CHEMOENZYMATIC SYNTHESIS OF BIOLOGICALLY ACTIVE NATURAL PRODUCTS

A THESIS SUBMITTED TO
THE GRADUATE SCHOOL OF NATURAL AND APPLIED SCIENCES
OF
MIDDLE EAST TECHNICAL UNIVERSITY

BY

ENGİN TURKUT

IN PARTIAL FULFILLMENT OF THE REQUIREMENT FOR THE DEGREE OF
MASTER OF SCIENCE
IN
THE DEPARTMENT OF CHEMISTRY

APRIL 2004

Approval of the Graduate School of Natural And Applied Sciences

_____

Prof. Dr. Canan Özgen

Director

I certify that this thesis satisfies all the requirements as a thesis for the degree of Master of Science.

_____

Prof. Dr. Hüseyin İşçi

Chairman of the Department

We certify that we have read this thesis and in our opinion it is fully adequate, in scope and quality, as a thesis for the degree of Master of Science.

_____

Prof. Dr. Cihangir Tanyeli

Supervisor

Examining Committee Members

Prof. Dr. Canan Ünaleroğlu _____

Prof. Dr. Cihangir Tanyeli _____

Assoc. Prof. Dr. Özdemir Doğan _____

Prof. Dr. İdris M. Akhmedov _____

Prof. Dr. Metin Balcı _____

ABSTRACT

CHEMOENZYMATIC SYNTHESIS OF BIOLOGICALLY ACTIVE NATURAL PRODUCTS

Turkut, Engin
M.S., Department of Chemistry
Supervisor: Prof. Dr. Cihangir Tanyeli
April 2004, 95 pages

Racemic metyhl 3-cyclohexene-1-carboxylate was resolved via enzymatic hydrolysis to afford the enantiomerically enriched 3-cyclohexene-1-carboxylic acid with PLE (*S*-configuration), HLE (*S*-configuration), CCL (*S*-configuration) and PPL (*R*-configuration) . The nucleoside's precursor, 5-(hydroxymethyl)-2-cyclohexen-1-ol **(19)**, was synthesized by iodolactonization, followed by iodine elimination and the reduction of the lactone.

In connection with this work, α,β-unsaturated and saturated cyclic ketones were selectively oxidized on α′- and α-positions using $Mn(OAc)_3$ and $Pb(OAc)_4$, respectively. The resultant racemic α′- and α-acetoxylated substrates were resolved into corresponding enantiomerically enriched α′- and α-hydroxylated and acetoxylated compounds via PLE hydrolysis.

Key words: Nucleosides, enzymatic hydrolysis, $Mn(OAc)_3$ and $Pb(OAc)_4$

ÖZ

BİYOLOJİK ACTİVİTEYE SAHİP DOĞAL ÜRÜNLERİN
KEMOENZİMATİK SENTEZİ

Turkut, Engin
Yüksek Lisans, Kimya Bölümü
Tez Yöneticisisi: Prof. Dr. Cihangir Tanyeli
Nisan 2004, 95 sayfa

Rasemik metil 3-siklohekzen-1-karboksilat, enantiyomerce zenginleşmiş 3-siklohekzen-1-karboksilik asiti elde etmek için PLE (*S*-konfigürasyon), HLE (*S*-konfigürasyon), CCL (*S*-konfigürasyon) ve PPL (R-konfigürasyon) kullanılarak enzimatik hidroliz yöntemiyle ayrıştırılmıştır. Nükleositler için gerekli başlangıç maddesi olan 5-(hidroksimetil)-2-siklohekzen-1-ol **(19),** bu asitin iyodolaktonizasyonu sonrasında iyot eliminasyonu ve lakton indirgenmesi yöntemleriyle sentezlenmiştir.

Bu konuyla ilgili ayrıca, α,β-Doymamış ve doymuş siklik ketonlar, $Mn(OAc)_3$ ve $Pb(OAc)_4$ kullanılarak α′- ve α-pozisyonlarından seçici olarak yükseltgendi. Sonuç ürün olan rasemik α′- ve α-asetoksilenmiş substratlar PLE hidrolizi ile enantiyomerce zenginleşmiş α′- ve α-hidroksilenmiş ve asetoksilenmiş maddelere dönüştürüldü.

Anahtar kelimeler: Nükleositler, enzimatik hidroliz, $Mn(OAc)_3$ ve $Pb(OAc)_4$

To My Family

ACKNOWLEDGEMENT

First and foremost, I would like to express my sincere thanks to my supervisor Prof. Dr. Cihangir Tanyeli for his continuing guidance, suggestions and encouragement throughout the research with great patience. It has been a great honour for me to work with him.

Also, I wish to express my thanks to Prof. Dr. İdris M. Akhmedov for his kind helpful suggestions.

In addition, I would like to thank all of the METU Organic Chemistry Reseach Group, especially, Olcay, Çiğdem and Timur for their help, friendship and cooperation.

Finally, I devoted this thesis to my parents, sister and friends for their endless love, encouragement and patience with great appreciation and gratitude.

TABLE OF CONTENTS

LIST OF SCHEMES

LIST OF FIGURES

LIST OF TABLES

LIST OF ABBREVIATIONS

DMSO: Dimethyl Sulfoxide

PLE: Pig Liver Esterase

PPL: Porcine Pancreatic Lipase

CCL: Lipase from *Candida Rugosa*

HLE: Horse Liver Esterase

DBU: 1,8-diazabicyclo[5.4.0]undec-7-ene

THF: Tetrahydrofuran

CHAPTER 1

INTRODUCTION

1.1 Asymmetric Synthesis

Asymmetric synthesis is a reaction or reaction sequence that selectively creates one configuration of one or more stereogenic elements by the action of a chiral reagent or auxiliary, acting as heterotopic faces, atoms or groups of a substrate.

Asymmetric synthesis, the selective of new chirality elements, has developed from a speciality followed by outsiders to an art cultured by some learned ones, and now may be considered a standard laboratory methodology for everybody's use. This development has taken place exponentially in the last two decades. Also the practitioners of pharmaceutical, vitamin, and agro synthesis need to produce enantiopure, rather than racemic active compounds.[1]

The field of asymmetric synthesis evolved from the study of diastereoselectivity in reactions of chiral compounds, through auxiliary-based methods for the synthesis of enantiomerically pure compounds, to asymmetric catalysis. In the former case, diastereomeric mixtures ensue, and an analytical technique such as chromatography is used for isomer purification. In the latter instance, enantiomers are the products and chiral stationary phases can be used for chromatographic purification. Furthermore, many methods have now been developed that generate numerous stereocenters in a single step. Highly selective reactions that produce one or more stereocenters with a high degree of selectivity (≥90%), along

modern purification techniques, allow the preparation – in a single step – of chiral substances in ≥98% ee for many reaction types.[2]

1.2 Why Scientists Do Asymmetric Synthesis

Nature produces a wide variety of chiral compounds. Interactions with these materials, which are very important in many fields such as the development of a pharmaceutical agent, often now require the development of a chiral compound to maximize the desired effects. Public perception, and associated legislation, surronding the development of pharmaceuticals, especially since the thalidomide **(1)** (Figure 1) tragedy, is also demanding enantioselective synthesis.[3,4] In addition, one of the enantiomers may carry all, or the majority, of the activity.[5,6] Thus, preparation of the active component not only reduces the amount of the inactive enantiomers, in essence, it is an impurity but can also make economic sense (the specific activity can be higher). Some of the arguments for use of a single enantiomer over a racemate are given in the Table 1.

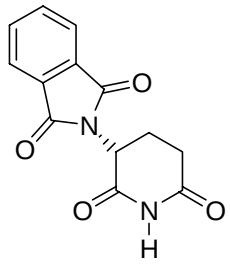


Figure 1. (*R*)-Thalidomide **(1)**
Sedative,
other isomer is teratogen

Table 1. Potential Benefits for use of a Single Enantiomer for Therapeutic Uses[7,8,9]

Properties of Racemate	Potential Benefits of Enantiomer
One enantiomer has exclusive property	Reduce dose and load on metabolism
Other enantiomer is toxic	Increased latitude in dose and broader usage
Enantiomers have different pharmokinetics	Better control of kinetics and dose
Enantiomers metabolized at different rates (in one person)	Wider latitude in dose setting; less variability in patient response
Enantiomers metabolized at different rates (different people)	Reduction in variability of patient response; larger confidence in dose selection
One enantiomer are prone to intersection with key detoxification pathways	Reduce interactions with other (common) drugs
One enantiomer is agonist, other antaagonist	Enhanced activity and reduction of dose
Enantiomers vary in spectra of pharmalogical action and tissue specificity	Increased specificity and reduced side effects for one enantiomer; use of other enantiomer for different indication

1.3 Methods to Achieve Asymmetric Synthesis

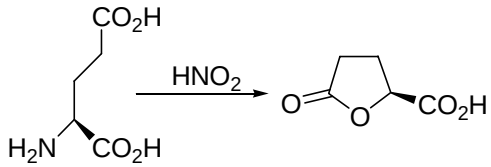
To do an enantioselective synthesis, at least one of the agents in the system must be chiral. There are two major methods to achieve this goal: resolution or asymmetric synthesis, which includes the use of a chiral starting material, chiral auxiliaries or reagents.

1.3.1 Chiral Entities

Before embarking on a synthesis, careful thought must be given to how a chiral center will be introduced into a molecule. The three major options are use of a chiral reagent (chemical or biological); use of a chiral environment; and use of a chiral starting material. Chirality can also be introduced in a temporary manner through the use of a chiral auxiliary, alhough this is a sub-class of chiral substrates.[10]

1.3.1.1 Chiral Substrates

The best scenario is to have a chiral starting material that can control the stereoselection of the reaction itself. Nature produces chiral materials and a number of these are available in quantity (vide infra).[11,12] These compounds make up the 'chiral pool'. If all of the parameters are favorable, this approach is the method of choice as it has the potential to eliminate resolutions or the necessity for an enantiospecific transformation in the synthetic design. An example of the use of a natural product for a starting material is the conversion of L-glutamic acid **(2)** to the chiral butrylactone **(3)** (Scheme 1).[13]



2 **3**

Scheme 1. The Conversion of L-Glutamic Acid **(2)** to the Chiral Butrylactone **(3)**

The chiral pool is not a stagnant pond. As enzymes and reagents are discovered and developed, they can be applied to provide large quantities of useful chiral starting materials.

1.3.1.2 Chiral Auxiliries

This class of compounds modifies the substrate molecule to introduce a stereogenic center that will influence the outcome of a reaction to provide an asymmetric synthesis. The auxiliary has to be put onto the substrate and removed. Although this involves two steps, concurrent protection of sensitive functionality can also take place, so that are inefficient sequence (protection and deprotection) is traded for another (auxiliary introduction and removal).

1.3.1.3 Self Regeneration of Stereocenters

There is a variation on the chiron approach. A chiral center from a starting material can be transferred to another part of the molecule. This new chiral center

then provides control for a stereoselective reaction, where a new center of asymmetry can be established, or the chirality at the center of the original starting material can be reestablished.[14]

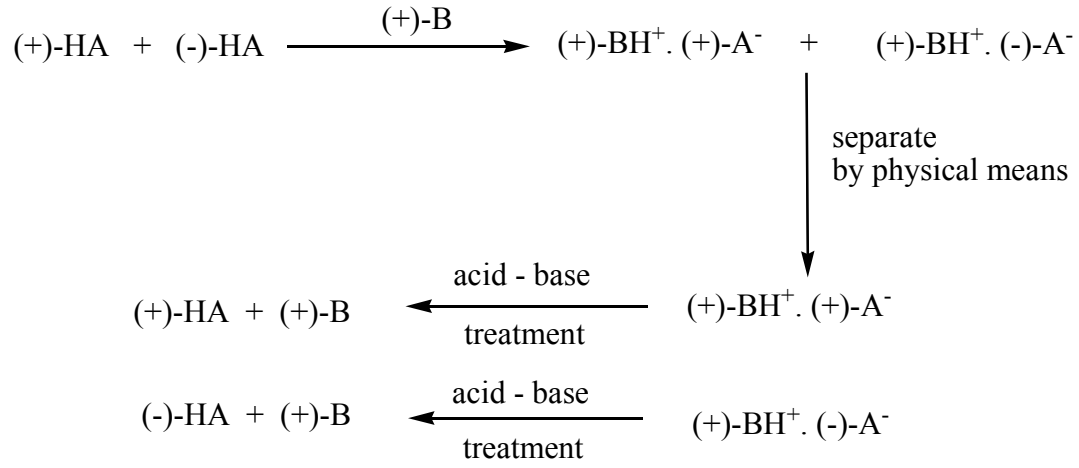
1.3.1.4 Chiral Reagents

Chiral reagents allow for the transfer of chirality from the reagent to the prochiral substrate. Almost all of these reactions involve the conversion of an sp^2 carbon to an sp^3 center. For example, reductions of carbonyl compounds, asymmetric hydrogenations, and asymmetric oxidations of alkenes are all of this type.

1.4 Resolution

Resolution and chirality are twins born on the day Louis Pasteur separated crystals of salts of D- and L-tartaric acid under his microscope. Since then, the separation of each enantiomer from a racemic mixture has been the primary means to synthesize optically active organic compounds. Only recently, the fast and explosive new developments in asymmetric synthesis involving the use of organometallic catalysts, enzymes, and chiral auxiliaries have begun to challenge the resolution approach. Through many years of evolution, the art of resolution has become a multidisciplinary science that includes diastereomeric, kinetic, dynamic, chromatographic, enzymatic components.

To effect a resolution, the compound must contain a functional group that can interact or react with another chiral agent to produce diastereomers. These diastereomers can then be separated by physical means. The resolution agent then needs to be removed from the subject material. Thus, two extra chemical steps may be necessary to use the resolution agent. In addition, as only 50% of a racemic mixture can be desired isomer, recovery is, at the very best, 50%. This destracts from the method. However, if the compound under investigation is an acid or a base, then it may be possible to form a salt that can be separated, although many factors can still affect the success of the approach (Scheme 2).[15-19]



Scheme 2. The Resolution

1.4.1 Kinetic Resolution

In this approach a substrate is acted on by a chiral agent to produce one enantiomer or diastereomer of the product at a much faster than the other isomer. In general, the enantiomeric excess of the starting material will increase as the reaction progresses, while the enantiomeric excess of a chiral product will decrease. As this is a resolution, only 50% of the substrate can be converted to the desired product unless it is meso.[20]

Kinetic resolution may be realized by chemical or enzymic methods; in the former case the reaction may be either catalytic or stoichiometric with respect to the optically active auxiliary; from an economic standpoint catalysis is obviously preferred. Kinetic resolutions and high E values are more commonly found with enzymic than chemical processes.

1.5 Enzymes

The use of enzymes and microorganisms in organic synthesis, especially in the production of chiral organic compounds, has grown significantly in recent years and has been accepted as an effective and practical alternative for certain synthetic organic transformations.

1.5.1 Enzyme Nomenclature

The IUB has classified enzymes into 6 main classes according to type of reaction catalysed.

- Oxidoreductases

catalyze oxidation/reduction reactions transferring hydrogen, oxygen, and/or electrons, between molecules. In this important class belong dehydrogenases (hydride transfer), oxidases (electron transfer to molecular oxygen), oxigenases (oxygen transfer from molecular oxygen), and peroxidases (electron transfer to peroxide).

