CHAPTER 1: LIPASES IN BIOTRANSFORMATIONS

The biocatalysts for organic synthesis are emerging as a powerful synthetic tool to complement other methodologies of modern synthetic organic chemistry. They have gained utility on account of their mild nature (environmentally friendly) and selectivity (chemo-, regio- and enantioselectivity), but yet applicable for reactions with a large number of non-natural substrates. Among the 6 broad classes of enzymes (oxidoreductases, transferases, hydrolases, lyases, isomerases and ligases), hydrolases have been the favorite class of enzymes as they are robust and also do not require any cofactors or coenzymes. Among the hydrolases themselves proteases and lipases have been extensively studied and commercially exploited in paper, leather, soap, detergents, dairy and food industries.

Lipases are also used in medicine (it is administered to patients suffering from cystic fibrosis) and lipase inhibitors (e.g. Tetrahydrolipstatin) hold great potential as antiobesity drugs. Lipases not only hydrolyze fat in the digestion tract or interesterify triglycerides but are also flexible catalysts for acylation/deacylation of wide range of synthetic substrates, such as aliphatic, alicyclic, bicyclic and aromatic esters. A wide range of lipases/esterases isolated from plants, animal or bacterial sources find use in organic/biochemical laboratories. Their molecular structure solved by X-ray crystallography has revealed a unique tertiary structure that exposes the catalytically active site only in the presence of lipid or in organic solvent phases. The active site covered by the amphiphilic peptidic lid seems to be the reason for this unique mechanism of activation at the water-lipid (organic layer) interface. There is a strong evidence that upon contact with lipid-water interface, the lid undergoes a conformational rearrangement rendering the active site accessible to the substrate.
Lipases are found to have wide tolerance for different substrates and hence useful in a plethora of synthetic reactions. They are sturdy with respect to other enzymes and withstand high temperature (in dry solvents they have been used for esterification at ≈100 °C), active in pH range 5-9, and act in nearly anhydrous organic solvents. Majority of the known applications in organic synthesis are acylation and deacylation under exceptionally mild conditions, synthesis of amides and peptides, regioselective functionalization of polyfunctional groups (polyols, sugars), transesterification of lipids, diastereoselective hydrolyses and esterification reactions, desymmetrization of meso diesters/diols, enantioselective synthesis of esters, half-esters, polyesters, acids, lactones, alcohols, diols, polyols, and amines.

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

CHAPTER 2: LIPASE CATALYZED CHEMO-/ REGIO- AND ENANTIOSELECTIVE HYDROLYSIS OF (±)-METHYL O-ACETYLMANDELATES AND (±)-threo-ETHYL 2,3-DIACETOXY-3-ARYL PROPIONATES.

This chapter deals with the lipase mediated resolution of compounds of commercial interest viz- (±)-methyl mandelates I a and b and (±)-threo-ethyl 2,3-dihydroxy-3-arylpropionates II a and b via their respective acetates. The purpose was to study the selectivity of the enzymes for the acetate/diacetate hydrolysis in the presence of carbmethoxy/ethoxy functions. The utility of enantiopure mandelates is well-proved whereas the enantiopure diols II a and b are of pharmaceutical interest. Both enantiomers of IIa are precursors of the anti-cancer agent taxol side chain while both enantiomers of IIb are the precursors of a clinically used anti-hypertensive drug, (+)-

