array}{c}
\text{39b} \\
\text{OH} \\
\text{EtCOO} \\
\text{O} \\
\text{O} \\
\end{array} 
\end{equation}

Seebach and coworkers\textsuperscript{57} successfully employed pig liver acetone-methylenechloride powder (PLAMP), a crude form of PLE, for asymmetric hydrolysis of a variety of acyclic and cyclic meso 2-nitro-1,3-diols 40a-45a to produce the corresponding optically active monoacetates 40b-45b in 90\%-97\% enantiomeric excess.

\begin{align*}
\text{40} & \quad \text{41} \\
\text{42} & \quad \text{43} \\
\text{44} & \quad \text{45} \\
\text{a) } R = \text{Ac} & \quad \text{b) } R = \text{H}
\end{align*}
Vandewalle et al.\textsuperscript{58} recently reported PLE catalyzed asymmetric hydrolysis of the meso diacetates 46a and 47a, which produced the monoacetates 46b and 47b in 95\% and 80\% enantiomeric excesses respectively. The monoacetate 47b is a precursor for compactin and mevinolin (Scheme 13).

\textbf{SCHEME 13:}

\begin{center}
\begin{tikzpicture}
\node (a) at (0,0) {\textbf{a}};
\node (b) at (1,0) {\textbf{b}};
\node (c) at (2,0) {\textbf{Compactin}};
\node (d) at (2,-1) {\textbf{& Mevinolin}};
\draw[->] (a) -- (b) node[midway,above] {PLE};
\draw[->] (b) -- (c) node[midway,above] {R = OCH\textsubscript{2}Ph};
\draw[->] (b) -- (d) node[midway,above] {R = OCH\textsubscript{2}Ph};
\end{tikzpicture}
\end{center}

46) \( R = \text{CH}_2\text{OCH}_2\text{Ph} \), 47) \( R = \text{OCH}_2\text{Ph} \)

Enantioselective hydrolysis of racemic esters and acylated racemic alcohols:

The potential of esterhydrolases as catalysts for kinetic resolution of racemic carboxylic acids and alcohols via enantioselective hydrolysis of their esters has been well exploited. Several commercially available esterases, lipases and proteases have been employed for this purpose. Prominent among them are PLE, PPL, PSL, PCL, CCL, ANL, HLE, lipase p 30, SAM II, chymotrypsin, etc.

\textbf{Racemic carboxylic acid esters:}

In 1968, Cohen and Milovanovic\textsuperscript{59} described a highly efficient kinetic resolution of diethyl \( \alpha \)-benzylsuccinate (48a). They
subjected the racemic diester 48a to the α-chymotrypsin catalyzed enantioselective hydrolysis and obtained the half ester (R)-48b and (S)-48a in very high optical purities (Eq. 5).

\[
\begin{align*}
\text{EtOOC} & \quad \text{CH}_2\text{Ph} \\
\text{COOEt} & \quad \text{CH}_2\text{Ph} \\
\text{CH}_2\text{Ph} & \quad \text{COOH} \\
\text{EtOOC} & \quad \text{CH}_2\text{Ph}
\end{align*}
\]

\[
\begin{align*}
\text{Rac-48a} & \quad \text{CHT} \quad \text{EtOOC} \\
\text{(R)-(++)-48b} & \quad \text{COOH} \\
\text{(S)-(--)-48a}
\end{align*}
\]

In 1982, Sih et al.\textsuperscript{60} reported kinetic resolution of racemic hydroxy ester 49a via PLE catalyzed hydrolysis (Eq. 6).

\[
\begin{align*}
\text{Rac-49a} & \quad \text{PLE} \quad \text{COOMe} \\
\text{(2R, 3R)-49a} & \quad \text{COOMe} \\
\text{(2S, 3S)-49b}
\end{align*}
\]

Morrow et al.\textsuperscript{61} resolved several racemic β-hydroxy-β-methyl alkanoic acid esters 50a to produce optically active acids (R)-50b and (S)-50a in 22-98% enantiomeric excess via PLE catalyzed hydrolysis (Eq. 7).