- Tranferases

catalyze the transfer of groups of atoms e.g. amino-, acetyl-, phosphoryl-, glycosyl-, etc. from a donor to a suitable acceptor.

- Hydrolases

catalyze the hydrolytic cleavage of bonds. Many commercially important enzymes belong to this class, e.g. proteases, amylases, acylases, lipases and esterases.

- Lyases

catalyze the non-hydrolytic cleavage of e.g. C-C, C-O, C-N bonds by elimination reactions leaving double bonds or in reverse adding groups to a double bond. Examples are fumarases, aspartases, decarboxylases, dehydratates, and aldolases.

- Isomerases

catalyze isomerisation and transfer reaction within one molecule. The most prominent member of this group is D-xylose ketolisomerase, commonly known as glucose isomerase.

- Ligases

catalyze the covalent joining of two molecules coupled with the hydrolysis of an energy rich bond in ATP or similar triphosphates. An example is γ-L-glutamyl-L-cysteine:glycine ligase (ADP forming) also found under the same glutathion synthetase.

Table 2 lists the chemical reaction types that can be mediated by enzymes or microorganisms.[21,22]

Table 2. Chemical Reaction Types Mediated By Enzymes or Microorganisms

Class	Reaction Type	Class	Reaction Type
Oxidation	Dehydrogenation of C-C	Hydrolysis	Hydrations of C-C unsaturation
	Epoxidation		Hydrations of epoxides
	Hydroxylation		Hydrolysis of amides
	Oxidation of hetero functions		Hydrolysis of esters
	Oxidation of alcohols		Hydrolysis of ethers
	Oxidation of aldehydes		Hydrolysis of lactams
	Oxidative deamination		Hydrolysis of lactones
	Oxidative degradation of alkyl chains		Aminations
	Oxidative degradation of carboxy alkyl chains		Hydrolysis of alkyl halides
	Oxidative degradation of oxoallyl chains		Hydrolysis of nitriles
	Oxidative removal of substituents	Condensations	Dehydrations
	Oxidative ring fission		Esterifications
Reductions	Dehydroxylations		Glycosidations
	Hydrogenations of C-C unsaturation		Lactonizations
	Reductions of aldehydes		N-acylations
	Reductions of ketones		O-acylations
	Reductions of heterofunctions	Isomerizations	Migrations of double bonds
	Reductions of organic acids		Migrations of oxygen functionality
	Reductive amination of substituents		Racemization
	Reductions of nitrogen compounds		Rearrangements
Formation of	C-C bonds	Transfers	Transesterifications
	Heteroatoms bonds		Transaminations
	Ring systems		Hydroxymethyl group transfers

1.5.2 Common Prejudices Against Enzymes

If one uses enzymes for the transformation of non-natural organic compounds, the following prejudices are frequently encountered.

a) Enzymes are sensitive

b) Enzymes are expensive

c) Enzymes are only active on their natural substrates

d) Enzymes work only in their natural enviroment

1.5.2.1 Advantages Of Enzymes

a) Enzymes are very efficient catalysts

Typically the rates of enzyme-mediated processes are accelerated, compared to those of the corresponding nonenzymatic reactions, by a factor of 10^8-10^{10}. The acceleration may even exceed a value of 10^{12}, which is far above the values that chemical catalyst are capable of achieving.[23]

b) Enzymes are enviromentally acceptable

Unlike heavy metals, for instance, enzymes are completely degraded in the environment.

c) Enzymes act under mild conditions

Enzymes act in a pH range of about 5-8, typically around 7, and in a temperature range 20-40°C, preferably at around 30°C.

d) Enzymes are compatible with each other.[24]

Since enzymes are generally function under the same or similar conditions, several biocatalytic reactions can be carried out in a reaction cascade in one flask. Thus sequential reactions are feasible by using multienzyme systems in order to simplify reaction processes, in particular if the isolation of an unstable intermediate can be omitted.

e) Enzymes are not bound to their natural role

They exhibit a high substrate tolerance by accepting a large variety of man-made unnatural substances and often they are not required to work in water.

f) Enzymes can catalyze a broad spectrum of reactions

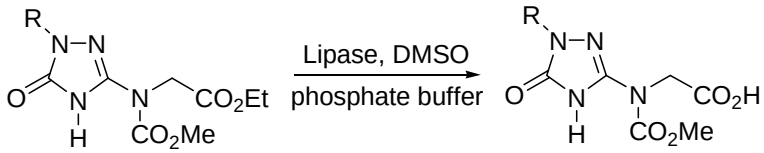
Like all catalysts, enzymes only accelerates a reaction, but they have no impact on the position of the thermodynamic equilibrium of the reaction.

Enzyme-catalyzed processes equivalent to almost every type of organic reaction are given in the Table 2.

Enzymes display three major types of selectivity:

a) Chemoselectivity

Since the purpose of an enzyme is to act on a single type of functional group, other sensitive functionalities, which would normally react to a certain extent under chemical catalysis, survive. For instance, enzymatic ester hydrolysis does not show any propensity for acetal-cleavage (Scheme 3).

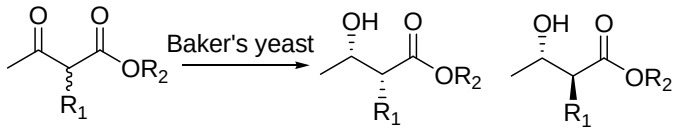


4 **5**

Scheme 3. Chemoselective Hydrolysis of Fatty Acid Ester[25]

b) Regioselectivity or Diastereoselectivity

Due to their complex three dimensional structure, enzymes may distinguish between functional groups which are chemically situated in different regions of the same substrate molecule (Scheme 4).[26,27]



R + *S*

6

2*R*,3*S*-syn

7

2*S*,3*S*-anti

8

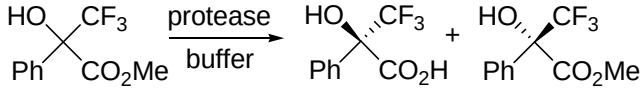
in situ racemization

$R_1 = CH_3$, $R_2 = C_2H_5$	100% ee, 83%	100% ee, 17%
$R_1 = CH_3$, $R_2 = Ph\text{-}CH_2$	100% ee, 67%	80% ee, 33%

Scheme 4. Reduction of α-Substituted β-Ketoesters Using Baker's Yeast

c) Enantioselectivity

Last but not least, almost all enzymes, are made from L-amino acids and thus are chiral catalysts. As a consequence, any type of chirality present in the substrate molecule is recognized upon the formation of the enzyme-substrate complex. Thus a prochiral substrate may be transformed into an optically active product and both enantiomers of a racemic substrate may react at different rates, affording a kinetic resolution. These latter properties collectively constitute the 'specificity' of an enzyme and represent its most important feature for selective and asymmetric exploitation.[28]



10 **11** **12**

Scheme 5. Resolution of Bulky Esters by Protease

1.5.2.2 Disadvantages of Enzymes

a) Enzymes are provided by Nature in only one enantiomeric form

Since there is no general way of creating mirror-image enzymes (from D-amino acids) it is impossible to invert the chiral induction of a given enzymatic reaction by choosing the 'other enantiomer' of the enzyme, a strategy which is possible if chiral chemical catalysts are involved.

b) Enzymes require narrow operation parameters

The obvious advantage of working under mild reaction conditions can sometimes turn into a drawback. If a reaction proceeds only slowly under given parameters of temperature or pH, there is only a narrow scope for alteration.

c) Enzymes display their highest catalytic activity in water

Due to its high boiling point and high heat of vaporization, water is usually the least desired solvent of choice for most organic reactions. Furthermore, the majority of organic compounds are only poorly soluble in aqeuos media. Thus shifting enzymatic reactions from an aqeous to an organic medium would be highly desired, but the unavoidable price one has to pay is usually some loss of activity, which is often in order of one magnitude.[29]

d) Enzymes are bound to their natural cofactors

It is a still unexplained paradox, that although enzymes are extremely flexible for accepting non-natural substrates, they are almost exclusively bound to their natural cofactors which serve as carriers of redox equivalents.

e) Enzymes are prone to inhibition phenomena

Many enzymatic reactions are prone to substrate or product inhibition, which causes the enzyme to cease to work at higher substrate and/or product concentrations, a factor which limits the efficiency of the process.[30]

f) Enzymes may cause allergies

Enzymes may cause allergic reactions. However, this may be minimized if enzymes are regraded as chemicals and handled with the same care.

1.6 Mechanistic Aspects

a) 'Lock and Key' Mechanism

The first proposal for a general mechanism of enzymatic action was developed by E. Fischer in 1894.[31] It assumes that an enzyme and its substrate mechanistically interact like a lock and a key, respectively (Figure 2). Although this

assumption was quite sophisticated at that time, it assumes a completely rigid enzyme structure.

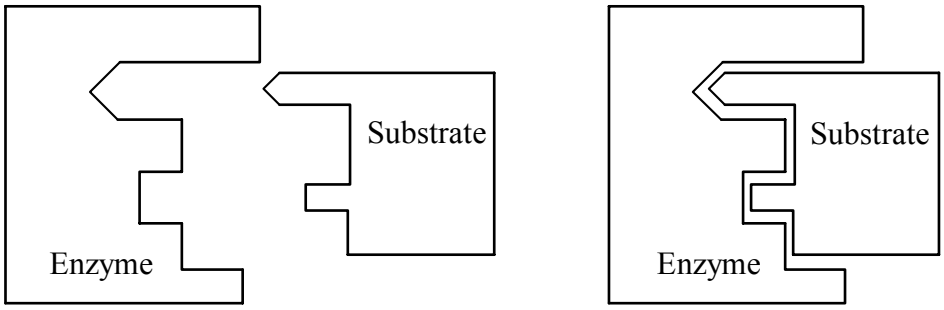


Figure 2. The Schematic Represantation of the Lock and The Key Mechanism

b) Induced-Fit Mechanism

This rationale, which takes into account that enzymes are not entirely rigid but rather represent delicate and soft structures, was developed by Koshland Jr. in the late 1960s.[32] It assumes that upon approach of the enzyme-substrate complex, the enzyme can change its conformation under the influence of the substrate structure so as to wrap itself around its guest (Figure 3).

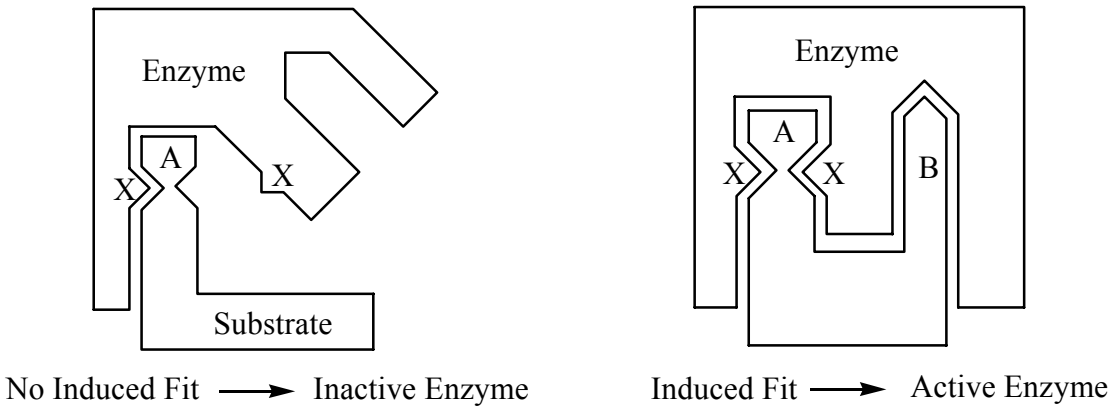


Figure 3. The Schematic Represantation of the Induced-Fit Mechanism

In Figure 3, A represents the reactive group of the substrate, X is the complementary reactive group(s) of the enzyme – the 'chemical operator'. Substrate part B forces the enzyme to adapt a different (active) conformation. Only then are the 'active' groups X of the enzyme positioned in the right way to effect catalysis.

c) Desolvation and Solvation-Substitution Theory

More recently, M. J. S. Dewar developed a different rationale[33] in attempting to explain the high conversion rates of enzymatic reactions, which are often substantially faster than the chemically-catalyzed equivalent processes.[34] The theory assumes that the kinetics of enzyme reactions have much in common with those of gas-phase reactions. If a substrate enters the active site of the enzyme, it replaces all of the water molecules at the active site of the enzyme. Then, a formal gas phase reaction take place which mimics two reaction partners interacting without a disturbing solvent. In solution, the water molecules impede the approach of the partners, hence the reaction rate is reduced.

d) Three–Point Attachment Rule

This widely used rationale to explain the enantioselectivity of enzymes was suggested by A. G. Ogston.[35] Since chirality is a quality of space, a substrate must be positioned firmly in three dimensions in order to achieve a high degree of enantioselection. As a consequence, there must be at least three different points of attachment of the substrate onto the active site.

This theory is exemplified for the discrimination of the enantiomers of a racemic substrate with its chirality located on a sp^3-carbon atom (Figure 4).

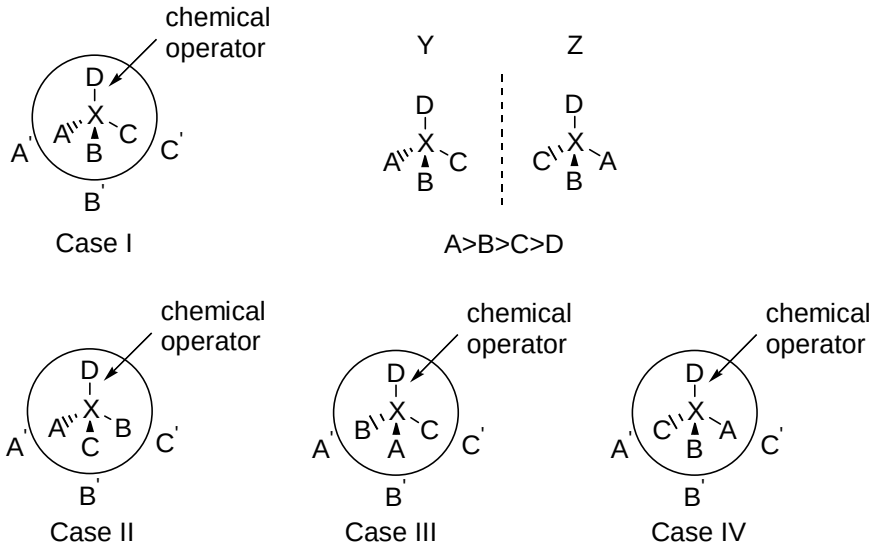


Figure 4. The Schematic Representation of the Enzymatic Enantiomer Discrimination

Case I: Enantiomer Y is a good substrate by allowing an optimal interaction of its groups (A, B, C) with their complementary binding site areas of the enzyme (A', B', C'). It ensures an optimal orientation of the reactive group (D) towards the chemical operator which is required for a successful transformation.

Case II to IV: Enantiomer Z is a poor substrate because optimal binding and orientation of the reactive group D is not possible. If a prochiral substrate (C), bearing two chemically identical but stereochemically different enantiotopic groups (A), is involved the same model can be applied to rationalize the favored transformation of one of two leading to an 'enantiotopos differentiation' (Figure 5).

