CHAPTER 3

MECHANISTIC STUDIES IN LIPASE CATALYZED HYDROLYSIS OF VICINAL DIACETATES:
CORRELATING THE ACTUAL AND OBSERVED REGIOSELECTIVITY / ENANTIOSELECTIVITY
3.1 INTRODUCTION

A large number of meso and unsymmetrical vicinal diacetates with a wide range of structural diversities have been enzymatically hydrolyzed to obtain chiral molecules.\(^1\) The enzymatic conversions of these diacetates have been carried out at acidic, neutral or basic pH depending upon the enzyme used. Since intramolecular acyl migrations under various reaction conditions are well documented,\(^2,3\) a major unresolved problem is the unambiguous determination of the actual and observed regioselectivities in enzymatic hydrolyses. For example, the literature reports on enzymatic hydrolysis of di- and triesters of glycerol reveal varying observations listed below.

(A) The enzyme directly recognizes the primary acetate.\(^4\) Different esters of glycerol e.g. 2-oleoyl dipalmitin, 2-oleoyl distearin, 2-palmitoyl diolein and 1-oleoyl dipalmitin, were studied for the \textit{in vitro} specificity of pancreatic lipase mediated hydrolysis. The course of the reaction, which was seen to be triglyceride to 1,2-diglyceride, to 2-monoglyceride, does not depend on the type of fatty acid, the degree of unsaturation or the chain length. The specificity is towards the hydrolysis or transesterification at the primary esters or alcoholic function.

(B) The enzyme directly recognizes the secondary acetate.\(^5\) Studies on the hydrolysis of secondary esters by pancreatic lipases were carried out on glycerol derivatives, \textit{viz} 1,3-\text{O}-benzylidene-2-oleate glycerol and 1,3-dialkylether-2-oleate. These upon treatment with pancreatic lipase showed hydrolysis of secondary ester and in the absence of non-specific lipases, the hydrolysis was relatively slow. In a third substrate, a trioleate of glycerol, wherein only the secondary oleate was labeled, some amount of the label was found to be hydrolyzed and the probability of label migration on the primary alcohol followed by hydrolysis was not discussed.
In another study, the objective was to test various aspects of specificities of lipolyses, with different derivatives of triglycerides such as 2-oleodipalmitin, 1-oleo dipalmitin and 1-palmitodiolein. The results indicated that

i. both primary esters are hydrolyzed more rapidly than the secondary ester, with the order of products formed

\[
\text{Triglyceride} \rightarrow 1,2\text{-diglyceride} \rightarrow 2\text{-monoglyceride}
\]

ii. formation of some (10-30%) of 1-monoglyceride with its original acid chain indicating that no isomerization (secondary ester to primary) occurs and the 1-monoglyceride is a result of direct hydrolysis of secondary ester.

(C) The enzyme first recognizes primary acetate followed by in-situ intramolecular acyl migration of the secondary ester to yield the secondary alcohol derivative.\(^4\) When 2-oleyl dipalmitin was used for the hydrolysis, the diglyceride formed was 2-oleoyl palmitin and further hydrolysis gave 1-monoolein. Thus, the course of hydrolysis of triglyceride is a series of directed stepwise reactions from triglyceride to 1,2-diglyceride to 2-monoglyceride, which could be followed by isomersation of 2-monoglyceride to 1-monoglyceride. The key results obtained in another study of triglyceride hydrolysis are summarized here:

i) The hydrolysis of esters of oleic acid by pancreatic lipase depends on the nature of the alcohol. The two factors that influence the rate of the reaction are (a) inductive effect and (b) steric hindrance.

ii) Lipolysis promoted by electrophilic substituent as might be expected from a reaction involving nucleophilic attack on the carbonyl e.g. slow hydrolysis of oleoyl oleate as compared to 2-fluoroethyl oleate or p-nitrobenzyl oleate. The operation of such an inductive effect explains the following sequence in rate of hydrolysis triglyceride> 1,2-diglyceride> 1,3-diglyceride> 1-monoglyceride> 2-monoglycride.
iii) The bulkiness of the carbinol group perhaps inhibits lipolysis, as vinyl oleate is hydrolyzed but isopropyl or phenyl oleate does not. This steric effect explains lipase specificity for α-chains of triglycerides. Electron-withdrawing substituents can counteract this hindrance since 1,3-difluoroisopropyl oleate and p-nitrophenyl oleate are slowly hydrolysed.

(D) Formation of a multiple point attachment of the substrate with enzyme.6 There could exist a possibility wherein the enzyme cavity may have more than one binding sites for the acetates, with some amino acid residues of the enzyme helping in simultaneous hydrolysis and shuffling of the hydrolyzed/unhydrolyzed acetyl moiety to aid the acyl migration.

E) The recognition of the primary acetate in antibody mediated hydrolysis7 of p-nitro substituted derivative of 10. An antibody-mediated hydrolysis of 2,3-diacetoxy-1-(p-nitrobenyloxy)glycerol in DMSO/aq. buffer at pH 8.0 was carried out. The hydrolysis was completely regioselective giving primary hydrolyzed product and also stereoselective (80% ee) at 36% conversion.

(F) Formation of 1:4 ratio of primary:secondary hydrolysed products.8

In lipase catalyzed hydrolysis9 of aliphatic diacetates having one primary acetate and other secondary acetate, only the secondary acetate was found to be hydrolyzed. This was proved to arise from the direct hydrolysis of secondary acetate and not as a result of post hydrolytic acetyl migration through a mixed ester intermediate. Another report10 has shown that the lipase catalyzed hydrolysis of threo-2,3-diacetoxybutanoic ester leads to both regio isomers of monoacetate, as a result of post hydrolytic acetyl migration on silica gel column.
3.2 DEFINITION OF THE PROBLEM

From the above observations it is obvious that there is no basis for clearly
delineating the specificities in lipase hydrolysis of vicinal diacetate substrates (for
convenience named as 1st-acyl and 2nd-acyl, see Figure 1). If a lipase is shown to be
regiospecific towards the hydrolysis of 1st-acyl, is this specificity a result of direct
hydrolysis of the 1st-acyl (ACTUAL SELECTIVITY) or the lipase hydrolyses 2nd-acyl
followed by the in-situ migration of 1st-acyl to give the apparent 1st-acyl hydrolysed
product (OBSERVED SELECTIVITY). A literature search revealed lack of any or
suitable direct and unambiguous methods to correlate the actual and observed
specificity in such enzymatic hydrolysis of vicinal diacetates. In order to delineate the
mechanisms of hydrolysis and migrations in enzymatic reactions, a simple
unambiguous protocol using labeling technique is presented here to validate the actual
and observed selectivities, along with a measure of enzyme selectivity efficiency.