\[
\begin{align*}
\text{Rac-50a} & \quad \text{PLE} \quad \text{HO} \\
\text{(R)-50b} & \quad \text{R''} \quad \text{Me} \\
\text{(S)-50a}
\end{align*}
\]

\[
\begin{align*}
\text{R'} = \text{CH}_2\text{CH}_3, \text{n-C}_6\text{H}_{11}, \text{CH}_2\text{CH}_2\text{OH}, \text{CH}_2\text{CH}_2\text{OCH}_2\text{Ph}, \text{CH}_2\text{CH(OMe)}_2 \\
\text{R''} = \text{Methyl or ethyl}
\end{align*}
\]
Ohta et al.\(^6\) employed PLE for resolution of several racemic \(\alpha\)-benzyl\(\alpha\)-methylealkanoic acid esters (51) and obtained the corresponding optically active acids and esters in 60-99% enantiomeric excess (Eq. 8).

\[
\begin{align*}
\text{Me} & \quad \text{OCH}_2\text{Ph} \\
\text{R} & \quad \text{COOMe} \\
\text{Rac-51} & \quad \text{PLE} \\
\text{Me} & \quad \text{OCH}_2\text{Ph} \\
\text{R} & \quad \text{COOMe} \\
\end{align*}
\]

Sih et al.\(^6\) prepared (S)-naproxen (52b) in 98% enantiomeric excess via CCL catalyzed enantioselective hydrolysis of corresponding racemic methyl ester 52a (Eq. 9).

\[
\begin{align*}
\text{MeOOCC} & \quad \text{CCL} \\
\text{MeO} & \quad \text{COOH} \\
\text{Rac-52a} & \quad \text{HOOCC} \\
\text{MeO} & \quad \text{COOMe} \\
\text{(S)-naproxen (52b)} & \quad \text{(R)-52a} \\
\end{align*}
\]

Bloch and coworkers\(^6\) described efficient resolution of several \(\alpha\)-arylpropionic acids via HLE catalyzed hydrolysis of their methyl esters. They prepared methyl ester of (S)-ibuprofen (53a) in >96% enantiomeric excess (Eq. 10).

\[
\begin{align*}
\text{MeOOCC} & \quad \text{HLE} \\
\text{MeOOC} & \quad \text{COOH} \\
\text{Rac-53a} & \quad \text{MeOOCC} \\
\text{MeOOC} & \quad \text{COOH} \\
\text{(S)-53a} & \quad \text{(R)-ibuprofen (53b)} \\
\end{align*}
\]
Sih and coworkers utilized ANL as catalyst for enantioselective hydrolysis of racemic ester 54a to produce the acid (R)-54b in 98% enantiomeric excess (Eq. 11).

\[
\text{COOMe} \quad \text{COOH}
\]

\[
\text{SCOPh} \quad \text{(ii)}
\]

\[
\text{Roc-54a} \quad \text{ANL} \quad \text{PhCOS} \quad \text{(R)-54b} \quad \text{COOMe} \quad \text{COOH} \quad \text{(S)-54a}
\]

(11)

Delinck and Margolin obtained both (S)- and (R)-acids 55b with high enantiomeric excess from lipase P and CCL catalyzed hydrolyses of racemic 55a respectively (Scheme 14).

SCHEME 14:

\[
\text{Lipase P}
\]

\[
\text{PhCOS} \quad \text{COOMe}
\]

\[
\text{CCL} \quad \text{HOOC}
\]

\[
\text{(S)-55b} \quad \text{Rac-55a} \quad \text{(R)-55b}
\]

Recently Sih and Gu reported kinetic resolution of racemic ester 56a via PPL catalyzed enantioselective hydrolysis to produce optically pure (S)-56b and (R)-56a (Eq. 12).

\[
\text{Rac-56a} \quad \text{PPL}
\]

\[
\text{COOEt}
\]

\[
\text{COOH} \quad \text{EtOOC}
\]

\[
\text{(S)-56b} \quad \text{(R)-56a}
\]

(12)

Kalaritis and coworkers described enantioselective hydrolysis of alkyl esters of several racemic α-substituted alkanoic acids 57a catalyzed by lipase P. They recovered the unhydrolyzed esters in 93-99% enantiomeric excess (Eq. 13).
Recently, Burgess et al.\textsuperscript{69} carried out resolution of several methyl sulfinyl acetates and propionates 58a via enantioselective hydrolysis catalyzed by \textit{Pseudomonas} K-10 lipase. They obtained the recovered esters in >95\% enantiomeric excess (Eq. 14).