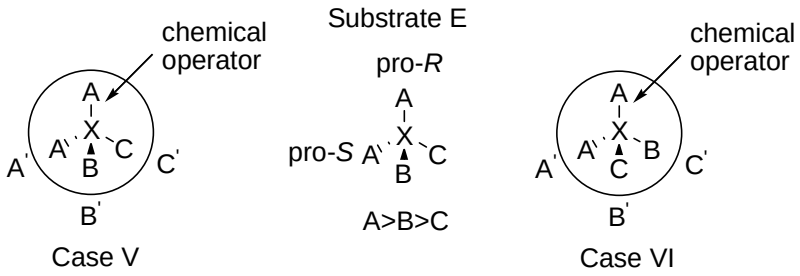


Figure 5. The Schematic Representation of the Enzymatic Enantiotopos Discrimination

Case V: It shows the good binding of a prochiral substrate (E) to the complementary enzyme's binding sites with the pro-(*R*) group out of the two reactive groups being positioned to the chemical operator.

Case VI: Positioning of the pro-(*S*) reactive group towards the chemical operator results in poor orientation of the other functions to their complementary sites, resulting in poor catalysis. As a consequence, the pro-(*R*) group is cleaved prefentially to its pro-(*S*) counterpart.

The ability of enzymes to distinguish between two enantiomeric faces of a prochiral substrate (F)- an 'enantioface differentiation' is illustrated in the Figure 6.

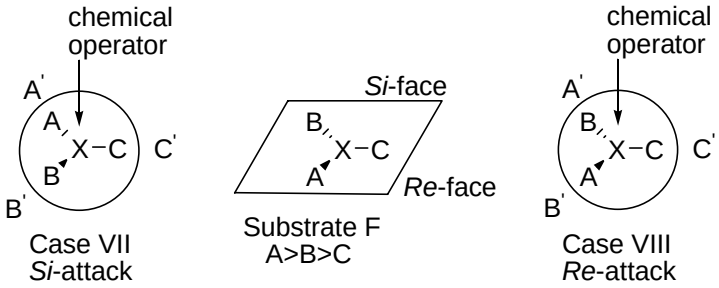


Figure 6. The Schematic Representation of Enzymatic Enantioface Discrimination

Case VII: An optimal match between the functional groups of substrate F leads to an attack of the chemical operator to the central atom X from the *Si*-side (Figure 7).

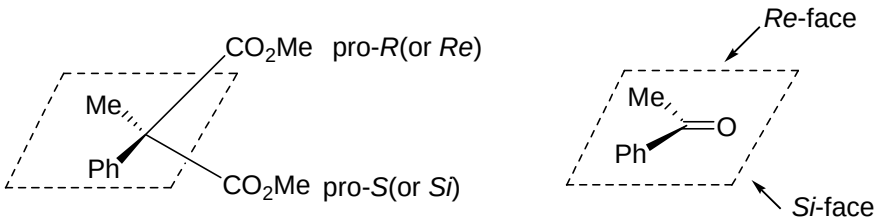


Figure 7. Enantiotopos and Face Nomenclature

Case VIII: The mirror image orientation of substrate F in the active site of the enzyme leads to a mismatch in the binding of the functional groups, thus an attack of the chemical operator, which would come from the *Re*-side in this case, is disfavored.

1.6.1 Kinetic Reasons for Selectivity

As in every catalytic reaction, an enzyme (E) accelerates the reaction by lowering the energy barrier between substrate (S) and product (P)-the activation energy (E_a).[36] The origin of this catalytic power – the rate acceleration – has generally attributed to transition-state stabilization of the reaction by the enzyme,[37] assuming that the catalyst binds more strongly to the transition state than to the ground state of the substrate, by a factor approximately equal to the rate acceleration (Figure 8).[38]

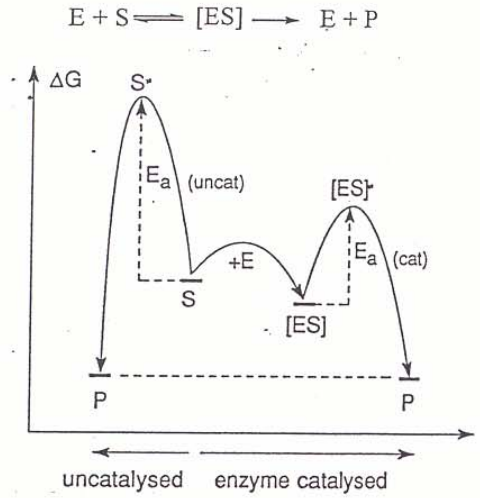


Figure 8. Energy Diagram of Catalyzed versus Uncatalyzed Reaction

Virtually all stereoselectivities of enzymes originate from the energy difference in the enzyme-transition-state complex $[ES]^{\neq}$ (Figure 9). Due to the chiral environment of the active site of the enzyme, diastereomeric enzyme substrate complexes [EA] and [EB] are formed, which possess different values of free energy (ΔG) for their respective transition state $[EA]^{\neq}$ and $[EB]^{\neq}$. The result is a difference in activation energy ($\Delta\Delta G^{\neq}$) for both of the enantiomeric substrates or the enantiomeric orientations, respectively. As a consequence, one enantiomer (or orientation) will be transformed faster than the other. This process is generally referred to a 'chiral recognition'.

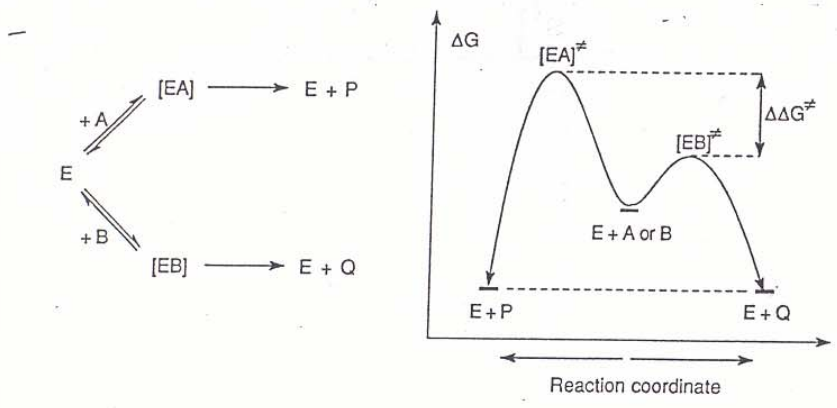


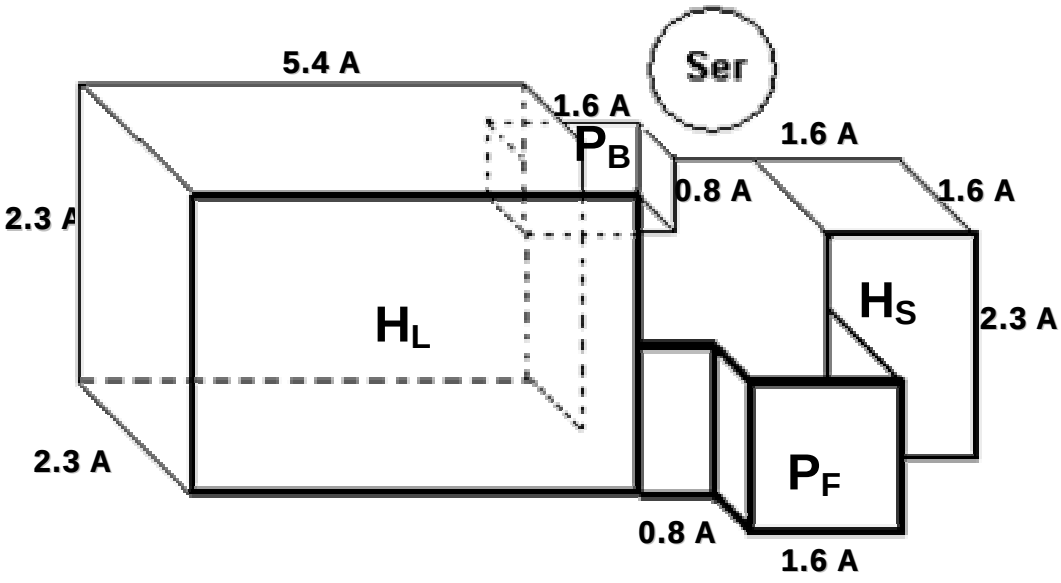
Figure 9. Energy Diagram for an Enzyme-Catalyzed Enantioselective Reaction

1.7 Hydrolase Type Enzymes

1.7.1 Pig Liver Esterase (PLE, E.C.3.1.1.1)

Pig liver esterase (PLE) is now one of the most useful enzymes for the preparation of valuable chiral synthons. In order to overcome its seemingly unpredictable specificity, a simple and easy-to-use active site model was developed by Jones et al. in 1990 (Scheme 6).[39]

In the past, PLE has demonstrated some patterns of stereochemical discrimination that were apparently anomalous and that undermined confidence in its logical utilization in asymmetric synthesis. In particular, PLE exhibited reversals in stereoselectivity, such as changing from *R* center to *S* center ester preference within structurally similar series of substrates that were triggered by apparently trivial changes in substrate size or structure.[40] To overcome this unpredictable and fickle stereochemical behavior, several approaches to develop an active-site model of the enzyme have been reported. While those models provided an excellent beginning toward rationalizing PLE's specificity, only limited structural range of substrates were worked. However, Jones's active site model was capable of interpreting all aspects of enzyme's specificity and that would be predictive value for new structures that were potential PLE substrates.



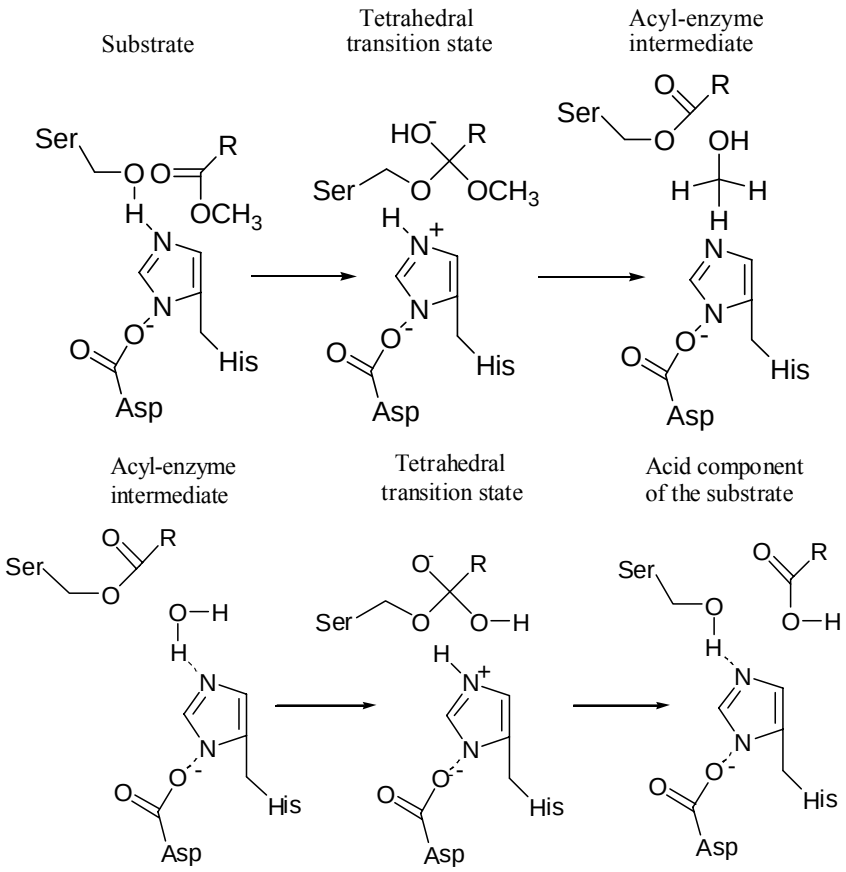
Scheme 6. Active Site Model for PLE

The boundaries of the model represent the physical constraints placed on the available substrate-binding volume by the amino acid residues of the enzyme. The important binding regions for specificity determinations are two hydrophobic pockets, H_L and H_S, and two pockets of more polar character, P_F and P_B. The best fit, if any, of a substrate is determined by locating the ester group to be hydrolyzed within the serine sphere and then placing, if possible, the remaining substrate moities in the H and P pockets according to the simple set of rules.

The model was constructed in cubic-space for several reasons. First, this approach has been used successfully in the development of active-site models for other enzymes.[41] Second, for an empirically derived model, a simple geometrical representations of the space is easiest to visualize and use. Furthermore, by creating the model in cubic space, any biochemical statement regarding the presence or absence of specific amino acid residues is avoided. Also, there is no temptation to align the bonds of the framework adopted, as is the case for diamond lattice[42] models.

The PLE available commercially is a mixture of isoenzymes. It was initially thought that the stereoselectivity reversals might be attributable to differences in the specificities of these isoenzymes. However, it has now been demonstrated that all the isoenzymes act in a more or less equivalent manner that for preparative purposes PLE can be used as though it were a single species.[43]

PLE is a serine hydrolase that catalyzes the hydrolysis of a wide range of ester structures with considerable specificity, and numerous stereoselective transformations of prochiral substrates[44] and some resolution of racemates[45] have been reported. PLE folds in a way that brings together histidine, aspartic acid and serine residues. Together, these residues constitute what is called the catalytic triad of the active site (Scheme 7).



Scheme 7. Catalytic Triad

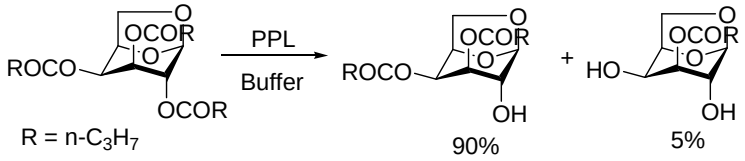
In the catalytic triad, after PLE has bound its substrate, the serine residue is ideally situated to attack the acyl carbon of the substrate. The serine residue is made more nucleophilic by transferring its proton to the imidazole nitrogen of the histidine residue. The imidazolium ion that is formed is stabilized by the polarizing effect of the carboxylate ion of the aspartic acid residue. Nucleophilic attack by the serine leads to an acylated serine through a tetrahedral intermediate. The new N-terminal end diffuses away and is replaced by a water molecule.

Regeneration of the active site of PLE is shown in the deacylation part. In this process water acts as the nucleophile and in a series of steps analogous to those in the acylation part, hydrolyzes the acyl-serine bond. Finally, the enzyme is ready to repeat the whole process.

1.7.2 Porcine Pancreatic Lipase (PPL, E.C. 3.1.1.3)

The cheapest and hence one of the most widely used lipases is isolated from porcine pancreas.[46-48] The crude preparation mostly used for biotransformations is called 'pancreatin' or 'steapsin' and it contains less than 5% protein. The main hydrolase-impurities are α-chymotrypsin, cholesterol esterase, carboxypeptidase B, phospho-lipases and other unknown hydrolases. Both of the former proteins – and also other unknown hydrolases – can impair the selectivity of a desired PPL-catalyzed ester hydrolysis by exhibiting a reaction of lower selectivity (or even of opposite stereochemisry). Thus, any models for PPL should be applied with great caution.[49,50] Indeed, it has been shown in some cases that these hydrolase impurities were responsible for the highly selective transformation of substrates, which were not, in fact, accepted by purified 'true PPL'.