\[
\begin{align*}
\text{I a/b } & \quad \text{II a/b} \\
\text{a = phenyl b = p-anisyl}
\end{align*}
\]
The enantiopure methylmandelates have been prepared by conventional resolution, asymmetric syntheses, or by microbial/enzymatic conversions. In microbial resolutions one of the isomers in racemate is utilized by the organism to give the other isomer (in high % ee) and the yield is less than 50% as one of the isomer is consumed. Enzymatic transformations on the hydroxy or on ester of the hydroxy function by esterification/transesterification give either low yields accompanied with moderate to high optical purity or high yields accompanied by low enantiopurity. Lipase catalyzed hydrolysis targeted on the acetoxy function of (±)-O-acetoxy methylmandelate by PLAP (pig liver acetone powder) is known to give moderate optical purity and enzymatic reduction of benzoylformic acid derivative employs costly cofactor requirement. Our approach was to selectively hydrolyze the acetate by using a different lipase (AmanoPS) in the presence of carbomethoxy moiety. As shown in scheme-1, (±)-methyl O-acetylmandelates 1 on AmanoPS catalyzed hydrolysis gave S-methylmandelate 2 and R-O-acetoxy methylmandelate 3, followed by deacetylation (of 3) gave S and R isomers of methylmandelates 2a/b and 4a/b in good yields and high optical purities. Selective targeting of the acetate function in the presence of carbomethoxy was then explored to investigate the regiospecific hydrolysis of 1,2-diacetates. We reasoned that by directing the lipase on the diacetoxy derivative of the diol [(±)-threo-ethyl 3-(4-methoxyphenyl)-2,3-dihydroxy-3-arylpropionates] the enzyme might have a better selectivity and hence we attempted enzymatic hydrolysis on (±)-threo-ethyl 2,3-diacetoxy-3-arylpropionates. The hydrolysis of (±)-threo-ethyl 3-(4-methoxyphenyl)-2,3-
diacetoxypropionate 7 with the enzyme powders, Amano PS and PLAP was highly chemo-, regio-, and enantioselective yielding (2S, 3R)-hydroxyacetate 8 in 42% and (2R, 3S)-diacetate 9 in 51% yield which on deacylation gave the optically pure diols (2S, 3R)-diol 10 and (2R, 3S)-diol 11 in 83% (98% ee) and 85% (86% ee) yield respectively.

Scheme 2

\[ \text{Ar} = \text{p-anisyl} \]

i) OsO₄, NMO, t-BuOH/H₂O  
ii) Ac₂O, Py  
iii) Lipase, buffer/solvent  
iv) K₂CO₃/EtOH

The specificity in hydrolysis is remarkable since it is not only chemoselective (acetate hydrolysis rather than terminal ester), but also accompanied by regioselectivity (C-2 acetate is hydrolyzed and not the C-3 acetate) and high enantioselectivity.
(hydrolysis of only 2S acetate). The optical purity of diols 10 and 11 was determined by optical rotation, and by $^1$H NMR with chiral shift reagent and MTPA-derivatives of the diols. Surprisingly conversion rates in enzymatic hydrolysis of (±)-ethyl 2,3-diacetoxy-3-phenylpropionate were very poor (10-15% conversion in 8 days) with both the enzymes and hence were not pursued further. In this chapter the above results will be discussed in detail along with the effect of different solvents and temperature parameters on the cited set of substrates and lipases.

In summary, this chapter 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 efficient chemo-, regio-, and enantioselective lipase catalyzed (AmanoPS and PLAP) hydrolysis of (±)-threo-ethyl 3-(4-methoxyphenyl)-2,3-diacetoxypropionate was performed to yield the optically pure diols in good yields and enantiopurity.

CHAPTER-3: MECHANISTIC STUDIES IN LIPASE CATALYZED HYDROLYSIS OF VICINAL DIACETATES: CORRELATING THE ACTUAL AND OBSERVED REGIOSELECTIVITY / ENANTIOSELECTIVITY.

A large number of meso and unsymmetrical vicinal diacetates with a wide range of structural diversities have been enzymatically hydrolyzed to obtain chiral molecules, but one of the problems still unaddressed is related to its regioselective action. As shown in the previous chapter, the chemo-, regio- and enantioselective lipase mediated hydrolysis observed in (±)-diacetate 1 using Amano PS and PLAP (to obtain chiral precursors of clinically used (+)-diltiazem in good yields and optical purities) gave (2S, 3R)-hydroxyacetate- 2 as one of the products. This could arise either from the direct hydrolysis of (±)-1 by the enzyme or the hydroxyacetate 2 can form via 5, through in-situ intramolecular migration. Literature survey revealed lack of any direct method to correlate the actual and observed regioselectivity in such enzymatic hydrolysis of
vicinal diacetates. This chapter reports for the first time a strategy to correlate the observed and actual regioselectivity in enzymatic hydrolysis.

The approach consists of an enzymatic hydrolysis of unsymmetrical diacetate, followed by labeling of the hydroxy function with CD$_3$COOD/DCC to give monolabeled diacetate and enzymatic rehydrolysis under the identical set of conditions. The amount of label lost will directly indicate the extent of regioselective action of the enzyme.