Schneider et al.\textsuperscript{70} reported the PLE catalyzed stereoselective hydrolysis of cyclopropane esters 59a. PLE hydrolyzes only \textit{trans}-IR-esters to produce the corresponding (1R, 2R) acids 59b in moderate enantiomeric excesses (Eq. 15).

Francalanci et al.\textsuperscript{71} employed steapsin for enantioselective hydrolysis of racemic \textit{n}-butyl $\beta,\gamma$-epoxybutyrate (60) which produced the unreacted ester (R)-60 in >95\% enantiomeric excess:
This (R)-epoxybutyrate 60 was converted into (R)-carnitine chloride (61) (Scheme 15).

**SCHEME 15:**

![Scheme 15](image)

Recently, Moretti et al.\textsuperscript{72} prepared optically active diesters 62 and 63 (whose chirality is only due to trivalent nitrogen atom) in 76 and 87% enantiomeric excesses via enantioselective hydrolysis of the corresponding racemic diesters catalyzed by lipase from *Rhizopus delemer* and PPL respectively.

![Diesters 62 and 63](image)

Quite recently, Jones and Toone\textsuperscript{73} have reported an efficient synthesis of optically active esters and acids 64-66 in >97% enantiomeric excess via enantioselective hydrolysis of the corresponding racemic esters using PLE.

![Compounds 64-66](image)

\[ R = \text{Me or H}, \quad X = \text{Br or H} \]
Zwanenburg et al.\textsuperscript{74} synthesized the acid, (-)-67b in homochiral form via PLE catalyzed enantioselective hydrolysis of racemic ester 67a (Eq. 16).

\[
\text{COOEt} \quad \text{PLE} \quad \text{COOEt} + \text{HOOC}
\]

\begin{equation}
\text{Rac-67a} \quad (+)\text{-67a} \quad (-)\text{-67b}
\end{equation}

Sih and Fulling\textsuperscript{75} reported enantioselective hydrolysis of racemic ketorolac acid ester (68a) catalyzed by protease from \textit{Streptomyces griseus} to produce both acid 68b and unreacted ester, 68a, in $>$96\% enantiomeric excess (Eq. 17).

\[
\text{COOMe} \quad \textit{S\textsuperscript{\textcircled{\textregistered}} griseus} \quad \text{COOH MeOOC,}
\]

\begin{equation}
\text{Rac-68a} \quad (S)\text{-68b} \quad (R)\text{-68a}
\end{equation}

\textbf{Acylated racemic alcohols:}

Biocatalytic kinetic resolution of racemic alcohols via enantioselective hydrolysis of their acyl derivatives using esterhydrolases as catalysts has become a major tool for the synthesis of homochiral alcohols. Several commercially available esterhydrolases such as PLE, PPL, PFL, CCL, lipase P, lipase P 30 SAM II, etc., are capable of catalyzing the hydrolysis in enantio-
selective manner.

Whitesides and Ladner\textsuperscript{76} reported PPL catalyzed enantioselective hydrolysis of several acylated epoxylcohols. They obtained (R)-(−)-glycidyl butyrate (69a), a versatile chiron, in 92\% enantiomeric excess (Eq. 18).

\begin{align*}
\text{Rac-69a} & \xrightarrow{\text{PPL}} (R)-69a \quad (S)-69b \\
\end{align*}

Ikekawa and coworkers\textsuperscript{77} reported resolution of binaphthol via enantioselective hydrolysis of various dialkanoates of binaphthol catalyzed by Bacillus sp. L-75 which produced the diol and diester in high enantiomeric excess. Subsequently, Kazlauskas\textsuperscript{78} reported a practical synthesis of both enantiomers of binaphthol via bovine pancreas acetone powder (BPAP) catalyzed hydrolysis of divalerate of (±)-binaphthol (70a) (Eq. 19).

\begin{align*}
\text{Rac-70a} & \xrightarrow{\text{BPAP}} (S)-70b \quad (R)-70a \\
& > 99\% \text{ e.e.} \quad > 98\% \text{ e.e.} \\
\end{align*}
Sakai and coworkers\textsuperscript{79,80} showed that PCL catalyzed hydrolysis of several racemic acetates of substituted cycloalkanols produced with very high enantioselectivity. The cycloalkanols 71-73 were obtained in >99\% enantiomeric excess.