Regioselective reactions are particularly important in the synthesis of biologically interesting carbohydrates, where selective protection and deprotection of hydroxyl groups is a central problem. Selective removal of acyl groups of peracylated carbohydrates from the anomeric[51] center or from primary hydroxyl groups,[52,53] leaving the secondary acyl groups intact, can be achieved with Porcine Pancreatic Lipase (Scheme 8).



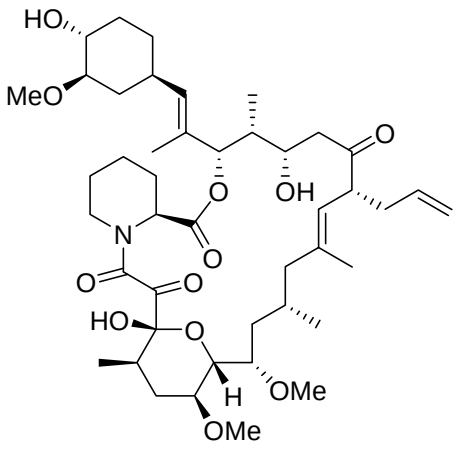
13 **14** **15**

Scheme 8. Regioselective Hydrolysis of Carbohydrate Esters By Porcine Pancreatic Lipase[54]

1.8 The Aim of The Work

The aim of the work is to synthesize the enantiomerically enriched precursor of the cyclohexanyl and cyclohexenyl nucleosides having the protection from resistance to hydrolysis since glycosidic bond cleavage is a frequently encountered degradative pathway of nucleoside antivirals and the cyclohexene ring in the nucleoside can be categorized as a bio(isostere) of a saturated furanose ring.

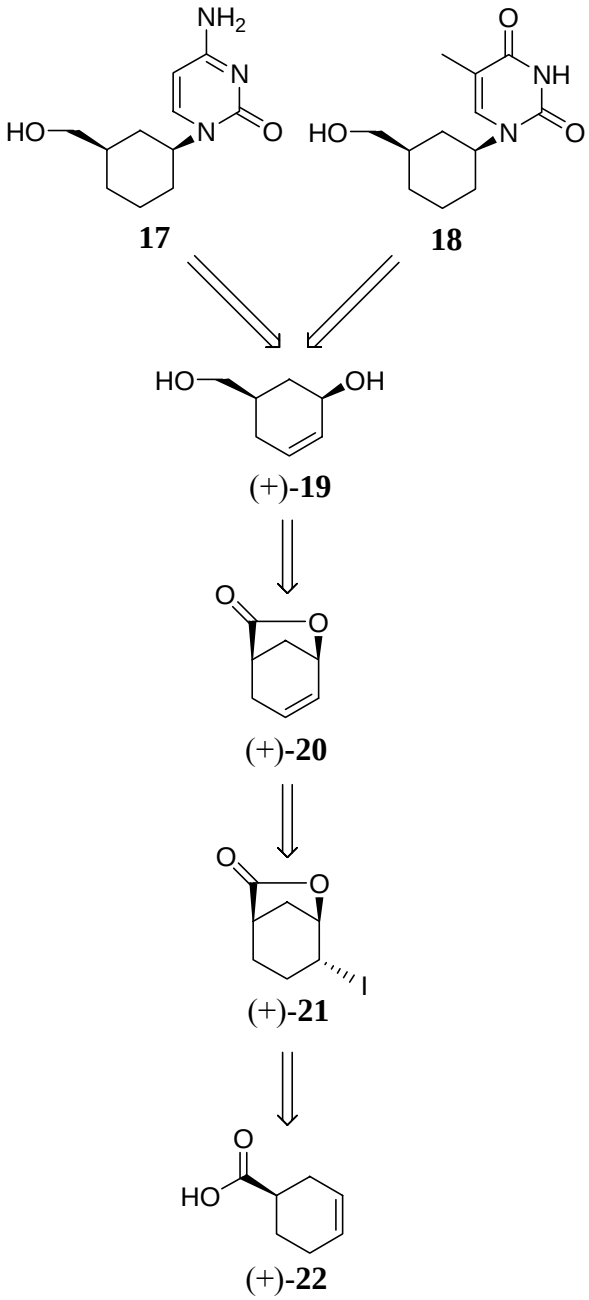
Apart from the usage of the 3-cyclohexene-1-carboxylic acid **(22)** in the synthesis of the cyclohexanyl and cyclohexenyl nucleosides, it can be also used in the synthesis of the unique immunosuppressant FK-506 **(16)**, isolated from *Streptomyces tsukubaensis*.



16

Scheme 9. FK-506

The aim of this work is shown retrosynthetically in the Scheme 10.



Scheme 10. Retrosynthesis of the Work

Our approach to enantiopure target molecule is to synthesize the enantiomerically enriched 3-cyclohexene carboxylic acid **(22)** by means of various enzymes, followed by iodolactonization, iodine elimination and lactone hydrolysis.

CHAPTER 2

RESULTS AND DISCUSSIONS

2.1 Perspective of the work

The development of new modified nucleosides as antiviral agents has remained a very active field of research.[55] Despite the fact that the carbocylic nucleosides have been extensively studied, few efforts have been directed toward the synthesis of six-membered carbocyclic analogues.[56]

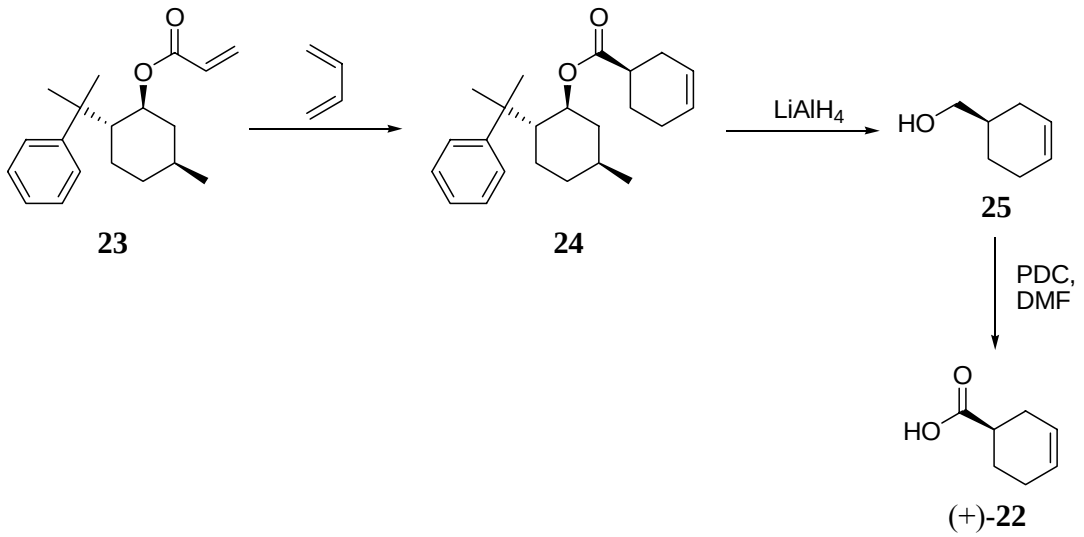
The major reasons which highlight the importance of six-membered carbocyclic nucleosides are:

- the protection from resistance to hydrolysis since glycosidic bond cleavage is a frequently encountered degradative pathway of nucleoside antivirals, particularly for 2', 3'-dideoxynucleosides[57] which have an ability to inhibit the cytopathic effect of the human immunodeficiency virus (HIV-1), the etiologic agent of AIDS.[58-61]

- a cyclohexene ring has structural properties that distinguish this ring system from common six-membered rings such as cyclohexane, cyclohexadiene, and benzene, and that allow us to categorize the cyclohexene ring as a bio(isotere) of a saturated furanose ring. Indeed, the conformational behaviour of a cyclohexene ring is similar to that of a saturated five-membered ring and this is caused by the presence of two sp^2-hybridized carbon atoms in the cyclohexene ring which reduce ring flexibility.[62]

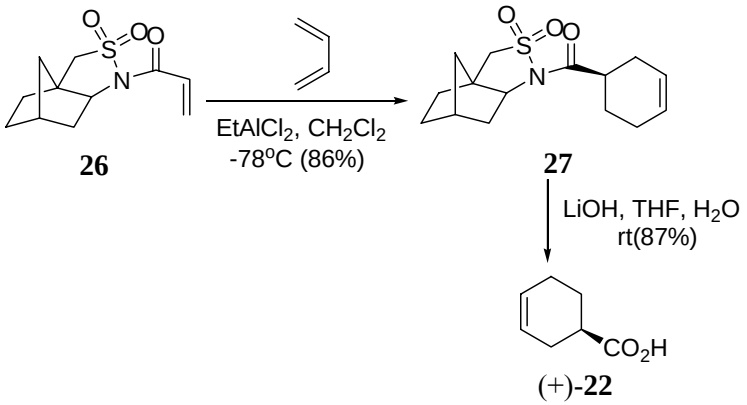
A recent article in the literature described the synthesis of several (±)-cis-substituted cyclohexenyl and cyclohexanyl nucleosides, starting from the commercially available precursor,[55] (±)-3-cyclohexene-1-carboxylic acid **(22)**, for which there are numerous number of asymmetric synthesis in the literature.

The asymmetric [4+2] cycloaddition between the acrylate ester of 8-phenylmenthol **(23)** and 1,3-butadiene and the reduction of the crude cycloadduct **24** with lithium aluminum hyride[63] gives (*R*)-3-cyclohexene-1-methanol **(25)**, treated with pyridinium dichromate in dimethyl formamide afforded the carboxylic acid (+)-**(22)**, which was estimated to be only approximately 73% enantiomerically pure (Scheme 11).[64]



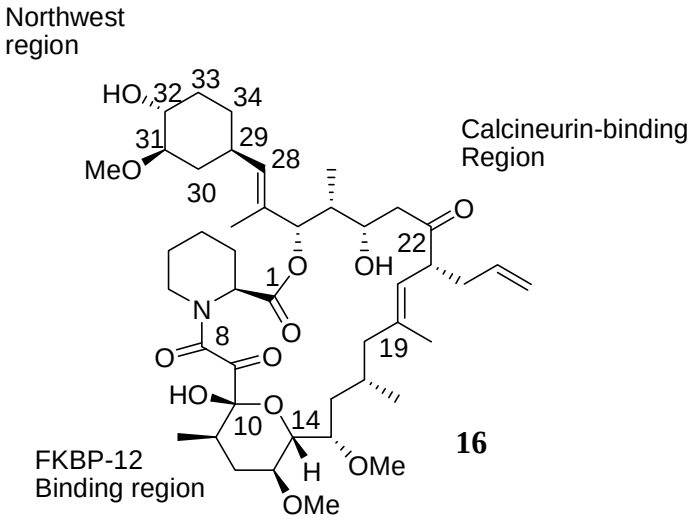
Scheme 11. Asymmetric Synthesis of (+)-**22**

A Lewis acid-catalyzed, asymmetric Diels-Alder reaction 1,3-butadiene with homochiral sultam **26** afforded the acid in good overall yield and 93% enantiomeric purity after base-promoted removal of the chiral auxiliary (Scheme 12).[65]



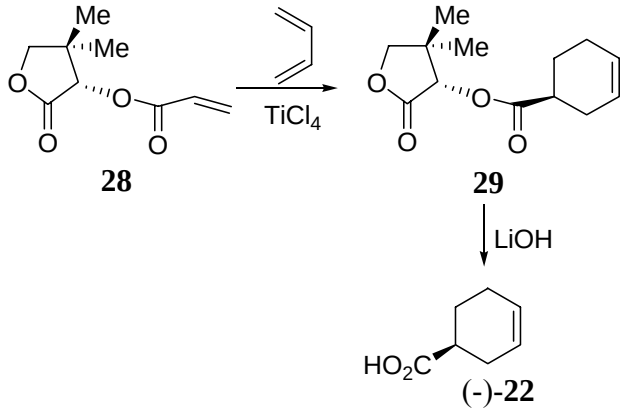
Scheme 12. A Lewis Acid-Catalyzed Asymmetric Synthesis of (+)-**22**

The unique immunosuppresant, 21-membered macrolactam FK-506 **(16)**, isolated from *Streptomyces tsukubaensis*, has C_{28}-C_{34} fragment that has been envisioned as an important builing block in the total synthesis of FK-506 and several non-racemic, multistep synthesis of this segment have been reported. Functionally FK-506 can be divided into three regions the FKBP-binding region, the calcineurin-binding region, and the northwest region. The intermolecular interactions between FK-506 and the FKBP-12 are mostly hydrophobic; only four intermolecular hydrogen-bonds are formed between the two molecules. It is known that as FK-506 and FKBP-12 bind more tightly they gain a greater affinity for calcineurin. This greater affintiy then leads to greater immunosuppresive activity. The northwest region of FK-506 does not interact with FKBP-12 or calcineurin. It was hoped that through modifications of this region the toxicity of FK-506 could be reduced.



Scheme 13. FK-506

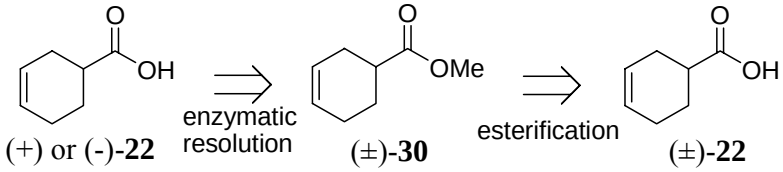
In the synthetic scheme, (*S*)-3-cyclohexene-1-carboxylic acid **(22)** was utilized for the synthesis of the C_{28}-C_{34} fragment of FK-506 starting from the $TiCl_4$-catalyzed asymmetric Diels-Alser reaction of 1,3-butadiene with the acrylic ester of the commercially available (*R*)-(-)-pantolactone. After removal (LiOH, THF, H_2O) of the chiral auxiliary, the crude (*S*)-acid was obtained (Scheme 14).[66]



Scheme 14. $TiCl_4$-Catalyzed Asymmetric Synthesis of (-)-**22**

2.2 Enzymatic Resolution of (±)-Metyhl 3-Cyclohexene-1-Carboxylate (30)

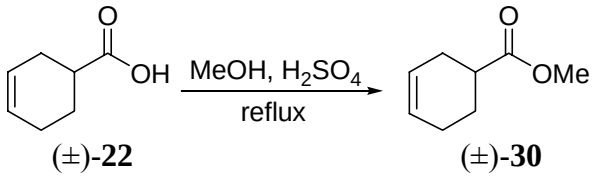
Our approach to enantiomerically enriched 3-cyclohexene-1-carboxylic acid **(22)** is that, firstly, esterification was carried out and then enzymatic resolution was performed. Scheme 15 shows the retrosynthetic pathway for the synthesis of enantiomerically enriched carboxylic acid.



Scheme 15. The Retrosynthetic Pathway For The Synthesis of Enantiomerically Enriched Carboxylic Acid

In order to get the target molecule, (1*R*,5*R*)-5-(hydroxymethyl)-2-cyclohexen-1-ol (**19)**, the commercially available racemic (±)-3-cyclohexene-1-carboxylic acid **(22)** was chosen since it is very appropriate for the enzymatic resolution after the esterification, the methyl ester is the most convenient functionality for the active site of enzymes, especially for PLE.

Firstly, for the esterification of the acid, a simple esterification procedure was carried out in the presence of MeOH and H_2SO_4 (Scheme 16).