3.2.1 Strategy

Our present strategy consists of an enzymatic hydrolysis of unsymmetrical
diacetate, followed by labeling with CD3COOD/DCC to give exclusive monolabeled
diacetate. Enzymatic rehydrolysis of the monolabeled diacetate under the identical set of reaction conditions aids in estimating the amount of label retention by $^1$H NMR spectroscopy. The complete loss of label would then indicate the actual and observed regioselectivity to be the same. The retention of the label would be a consequence of \textit{in-situ} intramolecular acyl migration and in such a condition, the actual and observed regioselectivity would be different. The amount of label lost will directly indicate the proportional extent of regioselective action of the enzyme.

3.3 RESULTS ON ACTUAL AND OBSERVED REGIOSELECTIVITY

3.3.1 Actual and Observed Regioselectivity in AmanoPS Reactions

As reported in Chapter 2, a remarkable chemo-, regio-, and enantioselective hydrolysis of the (±)-diacetate 1 was observed$^{11}$ using AmanoPS and pig liver acetone

\begin{center}
Scheme 1
\end{center}

\begin{align*}
(±)-1 & \xrightarrow{i \text{ or } ii} (2S, 3R)-2 + (2R, 3S)-3 \\
4 & \xrightarrow{i \text{ or } ii} (2S, 3R)-2
\end{align*}

$\text{Ar = p-Anisyl}$

\begin{align*}
5 & \text{ (5 and 6 not formed)} \\
6
\end{align*}

\begin{itemize}
  \item i) AmanoPS, $C_6H_5$:Petroleum ether (2:1), 50 mM sodium phosphate buffer pH 7.0 25° C; ii) PLAP, $C_6H_5$:Petroleum ether (2:1), 50 mM sodium phosphate buffer pH 8.0 37° C
\end{itemize}
powder (PLAP) to obtain chiral precursors of clinically used (+)-diltiazem in very good yields and optical purities. As depicted in Scheme 1, the (±)-diacetate 1 was enzymatically hydrolyzed using AmanoPS at its optimum pH (7.0), to obtain a mixture of (2S, 3R)-hydroxyacetate 2 and the unreacted (2R, 3S)-diacetate 3. The structural analysis of hydroxyacetate 2 using 1H NMR data revealed that the enzymes AmanoPS and PLAP are nearly 100% chemoselective as the carboethoxy function was not hydrolyzed and about 98-99% enantioselective in their action with substrate (±)-diacetate 1 to give (2S, 3R)-2. This was further supported by optical rotation and confirmed by spectral analysis of the complex with chiral shift reagent and Mosher's ester of 2. The enzymes were also seen to be ~100% regioselective in their action since only the C2-O-acetate of (±)-1 is hydrolyzed, keeping the C3-O-acetate intact. The column chromatographically purified (2S, 3R)-hydroxyacetate 2 (98% ee) was reacylated using CD3COOD and DCC as a coupling reagent to obtain 2S-deuterolabeled (2S, 3R) diacetate-4. The labeled diacetate 4 was resubjected to enzymatic hydrolysis using AmanoPS under the previous set of reaction conditions. The exclusive product formed in this experiment as identified by 1H NMR and the corresponding MTPA derivative was 2 in 75% chemical yield and nearly 100% ee. The rate of enzymatic hydrolysis for this optically pure enantiomer (2S, 3/?)-4 was relatively faster (24 h) than for the racemic (±)-1 (156-180 h).

In a second example to demonstrate the positive validity of this method, the (±)-glyceroldiacetate 10,12,13a was chosen and was subjected to biphasic enzymatic hydrolysis using AmanoPS at pH 7.0 (Scheme 2). This reaction furnished a product mixture as revealed by 1H NMR, (C1-hydroxy,C2-acetoxy) 11 and (C1-acetoxy,C2-hydroxy) 1213 in 9:1 ratio with a chemical yield of 55% in 2 h. In the control experiments without the enzyme AmanoPS at pH 7.0, the (±)-diacetate 10 remained unreacted, indicating the absence of any accompanying chemical hydrolysis.
The chemical shifts in $^1$H NMR of all the three compounds are listed below and show different shifts. The characteristic shift in each is of C2 proton that differentiates each one of them, particularly the two hydroxy-acetates. The spectral details are given later in the experimental section along with the respective spectra. The mixture of hydroxyacetates (C1-hydroxy,C2-acetoxy) 11 and (C1-acetoxy,C2-hydroxy) 12

\[
\begin{align*}
4.14-4.25 \text{ and } 4.30-4.40 & \quad 2.05 \\
5.15-5.30 & \quad 2.10 \\
3.62 &
\end{align*}
\]

\[
\begin{align*}
3.82 & \quad 2.02 \\
5.05 & \quad 3.97-4.10 \\
3.67 & \quad 3.45-3.62
\end{align*}
\]

thus obtained was mono-labeled with CD$_3$COOD/DCC to yield a mixture of primary labelled diacetate-13 and secondary labeled diacetate-14 in 9:1 product ratio (page 135). This mixture of mono-labeled diacetates in AmanoPS catalyzed hydrolysis yielded a product mixture of 11, 12 and 15 in 80:10:10 proportion (page 137). The $^1$H NMR spectral data of the crude reaction mixture and the column purified products revealed that there was no intramolecular acyl migration during silica gel column chromatography.
3.3.2 Actual and Observed Regioselectivity in PLAP Reactions

To examine whether these observations are general for other lipase hydrolysis, the enzymatic hydrolysis of (±)-1 and (±)-10 were studied with PLAP at its optimum pH (8.0). The optically pure mono-labeled diacetate 4 obtained via the PLAP catalyzed hydrolysis of (±)-1 (Scheme 1), after rehydrolysis with PLAP also revealed a complete loss of label as previously observed in case of AmanoPS catalyzed hydrolysis.