Roberts et al.\textsuperscript{81} reported enantioselective hydrolysis of acetate of racemic oct-1-yn-3-ol (74a) catalyzed by Mucor miehei lipase producing (3S)-oct-1-yn-3-ol (74b) in 80\% enantiomeric excess which was subsequently transformed into coriolic acid, an antifungal agent (Scheme 16).

\textbf{Scheme 16:}

\begin{center}
\begin{tikzcd}
\text{OAc} \text{ OH} \quad \text{ Mucor miehei} \\
\text{Coriolic acid \quad (S)-74b}
\end{tikzcd}
\end{center}

The racemic cyclopentenyl acetates 75 and 76 were resolved via enantioselective hydrolysis catalyzed by lipase P\textsuperscript{82} and \textit{Arthrobacter} lipase\textsuperscript{83} respectively, producing the corresponding alcohols and recovered esters in homochiral form (Scheme 17).
Recently, Nieduzak and Carr\textsuperscript{84} reported the synthesis of antiarrhythmic agent 77b via enantioselective hydrolysis of corresponding racemic acetate 77a catalyzed by ANL (Eq. 20).

\[
\text{AcO} \quad \text{Lipase P} \quad \xrightarrow{\text{ANL}} \quad \text{HO} + \text{AcO} \\
\text{Rac-75} \quad 99\% \text{ e.e.} \quad \text{Rac-76} \quad >95\% \text{ e.e.}
\]

Liang and Paquette\textsuperscript{85} described PPL catalyzed enantioselective hydrolysis of chloroacetate of racemic sulcatol 78a which produced unreacted chloroacetate (S)-78a in homochiral form (Eq. 21).

\[
\text{OCOCH}_2\text{Cl} \quad \xrightarrow{\text{PPL}} \quad \text{OH} + \text{CICH}_2\text{COO} \\
\text{Rac-78a} \quad (R)-\text{Sulcatol} \quad (S)-78a
\]
Wong and coworkers developed a chemoenzymatic route to optically pure versatile chiral synthons 79(a & b) using lipase LP-80 as catalyst for enantioselective hydrolysis (Eq. 22).

\[
\begin{align*}
\text{OAc} & \quad \text{OEt} \\
\text{R} & \quad \text{EtO}.
\end{align*}
\]

\[
\text{LP-80} \quad \text{R} = \text{N}_3, \text{F}, \text{Cl}
\]

Recently, Muljiani et al. reported Bacillus subtilis mediated enantioselective hydrolysis of racemic-80a, which produced the desired alcohol 80b in >98% enantiomeric excess (Eq. 23).

\[
\begin{align*}
\text{CCl}_3 & \quad \text{B. subtilis} \\
\text{Rac-80a} & \quad \text{OAc} \\
\text{Rac-80a} & \quad \text{OH}
\end{align*}
\]

Itoh et al. reported enzymatic resolution of racemic esters 81 via enantioselective hydrolysis catalyzed by lipase P, which gave the unreacted esters in >97% enantiomeric excess (Eq. 24).

\[
\begin{align*}
\text{OCOCH}_2\text{SR'} & \quad \text{CN} \\
\text{Rac-81a} & \quad \text{OH} \\
\text{Rac-81a} & \quad \text{CN}
\end{align*}
\]

\[
\begin{align*}
\text{OCOCH}_2\text{SR'} & \quad \text{CN} \\
\text{R} = \text{CH}_3, \text{Ph}, \text{CH}_2\text{CH}_2\text{Ph, CH} &= \text{CHPh}, \text{R'} = \text{CH}_3, \text{Ph}
\end{align*}
\]

86 Wong and coworkers.
87 Recently, Muljiani et al.
88 Itoh et al.
Esterification and transesterification:

The discovery\textsuperscript{89,90} of the fact that lipases retain their catalytic activity in organic media of low water content (<1\%) has great impact on the field of biotransformations. These lipase catalyzed reactions in organic media\textsuperscript{18} have become very important biotransformations for the production of enantiomerically enriched compounds.

The effect of various solvents on the stereoselectivity of lipases\textsuperscript{91-93} continues to be an interesting aspect of these biotransformations. Various solvents used for lipase catalyzed reactions are hydrocarbons (hexane, heptane, toluene, etc.) ethers (diethyl ether, diisopropyl ether, etc.), DMSO, DMF, pyridine, etc. As the literature is very vast it is very difficult to cover all the aspects, we have chosen few selected examples to indicate the importance of this class of biotransformations.

