CHAPTER 1

LIPASES IN BIOTRANSFORMATIONS

The universe is dissymmetrical; for if the whole of the bodies which compose the solar system were placed before a glass moving with their individual movements, the image in glass could not be superposed on reality. Life is dominated by dissymmetrical actions. I can foresee that all living species are primordially, in their structure, in their external forms, functions of cosmic dissymmetry.

Louis Pasteur
1.1 INTRODUCTION

Chirality is a geometrical attribute. An object that is not superimposable on its mirror image is said to be chiral. The most common type of chiral organic molecule contains a tetrahedral carbon atom attached to four different groups. Such a carbon is said to be a stereogenic center and the molecule exists in two stereoisomeric forms. Chirality is not a prerequisite for bioactivity but in bioactive molecules where a stereogenic center is present, great differences are observed for the activity of the enantiomers. This is a general phenomenon and applies to all bioactive substances, such as drugs, insecticides, herbicides, flavors, fragrances, pheromones and food additives.

1.1.1 Receptor Theory and Pharmaceuticals:

Most drugs are specific and their action is usually explained on the basis of receptor theory. This concept for bioactive agents was introduced by Langely (1906), in order to explain the observed effects of nicotine and curare on muscle tissues. Ehrlich later coined the term chemoreceptors to describe such binding sites. The chemoreceptor proteins that have affinities to ligands are analogous to enzyme substrate binding, the binding triggers regulation of vital functions as blood pressure, muscle contraction, gastric acid secretion etc. The overwhelming majority of naturally occurring drugs are chiral molecules, existing as single active enantiomer, whereas for many years it was common practice to market the synthetic drugs as racemates. But trend is towards rational drug design to produce complex molecules and hence there is an increased probability that they are chiral. In case of certain drugs eg. antihypertensive agents, when administered as racemates the distomer (inactive isomer) displays no side-effects. But in some other cases the distomer may even exhibit toxic side-effects e.g., the (S)-isomer of ketamine has anaesthetic and analgesic activity, but (R)-isomer is an hallucinating agent. The most well-known example is that
of thalidomide, formerly used as sedative (also immunomodulator) where the distomer is a teratogenic agent and causes fetal abnormalities. The grave side-effects due to the distomers have now made administering a single pure isomer very important. In short, different pharmacodynamics and pharmacokinetics of the eutomer (active isomer) and the distomer in a racemic drug can lead to a variety of side-effects attributable to inactive isomers. Thus it becomes very important to synthesize and administer chiral molecules in enantiomerically pure form, and asymmetric synthesis, kinetic resolution of racemates, preferential/diasteromeric crystallization have hence gained strategic importance.

1.1.2 Asymmetrical Synthesis versus Kinetic Resolution:

There are two possible approaches for the preparation of optically active products by chemical transformation of optically inactive starting materials: (i) kinetic resolution/diastereomeric crystallization and (ii) asymmetric synthesis. A kinetic resolution depends on the fact that two enantiomers of a racemate react at different rates with a chiral reagent or catalyst, such as an enzyme. A more modern example of enzymatic kinetic resolution is acylase catalyzed L-specific hydrolysis of racemic N-acetyl amino acids commercialized by Tanabe company. In diastereomeric crystallization, a solution of racemate is allowed to interact with a pure enantiomer (the resolving agent) thereby forming a mixture of diastereomers that can be separated by fractional crystallization. The most commonly used resolving agents are L-(+) tartaric acid, D(-) camphorsulfonic acid and various alkaloid bases. An asymmetric synthesis, on other hand, involves the creation of an asymmetric center by chiral auxiliary/reagent. Thus a kinetic resolution involves substrate selectivity while an asymmetric synthesis involves product selectivity.

A cursory appraisal of the relative economics of asymmetric synthesis versus kinetic resolution would seem to indicate a clear preference for the former since it has a
theoretical yield of 100% as compared to 50% for kinetic resolution. However, kinetic resolution has some advantage over asymmetric synthesis: (i) experimentally simple processes, (ii) tuning of the enantiomeric excess by adjusting the degree of conversion. The major disadvantage of kinetic resolution is that they require at least one extra step for racemization of the unwanted isomer for recycling to increase the yield. This can be circumvented if spontaneous in-situ racemization is carried to attain kinetic dynamic resolution.

1.1.3 Enantiomeric Purity Determination:

The importance of optical purity in the context of biological activity has created a growing need for accurate unequivocal methods for the determination of the optical purities.

(i) Optical Rotation

This classical method involves measurement of specific optical rotation \([\alpha]\)

\[
\text{Optical Purity} = \frac{[\alpha]}{[\alpha]_0} \times 100
\]

where \([\alpha]\) = specific optical rotation of the mixture and \([\alpha]_0\) = specific optical rotation of the pure enantiomer.

\[
\text{Enantiomeric excess (\% ee)} = \frac{R_{\text{isomer}} - S_{\text{isomer}}}{R_{\text{isomer}} + S_{\text{isomer}}} \times 100
\]

where \(R\) and \(S\) are the relative proportions (ratio) of the two enantiomers. In practice, this may often lead to some confusion since the optical rotation is dependent upon various conditions of measurements such as solvents, temperature, purity etc. and ambiguity may exist if the compound is new or not well documented in literature.
(ii) **HPLC Methods:**

As enantiomers have the same adsorption properties, they are not amenable to direct chromatographic separation on achiral adsorbents. However, this can be accomplished via the formation of diastereomers either by derivatization of sample with suitable chiral reagent\(^4\) or formation of transient diastereomers via the interaction of enantiomers with chiral stationary phase/mobile phase additive.\(^5\)

(iii) **GLC Methods:**

For compounds that are readily vaporized without decomposition, gas chromatography on chiral stationary phase constitutes an accurate and reliable method for enantiomeric purity determination. The technique has inherent advantages of simplicity, speed, reproducibility and sensitivity.\(^6\)

(iv) **NMR Methods:**

NMR is a widely used technique for enantiopurity determination and one well tested method involves conversion of mixture of enantiomers to a mixture of diastereomers by optically pure reagents eg. Mosher’s reagent.\(^7\) A closely related method employs the use of chiral Lanthanide shift reagents (LSRs),\(^8\) having the property of shifting the NMR signals of substrates via diastereomeric complex formation.