In a second set of experiments, the (±)-diacetate 10 was subjected to biphasic enzymatic hydrolysis using PLAP\textsuperscript{14} at pH 8.0 and the reaction upon 25% conversion (2 h)\textsuperscript{15} gave a mixture of (C 1-hydroxy,C 2-acetoxy) 11 and (C1-acetoxy,C2-hydroxy) 12, but in 1:9 proportion (reversal of AmanoPS expt) (Scheme 3). In control experiments without the enzyme PLAP at pH 8.0, the (±)-diacetate 10 remained unreacted. The column chromatographically purified compound 12 on coupling with CD\textsubscript{3}COOD/DCC

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i) AmanoPS, C\textsubscript{6}H\textsubscript{6};Petroleum ether (2:1), 50 mM sodium phosphate buffer pH 7.0 25°C
gave the secondary labeled diacetate 14 (page 139), which was resubjected to enzymatic hydrolysis using PLAP at pH 8.0. The reaction upon 25% completion (2 h) yielded a mixture of 12, 15 and 16 in 47:8:45 ratio (page 141). The $^1$H NMR spectral data of the crude reaction mixture and column purified products revealed that there was no intramolecular acyl migration during silica gel column chromatography.

**Scheme 3**

\[
\begin{align*}
10 & \xrightarrow{i} \begin{cases} 11 \quad (11:12 = 1:9) \\
12 \\
13 \quad (12:15:16 = 47:8:45) \\
14 \quad (13:14 = 9:1)
\end{cases} \\
15 \\
16
\end{align*}
\]

i) PLAP, $C_6H_6$:Petroleum ether (2:1), 50 mM sodium phosphate buffer pH 8.0 37° C
Similarly, the primary and secondary labeled mixture of diacetates $13+14$ (9:1) on PLAP catalyzed hydrolysis at pH 8.0 furnished a mixture of 11, 12 and 16 in 5:66:29 proportion.

3.4 DISCUSSION ON ACTUAL AND OBSERVED REGIOSELECTIVITY

3.4.1 Actual and Observed Regioselectivity in AmanoPS Reactions

It was thought appropriate to examine the nearly 100% regioselective action of AmanoPS on (±)-1 to give the hydroxyacetate 2 (Scheme 1), wherein, of the two acetates only C2 O-acetyl is hydrolyzed. In principle, the hydroxyacetate 2 can arise either from the direct hydrolysis of C2 O-acetate or via the hydroxyacetate-5, resulting from the hydrolysis of C3 O-acetate followed by in-situ intramolecular acyl migration (see Scheme 1). It is necessary to establish the real basis for the observed high regiospecificity. During the enzymatic hydrolysis of 2S-labeled diacetate-4, the exclusive product formed was 2 in high yield and optical purity as seen by PMR, optical rotation and mass spectral analysis. The rate of enzymatic hydrolysis for the optically pure enantiomer (2S, 3R)-4 was relatively faster than the diacetate (±)-1. It is possible that the opposite isomer might be acting as a competitive inhibitor. The fact that the labeled compound 6 was not obtained proves that the compound 2 is formed from diacetate 4 by direct hydrolysis. This implies that 1 → 2 conversion is also by direct hydrolysis and not via acyl migration in the initially formed intermediate 5 (Scheme 1). Thus the true and observed regioselectivity of the hydrolysis with AmanoPS at pH 7.0 with the (±)-diacetate 1 is same and the enzyme is nearly 100% regioselective in its activity. In control experiments without enzyme AmanoPS at pH 7.0, the (±)-diacetate 1 and diacetate 4 (Scheme 1) remained unreacted, proving the absence of any accompanying chemical hydrolysis.
During the AmanoPS catalyzed hydrolysis of (±)-diacetate 10, the products formed were (C1-hydroxy,C2-acetoxy) 11 (90%) and the (C1-acetoxy,C2-hydroxy) 12 (10%). In control experiments without the enzyme AmanoPS at pH 7.0, the (±)-diacetate 10 (Scheme 2) also remained unreacted, proving the absence of any accompanying chemical hydrolysis. The formation of 10% of (C1-acetoxy,C2-hydroxy) 12 can be explained due to two possibilities – (i) the enzyme is non-selective and hydrolyses both the 1° and 2° acetates such that along with the (C1-hydroxy,C2-acetoxy) 11, (C1-acetoxy,C2-hydroxy) 12 (10%) is also formed and (ii) the enzyme regioselectively hydrolyses 1° acetate to give exclusively (C1-hydroxy,C2-acetoxy) 11 followed by partial in-situ intramolecular acyl migration to give the (C1-acetoxy,C2-hydroxy) 12 in 10%. If the (C1-acetoxy,C2-hydroxy) 12 (10%) is formed from (C1-hydroxy,C2-acetoxy) 11 (90%) via in-situ intramolecular acyl migration, then one can follow the labeling technique wherein the retention of label would prove the presence of acyl migration. If we assume 12 in 10% arises from intramolecular acyl migration via 11, then it should be possible to deduce the ratio of products arising from the labeled diacetate mixture 13+14 (90:10). The calculated ratios of the so formed products would then be 11+12 (90:10) arising from primary labeled diacetate-13 and 15+16 (90:10) arising from 14. Further, calculating the overall ratios from combined mixture of labeled diacetates 13+14 would then give 11+12+15+16 in 81:9:9:1 and the observed results are 80:10:10 (Scheme 2) (the product 16 was not observed in the limits of NMR resolution). The observed results convincingly indicate that in the enzymatic hydrolysis of both 10 and 13+14, the enzyme AmanoPS recognizes the primary acetate in the substrate in nearly 100% regioselective (sn-1 specific) manner and 12 (10%) is formed by intramolecular acyl migration.
3.4.2 Actual and Observed Regioselectivity in PLAP Reactions

Similarly in PLAP catalyzed hydrolysis of the optically pure mono-labeled diacetate 4 obtained via the hydroxyacetate 2 (which in turn was obtained via PLAP catalyzed hydrolysis of (±)-1, rehydrolysis with PLAP at pH 8.0) also revealed a complete loss of label (Scheme 1), confirming that the actual and observed selectivity are same. In control experiments without the enzyme PLAP at pH 8.0, the diacetate (±)-1 and 4 remained unreacted, proving the absence of any accompanying chemical hydrolysis. The $^1$H NMR spectral data of the crude reaction products and the column purified products revealed that there was no intramolecular acyl migration during silica gel column chromatography.