Direct esterification of carboxylic acids and alcohols:

In 1985, Klibanov and coworkers\textsuperscript{94} reported direct esterification of various racemic $\alpha$-haloacids $82a$ with n-butanol in hexane catalyzed by CCL which resulted in production of optically active (R)-esters and (S)-acids (Eq. 25).

\[
\begin{align*}
\text{R}_{1}\text{CHXCOOH} & \xrightarrow{\text{CCL} \text{ n-BuOH}} \text{(R)}-\text{R}_{1}\text{CHXCOOBu} + \text{(S)}-\text{R}_{1}\text{CHXCOOH} \\
\text{Rac-}82a & \quad 82b \quad 82a
\end{align*}
\]

$R = \text{CH}_3, \text{n-C}_6\text{H}_{13}, \text{n-C}_{14}\text{H}_{29}; \text{X} = \text{Br, Cl, 4-Chlorophenoxy}$
Subsequently, Langrand et al.\textsuperscript{95} described direct acylation of various racemic alcohols 83a with lauric acid in hexane or heptane catalyzed by CCL, which gave the optically active esters and alcohols in high enantiomeric excess (Eq. 26).

$$
\begin{array}{c}
\text{Rac-83a} \xrightarrow{\text{CCL}} \text{83b} + \text{(-)-83a} \\
\end{array}
$$

\[ R = \text{CH}_3, \text{C}_2\text{H}_5, \text{n-C}_3\text{H}_7, \text{t-C}_4\text{H}_9 \]

Ring opening of prochiral cyclic anhydrides:

Oda et al.\textsuperscript{96} reported asymmetric ring opening of various 3-substituted glutaric anhydrides (84a) with alcohols catalyzed by PFL producing (R)-half esters 84b in 70-91% enantiomeric excess (Eq. 27).

$$
\begin{array}{c}
\text{84a} + \text{R' OH} \xrightarrow{\text{PFL}} \text{84b} \\
\end{array}
$$

\[ R' = \text{CH}_3, \text{C}_2\text{H}_5, \text{n-C}_3\text{H}_7, \text{i-C}_3\text{H}_7 \]

Transesterification of racemic and prochiral or meso alcohols:

Racemic alcohols:

Kinetic resolution of racemic alcohols via lipase catalyzed transesterification was first reported by Klibanov et al. in 1985, which marked the beginning of a new field of biotransformations.\textsuperscript{94}
They subjected various racemic alcohols 85a to transesterification with trichloroethyl butyrate catalyzed by PPL, producing optically active esters and alcohols in 57-100% enantiomeric excess (Eq. 28).

\[ \text{Rac-85a} \overset{\text{PPL}}{\underset{n-\text{PrCOOCH}_2\text{CCl}_3}{\xrightarrow{\text{Ether/Heptane}}} \text{OCOPr}^n + \text{85b}} \]

\[ R = \text{CH}_3, \text{C}_2\text{H}_5; \text{R}^\prime = \text{alkyl, phenyl} \]

Several important chiral molecules, for example 86-90, have been resolved via enantioselective transesterification catalyzed by various lipases in organic media.\(^{97-101}\)

Prochiral and meso diols:

In 1986, Tombo et al.\(^{102}\) showed that PPL catalyzed transesterification of prochiral diol 91a produces corresponding optically active monoacetate 91b while hydrolysis of corresponding diacetate 91c catalyzed by the same enzyme gives optically active monoacetyl diol 91b (R = alkyl) but with opposite stereochemistry, thus providing access to both the enantiomers. Later, Wong and coworkers\(^{103}\) reported similar results (R = OCH\(_2\)Ph) using PSL as
the enzyme (Scheme 18).