### 1.2 BIOTRANSFORMATION

Biotransformations involve the use of biological methods to effect chemical reactions and form a bridge between chemistry and biology. Here biological systems are employed to consequent chemical changes on compounds that are not their natural substrates. This distinguishes biotransformation from biosynthesis, which involves action of biological systems in their normal habitat upon their natural substrates. Fermentation is a powerful technique for the production of alcohol, acids, antibiotics,
amino acids, and nucleic acid related compounds. In this technique, the product is the result of a complex metabolism of the microorganism, based on inexpensive carbon and nitrogen sources. In microbial transformations, it is not necessary to use active cells and the microorganism is akin to a bag of enzymes, requiring a suitable substrate instead of the carbon and nitrogen sources required for fermentation. In some biotransformations the synthetic substrate may resemble the natural substrates while in some others (e.g., xenobiotic transformation) it may be completely unrelated to the natural system. Whilst both may be used for synthetic purposes, the former can focus some light on structural and mechanistic features of biosynthesis. This type of study could be useful for the synthetic chemist in planning the retrosynthetic approaches. In biotransformations, isolated enzyme systems or intact whole organisms may also be used. Many biotransformations are not only chemo- and regioselective but are also enantioselective allowing the production of chiral materials from racemic mixtures. The conditions for biotransformations are mild and in majority of cases do not require the protection of other functional groups. Furthermore the features governing their regioselectivity differ from those controlling the chemical specificity and indeed it is possible to obtain biotransformations at centers that are chemically non-reactive (e.g. in Figure 1, compound 1 is transformed to 2 by hydroxylation at the chemically unreactive C-11 site). From a commercial point of view some biotransformations can be cheaper and more direct than their chemical analogues, whilst the transformations
proceed under the conditions that are normally regarded as "environmentally friendly". There are many chemical reactions for which there are no equivalent biotransformation steps and the chemist should be versatile to use biotransformations in combination with the conventional chemical reagents in a synthesis. There is a vast amount of literature available on diverse aspects of biotransformation, a recent monograph by J. R. Hanson\textsuperscript{9} gives an elegant overview of biotransformations in organic chemistry.

A particularly useful biotransformation early reported was the chiral acetoin condensation mediated by yeast. The addition of benzaldehyde (3) to the fermentation broth gave the ketol (4) by condensation with the acetaldehyde. This led in 1934 to a commercial synthesis of the alkaloid, ephedrine (5, Figure 2).

![Figure 2]  
*Figure 2*

Another industrially useful application developed during the 1930's was the synthesis of vitamin C (8), which utilized the bacterial oxidation of D-sorbitol (6) to L-sorbose (7) by *Acetobacter suboxydans* (*Acetobacter xylinum*). The other chemical steps involve protection of L-sorbose as its bisacetonide, oxidation and deprotection to form 2-keto-L-gluconic acid, which is then converted to ascorbic acid (Figure 3, vitamin C).

![Figure 3]  
*Figure 3*
A versatile bioprocess for hydration that has been scaled-up to operate at the tonnage level, is the hydrolysis of acrylonitrile (9, Figure 4) to acrylamide (10) by the bacterium, *Rhodococcus rhodochrous*. The conditions are considerably milder than those required for the chemical hydrolysis, this material is one of the famous monomer used in polymer industry.

An interesting development with an impact on biotransformations involves the production of catalytic antibodies. An antibody possesses the property of molecular recognition by which it binds strongly to specific molecular structures - hapten and in fact antibodies are stimulated by the recognition of particular structural features in a macromolecule. This avidity of antibodies can be exploited to develop them for binding and stabilizing the transition state of a reaction to achieve catalysis. Since the transition states are unstable, the antibodies are raised to a stable transition state analog. Such antibodies in presence of suitable reactants exhibit catalytic activity and are termed "abzymes". For example, a tetrahedral phosphonate (11, Figure 5) mimics the tetrahedral intermediate (12) in ester hydrolysis. The catalytic antibody elicited by exposure to (13) catalyzes the hydrolysis of the ester (14).
1.2.1 Classes of Enzymes

Enzymes are classified by rules prescribed by the commission on Enzymes of the International Union of Biochemistry, according to which each enzyme is designated by four numbers; the main class, the subclass, sub-subclass, and the serial number. There are six main classes as shown:

1. **Oxidoreductases**: These enzymes mediate oxidation and reduction, including the insertion of oxygen to alkenes. This group also includes enzymes that are responsible for the addition or removal of hydrogen.

2. **Transferases**: These enzymes are involved in the transfer of one group, such as an acyl or a sugar unit from one substrate to another.

3. **Hydrolases**: This group includes the enzymes that mediate the hydrolysis or formation of amides, epoxides, esters and nitriles.

4. **Lyases**: These are group of enzymes that fragment larger molecules with the elimination of smaller units.

5. **Isomerases**: These enzymes are involved in epimerization, racemization and other isomerization reactions.

6. **Ligases**: This group includes the enzymes responsible for the formation of C-C, C-O, C-S and C-N bonds.

**Units**: The international unit (I.U.) of any enzyme activity is described as μmoles of substrate utilized per minute or μmoles of product formed per minute and the specific activity is defined as activity per mg of enzyme.

1.2.2 Hydrolases

1.2.2a Esterases and Lipases

Hydrolysis of amides and esters by enzymatic methods (proteases, lipases, and esterases) are now routine biotransformations. Furthermore, the general mechanisms by which some of these operate are quite well known and their scope has been
investigated to the extent that some predictive models are available. The molecular machinery of lipases is much like that of the serine protease's and consists of a catalytic triad of amino acids-serine, histidine, and aspartic (or glutamic) acid operating through a charge-relay system via hydrogen bonds (Figure 6). The function of the buried aspartate group is to polarize the imidazole ring of histidine, since the remote negative charge induces a positive charge adjacent to it. This facilitates the proton transfer along the hydrogen bonds, and the hydroxy proton is finally bound to histidine. The active serine residue is now capable of attacking the scissile ester or peptide bond. The tetrahedral intermediate 15 is formed. After displacement of the leaving group R-XH by another nucleophile R-YH, histidine once again, at first deprotonates the new nucleophile with concomitant release of a proton to aspartate, and the activated species attacks the acylserine in the precisely reverse fashion as before. The resulting

\[ \text{Figure 6: Mechanism of lipase/esterase and capture of acyl/enzyme complex with various nucleophiles.} \]
tetrahedral intermediate 16 collapses to a new carbonyl compound and regenerates the serine-OH. The catalytic cycle may resume, enabling a variety of nucleophiles to participate in this process, such as water resulting in hydrolysis, alcohol in esterification or transesterification, an amine offering amidation, hydrogen peroxide leading to formation of a peracid (which can epoxidize an available olefin). The structural shape of protein creates hydrophilic or lipophilic pockets within the enzyme active site, which leads to chemo-, regio-, enantio-, and diastereoselectivity.

In general, cells produce lipases to hydrolyze the extracellular fats and lipases are specially structured to act at water/organic interface. For this reason lipases appear to have optimum property among the enzymes to operate in organic solvents, in this case the interface is between the insoluble enzyme with its essential water of hydration and the organic solvent containing the acylating agent. Their broad synthetic potential is largely due to the fact that lipases, in contrast to most other enzymes, accept a wide range of substrates. They are stable in non-aqueous organic solvents and depending on the solvent system used, they can be employed for hydrolysis or esterification reactions. In addition, lipases can accommodate a wide range of substrates other than triglycerides such as aliphatic, alicyclic, bicyclic, and aromatic esters including the esters based on organometallic sandwich compounds. Lipases react with high regio- and enantioselectivity and although this view is clearly oversimplified and not valid for all types of esterases and lipases, it can explain the majority of the known applications of these enzymes in organic synthesis:

- Simple acylation and deacylation reactions under exceptionally mild conditions.
- Synthesis of amides and peptides.
- Transesterification, diastereoselective esterifications or hydrolyses of lipids
- Regioselective reactions on polyfunctional compounds (polyols, sugars)
- Highly enantioselective synthesis of esters, half-esters, acids, lactones, and polyesters.
- Highly enantioselective synthesis of alcohols, diols, polyols, and amines.