The PLAP catalyzed hydrolysis of (±) glycerol diacetate 10 at pH 8.0 gave (C1-hydroxy,C2-acetoxy) 11 (10%) and (C1-acetoxy,C2-hydroxy) 12 (90%) (Scheme 3). This is interesting since the ratio of the products is exactly the opposite of that obtained in the AmanoPS hydrolysis of (±)-glycerol diacetate 10. In this case also, it is possible that either PLAP hydrolyses both 1° and 2° acetates with different rates or the enzyme might hydrolyze 1° acetate exclusively followed by in-situ intramolecular acyl migration of 2° acetate to the 1° hydroxy to give 90% of (C1-acetoxy,C2-hydroxy) 12. Further, upon labeling the (C1-acetoxy,C2-hydroxy) 12 with CD$_3$COOD gave the monolabeled diacetate-14 which on rehydrolysis under same conditions gave the mixture 12+15+16 in 47:8:45 ratio. As observed above almost equal amounts of compounds with secondary hydroxyls 12 and 16 were formed and in 16 the label was retained as a result of acyl migration from the 2° position of 14. It is interesting to ponder on the surprising formation of unlabeled 12. As yet sn-2 specific lipases are unknown in the literature and hence it is unlikely that this arises from a direct hydrolysis of secondary acetate in 14. Alternatively, it could arise as a result of retaining back of the primary
unlabeled acyl group from a transitory intermediate set up during the migration of the secondary acyl group. Similarly, the mixture 11+12 (obtained from AmanoPS hydrolysis of (±) glycerol diacetate 10) upon labeling gave the monolabeled diacetates 13+14 (9:1), which on rehydrolysis under similar set of conditions (PLAP, pH 8.0) gave a mixture of 11+12+16 in 5:66:29 ratio. These results clearly suggest an intramolecular acyl migration in PLAP induced hydrolysis of both 14 and 13+14. In the absence of PLAP, the mixture of hydroxy acetates 11+12 (9:1) at pH 8.0 showed very slow intramolecular acyl migration to yield 12 (24 h, 60%). Hence in PLAP catalyzed hydrolysis of (±)-10, the observed intramolecular acyl migration seems to be a synergic effect of enzyme and pH. The observed difference in the amount of label retained in mixtures 12+15+16 and 11+12+16 in the above mentioned two experiments is interesting. It is possible that during the formation and cleavage of an unsymmetrical intermediate of substrate with the enzyme PLAP, the amount of label retained may depend on its position (1° or 2°) in the substrate. In PLAP catalyzed hydrolysis of (±)-10 at pH 8.0, the observed regioselectivity is very high and the actual selectivity differs markedly depending upon the intramolecular acyl migration or multiple point attachment to the enzyme and hydrolysis. Recent results also suggest that sn2-regioselectivity stems from acyl migration and an actual sn2-specific lipase may not exist.

3.5 APPLICATION TO meso DIACETATES

3.5.1 Background

The meso diacetates also are potential candidates for enzymatic desymmetrization, to provide optically pure hydroxyacetates and a similar problem is faced for determining the actual and observed enantioselectivities in enzymatic hydrolysis. For e.g. the enantioselective hydrolysis of a meso diacetate, cis 1,4-
diacetoxy-2-cyclopent-2-ene (17) is reported with Lipozyme lipase. In another example, lipase mediated hydrolysis of 1,2-diacetoxy-2-cyclopentane (21) revealed varying observations viz: (a) formation of enantiopure (1R,2S)-1-acetoxy,2-hydroxy-2-cyclopentane and (b) formation of optically inactive (racemic) 1-acetoxy-2-hydroxy-2-cyclopentane. We opted to extend our labeling technique to correlate the actual and observed enantioselectivities in lipase mediated hydrolysis of above mentioned both the meso diacetates.

### 3.5.2 Results on Actual and Observed Enantioselectivity in meso Diacetates

1,4-Diacetoxy-2-cyclopent-2-ene (17) when hydrolyzed with Lipozyme at pH 7.0 in a biphasic system gave the (1R, 4S)-hydroxyacetate 18 with >92% ee (Scheme 4a).

**Scheme 4a**

![Scheme 4a diagram](image)

**Scheme 4b**

![Scheme 4b diagram](image)
These results are consistent with that observed in literature. Among the enzymes screened were Lipozyme, PLAP and AmanoPS, of which Lipozyme showed good results. The labeling technique was extended to find the actual versus observed enantioselectivity of the enzyme on the diacetate 17. It was observed that on labeling the 4S-hydroxy function by CD$_3$COOD/DCC coupling led to the exclusive monolabeled diacetate 19, which underwent Lipozyme mediated biphasic hydrolysis to give back the (1R, 4S)-hydroxycetate 18.

The diacetate 21 was subjected to Amano PS catalyzed biphasic hydrolysis at pH 7.0. At 60% conversion, a product mixture of hydroxyacetate 22 plus 23 and ureacted diacetate 21 in 12 h (Scheme 4b) was obtained. The product analysis by $^1$H NMR indicated the product to be hydroxy acetate, but product (mixture) was optically inactive. This was then subjected to oxidation by oxalyl chloride and DMSO in dry CH$_2$Cl$_2$ to give (±)-2-acetoxy cyclopentanone (24+25) in 80% yield. The (±)-ketoacetate 24+25 on biphasic Amano PS catalyzed hydrolysis under similar set of conditions was stopped at 45% conversion (by PMR, page 150), to obtain the starting material ketoacetate 27 (55%) and 2-hydroxycyclopentanone (26) (45%). Further column chromatography gave pure 2-acetoxy cyclopentanone (27) and was found to be optically active. The measured optical rotation was $+33.4^\circ$ (c 1, CHCl$_3$) and by comparison to the literature reports, this isomer was assigned S-configuration. The 2-hydroxycyclopentanone (26) could not be isolated and further experiments are needed. The present method could be a potential technique to obtain both the enantiomers of 2-hydroxycyclopentanones (26) and 2-acetoxy cyclopentanones (27) in high optical purities and these are useful starting materials for syntheses of many naturally occurring bioactive products.
3.5.3 Discussion on Actual and Observed Enantioselectivity in meso Diacetates

It was reasoned that either the observed enantiomer of hydroxy acetate (1R, 4S)-18 is a result of direct hydrolysis of 'pro-4S-acetate' of the meso diacetate 17 or the enzyme recognizes the 'pro-1R-acetate' followed by complete or nearly complete in-situ acyl migration. The fact that during the enzymatic rehydrolysis of monolabeled diacetate-19, the label was totally lost points to direct recognition of the pro-4S-acetoxy function by the enzyme. Hence the observed and actual enantioselectivities of Lipozyme are same with this meso diacetate-17.