**SCHEME 18:**

\[
\begin{align*}
\text{R} & \quad \text{Lipase} \\
\text{AcOR'} & \\
\text{HO} & \quad \text{OH} & \quad \text{HO} & \quad \text{OH} & \quad \text{Ac} & \quad \text{AcO} & \quad \text{AcO} & \quad \text{OH} & \quad \text{AcO} & \quad \text{AcO} \\
\text{91a} & & \text{91b} & & \text{91c} \\
\end{align*}
\]

\[R = \text{Alkyl, } \text{OCH}_2\text{Ph}; \text{ R'} = \text{CH}_3, \text{ CH=CH}_2.\]

Similarly, meso diols can also be asymmetrized via lipase mediated acylation in organic media. Using this methodology, several synthetically useful chiral molecules 92-96 were synthesized in homochiral form.104-107

Other biotransformations useful in asymmetric synthesis:

Among the synthetically useful biotransformations affected by various types of enzymes, the biocatalytic reductions and oxidations catalyzed by oxidoreductases or whole cells, and carbon-carbon bond forming reactions catalyzed by aldolases, ketolases, oxynitrilases, etc., are the most important transfor-
mations in the context of asymmetric synthesis. The biocatalytic carbon-carbon bond cleaving reactions catalyzed by whole cells are the biotransformations that seem to open new avenues in the field of asymmetric synthesis.

**Asymmetric synthesis via biocatalytic reductions:**

Biocatalytic reductions of prochiral carbonyl groups and heterotopic carbon-carbon double bonds are very important biotransformations since they result in the production of enantio-merically enriched chiral synthons. Several microorganisms and isolated enzymes have been in use for this purpose. Baker's Yeast (*Saccharomyces cerevisiae*)\(^{108,109}\) catalyzed reductions are the best among several biocatalytic asymmetric reductions (Scheme 19).

**SCHEME 19:**
Asymmetric synthesis via biocatalytic oxidations:

Biocatalytic oxidation of alcohols, hydroxylations at activated and unactivated carbons, epoxidation and Baeyer-Villiger oxidation of ketones are among the most useful biotransformations. Some selected examples have been mentioned below (Eq. 29-31, Scheme 20).

Oxidation of alcohols:\textsuperscript{110}

\[
\text{HO''} \rightarrow \text{OH} \quad \text{(29)}
\]

Hydroxylation at activated and unactivated carbons:\textsuperscript{111,112}

SCHEME 20:

Epoxidation of olefins:\textsuperscript{113}

\[
\text{R} \quad \text{Pseudomonas} \quad \text{OH} \quad \text{OH} \\
\text{R} \quad \text{putida 39D} \quad \text{HOOC} \quad \text{OH}
\]

\( R = \text{F, Cl, Br, alkyl} \)
Baeyer-Villiger oxidation of ketones:

\[
\text{Acinetobacter} \quad \text{O} \quad \text{Acinetobacter} \quad \text{O} + \quad \text{Acinetobacter} \quad \text{O}
\]

Asymmetric synthesis via biocatalytic C-C bond forming reactions:

Aldol reaction:

The rabbit muscle aldolase (RAMA) catalyzed aldol reaction between dihydroxyacetone phosphate (DHAP) and aldehydes that produces optically active molecules has been extensively studied and utilized by Whitesides\textsuperscript{115} and Wong\textsuperscript{116} (Eq. 32).

\[
\begin{align*}
\text{H} & \quad \text{H} \\
\text{O} & \quad \text{O} \\
\text{R} & \quad \text{R} \\
\text{OP}_3^- & \quad \text{OP}_3^- \\
\end{align*}
\]

Cyanohydrin formation:

The enzymatic asymmetric addition of HCN to aldehydes\textsuperscript{117} catalyzed by oxynitrilases has proved to be one of the highly useful biotransformations (Eq. 33).

\[
\text{RCHO} + \text{HCN} \xrightarrow{\text{oxynitrilase}} \text{CN} \quad \text{H} \quad \text{OH} \quad \text{R}
\]
Asymmetric synthesis via biocatalytic C-C bond cleavage:

Decarboxylation:

Enzymatic cleavage of a carbon-carbon bond is relatively new to organic synthesis. The recently reported\textsuperscript{118} asymmetric decarboxylation of $\alpha$-disubstituted malonic acids catalyzed by microorganism, *Alcaligenes bronchisepticus*, seems to be a highly useful biotransformation (Eq. 34).

\[
\begin{align*}
\text{Me} & \text{COOH} \xrightarrow{\text{A bronchisepticus}} \text{Me} \text{COOH} \\
\text{Ar} & \text{COOH} \quad \text{Ar} \text{COOH} \\
\end{align*}
\]

Ar = Phenyl, 4-anisyl, 6-methoxy-2-naphthyl, 4-chlorophenyl, 2-thienyl