In neat organic solvent, the enzymes retain the minimum amount of water which is necessary for their catalytic activity. Molecules of hydrophilic solvents can enter the inner core of the macromolecule and eventually destroy the functional structure. Water-immiscible solvents, containing water below the solubility limit (ca. 0.02 to 10% by weight) are suitable for dry enzymes. In other cases, more polar solvents or cosolvents improve the success of transformation. The use of organic solvents is seen to enhance the enantioselectivity and thermostability of the enzymes, probably due to restricted conformational flexibility. Conversely, some enzymes can lose or alter their enantioselectivity in organic media indicating that the conformation of the enzymes is dependent on the polarity of the medium and that individually optimized reaction conditions have to be developed for each transformation. From all the above discussion it is evident that in an adequate organic solvent, the catalytic activity can be of comparable order in aqueous medium with low water media, but overall, the rates of such (in organic media) reactions are slow.

However, irrespective of the aforementioned complications, the use of organic solvents is of great importance, since this adds a new perspective to the applicability of the enzymes for organic synthesis. The advantages are obvious: (i) high solubility of most organic substrates, (ii) transformations of water-sensitive substrates in organic solvents, (iii) choice of a wide range of nucleophiles (Figure 7, HY-R) for esterifications, aminolysis, oximolysis, or thiolysis etc. (iv) suppression of product or substrate inhibition when they can be retained in organic phase, (v) greater stability of the biocatalyst, (vi) ease of operation and easy removal of the insoluble catalyst.
1.2.2b Occurrence and Preparation

Lipases are ubiquitous enzymes and have been found in most organisms from the microbial,\textsuperscript{16-18} plant\textsuperscript{19,20} and animal kingdom.\textsuperscript{21,22} They are prepared either by extraction from animal or plant tissue, or by cultivation of microorganisms. Usually lipases are just one member of a “hydrolytic enzyme cocktail” elaborated by an organism with the objective to sustain its growth. Often the lipases must be separated from other enzymes such as estrases and proteases occurring in the crude preparations. Purification protocols are often laborious as the affinity of lipases is high not only in the oil/water boundary, but at any interphase of lower polarity than water (e.g. water-immiscible organic solvents, glass and plastic surfaces, and air bubbles); lipases may irreversibly adsorb and denature at such interphases.\textsuperscript{23}

1.2.2c Interaction of Lipases with Lipids

Since lipase catalytic action is strictly dependent upon the presence of a lipid interface, lipolytic enzymes provide a valuable model for studying protein-lipid interactions.\textsuperscript{24} Most data dealing with the surface properties of lipases were obtained with the monolayer technique, by recording (either independently or simultaneously) the lipolytic activity, the amount of the protein adsorbed to the lipid monolayer, and the variations in surface pressure following protein adsorption. Several non-enzymatic proteins were used as controls in order to determine how lipase behavior differs from that of other proteins.

1.2.2d Structure and Mechanism

In 1958, Sarda and Desnuelle\textsuperscript{25} defined lipase in kinetic terms, based on the phenomenon of interfacial activation. They suggested that the activity of lipases is low on monomeric substrates but strongly enhanced once an aggregated “supersubstrate” (such as an emulsion or micellar solution for instance) is formed above its saturation limit. This property is different from the usual esterases acting on water-soluble
carboxylic ester molecules, and for long time lipases were considered as a special category of esterases which are highly efficient at hydrolyzing molecules aggregated in water.

The first two lipase structures were solved in 1990 by X-ray crystallography which revealed a unique mechanism, unlike that of any other enzyme. Their three dimensional structures suggested that interfacial activation is due to the presence of an amphiphilic peptidic loop covering the active site of the enzyme in solution, just like a lid or a flap. From the X-ray structure of cocrystals between lipases and substrate analogue, there is a strong evidence that, upon contact with a lipid/water interface, the lid undergoes a conformational rearrangement which renders the activation site accessible to the substrate (Figure 7).

There seems to be some evidence which suggests that not all lipases subscribe to the phenomenon of interfacial activation. The lipases from *P. glummae* and *C. antartica* (type B), whose tertiary structure is known, have an amphiphilic lid

![Figure 7](image-url)
covering the active site but do not show interfacial activation. The lipase of the guinea pig enzyme features a mini-lid composing of only five amino acid residues and it shows no interfacial activation. Thus, neither the phenomenon of interfacial activation nor the presence of a lid domain are appropriate criteria to classify an esterase into lipase subfamily. For classifying an esterase as a lipase, the safest experimental evidence is to find out whether or not it can hydrolyze long-chain acyl glycerols. Till date, 12 lipases whose structure have been elucidated, are members of the “α/β hydrolase fold” family with a common architecture composed of α-helices and β-strands.

In spite of these similarities, subtle variations in the architecture of the substrate-binding site may have strong effect on the temperature and the stability of lipase in a solvent and hence its catalytic properties. These differences are of paramount importance for the selection of the individual lipase for a desired application.

1.3 LIPASES AS BIOCATALYSTS IN ORGANIC SYNTHESIS

Considering their specific and limited function in metabolism, one should expect lipases to be of limited interest for the organic chemist. However, chemists have discovered lipases to be one of the most versatile classes of biocatalysts in organic synthesis for a few simple reasons:

1. Owing to the large enzyme structural domains required for the acyl group binding and the unpronounced structural features of acyl chains, lipases can accommodate a wide variety of synthetic substrates, while still showing chemo-, regio, and/or stereoselectivity.

2. Lipases act at the water/lipid boundary, which exhibits high interfacial energy. To withstand the denaturing effect of the interface, lipases have evolved unusually stable structures that may survive even the effect of organic solvents.
3. The free energy of fat hydrolysis is close to 0 kJ mol\(^{-1}\).\(^{34}\) As a result, thermodynamic equilibria are largely governed by the reactant concentrations, and lipase catalyzed ester hydrolysis in water can easily be reversed, in non-aqueous media, into ester synthesis or transesterification.

4. The acyl lipase formed in the first step of the enzymatic reaction can formally be considered as an acylating agent. The wide substrate specificity of this enzyme class allows acylation of nucleophiles other than those with hydroxyl groups, for example, hydroperoxides, thiols, and amines.

As a result of this unique combination of properties, chemists have used lipases for a plethora of synthetic reactions.\(^{35}\) Following a thorough survey of the literature on chiral resolutions with lipases from \emph{Candida rugosa} (CRL) and \emph{Pseudomonas cepacia} (PCL), Kazlauskas et al. put forward tentative rules for the enantiopreference of these two enzymes based on the spatial requirements of the substituents on the reagent.\(^{36}\)

The basics of "Kazlauskas rules", are presented in Figure 8 and the literature has shown that the rule is highly predictive for lipase action on secondary alcohols, but less accurate for lipase-catalyzed transformations of primary alcohols and acids.