(±)-**22** (±)-**30**

Scheme 16. Esterification of (±)-**22**

However, this procedure did not work. In fact, it is understood that a bicyclic saturated lactone was formed from this reaction and due to the unexpected products and isolation problems, it could not be identified by means of NMR, IR and other techniques except HRMS data which is matched with molecular mass of the lactone (Figure 6).

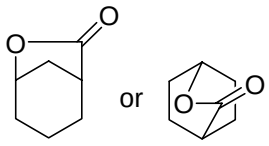
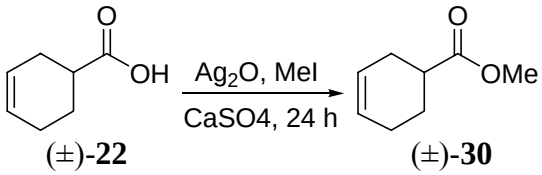


Figure 10. Examples of Saturated Bicylic Lactone

As an alternative way, in which the carboxylic acid was allowed to react with Ag_2O, MeI and $CaSO_4$, was performed to give the desired methyl ester of the carboxylic acid (Scheme 17).



Scheme 17. Esterification of (±)-**22** With Ag_2O and MeI

The reaction was monitored by TLC (Silica Gel, EtOAc/Hex 1:3). After the work-up, the desired product, (±)-metyhl 3-cyclohexene-1-carboxylate **(30)**, was obtained as a yellowish colored oil in quantitative yield.

The product was identified by using NMR spectroscopy. From the ^{1}H-NMR spectrum it is observed that a multiplet between 1.57-1.68 ppm, a multiplet between 1.89-1.98 ppm, a multiplet between 1.98-2.09 ppm, a multiplet between 2.18-2.25 ppm, a multiplet between 2.45-2.56 ppm, a singlet at 3.61 ppm for the methyl group and a triplet (J=13.2 and 11.7Hz) at 5.61 for the protons of the double bonds (Figure 11). From the ^{13}C-NMR spectrum, it is observed that eight singlets at 24.8 ppm for C_5, 25.4 ppm for C_2, 27.8 for C_6, 39.6 ppm for C_1, 51.9 ppm for the carbon of methyl group, 125.5 ppm for C_3, 127.0 ppm for C_4, 176.7 ppm for the carbon of carbonyl group (Figure 12).

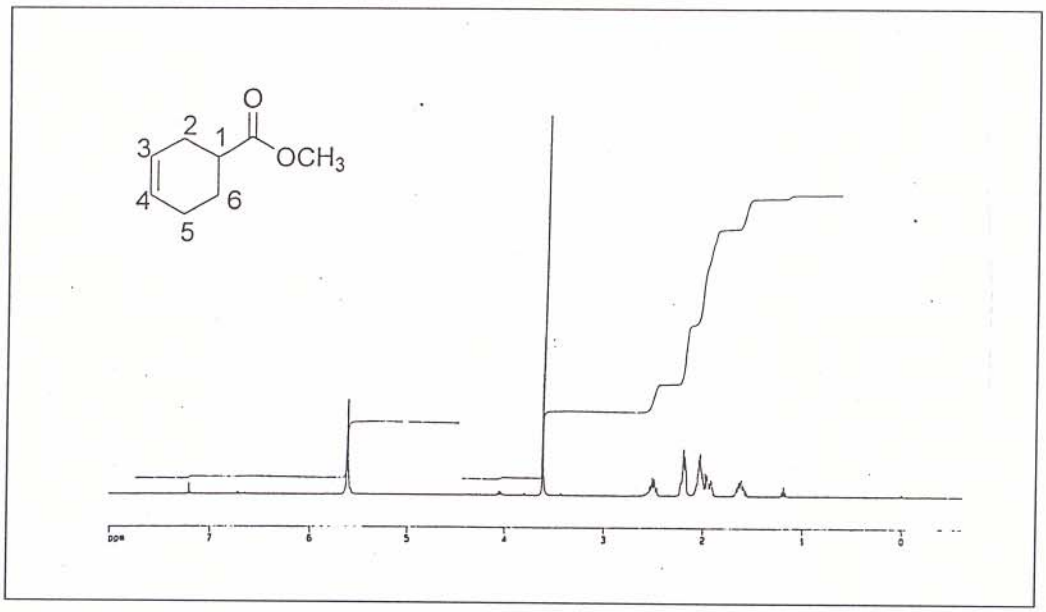


Figure 11. ^{1}H-NMR Spectrum of (±)-Metyhl 3-Cyclohexene-1-carboxylate **(30)**

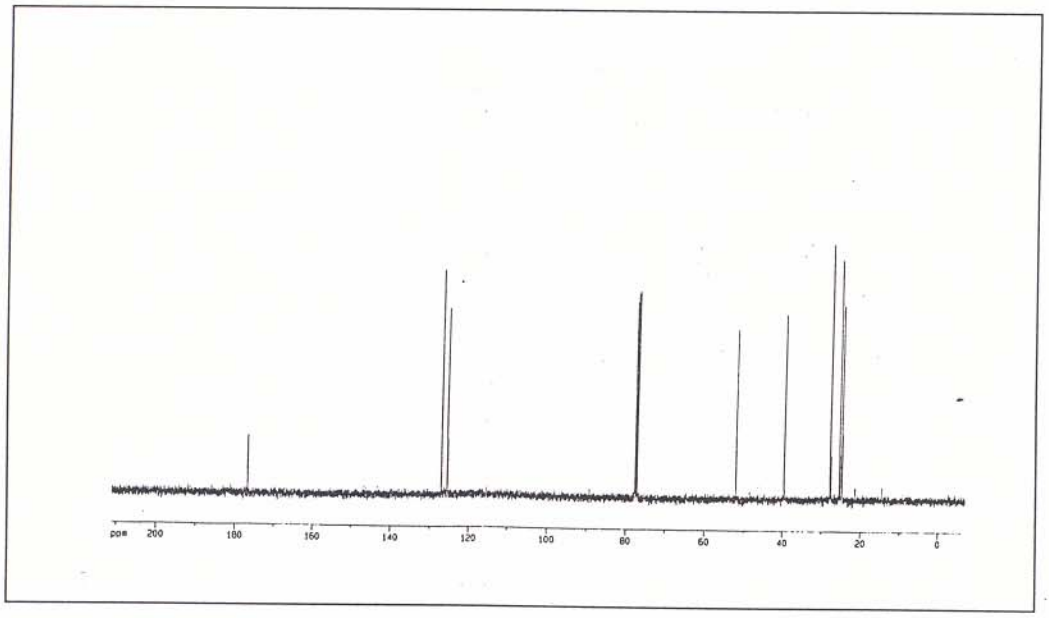
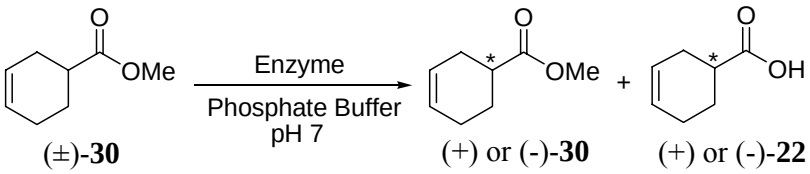


Figure 12. ^{13}C-NMR Spectrum of (±)-Metyhl 3-Cyclohexene-1-carboxylate **(30)**



(±)-**30** (+) or (-)-**30** (+) or (-)-**22**

Scheme 18. Enzymatic Hydrolysis of (±)-**30**

All enzymatic reactions were carried out in phosphate buffer (pH 7) at room temperature. The mixture was stirred at room temperature in the presence of enzyme. The reaction was monitored by TLC and when approximately 50% conversion was attained, the crude product was separated by flash column chromatography to afford the optically active carboxylic acid and the ester (Scheme 18). The products were elucidated by using NMR spectroscopy. From the ^{1}H-NMR spectrum of the 3-cyclohexene-1-carboxylic acid **(22),** it is observed that a multiplet between 1.64-1.71 ppm, a multiplet between 1.91-2.01 ppm, a multiplet between 2.01-2.13 ppm, a doublet (J=5.9Hz) at 2.22 ppm, a multiplet between 2.39-2.59 ppm, a triplet (J=12 Hz) at 5.60 ppm for the protons of the double bond and a broad singlet at 11.4 ppm dor the proton of the carboxylic acid functionality (Figure 13). From the ^{13}C-NMR spectrum, the signals are observed at 24.7 ppm for C_6, 25.2 ppm for C_5, 27.6 ppm for C_2, 39.5 ppm for C_1 ppm, 125.4 ppm for C_4, 127.1 ppm for C_3, 182.4 ppm for the carbon of carboxylic acid functionality (Figure 14). The optical rotation of enantiomerically pure (*S*)-3-cyclohexene-1-carboxylic acid **(22)** is -95 for c=7.0 in MeOH.[66]

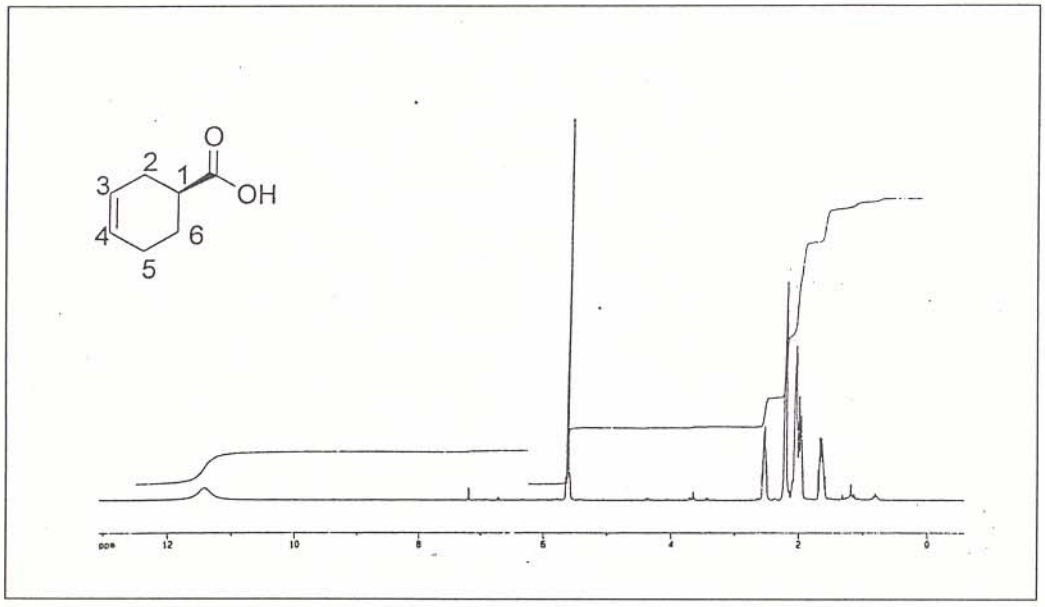


Figure 13. ^{1}H-NMR Spectrum of 3-Cyclohexene-1-carboxylic Acid **(22)**

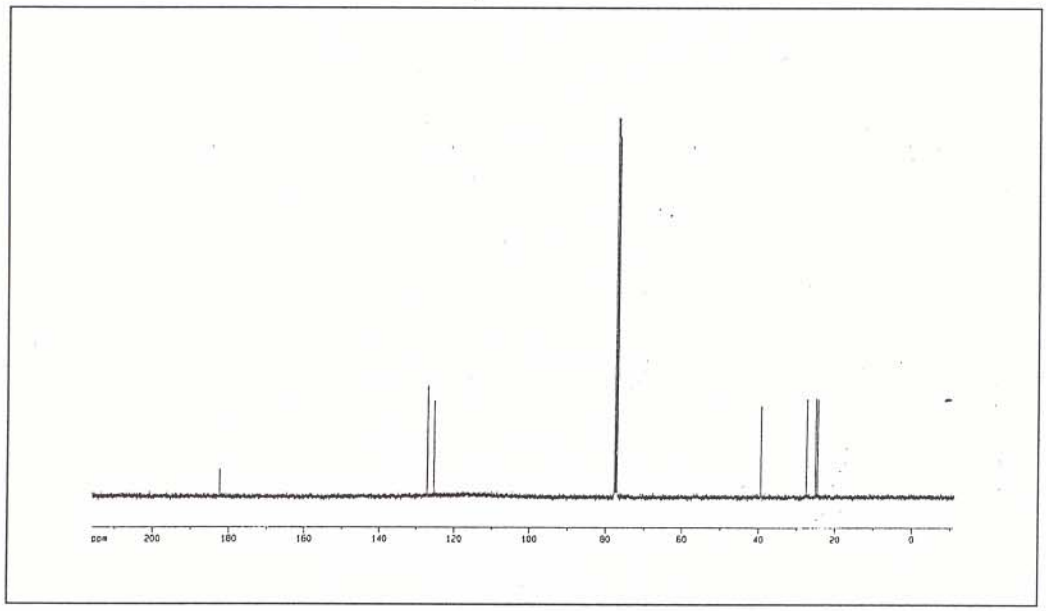


Figure 14. ^{13}C-NMR Spectrum of 3-Cyclohexene-1-carboxylic Acid **(22)**

During the course of the study on the biotransformation of (±)-metyhl 3-cyclohexene-1-carboxylate **(30)** screening reactions were first completed with various hydrolases (i.e. PLE, CCL, HLE and PPL) using substrate:enzyme ratio from 1:1 to 1:0.5. Among the hydrolases studied, PLE and HLE proved suitable for the enantioselective hydrolysis of this substrate. The observed promising preliminary results directed us towards catalytic reactions on the study. Thus, PLE and HLE catalyzed reactions afforded (*S*)-configurated carboxylic acids with 95% ee and 94% ee, respectively. In contrast to this, PPL yielded (*R*)-configurated carboxylic acid with 72% ee and showed unusual versatility and diversity in the enantioselective synthesis of the substrate. Unfortunately, the enantiomeric excesses of the unreacted methyl ester of the carboxylic acid was not determined due to the mismatching of the HPLC column type.