This is depicted in scheme 1 the (2S, 3R)-hydroxyacetate-2 was acylated using CD$_3$COOD/ DCC to obtain monolabeled diacetate-4, which was rehydrolysed enzymatically under the identical conditions. The reaction furnished 2 (75%, ~100% ee) thus proving that the actual and observed regioselectivity is same (6 not being formed via in-situ acyl migration). To demonstrate the positive validity of this method, the glyceroldiacetate (±)-7 (Scheme 2) was chosen for hydrolysis with Amano PS and PLAP at pH 7.0 and 8.0 respectively. The reaction with Amano PS and PLAP furnished products 8 and 9 in 9:1 proportion (55% yield) and 1:9 proportion (25% yield) respectively. On labelling the compounds with CD$_3$COOD to give 10+11 (9:1), and on rehydrolysis with Amano PS under identical set of conditions gave products 8+9+12 in
Scheme 2 #

\[ \begin{align*}
\text{80:10:10 proportion. Similarly, the labelled diacetate } & 11 \text{ on hydrolysis with PLAP at pH 8.0 gave products } 9+12+13 \text{ in 47:8:45 proportion and } 10+11 \text{ (9:1) gave 8+9+13 in 5:66:29 proportion. These results indicate that Amano PS recognizes the primary acetate in nearly 100% regioselective manner, with 9 (10%) formed by intramolecular acyl migration and an } \textit{in-situ} \text{ intramolecular acyl migration is observed in Amano PS and PLAP induced hydrolysis of } 10+11 \text{ and } 11. \text{Preliminary studies on cyclic meso diacetates have also been carried out, in one of the cases the actual and observed enantioselectivity is proved to be same. In yet another case the results indicate the actual and observed enantioselectivity may be different and further work is in progress.}
\end{align*} \]

In summary, this chapter demonstrates a first simple method to correlate the actual and observed regioselectivity in enzymatic hydrolysis of unsymmetrical
diacetates. By suitable manipulation of reaction conditions (enzyme source, pH, solvent, temp etc) it may be possible to obtain either of the regioisomers by completely preventing or forcing the acyl migration. This method will be also useful in assessing the actual and observed regioselectivities in polyacylated systems like sugars and stereoselectivities in meso-diacetates.

CHAPTER-4: FACILE SYNTHESIS OF CHAETOMELLIC ACID A: RAS FARNESYL-PROTEIN TRANSFERASE INHIBITOR AND ITS ANALOGUES.

The present chapter deals with the facile synthesis of a potent anti-cancer agent: Chaetomellic acid A, and its derivatives with an attempt towards lipase mediated resolution. Chaetomellic acid A anhydride (1) was isolated in 1993 from Chaetomella acutiseta by a Merck group and shown that its dianionic form 2 is a highly specific inhibitor of ras farnesyl-protein transferase.

Till now eight syntheses of 1 have been reported, ranging from 18 to 83% overall yields by various approaches including the one that is reported in this chapter. In the present work the synthesis of Chaetomellic Acid A (1) was completed by Wittig reaction of the ylide adduct 6 as shown in scheme with the tetradecyl aldehyde. The ylides 5 and 6 were prepared so as to attempt for the first time their ability to condense with aliphatic aldehydes (in literature studies with 5 for condensation with benzaldehyde and other aliphatic aldehydes had met with failure) and design a convenient method to obtain the diverse menu of dialkyl substituted maleic anhydrides. The ylide 6 (relatively less stable) smoothly condensed with tetradecanal in glacial acetic acid under reflux conditions to yield the geometrical isomers 7 and 8 in 70% yield. Since the isolated yield of 6 was only 50%, one-pot reaction of the imide 4, TPP and tetradeacanal in acetic acid was attempted to successfully yield 9 via 6 and 7+8 in 91% yield offering both, condensation and isomerization of the double bond (exo to endo) and subsequently giving 1.
Racemic analogues of 1 were prepared to study the structure-activity relationship by attempts of their resolution by lipases. Consequently chaetomellic acid was chemoselectively brominated at allylic position with NBS/CCl₄ to obtain bromo derivative 10 generating a new chiral centre. This was then subjected to allylic substitution under alkaline conditions to give the hydroxy derivative 11. The (±)-hydroxy-chaetomellic acid-11 was acetylated with NaOAc/Ac₂O offered acetoxy-12 derivative which was subjected for enzymatic resolution with lipases, AmanoPS and PLAP so as to furnish the enantiopure alcohols of 11, but all our attempts met with failure since the substrate is not acted upon by the enzymes.
In summary this chapter demonstrates, for the first time that the citraconimide-TPP adduct condenses with (aliphatic aldehyde) tetradecyl aldehyde providing a facile two-step synthesis of 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. Studies on the biological activity testing of these novel analogues is under progress.