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{kazlauskas_rules}
\caption{"Kazlauskas rules" (M = medium-sized substituent, L = large substituent)}
\end{figure}

\subsection*{1.3.1 Transformations of Prochiral and \textit{meso} Substrates ("Meso Trick")}

In kinetic resolutions, theoretical yields are limited to 50%. Yields of up to 100% are possible with \textit{meso} -diesters or diol substrates, which undergo enantioselective hydrolysis or transesterification through enantiotopic group differentiation.
a) **Synthesis of Chiral Dicarboxylic Acid Monoesters**

Meso compounds have to fit in and accommodate the active center in an optimum manner and only then the catalytical process creates a chiral molecule.\(^{41,42}\)

\[
\begin{align*}
R_1\text{CO}_2\text{R} & \xrightarrow{\text{biocatalyst}} R_1\text{CO}_2\text{H} \\
17a-f & \quad 18a-f
\end{align*}
\]

Enantioselective Hydrolysis of Malonate Diesters

\[
\begin{align*}
R_2\text{CO}_2\text{R} & \xrightarrow{\text{biocatalyst}} R_2\text{CO}_2\text{Me} \\
19a-f & \quad 20a-f
\end{align*}
\]

Enantioselective Hydrolysis of Glutarate Diesters

**Figure 9**

The result is that, at least theoretically, an achiral compound can be quantitatively converted into an optically pure product. This concept has been realized with a great number of acyclic, alicyclic and heterocyclic substrates. Most of the work on acyclic compounds has been devoted to derivatives of malonic (Figure 9, Table 1) and glutaric acids (Figure 9, Table 2) for the synthesis of their respective monoesters, which find use as valuable intermediates of amino acids and barbiturates.

**Table 1 Enantioselective Hydrolysis of Malonate Diesters**

<table>
<thead>
<tr>
<th>Sub</th>
<th>R1</th>
<th>R2</th>
<th>Product</th>
<th>yield</th>
<th>% ee</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>17a</td>
<td>alkyl</td>
<td>alkyl</td>
<td>18a</td>
<td>-</td>
<td>38-90</td>
<td>37</td>
</tr>
<tr>
<td>17b</td>
<td>Me</td>
<td>OH</td>
<td>18b</td>
<td>82</td>
<td>46</td>
<td>38</td>
</tr>
<tr>
<td>17c</td>
<td>CH$_2$O Me</td>
<td>Me</td>
<td>18c</td>
<td>86</td>
<td>21</td>
<td>39</td>
</tr>
<tr>
<td>17e</td>
<td>F</td>
<td>H, alkyl</td>
<td>18e</td>
<td>-</td>
<td>46-93</td>
<td>40</td>
</tr>
</tbody>
</table>

**Table 2 Enantioselective Hydrolysis of Glutarate Diesters**

<table>
<thead>
<tr>
<th>Sub</th>
<th>R1</th>
<th>R2</th>
<th>Product</th>
<th>yield</th>
<th>% ee</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>19a</td>
<td>H</td>
<td>Me</td>
<td>20a</td>
<td>-</td>
<td>90</td>
<td>43</td>
</tr>
<tr>
<td>19b</td>
<td>OH</td>
<td>Me</td>
<td>20b</td>
<td>82</td>
<td>&gt;99</td>
<td>46</td>
</tr>
<tr>
<td>19c</td>
<td>NHR3</td>
<td>H</td>
<td>20c</td>
<td>93</td>
<td>54-99</td>
<td>47</td>
</tr>
</tbody>
</table>

The enzyme catalyzed hydrolysis of cyclic meso-diesters has found broadest application in the preparation of synthetically useful chiral building blocks. Most of
these reactions are carried out with PLE, some of the representative examples are listed in Table 3.

Table 3 Enantioselective Hydrolysis of Alicyclic and Heterocyclic meso-Diesters

<table>
<thead>
<tr>
<th>Entry</th>
<th>Product</th>
<th>Yield (%)</th>
<th>ee (%)</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>21</td>
<td></td>
<td>17-100</td>
<td>17-100</td>
<td>38</td>
</tr>
<tr>
<td>22</td>
<td></td>
<td>98</td>
<td>98</td>
<td>38</td>
</tr>
<tr>
<td>23</td>
<td></td>
<td>86</td>
<td>88</td>
<td>43</td>
</tr>
<tr>
<td>24</td>
<td></td>
<td>86</td>
<td>75</td>
<td>44</td>
</tr>
<tr>
<td>25</td>
<td></td>
<td>82</td>
<td>&gt;98</td>
<td>44</td>
</tr>
<tr>
<td>26</td>
<td></td>
<td>85</td>
<td>&lt;10</td>
<td>45</td>
</tr>
<tr>
<td>27</td>
<td></td>
<td>30</td>
<td>100</td>
<td>46</td>
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<tr>
<td>28</td>
<td></td>
<td>100</td>
<td>85</td>
<td>47</td>
</tr>
</tbody>
</table>
b) *Synthesis of Chiral Monoacyl Diols*

Instead of hydrolyzing a prochiral or meso diester, the corresponding diacetate or diol can be employed. In certain cases, the more readily available diols and diacetates are used and in consequence, the stereochemical complementarity of the synthetic or hydrolytic action of the enzymes can be exploited to the full extent. The 3-O-protected glycerols\(^{49}\) or their diacetates,\(^{50}\) can be transformed into optically active 32 and 33 (e.g. R = OBn, Figure 10), this route provides an highly efficient access to chiral glycerol derivatives. Previous syntheses of these from carbohydrate precursors including laborious protective group technique are no longer required. These compounds are important intermediates in synthesis of many drugs, e.g. \(\beta\)-blockers, platelet activating factor (PAF), and many other biologically important compounds. Monoacyl diols of (Figure 11) 34 and 35 were obtained in excellent optical purity and high yields with various enzymes as PFL, PPL or PLE.\(^{50}\) Some examples of the different chiral alicyclic and other heterocyclic diols/diacetates that have been prepared
are cited in the Table 4. In synthetic mode, the reactions are carried in anhydrous inert solvents e.g. toluene, diisopropyl ether, or ethyl acetate, the later serving as solvent and acyl donor. The different types of acyl donors used are vinyl acetate, propenyl acetate, or anhydrides. The concept has been realized with various 2-alkypropane-1,3-diols 30 (R = alkyl, aryl, etc) as shown in the scheme above.