Table 3. Enzymatic Hydrolysis of the Ester **30** Using Hydrolase-Type Enzymes

	ACID			ESTER		
ENZYME	YIELD(%)	ee(%)	$[\alpha]^{20}_D$	YIELD(%)	ee(%)	$[\alpha]^{20}_D$
PLE	36	95(*S*)	-91.1	37	ND	+83.0
HLE	38	94(*S*)	-90.2	39	ND	+82.1
CCL	32	44(*S*)	-42.2	33	ND	+38.4
PPL	34	72(*R*)	+69.1	35	ND	-62.9

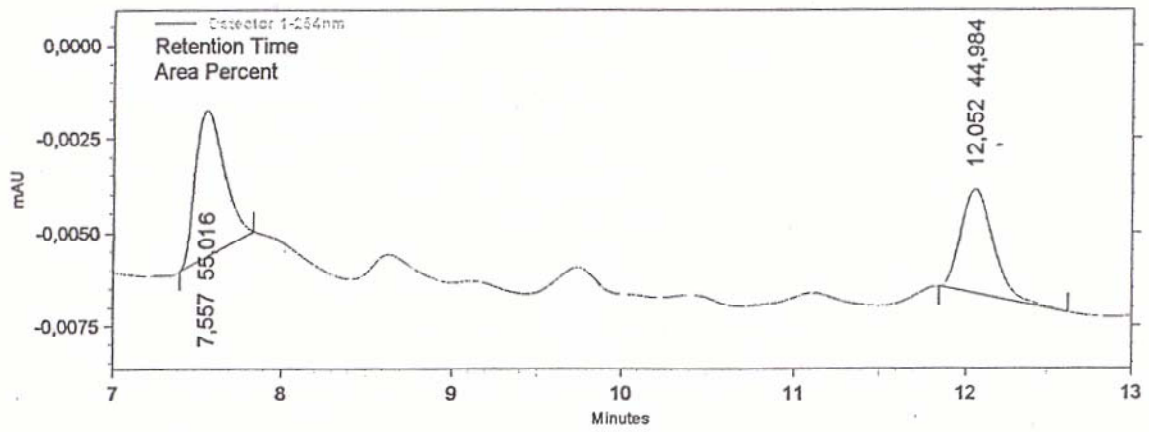


Figure 15. The HPLC-Chromatogram of Racemic 3-Cyclohexene-1-carboxylic Acid

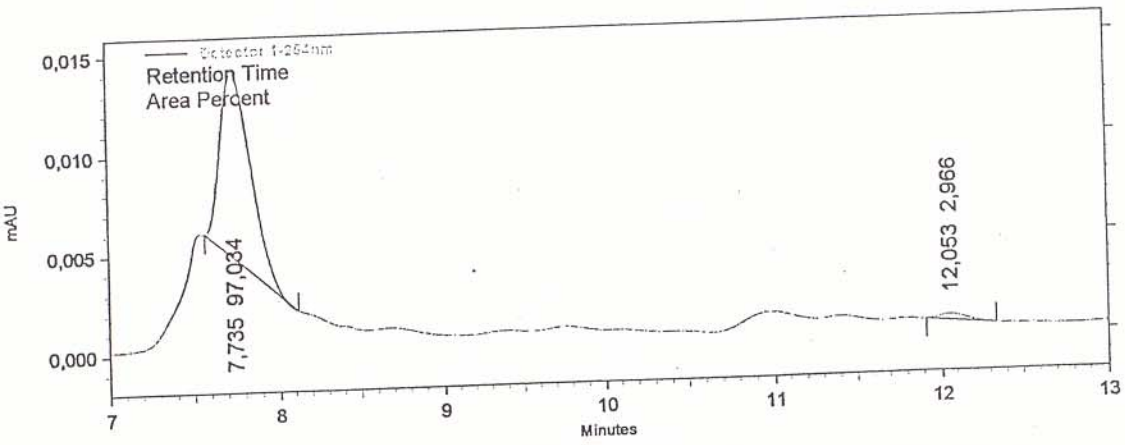


Figure 16. The HPLC-Chromatogram of (-)-**22** from PLE Hydrolysis

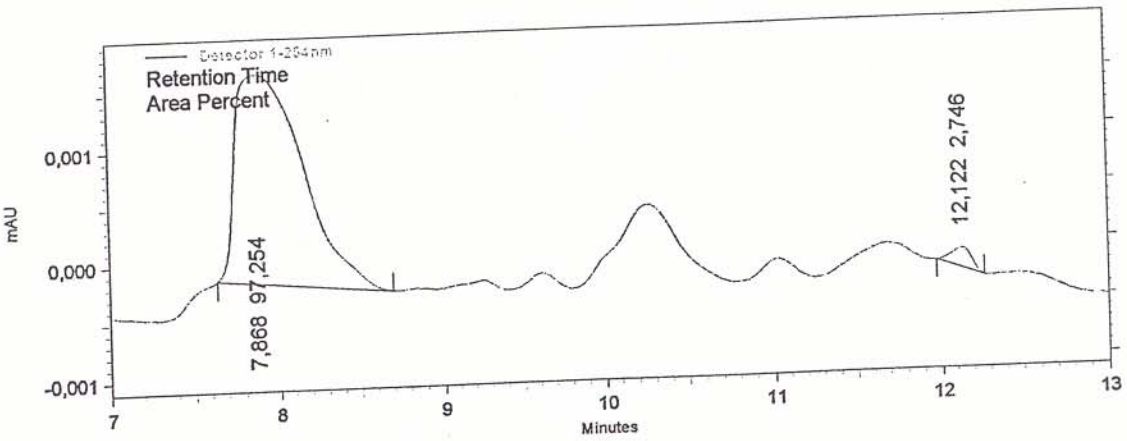


Figure 17. The HPLC-Chromatogram of (-)-**22** from HLE Hydrolysis

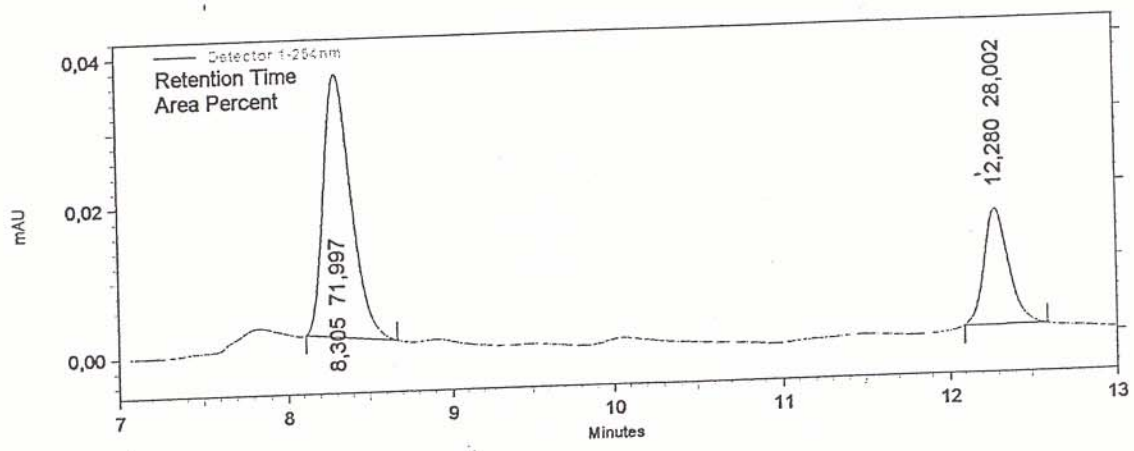


Figure 18. The HPLC-Chromatogram of (-)-**22** from CCL Hydrolysis

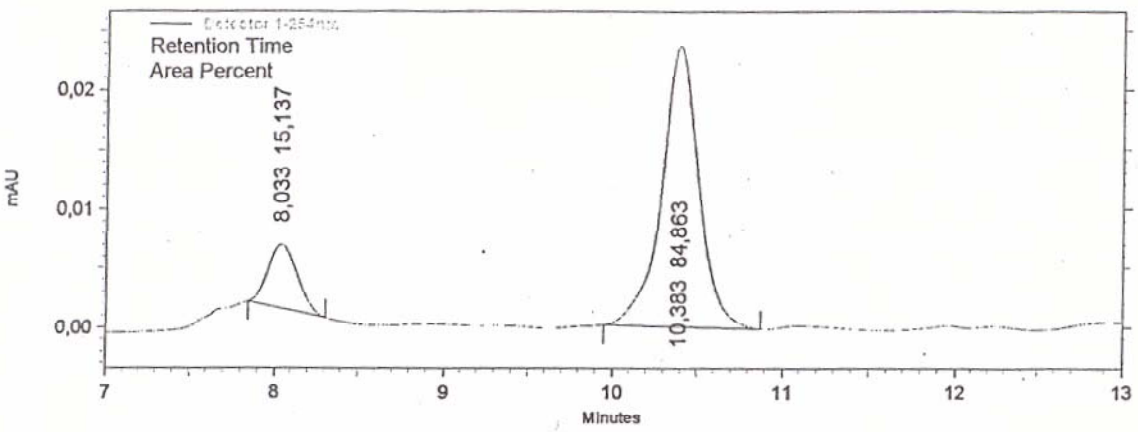
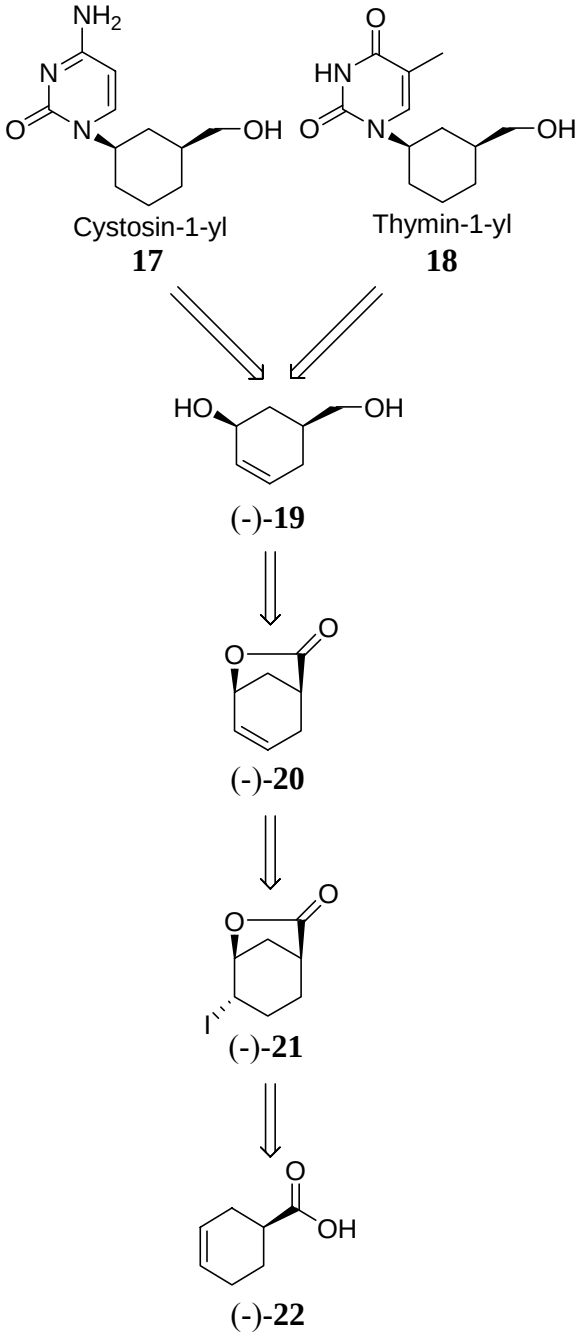


Figure 19. The HPLC-Chromatogram of (+)-**22** from PPL Hydrolysis

2.3 The Synthesis of the Nucleoside's Precursor

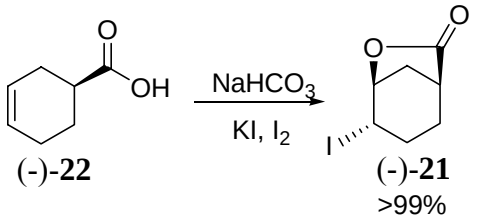
Once we obtained the enantiomerically enriched carboxylic acid, the next step is to synthesis of the nucleoside's precursor which is (-)-5-(hydroxymethyl)-2-cyclohexen-1-ol (**19)**.



Scheme 19. The Retrosynthesis of The Nucleoside's Precursor

2.3.1 Synthesis of (-)-2-Iodo-7-oxabicyclo[3.2.1]-octan-6-one (21)

In the first step, the optically active carboxylic acid was treated with $NaHCO_3$, KI and I_2. The reaction stirred for 24 h at room temperature. After work-up, the desired product was obtained as a white solid with quantitative yield because the geometry of the carboxylic acid does not allow to form another product (Scheme 20).



(-)-**22** (-)-**21** >99%

Scheme 20. Synthesis of (-)-**21**

The product was identified by using NMR spectroscopy. From the ^{1}H-NMR spectrum, it is observed that a multiplet between 1.72-1.79 ppm, a multiplet between 1.79-1.89 ppm, a doublet of doublet (J=16.4 and 5.1Hz) at 2.05 ppm, a multiplet between 2.28-2.44 ppm, a broad singlet at 2.60 ppm, a doublet (J=12.3 Hz) at 2.73 ppm, a broad triplet (J=4.6 and 5.4 Hz) at 4.76 ppm from the proton of C_2, a broad triplet (J=4.5Hz) at 4.44 ppm from the proton of C_1 (Figure 20). From the ^{13}C-NMR spectrum, the signals are observed at 25.5 ppm for C_4, 26.2 ppm for C_2, 32.1 ppm for C_3, 36.8 ppm for C_8, 40.9 ppm for C_5, 82.6 ppm for C_1 and 180.1 ppm from the carbonyl carbon (Figure 21). The melting point of (-)-2-iodo-7-oxabicyclo[3.2.1]-octan-6-one **(21)** is 132°C (lit. mp 135-136°C)[67] and has $[\alpha]^{25}_{D}$= -37.81° for c=1.92 in $CHCl_3$.[68]

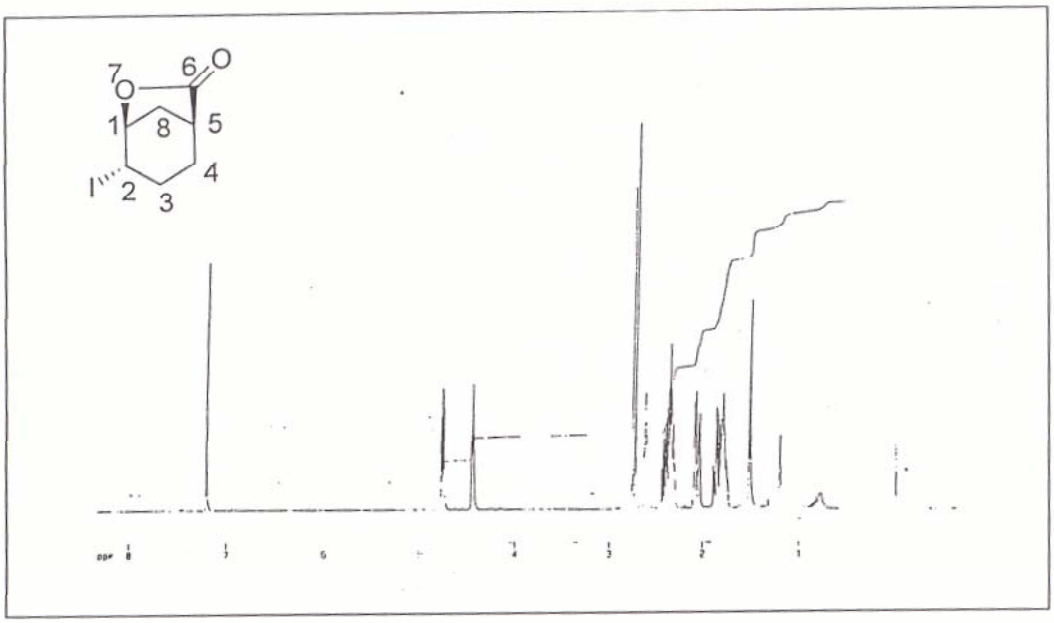


Figure 20. ^{1}H-NMR Spectrum of (-)-2-Iodo-7-oxabicyclo[3.2.1]-octan-6-one **(21)**