Upon AmanoPS biphasic hydrolysis of diacetate-21, the hydroxy-acetates formed 22 plus 23 (identified by 1H NMR) were found to be optically inactive. The racemic hydroxyacetate 22 plus 23 could be a result of non-selective random hydrolysis of both acetates. Alternatively the enzyme if believed to be enantioselctive in its hydrolysis, the product may form by instantaneous in-situ acyl migration in a vicinal cis-cyclic system. The acyl migration could be kinetically favored such that both the enantiomers so formed would be in equal proportions giving the racemic hydroxyacetate 22 plus 23. In this case, our labeling technique would prove to be ineffective, since the label would be on both the enantiomers of the resultant diacetate and hydrolysis would result in only shuffling of the label. The labeled hydroxyacetate would then be in 1:1 ratio, not enabling us to draw any definite inference. Hence it was planned to convert the hydroxy to keto by Swern oxidation to obtain (±)-2-acetoxycyclopentanone (24+25). If the enzyme were to be non-selective then one would get optically inactive 2-hydroxy cyclopentanone. If the enzyme is selective towards hydrolysis of one of the enantiomer and if the reactions are stopped at < 50% conversions, products would consist of optically pure 2-hydroxycyclopentanone (26) and the unreacted enantiopure 2-acetoxycyclo pentanone (27). The (±)-2-acetoxycyclopentanone (24+25) upon
AmanoPS hydrolysis offered the starting material keto-acetate 27 (55%) and 2-hydroxy cyclopentanone 26 (45%) as analyzed by $^1$H NMR. Column chromatographic purification gave pure 2-acetoxycyclopentanone (27) in 49% overall yield and was found to be optically active. The rotation was measured to be $+33.4^\circ$ (c 1, CHCl$_3$) which by comparison with the literature$^{19}$ was assigned S-configuration. This clearly indicates that the AmanoPS is enantioselective towards the ketoacetate-24+25, which is derived from the meso diacetate 21. It is tempting to extend the enantiospecificity of the AmanoPS from the substrate keto-acetate 24+25 to the meso diacetate 21, and the enzyme perhaps enantioselectively hydrolyzes one of the acetates in meso diacetate 21 followed by rapid intramolecular acyl migration to give the optically inactive hydroxyacetates 22+23. Although the substrate structure of (±)-2-acetoxycyclopentanone (24+25) has been changed with respect to the parent diacetate 21, the results may lead to some idea about the enantioselectivities of the enzyme towards the meso diacetates.

3.6 SUMMARY

In summary, with two unsymmetrical diacetates as examples, we have demonstrated a first simple method to correlate the actual and observed regioselectivity in enzymatic hydrolysis of unsymmetrical diacetates and to measure the enzyme selectivity efficiency.$^{20}$ The simple method consists of enzymatic hydrolysis of unsymmetrical diacetate followed by labeling of the formed hydroxyacetate with CD$_3$COOD/DCC, and enzymatic rehydrolysis of labeled compound under the identical reaction conditions to estimate the amount of label retained by $^1$H NMR spectroscopy. The isotopic effect on rate and selectivity of enzymatic hydrolysis has not been taken into account in this method. The labeled compounds were characterized by using $^1$H NMR and mass spectral data, while the quantitative estimation of the label was performed using $^1$H NMR data (relative integrations of methyl group from -OAc). The
amount of label lost directly indicates the extent of regioselective action of the enzyme. By suitable manipulations in reaction conditions (enzyme type, solvent system, pH range and temperature) it may be possible to get both the isomers with high regioselectivity by complete prevention or forcing the acyl migration. The same strategy has been also extended to meso diacetate 17 to show the actual and observed enantioselectivities by exclusively labeling the pro-R or pro-S acetate. In yet another strategy on meso diacetates, it is plausible to correlate the actual and observed enantioselectivities and more work in this direction is necessary. The strategy used is, enzymatic hydrolysis of a meso diacetate to give the racemic hydroxyacetate, followed by converting the hydroxy function to its keto derivative (in this case) and carrying out the enzymatic rehydrolysis under same conditions. The formation of optically pure hydroxyketone/ unreacted keto-acetate leads to the inference that the observed and actual enantioselectivities are different due to the acyl migration. The labeling strategy may also be useful for assessing the actual and observed regioselectivities in enzymatic hydrolysis of polyacylated systems including sugars.

3.7 EXPERIMENTAL SECTION

DCC and CD$_3$COOD (99.5%) were obtained from Aldrich Chemical Co. The substrate diacetates (±)-1$^1$, (±)-10$^{12,13a}$ and (2S, 3R)-hydroxyacetate 2$^{11}$ were prepared as reported before. The biphasic enzymatic hydrolysis of (±)-1 and (-)-4 using AmanoPS (800 U) and PLAP (20 U) were carried out using known$^{11}$ procedures. The activity of lipase powder has been expressed in terms of units, with 1 unit corresponding to micromoles of butyric acid (estimation by GC) liberated from glyceryl tributyrate per minute per milligram of enzyme powder. The term usual work-up refers to extraction with ethyl acetate, washing the organic layer with water, and brine, drying of organic layer over Na$_2$SO$_4$, and concentration under vacuo.
Preparation of 1,3-dioxolone-4-methanol-2,2-dimethyl-glycerol (7): To a 500 mL two-necked RB flask with dean-stark apparatus acetone (80 mL, 1.020 mol) and glycerol (25 g, 275 mmol) was charged and to it low boiling (40-60 fraction, 80 mL) of petroleum ether was added along with cat. amount of p-TSA (750 mg) and the reaction mixture was stirred under reflux, the formed water was removed azeotropically and the heating was stopped after 24 h. The reaction mixture on cooling was stirred with NaOAc (750 mg) for 45 min and filtered, concentrated and distilled under reduced pressure (100 °C at 12 mm of Hg) to give the product as colorless oil in 85% yield.

Preparation of 1,3-dioxolone-4-benzylmethyl-2,2-dimethyl-glycerol (8): In a three necked-flask NaH (60% suspension in paraffin oil, 2 g, 48 mmol) was washed with petroleum ether (20 mL X 3) and dried under vacuum, a slurry was made by adding anhyd. THF (30 mL). To this suspension a solution of acetonide 7 (5.4 g, 36 mmol) in anhyd. THF (15 mL) was slowly added at 0 °C and stirred for 1 h. BnBr (5.5 mL, 45 mmol) was added at rt and stirred overnight. The reaction was quenched by adding methanol, then reaction mixture was filtered over celite bed, concentrated, to this ethyl acetate was added and water wash, followed by brine, drying over Na₂SO₄ and concentrating under vacuo gave yellowish oil in 82% yield.

1-Benzylglycerol 9: The solution of acetonide 8 (2.22 g, 10 mmol) in 15% acetic acid (100 mL) in distilled water was stirred for 4-5 h at 90°C. The reaction mixture was cooled and extracted with ethyl acetate (30 mL X 3) and this was then washed with water, 10% bicarbonate solution, water and then with brine. The organic layer was dried over Na₂SO₄ and concentrated under vacuo to give colorless oil in quantitative yield.