Table 4: Enzymatic Synthesis of Chiral Alicyclic and Heterocyclic Monoacetates

<table>
<thead>
<tr>
<th>Entry</th>
<th>Product</th>
<th>Biocatalyst</th>
<th>Yield (%)</th>
<th>ee (%)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>36</td>
<td><img src="image" alt="Structure" /></td>
<td>SAM-II, PPL</td>
<td>20-90</td>
<td>80-95</td>
<td>51</td>
</tr>
<tr>
<td>37</td>
<td><img src="image" alt="Structure" /></td>
<td>PPL</td>
<td>55-94</td>
<td>50-96</td>
<td>51</td>
</tr>
<tr>
<td>38</td>
<td><img src="image" alt="Structure" /></td>
<td>PLE, PPL, CCL, LPF</td>
<td>23-84</td>
<td>33-99</td>
<td>52</td>
</tr>
<tr>
<td>39</td>
<td><img src="image" alt="Structure" /></td>
<td>RDL</td>
<td>69</td>
<td>99</td>
<td>53</td>
</tr>
<tr>
<td>40</td>
<td><img src="image" alt="Structure" /></td>
<td>PLE, PPL, CCL, others</td>
<td>~90</td>
<td>50-100</td>
<td>54</td>
</tr>
<tr>
<td>41</td>
<td><img src="image" alt="Structure" /></td>
<td>PPL</td>
<td>84</td>
<td>55</td>
<td>44</td>
</tr>
<tr>
<td>42</td>
<td><img src="image" alt="Structure" /></td>
<td>PLE</td>
<td>50-80</td>
<td>&gt;98</td>
<td>55</td>
</tr>
</tbody>
</table>
1.3.2 Kinetically Controlled Transformations of Racemic Substrates

a) *Synthesis of optically Active Acids and Esters via Hydrolysis*

The essential requirements for a successful separation of a racemate by esterases and lipases are sufficiently different rate constants for the transformations of the enantiomers, either in the synthetic direction, or driven in the reverse towards

![Chemical structure](image)

*Figure 12*

hydrolysis (Figure 12). Under optimum conditions, i.e., at complete enantiospecificity of the enzyme, the reaction will stop at 50% conversion. If the unwanted enantiomer cannot be recycled by racemization, 50% of the product gets discarded. On the other hand, compared to the classical approaches via diastereomers or tedious chromatographic procedures, the enzymatic approach has a great advantage that only a small amount of the biocatalyst may be needed instead of molar quantities of a chiral reagent. Table 5 gives an overall view of the enantioselective hydrolysis of the representative alicyclic and heterocyclic compounds. Using PLE, out of a mixture of diastereomeric, racemic chrysanthemum esters (R = Me) the *trans*-acid 48 has been obtained with high diastereselectivity. When pure *trans*-chrysanthemum esters were subjected for hydrolysis (50% conversion), the (1R, 2R)-48 (70-80% ee) were obtained.
The enzymatic route to norborane esters and half esters 50 and 52 provides a very simple and effective alternative to asymmetric Diels-Alder reaction.

Table-5: Enantioselective Hydrolysis of Alicyclic and Heterocyclic Esters

<table>
<thead>
<tr>
<th>Entry</th>
<th>Product</th>
<th>Biocatalyst</th>
<th>Yield (%)</th>
<th>ee (%)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>48</td>
<td>![Product Image] (R = Me, Cl)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>49</td>
<td>![Product Image] (R1, R2 = H, Me)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>50</td>
<td>![Product Image]</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>51</td>
<td>![Product Image] (X = N, S, CH₂)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>52</td>
<td>![Product Image]</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>53</td>
<td>![Product Image] (X = N, S, CH₂)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

b) Cyclic and Aliphatic Monohydroxy Substrates

The principal requirements for the resolution of the racemates have been mentioned in the previous section. However, in the case of simple secondary alcohols, an additional consequence of the kinetically controlled reaction namely, double resolution strategies, can be readily exploited. If the ester synthesis from a given alcohol and an appropriate acyl donor does not lead to a product of high % ee, this ester can be subjected to a second enzyme-catalyzed transesterification by an achiral
alcohol. Since, both reactions can be carried out in organic media, the conformation of
the enzyme remains the same, and the faster reacting enantiomer in the synthetic
direction will also be the faster released by transfer to an achiral alcohol. The strategy
is outlined in the Figure 13. Under suitable kinetic control in both directions, products
of very high % ee can be obtained by this two-step procedure.

**Figure 13**

Aliphatic secondary alcohols constitute a class of industrially important aroma
and flavor compounds. Hence much work has been devoted to explore optimum
conditions for their preparation from chiral precursors. This includes the search for the
best enzyme and acyl donor,\(^\text{62}\) optimum conversion rates,\(^\text{63}\) solvents,\(^\text{62}\) and
temperature.\(^\text{64}\) The effect of temperature is interesting, since PPL produced esters, at
70 °C with higher % ee than at 40 °C.\(^\text{65}\) Pheromones are semiochemicals and used for
intraspecific communications, which have to be synthesized with very high % ee
because of possible interfering effects of other isomer.

Among the cyclic monohydroxy substrates as shown in Table 6 enantioselective
preparation of 2,3-epoxy alcohols 54 is of great synthetic value.\(^\text{66}\) Studies on variation
of chain length,\(^\text{59}\) co-solvents,\(^\text{71}\) and temperatures with respect to efficiency of
resolution\(^\text{72}\) has been extensively done. Entries 58, 59 and 60 are further examples for
the utilities of the lipases in the field of cyclopentanoid precursors to natural products.
The biocatalytic resolution of cyclohexanols 61 is an attractive route to chiral auxiliaries
and reagents like menthol or phenylmenthol.\(^\text{73}\) Entry \((S)-62\ (n=1, R=Alkyl) deserves a
special attention, the substrate is an enol ester, hence hydrolysis of the ester moiety and transfer of the proton to the double bond have to occur from the same direction. The ketone $S\cdot(62)$ is obviously released without formation of an intermediary enol.\(^74\)

**Table-6: Enantioselective Synthesis of Alicyclic Alcohols**

<table>
<thead>
<tr>
<th>Entry</th>
<th>Product</th>
<th>Biocatalyst</th>
<th>Yield (%)</th>
<th>ee (%)</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>54</td>
<td><img src="image" alt="Chemical Structure" /></td>
<td>PPL</td>
<td>30-60</td>
<td>30-95</td>
<td>59</td>
</tr>
<tr>
<td>55</td>
<td><img src="image" alt="Chemical Structure" /></td>
<td>PPL</td>
<td>50</td>
<td>&gt;98</td>
<td>65</td>
</tr>
<tr>
<td>56</td>
<td><img src="image" alt="Chemical Structure" /></td>
<td>PPL</td>
<td>50</td>
<td>0 (n=1) &gt;97 (n=2)</td>
<td>59</td>
</tr>
<tr>
<td>57</td>
<td><img src="image" alt="Chemical Structure" /></td>
<td>PL 266, others</td>
<td>30</td>
<td>93-95</td>
<td>67</td>
</tr>
<tr>
<td>58</td>
<td><img src="image" alt="Chemical Structure" /></td>
<td>Penicillin acylase</td>
<td>30-43</td>
<td>90</td>
<td>68</td>
</tr>
<tr>
<td>59</td>
<td><img src="image" alt="Chemical Structure" /></td>
<td>Lipase P</td>
<td>42</td>
<td>&gt;99</td>
<td>69</td>
</tr>
<tr>
<td>60</td>
<td><img src="image" alt="Chemical Structure" /></td>
<td>Arthrobacter lipase</td>
<td>&lt;50</td>
<td>&gt;95</td>
<td>70</td>
</tr>
<tr>
<td>61</td>
<td><img src="image" alt="Chemical Structure" /></td>
<td>SAM-II, PLE, CCL</td>
<td>10-88</td>
<td>36-98</td>
<td>73</td>
</tr>
</tbody>
</table>
1.3.3 Intra- and Intermolecular Transesterification of Hydroxy Acids