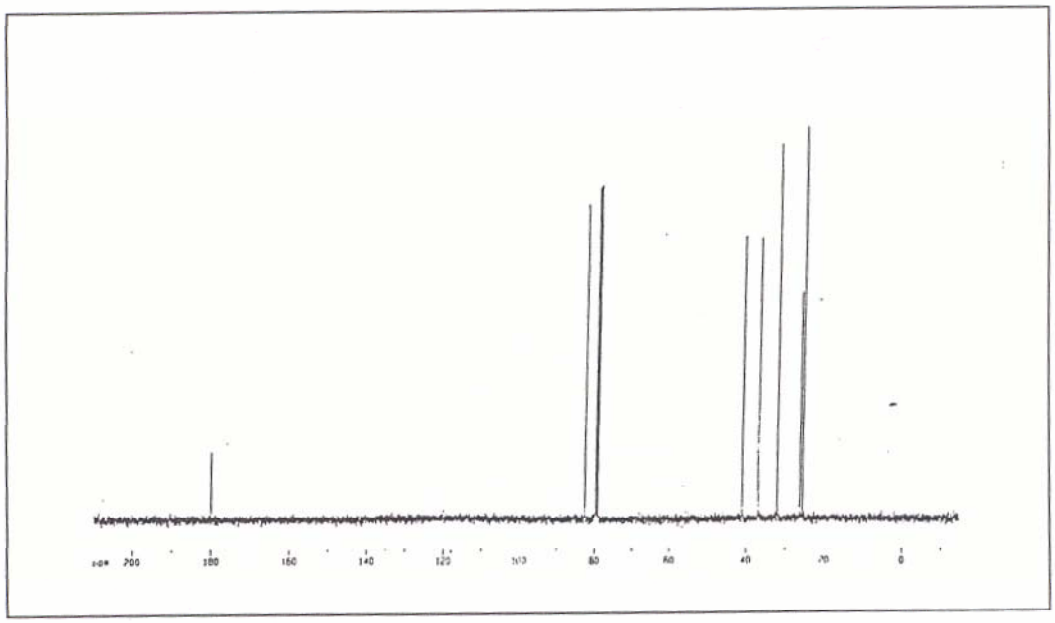
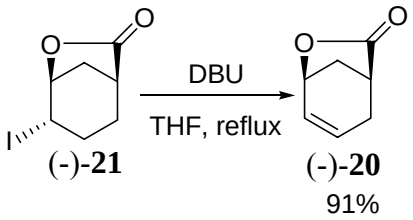


Figure 21. ^{13}C-NMR Spectrum of (-)-2-Iodo-7-oxabicyclo[3.2.1]-octan-6-one **(21)**

2.3.2 Synthesis of (-)-7-Oxabicyclo[3.2.1]oct-2-en-6-one (20)

The next step is to eliminate the iodide using 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU), and to afford the unsaturated lactone **(20)** (Scheme 21).



Scheme 21. Synthesis of (-)-**20**

The product was identified by using NMR spectroscopy. From the ^{1}H-NMR spectrum, it is observed that a doublet (J=11.2Hz) at 2.03 ppm, a multiplet between 2.34-2.51 ppm, a broad singlet at 2.85 ppm, a triplet (J=5.3Hz) at 4.69 ppm for the proton of C_1, multiplet at 5.78 ppm for the proton of C_3, a multiplet at 6.12 ppm for the proton of C_2 (Figure 22). From the ^{13}C-NMR spectrum, the signals are observed at 29.5 ppm for C_4, 34.8 ppm for C_8, 38.4 ppm for C_5, 73.7 ppm for C_1, 129.7 ppm for C_3, 130.6 ppm for C_2 and 179.8 ppm for the carbon of carbonyl group (Figure 23). (-)-7-oxabicyclo[3.2.1]oct-2-en-6-one **(20)** has $[\alpha]^{24}_{D}$= -182.12° for c=4.8 in $CHCl_3$.[68]

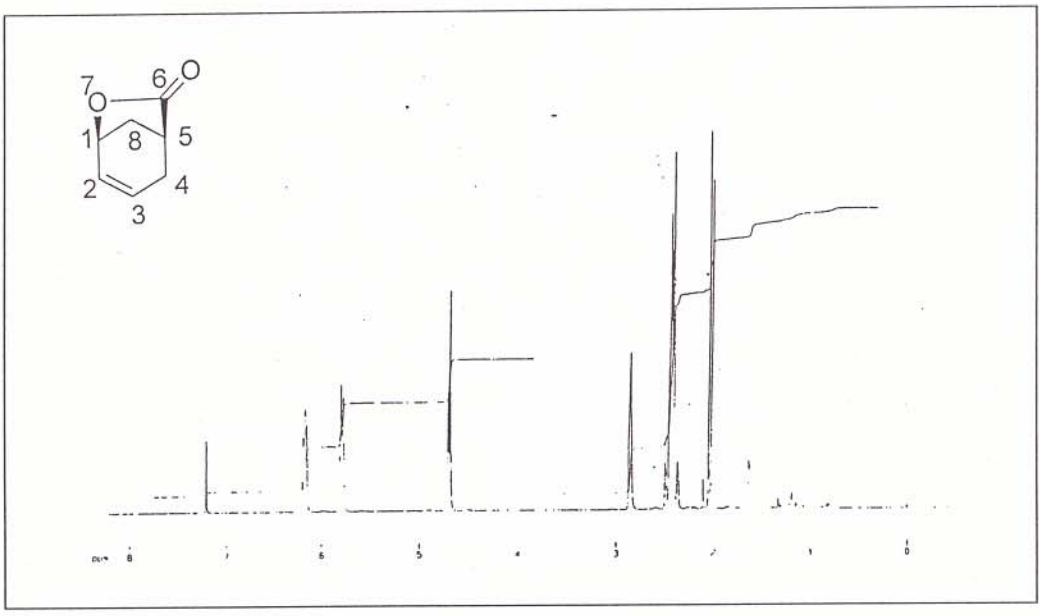


Figure 22. ^{1}H-NMR Spectrum of (-)-7-Oxabicyclo[3.2.1]oct-2-en-6-one **(20)**

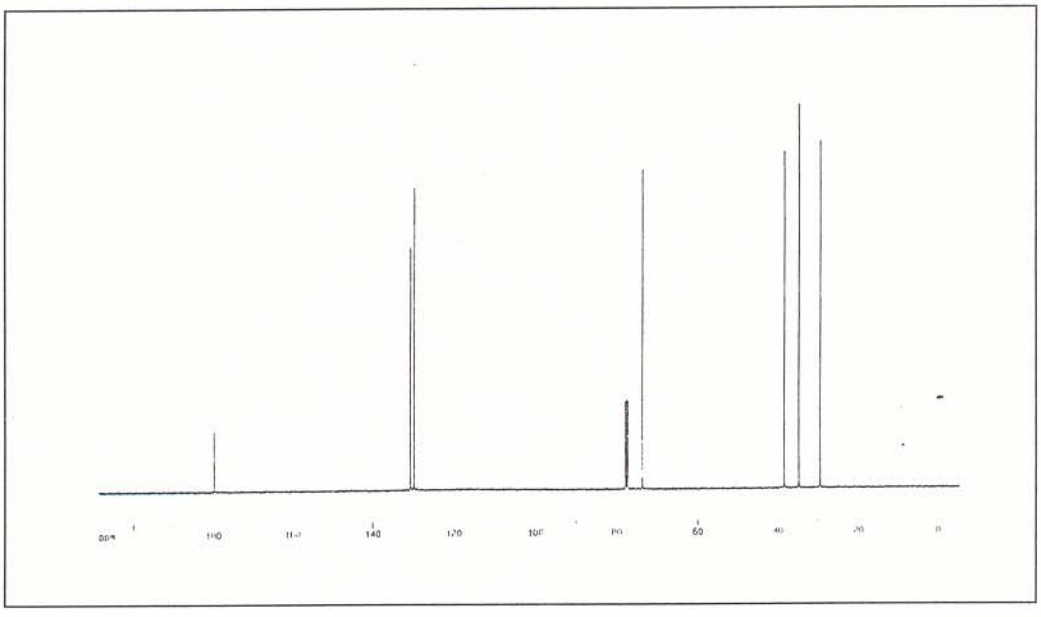
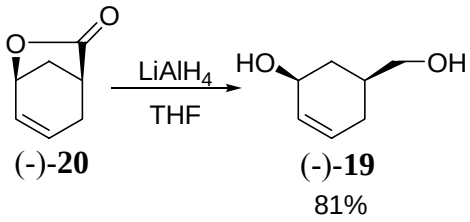


Figure 23. ^{13}C-NMR Spectrum of (-)-7-Oxabicyclo[3.2.1]oct-2-en-6-one **(20)**

2.3.3 Synthesis of (-)-5-(Hydroxymethyl)-2-cyclohexen-1-ol (19)

The final step, the reduction of unsaturated lactone with lithium aluminum hydride in THF, gives the desired product for the synthesis of nucleosides with 81% yield as a colorless semisolid (Scheme 22).



(-)-**20** (-)-**19**

81%

Scheme 22. Synthesis of (-)-**19**

The product was identified by using NMR spectroscopy. From the ^{1}H-NMR spectrum, it is observed that a multiplet between 1.15-1.27 ppm for the proton of C_5, a multiplet between 1.65-1.77 ppm for the proton of C_4, a multiplet between 1.77-1.88 ppm for the other proton of C_4, a multiplet between 1.96-2.11 ppm for the protons of C_6, a broad singlet at 2.89 ppm for the protons of the hydroxyl groups, a multiplet between 3.40-3.53 ppm for the protons of the methylene group attached to the hydroxyl group, a multiplet between 4.29-4.39 ppm for the proton of C_1, a broad doublet (J=10.2 Hz) between 5.58-5.64 ppm for the proton of C_3, a multiplet between 5.64-5.73 ppm for the proton of C_2 (Figure 24). From the C-NMR spectrum, the signals are observed at 28.4 ppm for C_4, 35.53 ppm for C_5, 35.56 ppm for C_6, 67.3 ppm for methylene carbon attached to the hydroxyl group, 67.4 ppm for C_1, 128.5 ppm for C_3, 131.4 ppm for C_2 (Figure 25). (-)-(1*S*,5*S*)-5-(hydroxymethyl)-2-cyclohexen-1-ol **(19)** has $[\alpha]^{23}_{D}$= -20.3 for c=1.46 in MeOH.[69]

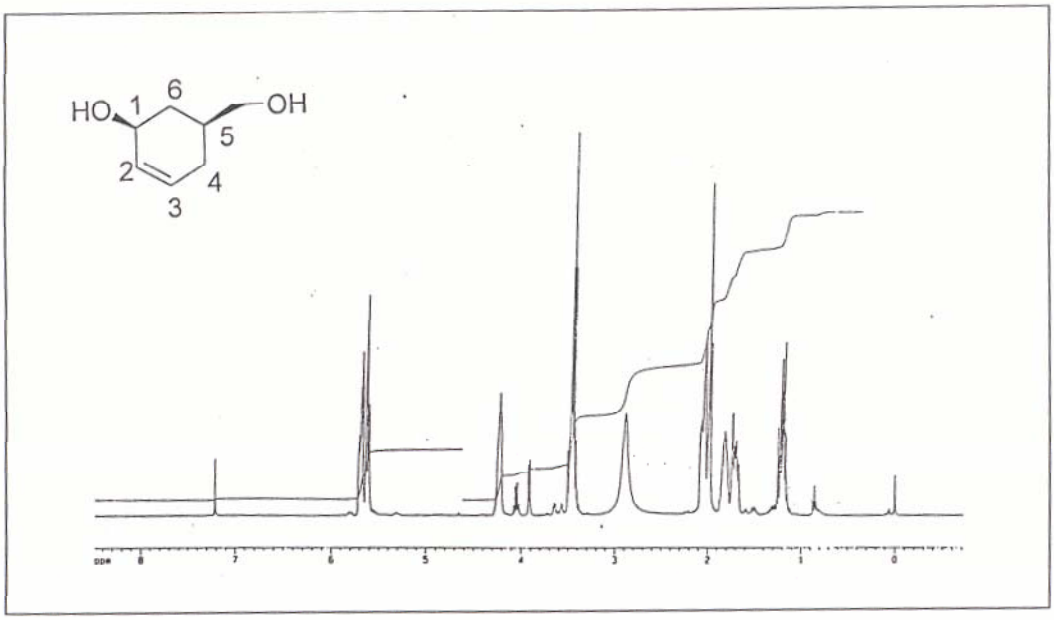


Figure 24. ^{1}H-NMR Spectrum of (-)-5-(Hydroxymethyl)-2-cyclohexen-1-ol **(19)**

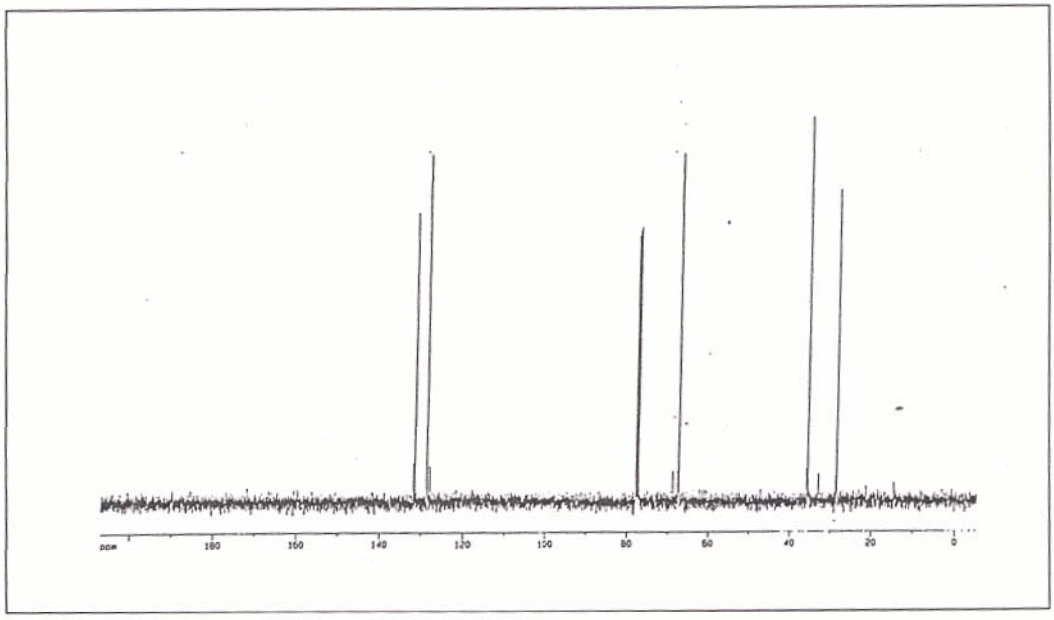


Figure 25. ^{13}C-NMR Spectrum of (-)-5-(Hydroxymethyl)-2-cyclohexen-1-ol **(19)**

CHAPTER 3

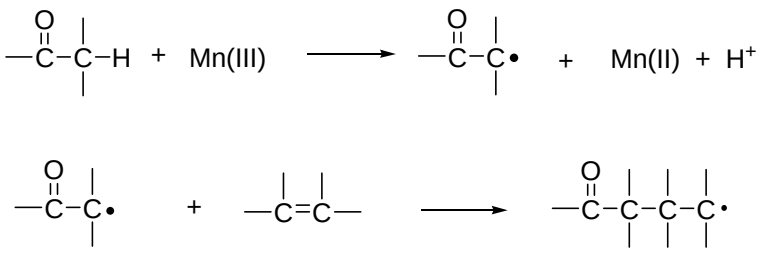
INTRODUCTION

3.1 Manganese(III) Acetate Oxidations

Oxidations with manganese(III) acetate can be broadly divided into two classes:

1. Direct inner- or outer-sphere one-electron oxidation of the substrate- after formation of an inner- or outer-sphere substrate-Mn(III) complexes. Often subsequent oxidation of an intermediate radical is product determining. Numerous examples can be found in oxidations of alcohols, amino and thio-compounds, carboxylic acids and certain aromatics.