1,2-Diacetyl-3-benzylglycerol (10): To a stirred solution of diol (1.82 g, 10 mmol) in pyridine (20 mL) was added acetic anhydride (15 mL) and the reaction mixture was kept in dark for 24 h at rt. On usual aqueous work-up, ether extraction followed by
concentration and silica-gel column chromatography purification gave pure diacetate 
10, 2.40 g (90.2% yield).

**AmanoPS Catalyzed Biphasic Hydrolysis of 10:** A solution of diacetate 10 (1 mmol) 
in petroleum ether:benzene (2:1) mixture (20 mL) was added to a suspension of 
AmanoPS (125 mg) in 50 mM sodium phosphate buffer (10 mL) pH 7.0 at 25 °C. After 
2 h, the reaction mixture was filtered through celite and on usual work-up followed by 
silica gel column chromatographic removal of the unreacted diacetate (elution with 20% 
ethyl acetate : pet ether), furnished 11+12 (9:1) as a thick oil in 55% yield. In the 
spectral data only signals due to the major component 11 have been listed below. 
Similarly 13+14 on AmanoPS catalyzed hydrolysis furnished a mixture of 11+12+15 as 
a thick oil in 80:10:10 proportion.

**PLAP Catalyzed Biphasic Hydrolysis of 10:** A solution of diacetate 10 (5 mmol) in 
petroleum ether:benzene (2:1) mixture (150 mL) was added to a suspension of PLAP 
(500 mg) in 50 mM sodium phosphate buffer (75 mL) pH 8.0 at 25 °C. The pH was 
maintained at 8.0 using auto-stat with 0.1 M NaOH solution. At the end of 2 h, the 
reaction mixture was filtered through celite and on usual work-up followed by silica gel 
column chromatographic purification (elution with 20% ethyl acetate:pet ether), first 
gave pure 12 as a thick oil (20% yield) and then a mixture of 11 and 12 in 5% yield. 
Similarly 13 on PLAP catalyzed furnished a mixture of 12+15+16 as thick oil in 47:8:45 
proportion. While 13+14 (9:1) on PLAP catalyzed hydrolysis furnished a mixture of 
11+12+16 as thick oil in 5:66:29 proportion.

**Labeling of hydroxyacetate-2 with CD₃COOD/DCC:** To a stirred solution of 
hydroxyacetate 2 (1 mmol), CD₃COOD (72 mg, 1.2 mmol), and cat. amount of DMAP in 
EDC (5 mL) was added a solution of DCC (247 mg, 1.2 mmol) in EDC (2 mL) in a 
dropwise fashion at rt. The reaction mixture was further stirred at rt for 45 min and then 
filtered through celite, the residue was washed with EDC and the organic layer was
concentrated in vacuo. The residue on usual work-up followed by silica gel column chromatographic purification (elution with 10% ethyl acetate:pet ether) gave the corresponding mono-labeled compound 4 in 90-95% yields as a thick oil. By following a similar procedure, 11+12 (9:1) gave the mixture of 13+14 (9:1) as thick oil, 11 gave 14 as thick oil and 18 gave 19 as a thick oil, all in 90-95% yield.

1,4-Diacetoxy cyclopent-2-ene 17: To a stirred solution of diol (1.0 g, 10 mmol) in pyridine (20 mL) was added acetic anhydride (15 mL) and the reaction mixture was kept in dark for 24 h at rt. On usual aqueous work-up, ether extraction followed by concentration and column purification gave pure diacetate, 1.67 g (91.0% yield).

1-Acetoxy-4-hydroxycyclopent-2-ene (18): A solution of (±)-diacetate 17 (184 mg, 1 mmol) in petroleum ether: benzene (2:1) mixture (10 mL) was added to a suspension of Lipozyme lipase (200 mg) in 5 mM aq. sodium phosphate (5 mL) at pH 7.0. The reaction mixture was stirred at 25 °C for 36 h after which it was filtered through celite and extracted with ethyl acetate (25 mL x 3). The combined organic layer after usual work-up and concentration in vacuo gave an oily residue, which was subjected to column chromatography. Elution with 12% ethyl acetate:pet ether gave diacetate (17), 64.5 mg (35% yield) and with 18% ethyl acetate:pet ether gave hydroxy-aceate (18) 118 mg (60.0% yield): \[ \alpha \] \text{D} = +66.4° (c 1.0, CHCl₃) [lit. +66.3° c 1.0, CHCl₃ >99%ee]¹⁶

1,2-Dihydroxycyclopentan (20): To a stirred solution of cyclopentene (1.15 g, 17 mmol) in t-butanol:water (125 mL) was added K₃FeCN₆ (16.8 g, 51 mmol) and K₂CO₃ (7.04 g, 51 mmol) the reaction mixture was cooled to 0 °C. To this mixture, was added dropwise a solution of OsO₄ (120 mg, 0.03 equiv.) in t-butanol. The reaction mixture was stirred at rt. for 2 days, and reaction was quenched with an aqueous solution of sodium sulphite (50 mL, 20%). The mixture was stirred at rt. for 1 h, filtered through
celite, concentrated in vacuo and extracted with ethyl acetate (50 mL x 3). The organic layer upon usual work-up gave a residue which was chromatographed on silica gel to obtain pure diol 20, 1.13 g (65.0% yield).

1,2-Diacetoxy cyclopentane (21): To a stirred solution of diol 20 (1.02 g, 10 mmol) in pyridine (20 mL) was added acetic anhydride (15 mL) and the reaction mixture was kept in dark for 24 h at rt. On usual aqueous work-up, ether extraction followed by concentration and silica-gel column chromatographic purification gave pure diacetate 21, 1.57 g (84.5% yield).

1-Acetoxy-2-hydroxycyclopentane (22 and 23): A solution of (±)-diacetate 21 (186 mg, 1 mmol) in petroleum ether: benzene (2:1) mixture (10 mL) was added to a suspension of Amano PS lipase (100 mg) in 5 mM aq. sodium phosphate (5 mL) at pH 7.0. The reaction mixture was stirred at 25 °C for 12 h after which it was filtered through celite and extracted with ethyl acetate (15 mL x 3). The combined organic layer after usual work-up and concentration in vacuo gave an oily residue which was subjected to silica-gel column chromatography. Elution with 10% ethyl acetate:pet ether gave diacetoxy 21, 65.1 mg (35% yield) and with 15% ethyl acetate:pet ether gave hydroxy-acetate 22 and 23 86.5 mg (60.0% yield)); [α]_D^{25} = 0° (c 1.0, CHCl_3).