If the substrate is a hydroxy acid or a hydroxy ester such as 63, the remote OH-group can act as an internal nucleophile onto the primarily formed acyl-enzyme. This reaction becomes feasible when water is replaced by an organic solvent as the reaction medium. Since hydroxy acids 63 (R = H, Figure 14) are prone to spontaneous lactonization, the more stable methyl esters were subjected to enzymatic

![Figure 14](image)

Figure 14

transesterification in diethylether or hexane. Using PPL, at low conversion rates (36%) for example (S)-(-)-γ-methylbutyrolactone 64 (R¹ = Me, R² = H) is obtained with high optical purity (97% ee). At high conversion rates (60%) the recovered (S)-4-OH ester 63 is of high optical purity (97% ee), and the corresponding R-(+)-lactone is available by acid treatment. Up to α-dodecalactone high optical purities (82-98% ee) have been obtained whereas the α-substituted derivatives of the esters 63 are poor substrates. PFL yields γ-lactones 64 with opposite configuration, but their % ee is low (10-45%). Enzymatic (PPL or PFL) intramolecular transesterification of the symmetrical hydroxy diester (meso-substrate, Figure 16) 65 leads to (S)-(−)-66 (>98% ee, 100% conversion) in an enantioconvergent manner.
If the hydroxy group is located at more remote positions, only very few lipases are able to catalyze lactonization, and intermolecular oligomerization becomes the preferred pathway. In general, if a ring containing five annular atoms can be formed,

![Figure 15](image)

**Figure 15**

this is the exclusive product; if of six or seven atoms, either the ring or the chain polymer is formed — in some instances both.\(^77\) For example, methyl 9-hydroxydecanoate (67, Figure 16) is converted to the corresponding macrocyclic lactone (S)-68 (100% ee) only by PFL;\(^78\) others catalyze almost exclusively the formation of cyclic dimers of type 71\(^79\) and acyclic oligomers of type 72 (Figure 17), respectively. However, careful optimization of the reaction conditions provided the whole series of C(12) to C(16) lactones 70 [70-80% yield for C(16) and C(15)] from the
corresponding ω-hydroxy acids.\textsuperscript{78} With shorter chain hydroxy acids the formation of oligomers 72 is the preferred reaction.\textsuperscript{77} With α,ω-di acids (m= 2-12) and α,ω-dialcohols (n= 5-16) and lipases from \textit{Candida cylindracea} (OF-360) or \textit{Pseudomonas} sp. (AK and K-10) the intermolecular transesterification leads to macrocyclic mono-and dilactones 73 and 74 (Figure 18) in isooctane at 65 °C. Lower temperatures favor the production of linear oligomers\textsuperscript{80} with the varying ratio of 73/74 depending on the enzyme used.

1.3.4 Enzyme Catalyzed Acylation and Deacylation of Polyhydroxy Compounds

The selective manipulation of individual hydroxy groups in complex sugar moieties is still a challenging problem. Up to now, the selective introduction of acid or base labile blocking groups into the polyfunctional molecules, often in cumbersome multisteps, is the most commonly employed strategy. Recent advances using lipases and activated esters of organic acids as acylating agents provide a new solution for this unsatisfactorily solved problem. Thus, for example, treatment of glucose 75, galactose, or mannose, in dry pyridine with PPL in the presence of 2,2,2-trichloroethyl butyrate or acetate resulted in almost exclusive acyl transfer to the primary hydroxyl group and gave the 6-O-butyryl (acetyl) derivatives 76 (Figure 19) of the corresponding sugars in 50-91% yield.\textsuperscript{81} In the case of fructose, the two primary hydroxyl groups are not well distinguished and a mixture of 1-O-acetyl- (71%) and 6-O-acetylfructose (29%) was obtained. PPL also accepts triolein as an acyl donor. Similar results were reported with

\[ \text{CH}_2\text{OH} \]
\[ \text{OH} \rightarrow \text{CH}_3\text{OC(O)}\text{Pr} \]
\[ \text{lipase} \]

\( \text{Figure 19} \)
medium and long chain fatty acids (C\textsubscript{10} to C\textsubscript{18} acids) and sugars or glucosides using lipases from \textit{Candida antarctica}\textsuperscript{82} (up to 90\% yield) and many other microorganisms. The resulting 6-O-monoesters represent an industrially important class of nonionic surfactants and emulsifiers. Since the embedding of substrates into the active center of many lipases is identical in aqueous and nonaqueous media, it follows, that CCL leaves preferentially the primary function of fully acylated sugars.\textsuperscript{83}

Protected sugars (acetonides) can also be selectively deacylated with CCL or PPL. For example, CCL hydrolyzes a secondary butyrate from diacetone D-glucose or liberates the 5-OH of (Figure 20) \textsuperscript{77}. If the 5-OH is blocked by a pivolate, both enzymes are able to hydrolyze the secondary butyrate\textsuperscript{83} \textsuperscript{78}. The regioselective hydrolysis of peracetylated furanose to 5-OH sugars (40-96\% yield) is possible with CCL and ANL.\textsuperscript{84}). Tetraocta(penta)noates of methyl D-glucosides, galactosides or mannosides react similarly yielding the 6-OH products, irrespective of the \(\alpha\) or \(\beta\)-anomers used. PPL releases the 1-OH of pentaacetates of glucose and mannose with a significant preference for the \(\alpha\)-anomer. Under the same conditions CCL cleaved the 4- and 6-O-acetyl groups. Acylation of methyl furanosides or pyranosides occur at the primary position using trifluoroethyl acetate as the acyl donor. When this position is already blocked (enzymatically acylated or chemically alkylated), CVL selectively acylates the 3-OH of the above substrates, while PPL has an overwhelming preference for the 2-OH.\textsuperscript{81}
Glycals 79 and 80 and, in particular, their esters are interesting chiral intermediates.\textsuperscript{85} CCL readily acylates the primary hydroxy group yielding (Figure 21)

\[ \text{Figure 21} \]

81, but PFL in combination with enol acetates leads to 3,6-di-O-acetyl derivatives\textsuperscript{86} 82. The PFL-catalyzed hydrolysis of the corresponding triacetate makes the 4,6-di-O-acetyl D-glucal available.\textsuperscript{86} Optically active glycerols have been prepared by \( \alpha \)-monobenzylation via lipase-catalyzed transesterification. Similarly diastereo- and enantioselective esterification of butane-2,3-diol has been reported with Amano PS (91\% de, 98\% ee). The full differentiation of all hydroxy groups is possible by using this biocatalytic approach.

1.3.5 Nitrogen Containing Substrates; Formation of Peptide Bonds

The hydrolytic activity of lipases and esterases can be exploited for the preparation of optically active amino acids. Two major approaches, namely the

\[ \text{Figure 22} \]
hydrolysis of esters of N-protected amino acids with lipases/esterases (83 to 84, Figure 22) or the cleavage of amide bond of N-acetyl amino acids by proteases (86 to 87) are the most commonly employed. Conversion efficiencies with ANL or PFL were low to good (14-44%), yielding the corresponding amino acids 84 with acceptable optical purities (85-96% ee). This process is particularly well suited for the synthesis of unnatural amino acids. Some examples of enantioselective hydrolysis of diesters are the synthesis of glutamic acid half-esters (PFL 100% ee), the preparation of optically active oxaziridine (R)-89 (45-87% ee) and N-chloroazirine 90 with various enzymes.89 The (2R) hydroxy ester 91 (93% ee) which is the building block for angiotensin converting enzyme inhibitor CV-5975, has been obtained with 86% conversion from its racemic ethyl ester with lipase PN from Phycomyces nitens.90 Another interesting reaction carried out in organic media, is the lipase-catalyzed alcoholysis of the oxazol-5(4H)-one 92. When 92 is treated with butanol in presence of MML, (S)-94 is obtained (57% ee at 45% conversion) together with the unreacted azlactone 93 (Figure 24).

Since the latter showed no optical rotation, in situ racemization of 93 might have occurred.91 Once optimized, this could be a promising, alternative, high yielding route to
chiral amino acids. If amines act as nucleophiles in the intermediary acyl-enzyme, the synthesis of amides becomes possible in low water media. A simple application of this kind is the enantioselective synthesis of a number of (S)-amides from racemic ethyl 2-chloropropionate catalyzed by CCL. The procedure works well with primary amides, while the secondary amides do not react (38-81% yield, 30-95%).

Unlike the solid phase synthesis of peptides, a scale-up of enzyme-catalyzed reactions is more readily achieved at least for small peptides. Further, the synthetic activity of many lipases in anhydrous media is greater than that of the proteases and amidases. Peptide synthesis can be also carried out with PPL, PLE or CCL in ether or ethyl acetate, containing pyridine or 5-10% aqueous buffer. Lipases entrapped in Amberlite XAD-8 in combination with glycerol esters of N-protected amino acids as acyl donors reduce the long reaction times.

1.3.6 Organometallic Substrates

The number of biotransformations on organometallic substrates is rather limited. An interesting observation has been made, when tributylstanny! ethers were used in transesterification reactions.

\[
\text{R}^1\text{CO}_2\text{Et} + \text{R}_2\text{OSnBu}_3 \rightarrow \text{R}^1\text{CO}_2\text{R}^2 + \text{EtOSnBu}_3
\]

In this case, CV lipase and PPL are not only able to utilize the organometallic ethers, but in addition they proved to be even more active than the free alcohols (e.g. hexanol). The reason for this enhanced reactivity has not been fully elucidated. The first example of successful resolution of a racemic organometallic substrate is provided.
by the kinetically controlled enantoselective hydrolysis of (±)-95 (Figure 25) into the chiral crystalline acid 96 (85% ee at 40% conversion).

Another example of PFL-catalyzed enantioselective esterification is the resolution of the racemic mixture of 97 (Figure 26) to get the air and light sensitive (S)-alcohol 98 in optically pure (100 ee, 47% yield) form using, isoprenyl acetate as the acyl donor.94

$$\text{Cr(CO)}_3 \text{X} \xrightarrow{\text{biocatalyst}} \text{Cr(CO)}_3 \text{X} + \text{Cr(CO)}_3 \text{X}$$

Figure 26

$X = \text{OMe, Me, OSiMe}_3$

1.4 INDUSTRIAL APPLICATIONS OF LIPASES

1.4.1 Food Industry

The bulk of fats and oils produced every year are mostly used in food and renewable chemical feedstock and for non-food related applications. Many lipases exhibit sn-1,3 specificity, and can therefore be used to regioselectively interesterify positions 1 and 3 of a natural glyceride: however, the tendency towards acyl migration from sn-2 to the sn-1 or sn-3 positions must be suppressed. Some companies in Japan are operating on enzyme based processes for the preparation of highly pure unsaturated fatty acids, using lipase from Candida rugosa.95

Transesterified Triglycerides: The melting point of an oil can be modulated by the degree of catalytic hydrogenation of double bonds in unsaturated fatty acids, viz: for the preparation of margarines and shortenings of plant oils. Alternatively, the desired melting behaviour can be achieved through interesterification of suitable
triglyceride mixtures with the use of sn-1,3 specific lipases or a combination of both procedures.\textsuperscript{96}

*Improved Spreadability:* Since the melting point of cocoa butter is around the human body temperature (37 °C), cocoa butter is well suited as a matrix for suppositories. The main application is in the production of chocolates, where the rapid melting conveys a desirable “mouth feel”. Cocoa-butter can be prepared either chemically or by lipase catalysis through interesetrification of suitable natural triglycerides.

*Monoglycerides:* Monoglycerides are mild emulsifiers (HLB value 3.4, HLB= hydrophobic lipophilic balance) permitted for use as food additives. Industrial applications include emulsifications in food, cosmetics, and drug preparations.\textsuperscript{97} Many reports deal with the preparation of monoglycerides through lipase catalysis, but the key problem remains the formation of mixtures of mono- and diglycerides.\textsuperscript{98}

*Dairy Industry:* Rennet paste, isolated from the stomach of ruminating animals such as cows or goats, is an example of an enzyme mixture traditionally used for the preparation of cheese.\textsuperscript{84} The active component of rennet is chymosin, an aspartate protease involved in the clotting of milk through the hydrolysis of $\kappa$-caesin; however, lipases and esterases contained in rennet also contribute to cheese ripening. Depending on the chain-length specificity of a given lipase, its addition to a milk product may enhance the flavor of the cheese, accelerate the cheese ripening, or assist in the preparation of “enzyme modified cheese” (EMC). EMC is produced from cheese curd by the addition of the lipases at elevated temperatures, increasing the content of free fatty acids about tenfold.
1.4.2 Lipases in Detergents

Lipases are known to remove fat stains when used in detergent formulations and also to generate peracid bleach by perhydrolysis. Standard wash components contain anionic and nonionic surfactants, oxidants, and complexing agents at a pH of about 10 and temperatures around 50 °C, which is a rather hostile environment for enzymes. As a result, screening for lipases under these conditions have been carried out. The product 'Lipolase', a recombinant fungal lipase from *Humicola lanuginosa* expressed in a host strain of *Aspergillus oryzae*, was produced on industrial scale. This was probably the first genetically engineered protein to obtain permission by regulatory bodies in Europe to be used by consumer products and be discharged in the environment after use.

Soaps and Fatty Acids: A major application of triglycerides is in the preparation of soaps (from fats and oils), by enzyme-based processes for obtaining highly pure unsaturated fatty acids, using lipase from *Candida rugosa*.95

1.4.3 Lipases in Paper Manufacture

Nihon Seishi Co., a paper manufacturer in Japan, has developed a process by which the triglycerides contained in raw lumber are hydrolyzed by the addition of lipase. This resulted in better pitch control, that is, an easier processing of the lumber to low-grade paper. The process is carried out at a scale of several hundred tons of lumber per day, and other paper manufacturers are now using the similar procedures.

1.4.4 Lipases in Medicine

Substitution Therapy: Exocrine pancreatic insufficiency, often found in cystic fibrosis patients, results in two major problems: malnutrition and steatorrhea. These problems can be partly solved by administration of porcine pancreatic lipase extracts as a replacement therapy. In the past such preparations were far from satisfactory, since a
large proportion of the enzyme administered were denatured in the stomach due to acidity and gastric juice. With the advent of genetic engineering techniques, human gastric lipase (HGL) cDNA have been synthesized to overcome these shortcomings.