1-Oxo-2-acetoxy cyclopentane (24 and 25): A solution of oxalyl chloride (0.2 mL, 2.2 mmol) in dry CH_2Cl_2 (5 mL) was placed in a two necked RB flask kept under argon at −60 °C, to it a solution of DMSO (0.35 mL) in dry CH_2Cl_2 (2 mL) was added dropwise over a period of 5 min. hydroxyacetate solution (288 mg, 2 mmol) in dry CH_2Cl_2 (5 mL) was charged dropwise over period of 5 min and stirred at −60 °C for a period of 15 min, TEA (1.5 mL, 10 mmol) was added and stirred at rt for 5 min. Water (5 mL) was added to it and extracted with CH_2Cl_2 (7 mL x 3), and the organic layer was washed with brine, dried over Na_2SO_4 and concentrated under vacuo and silica-gel column chromatographed to give colorless oil in 229 mg, 80% yield.
AmanoPS Catalyzed Biphasic Hydrolysis of 1-oxo-2-acetoxy-cyclopentane (24+25): A solution of (±)-ketoacetate 24+25 (215 mg, 1.5 mmol) in petroleum ether:benzene (2:1) mixture (20 mL) was added to a suspension of Amano PS lipase (100 mg) in 5 mM aq. sodium phosphate (5 mL) at pH 7.0. The reaction mixture was stirred at 25 °C for 10 h after which it was filtered through celite and extracted with ethyl acetate (15 mL x 3). The combined organic layer after usual work-up and concentration in vacuo gave an oily residue, which was subjected to column chromatography. The spectral analysis showed 45% conversion (45% of hydroxyketone 26) and 55% of unreacted acetoxyketone 27. Elution with 10% ethyl acetate:pet ether gave acetoxyketone 27 105.5 mg (49% yield): [α]_D^25 = +33.4° (c 1.0, CHCl3). We were unable to isolate pure hydroxyketone 26 and further work is in progress.
<table>
<thead>
<tr>
<th>Structure (No.)</th>
<th>IR (cm⁻¹)</th>
<th>PMR (δ)</th>
<th>CMR (δ) and Mass spectral data</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="image" alt="Structure 4" /></td>
<td>4: IR (neat) ν_max 1740, 1730, 1720 cm⁻¹. ¹H NMR (CDCl₃, 200 MHz) δ 1.18 (t, J = 7.0 Hz, 3H), 2.10 (s, 3H), 3.80 (s, 3H), 4.15 (q, J = 7.0 Hz, 2H), 5.30 (d, J = 4.4 Hz, 1H), 6.22 (d, J = 4.4 Hz, 1H), 6.88(d, J = 8.8 Hz, 2H), 7.32 (d, J = 8.8 Hz, 2H). MS (m/e) 327, 264, 222, 179, 151, 137, 121.</td>
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<tr>
<td><img src="image" alt="Structure 7" /></td>
<td>7: ¹H NMR (CDCl₃, 200 MHz) δ 1.36 (s, 3H), 1.43 (s, 3H), 2.25 (bs, 1H), 3.58 (dd, J = 3.5 &amp; 3.2 Hz, 1H), 3.72 (dd, J = 3.4 &amp; 3.5 Hz, 1H), 3.78 (dd, J = 5.4 &amp; 5.6 Hz, 1H), 4.03 (dd, J = 5.4 &amp; 5.6 Hz, 1H) 4.15-4.30 (dd, J = 6 Hz, 1H).</td>
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<tr>
<td><img src="image" alt="Structure 8" /></td>
<td>8: ¹H NMR (CDCl₃, 200 MHz) δ 1.38 (s, 3H), 1.43 (s, 3H), 3.48 (dd, J = 4.8 &amp; 4.8 Hz, 1H), 3.58 (dd, J = 4.9 &amp; 4.9 Hz, 1H), 3.73 (dd, J = 5.8 &amp; 6.1 Hz, 1H), 4.07 (dd, J = 5.8 &amp; 5.8 Hz, 1H), 4.32 (quin, J = 6 Hz, 1H), 4.58 (d, J = 1.5 Hz, 2H), 7.20-7.40 (m, 5H).</td>
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<tr>
<td><img src="image" alt="Structure 9" /></td>
<td>9: ¹H NMR (CDCl₃, 200 MHz) δ 3.50-3.60 (m, 2H), 3.60-3.80 (m, 2H), 3.85-4.00 (m, 1H), 4.55 (s, 2H), 5.00-5.50 (bs, 2H), 7.35 (bs, 5H).</td>
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<tr>
<td><img src="image" alt="Structure 10" /></td>
<td>10: ¹H NMR (CDCl₃, 200 MHz) δ 2.05 (s, 3H), 2.10 (s, 3H), 3.62 (d, J = 5 Hz, 2H), 4.14-4.25 (dd, J = 12 &amp; 7 Hz, 1H), 4.30-4.40 (dd, J = 12 &amp; 5 Hz, 1H), 4.55 (d, J = 2 Hz, 2H), 5.15-5.30 (m, 1H), 7.25-7.40 (m, 5H), ¹³C NMR (CDCl₃, 50 MHz): 20.4, 20.7, 62.6, 68.0, 70.2, 73.1, 127.5, 127.6, 128.3, 137.7, 169.9, 170.2.</td>
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<tr>
<td>Structure</td>
<td>Description</td>
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<tr>
<td><img src="image" alt="Structure 11" /></td>
<td>11: IR (neat) $v_{max}$ 3440, 1730 cm$^{-1}$. $^1$H NMR (CDCl$_3$, 200 MHz) $\delta$ 2.12 (s, 3H), 3.67 (d, $J = 5.5$ Hz, 2H), 3.82 (d, $J = 5.5$ Hz, 2H), 4.56 (d, $J = 2.5$ Hz, 2H), 5.05 (quin, $J = 5.5$ Hz, 1H), 7.20-7.45 (m, 5H). $^{13}$C NMR (CDCl$_3$, 50 MHz): 20.8, 62.0, 68.7, 73.2 (2C), 127.4, 127.6, 128.2, 137.6, 170.6. MS (m/e) 225, 207, 154, 137, 117, 105, 91.</td>
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<tr>
<td><img src="image" alt="Structure 12" /></td>
<td>12: IR (neat) $v_{max}$ 3440, 1720 cm$^{-1}$. $^1$H NMR (CDCl$_3$, 200 MHz) $\delta$ 2.08 (s, 3H), 2.60 (bs, 1H), 3.45-3.62 (m, 2H), 3.97-4.10 (m, 1H), 4.08-4.26 (m, 2H), 4.58 (s, 2H), 7.20-7.48 (m, 5H). $^{13}$C NMR (CDCl$_3$, 50 MHz) 21.0, 65.8, 68.9, 71.2, 73.6, 128.0 (2C), 128.7, 138.0, 178.3.</td>
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<tr>
<td><img src="image" alt="Structure 13" /></td>
<td>13: $^1$H NMR (CDCl$_3$, 200 MHz) $\delta$ 2.10 (s, 3H), 3.60 (d, $J = 5$ Hz, 2H), 4.12-4.25 (dd, $J = 12$ &amp; 7 Hz, 1H), 4.30-4.42 (dd, $J = 12$ &amp; 5 Hz, 1H), 4.54 (d, $J = 2$ Hz, 2H), 5.23 (q, $J = 6$ Hz 1H), 7.20-7.45 (m, 5H); MS (m/e) 270, 210, 181, 162, 137, 117, 91, 43.</td>
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<tr>
<td><img src="image" alt="Structure 14" /></td>
<td>14: $^1$H NMR (CDCl$_3$, 200 MHz) $\delta$ 2.06 (s, 3H), 3.60 (d, $J = 5$ Hz, 2H), 4.12-4.26 (dd, $J = 12$ &amp; 7 Hz, 1H), 4.28-4.40 (dd, $J = 12$ &amp; 5 Hz, 1H), 4.56 (d, $J = 2$ Hz, 2H), 5.16-5.30 (m, 1H), 7.25-7.45 (m, 5H); MS (m/e) 270, 207, 181, 162, 120, 91, 46.</td>
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<td><img src="image" alt="Structure 17" /></td>
<td>17: $^1$H NMR (CDCl$_3$, 200 MHz) $\delta$ 1.75 (td, $J = 15$ &amp; 5 Hz, 1H), 2.07 (s, 6H), 2.88 (td, $J = 15$ &amp; 7.5 Hz, 1H), 5.50-5.60 (m, 2H), 6.10 (s, 2H).</td>
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</tbody>
</table>
18: $^1$H NMR (CDCl$_3$, 200 MHz) δ 1.65 (td, $J = 15$ & 5 Hz, 1H), 1.88 (bs, 1H), 2.05 (s, 3H), 2.80 (td, $J = 15$ & 7.5 Hz, 1H), 4.67-4.78 (m, 1H), 5.45-5.55 (m, 1H), 5.98 (d, $J = 8$ Hz, 1H), 6.12 (d, $J = 8$ Hz, 1H). MS (m/e) 142, 125, 111, 98, 81, 70, 60, 54. IR (neat) $\nu_{\text{max}}$ 3400, 1720 cm$^{-1}$.