Lipase Inhibitors as Antiobesity Agents: Conventional treatment for obesity has focused largely on strategies to control energy intake. Under clinical circumstances, the use of an inhibitor of digestive lipase, which reduces dietary fat adsorption, holds great promise as an antiobesity agent. Tetrahydrolipstatin, derived from lipstatin produced by Strptomyces toxytricini, acts in vitro as a potent inhibitor of pancreatic and gastric lipases as well as cholesterol ester hydrolase. THL inhibits hydrolysis and thus the adsorption of triacylglycerols in the duodenum; it is being developed as an antiobesity agent by Hoffmann - La Roche. It could be demonstrated that an intraduodenal infusion of THL in humans strongly reduces the activity of pancreatic lipase, both by reducing its catalytic activity and pancreatic secretion.

1.5 RECENT TRENDS AND FUTURE IN LIPASE RESEARCH AND APPLICATIONS

1.5.1 Immobilization and Modification of the Biocatalyst

For practical and economic reasons it is often advantageous to use enzymes immobilized on solid supports. Such catalysts are particularly easy to handle and can be easily recovered at any time of the solutions. Furthermore, many of the immobilized enzymes can be repeatedly used without considerable loss of activity. Especially for large-scale preparations, the increased lifetime and enhanced stability of the modified proteins compensate for the lowered conversion rates. Sometimes the enantioselectivity may be enhanced. Approved systems are, for example, PLE on Eupergit C, α-chymotrypsin on silica, Rhizopus japonicus lipase or CCL on anion exchange resins (Duolite A-568 Dowex MWA-1) or graft-polymerized lipoprotein lipase
with N-vinylpyrrolidone or polystyrene. Other useful supports include methacrylic polymers containing hydroxy groups, Celite, PVC, chitosan, chitin, agarose, sepharose, hollow fibres, respectively. CCL was covalently bound to several surface-treated ceramic supports. Some of these systems can be enclosed in continuous flow reactors where they are capable of ester synthesis, hydrolysis or transesterification. A recent technique uses ferromagnetic-modified lipase from *Pseudomonas fragi* which can be recovered from the colloidal solution by application of strong magnetic field. For the use of enzymes in organic solvents, the catalysts can be made soluble by several procedures. Coating of lipase from *P. fragi* with a synthetic dialkyl amphiphile resulted in lipase-lipid complex which is freely soluble in iso-octane. Most commonly employed modification of the enzymes is that with polyethylene glycol, resulting in powders that are soluble in benzene or chlorinated hydrocarbons. This concept has been extended to soluble magnetic lipases. Other useful techniques are microencapsulation, or enclosure of enzymes into dialysis membranes which is recommended as an operationally simple and effective procedure. Other techniques, which overcome problems connected with low solubility of substrates, are the use of surfactants and reversed micelles.

### 1.5.2 Highly Pure Lipases

Lipases hold considerable promise in synthetic organic chemistry and have already found practical applications in detergents, oleochemistry, cheese production, medical therapy, and industrial synthesis of speciality chemicals. By now, lipases from over 30 biological sources have been cloned, sequenced, and expressed in host organisms. Pure recombinant lipases from *Humicola languinosa*, *Pseudomonas pseudoalcaligenes*, *Pseudomonas aeruginosa*, *Mucor miehi*, *Candida antartica* (type B), *Bacillus thermocatenulatus*, and other sources are now commercially available in free or immobilized form or as part of a screening set (e.g. Chirazyme from Boehringer...
Mannheim). Even cross-linked crystals of *Candida rugosa* and *Psedomonas cepacia* lipase (ChiroCLEC) are commercially available from Altus (Cambridge, MA). The tertiary structures of twelve lipases have been resolved and this now allows a more rational approach to modify lipases for specific applications.

### 1.5.3 Protein Engineering of Lipases

With increasing knowledge on lipase structure and function, it has become clear that substrate-binding domains vary greatly from one lipase to another. As detergents are still the commercially most important field of lipase applications, most pertinent patents deal with enhancing lipase stability and activity in a household detergent matrix. The chain-length specificity observed in *Rhizopus delemar* lipase is related to the steric effects involved in the binding of acyl groups. Indeed, site-directed mutagenesis of lipase leading to sterically demanding points of acyl chain led to a significant shift in the preference of the mutant lipase for the hydrolysis of medium-chain triglycerides.

### 1.5.4 Combinatorial Designs

In view of their broad applications and the rapidly advancing knowledge about their structure and function, lipases are interesting candidates for combinatorial approaches to modify their stability and substrate specificity. The esterase activity of *Rhizomucor miehei* lipase could already be enhanced with this technique. Recently, evolutionary screening of proteases led to an enhanced solvent stability and modified substrate specificity. It is safe to predict that before long lipases will become an eminent example of those enzymes which have been modified by rational and combinatorial design for use in industrial processes.
1.6 SUMMARY

As described in this chapter, lipolytic and esterolytic enzymes have found broadest application in almost any area of organic synthesis. Besides finding new applications and new enzymes from different organisms, in the forthcoming years organic chemist has to become familiar with enzyme kinetics and their routine exploitation for the production of highly optically active products. Another promising field of research will be the improvement of the disposable biocatalyst by the enantioselective inhibition or genetic manipulations of the producing organism. However the most important aspects are: (i) the availability of more exact three dimensional protein structures, (ii) the detailed elucidation of the catalytic processes which have to be correlated to functional, three dimensional models useful for providing the chemist with reliable predictions on the stereochemical course of an intended reaction. Furthermore, new approaches via catalytic antibodies (abzymes), together with the methods of site-directed mutagenesis of proteins are a new and promising route towards tailor-made enzymes for specific synthetic problems.

1.7 PRESENT WORK

This chapter presented an overview of biocatalysts in organic synthesis with special reference to lipase-catalyzed reactions and their applications.

Chapter 2 demonstrates (i) an efficient AmanoPS catalyzed resolution of (±)-methyl O-acetylmandelates in high yields (45-50%) and optical purities (90-99% ee) and (ii) an exceptional chemo-, regio-, and enantioselective lipase catalyzed (AmanoPS and PLAP) hydrolysis of (±)-threo-ethyl 3-(4-methoxyphenyl)-2,3-diacetoxy propionate to yield the optically pure diols in good yields (83-85%) and enantiopurity (86-98 %ee).
Chapter 3 demonstrates a first simple method to correlate the actual and observed regioselectivity/enantioselectivity in enzymatic hydrolysis of unsymmetrical/meso diacetates. By suitable manipulation of reaction conditions (enzyme source, pH, solvent, temp etc) it may be possible to obtain either of the isomers by completely preventing or forcing the acyl migration. This method will be also useful in assessing the actual and observed regioselectivities in polyacylated systems including sugars.

Chapter 4 deals with a facile two-step synthesis of Ras Farnesyl-Protein Transferase Inhibitor, chaetomellic acid A with 89% overall yield and preparation of its racemic analogues. This is a new convenient and efficient method to model a variety of other dialkyl-substituted maleic anhydride derivatives. The results on biological screenings of these novel analogues are awaited.
1.8 REFERENCES