19: $^1$H NMR (CDCl$_3$, 200 MHz) δ δ 1.75 (td, $J = 15$ & 5 Hz, 1H), 2.07 (s, 3H), 2.80 (td, $J = 15$ & 7.5 Hz, 1H), 5.50-5.60 (m, 2H), 6.10 (s, 2H).

20: $^1$H NMR (CDCl$_3$, 200 MHz) δ 1.35-2.00 (complex m, 6H), 3.75-4.15 (m, 2H). IR (neat) $\nu_{\text{max}}$ 3400 cm$^{-1}$.

21: $^1$H NMR (CDCl$_3$, 200 MHz) δ 1.50-2.10 (m, 6H), 2.04 (s, 6H), 5.00-5.25 (m, 2H). IR (neat) $\nu_{\text{max}}$ 3380, 1690 cm$^{-1}$.

22+23: 1.40-2.05 (m, 6H), 2.08 (s, 3H), 2.25 (bs, 1H), 4.15 (q, $J = 4.8$ Hz, 1H), 4.87-5.03 (m, 1H). IR (neat) $\nu_{\text{max}}$ = 3320, 1695 cm$^{-1}$.

24+25: $^1$H NMR (CDCl$_3$, 200 MHz) δ 1.70-2.00 (m, 2H), 2.13 (s, 3H), 2.20-2.50 (m, 4H), 5.07 (t, $J = 8.8$ Hz, 1H); $^{13}$C NMR (CDCl$_3$, 50 MHz): 17.0, 20.5, 28.2, 34.6, 75.5, 169.9, 212.2. MS (m/e) 143, 142, 99, 86, 71. IR (neat) $\nu_{\text{max}}$ 1749 cm$^{-1}$.

Please see page 150.
MASS SPECTRUM
04/17/97 12:00:00 + 2:10
SAMPLE: NPH-11 OF DR N.P. ARCADE, OCS, 2223(D:400)

DATA: ARD #32
BASE M/E: 137
R/I C: 4143248.

Ar
\[ \text{OAc} \]
\[ \text{CO}_2\text{Et} \]
\[ \text{OCOCOD}_3 \]

4 MW 327
(11:12 = 9:1)
GLY ISS/AMA

\( \text{expected (11:12:15:16 = 81:9:9:1)} \)
\( \text{observed (11:12:15 = 80:10:10)} \)
expected \((11:12:15:16 = 81:9:9:1)\)
observed \((11:12:15 = 80:10:10)\)

MW 224 and 227
\[
\text{OCOCH}_3
\begin{array}{c}
\text{OH} \\
\text{OCH}_2\text{Ph}
\end{array} + \begin{array}{c}
\text{OH} \\
\text{OCOCD}_3 \\
\text{OCH}_2\text{Ph} \\
\text{OCH}_2\text{Ph}
\end{array}
\]

\[
\text{12} \quad \text{15} \quad \text{16}
\]

\[
(12:15:16 = 47:8:45)
\]
(12:15:16 = 47:8:45)

MW 224 and 227
(11:12:16 = 5:66:29)
3.8 REFERENCES AND NOTES


7. Ikeda, K.; Achiwa, K. Bioorg. Med. Chem. Lett. 1997, 7, 225. The $^1$H NMR data reported by the authors for the p-nitro derivative of 11 fits in well with the alternate...
structure, ie p-nitro derivative of 12 and our method will be directly useful to confirm actual and observed selectivities in this example.


14 The AmanoPS and PLAP catalyzed hydrolysis of (+)-10 was non-stereoselective, see Taterie, N. H.; Bailey, R. A.; Kates, M. Arch. Biochem. Biophys. 1958, 78, 319.

15 PLAP catalyzed hydrolysis of (+)-10 was arrested when the corresponding diol formation was detected on tlc (~25% conversion, 2 h).