2. Indirect oxidation of the substrate after formation of an intermediate adduct free radical from interaction of manganese(III) acetate and an enolizable compound and subsequent addition or substitution of this radical to the substrate. Manganese(III) acetate deals with addition reaction of compounds having α-hydrogen atom to a carbonyl group with olefinic and aromatic unsaturated systems (Scheme 23).



Scheme 23. Indirect Oxidation with Manganese(III) Acetate

Manganese(III) acetate, bear many similarities with respect to a given substrate class with other one-electron oxidants like Co(III), Ce(IV) and some two-electron oxidants like Tl(III) and Pb(IV). However, lower reactivity and higher selectivities is observed with manganese(III) acetate compared with the other oxidizing agents. Many of these reactions proceed according to the simplified scheme shown below:

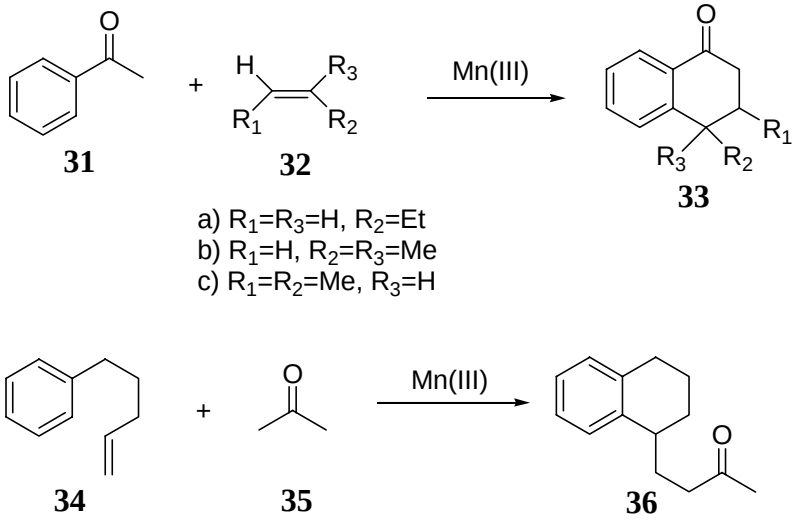
$$Mn(III) + \text{substrate} \rightarrow \text{intermediate radical } R\cdot + Mn(II)$$
$$Mn(III) + \text{intermediate radical } R\cdot \rightarrow \text{product} + Mn(II)$$

Scheme 24. The Simplified Reaction of Manganese(III) Acetate

Complications may arise in the presence of water, since water causes disproportionation of trivalent manganese into Mn(IV) and Mn(II) and alternative two electron oxidants may take place by Mn(IV).[70]

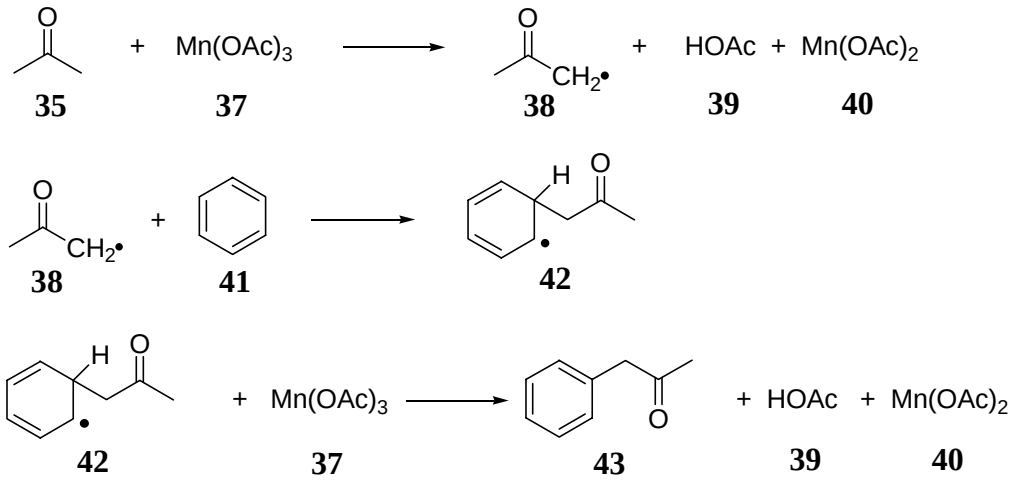
3.1.1 Examples of Manganese(III) Acetate Oxidations

Ketones, esters and aldehydes are oxidized by manganese(III) acetate to give oxoalkyl radicals that can add to olefins to form a variety of interesting compounds (Scheme 25).[71]



Scheme 25. Examples of Manganese(III) Acetate Oxidations

Manganese(III) acetate can be used in the aromatic substitution reactions. A number of methods have been reported for aromatic substitution reactions by radical generated by $Mn(OAc)_3$. Thus, carboxymethyl, acetonyl, and nitro methyl radicals readily substitute on the aromatic ring of suitable substrates. The substitution reactions of this type require two equivalents of $Mn(OAc)_3$ (Scheme 26).[72]



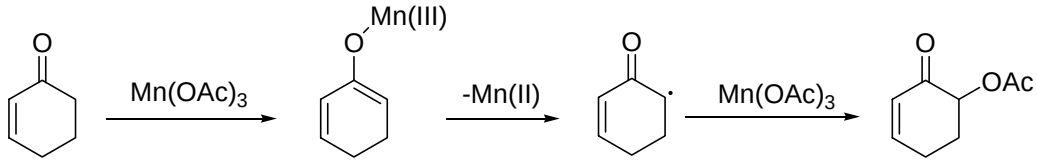
Scheme 26. The Substitution Reaction For Manganese(III) Acetate

In addition to its use in oxidative addition and substitution reactions, $Mn(OAc)_3$ constitutes a mild one-electron oxidant. Direct inner or outer-sphere one-electron oxidants with $Mn(OAc)_3$ in many cases proceed through the primary formation of an intermediate radical. The fate of this primary radical depends on the nature of the substrate and reaction conditions. Thus, with excess Mn(III) in many cases it is rapidly oxidized in a ligand transfer reaction to an acetate. However, the primary radical may dimerize, disproportionate, lose a proton, or enter in a sequence of transfer or addition reactions with other compounds, in a one-step procedure from substrates to products which otherwise require a multistep sequence.

Compounds that contain enolizable carbonyl groups are readily oxidized to α-keto radicals. In the absence of olefins or aromatics and depending on reaction conditions, these radicals can be further oxidized or couple to dimers. Thus, at high Mn(III) / substrate ratios in the presence of acetic acid mostly re-formed.[73] Dimer formation is favored at low Mn(III)/substrates ratios, high temperatures, and absence of acetic acid.[70,74]

3.1.2 The Suggested Mechanism for the Manganese(III) Acetate Oxidations of Enones

The reaction presumably proceeds via the formation of the Mn(III) enolate **45**, which loses Mn(II) upon one-electron oxidation to give α-keto radical **46**. The resultant tertiary radical is prone to further oxidation by another equivalent of $Mn(OAc)_3$. The final oxidation step provides α'-acetoxy-α,β-unsaturated ketones **47** (Scheme 27).



44 **45** **46** **47**

Scheme 27. The Suggested Mechanism for the $Mn(OAc)_3$ Oxidations of Enones

3.2 Lead(IV) Acetate Oxidations

For the oxidation of organic compounds, many reaction mechanisms seem possible. Besides lead tetraacetate itself, acetoxy radicals, acetoxy cations, and $Pb(OAc)_3^+$ ions have been regarded as the oxidizing species.

Lead tetraacetate itself could react as an electrophile using empty orbitals of the lead. A transient increase in the coordination number of the lead is indicated by the rapid exchange of the acetoxy groups between lead tetraacetate and acetic acid or acetic anhyride,[75] but this can also be explained by a reversible dissociation of $Pb(OAc)_4$. However, the solubility of lead tetraacetate in acetic acid is decreased rather than increased by sodium or potassium acetate.[75,76] Thus the complex anions $Pb(OAc)_5^-$ or $Pb(OAc)_6^{2-}$ are not present in appreciable concentrations. Using empty orbitals of the lead, the unshared electron pair of an alcohol or phenol could initiate the oxidation of these substances, as could the formation of a π-complex with an olefin.[77]

Acetoxy radicals have been regarded as intermediates in many tetraacetate oxidations. They might be formed by dissociation, the lead tetraacetate losing one or two acetoxy radicals. In the first case, the trivalent lead formed would have to be very short lived and should itself have radical character.

Acetoxy cations have been postulated as an active species, particularly by Mosher and Kehr.[78] However, acetoxy cations certainly do not exist free. Moreover, the result of the reaction of lead tetraacetate with organomercury compounds makes it unlikely that acetoxy cations are produced at all.

On the other hand, many oxidations can be understood by assuming that lead tetraacetate loses an acetate ion, forming $Pb(OAc)_3^+$. An electrophilic attack of the latter forms an organolead compound with the substrate. Loss of lead diacetate completes the reaction, and an AcO^+ moiety has been transferred without ever existing as a species.

In other cases the organolead compound $R\text{-}Pb(OAc)_3$ loses $Pb(OAc)_3^-$ which seems to be a good leaving group.[79] The electrophilic introduction of $Pb(OAc)_3^+$ followed by its loss as anion corresponds to the loss of one electron pair.[80]

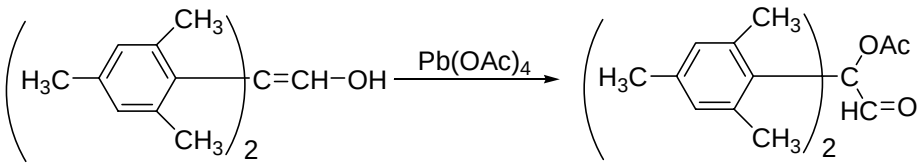
Depending on substrate and reaction conditions, different mechanisms might be operative. The solvent is especially important as pointed out by Barron *et al.*[81] The solvent used most frequently, acetic acid, has an intermediate dielectric constant and might equally favor ionic and radical mechanisms.

3.2.1 Reactions of Lead(IV) Acetate with C-H Bonds

Reactions of lead tetraacetate with C-H bonds usually result in substitution by an acetoxy group, but dehydrogenation and other reactions are also known. Generally, only activated C-H bonds are attacked. C-H bonds are activated toward lead tetraacetate by adjacent carbonyl groups, aromatic rings, or C=C double bonds.

Lead tetraacetate converts carbonyl compounds to α-acetoxy derivatives. The reactivity increases in the sequence: acid anhydride < ester < ketone. It is especially

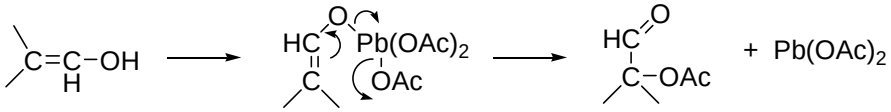
high when two activating groups are adjacent to the C-H bond. The tendency to enolize increases in the same sequence, so that one is tempted to assume that the enols are the species that are actually attacked. This assumption is supported by a number of observations. The first comes from Fuson's[82] studies of compounds which can exist only as phenols. In acetic acid at 40ºC they are oxidized almost quantitatively to α-acetoxy aldehydes (Scheme 28).



48 **49**

Scheme 28. The Oxidation of the Enol to α-Acetoxy Aldehydes with $Pb(OAc)_4$

The mechanism should be as below:



Scheme 29. The Mechanism of the Oxidation with $Pb(OAc)_4$

Second, Ichikawa and Yanaguchi[83] found that the rate of oxidation of ketones depends only on the concentration of ketones, not on that of lead tetraacetate. As with the bromination of ketones, the rate determining step is the enolization.

Third, Henbest and associates[84] observed that the oxidation of ketones is strongly accelerated by boron trifluoride, so much that it can be accomplished in benzene at room temperature. They explain the catalysis as an acceleration of enol formation due to the boron trifluoride. In addition, however, boron trifluoride could increase the dissociation of lead tetraacetate and thus the formation of the cation $Pb(OAc)_3^+$.

If a radical mechanism was operative, one would expect the formation of dehydrodimers R-CO-CHR'-CHR'-CO-R. Simple ketones do not give such products in the lead tetraacetate oxidation, but they do when treated with diacylperoxides.

The lead tetraacetate oxidation is a general method for the preparation of α-acetoxy-ketones. In addition to mono-acetoxylation, some di-acetoxylation may occur, α-α' where such position are available. Correspondingly, α-acetoxy ketones react to yield α,α'-diacetoxy compounds. The reactions are faster in acetic acid but give better yields in benzene.

CHAPTER 4

RESULTS AND DISCUSSIONS

4.1 Perspective of the Work

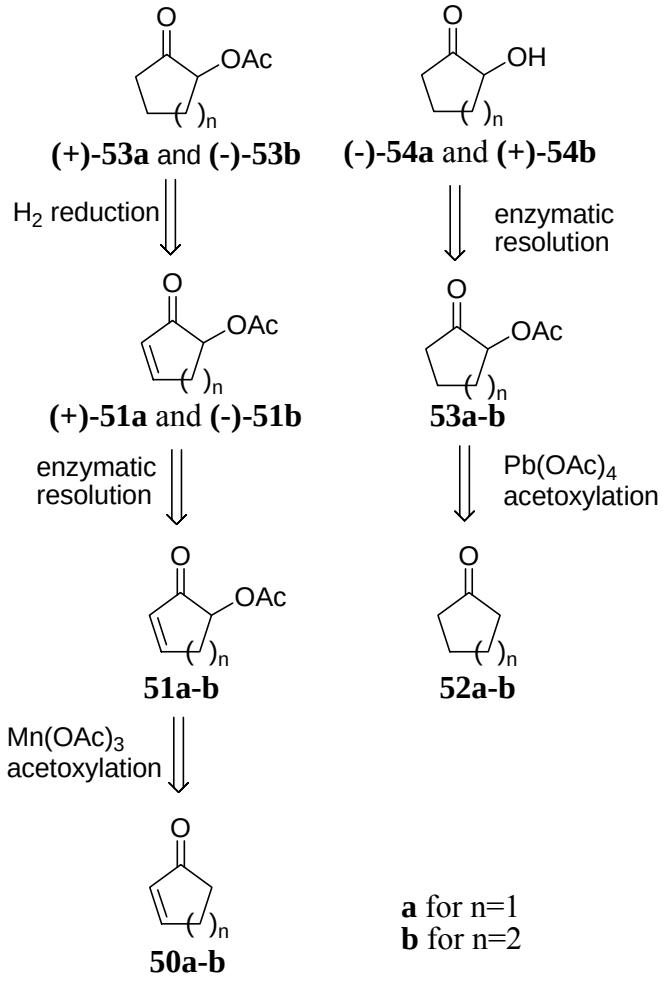
Synthetic methods for the selective oxidation of common functional groups possess a central position in the synthesis of various complex natural products. In particular, the utilities of (±)-2-hydroxy and (±)-2-acetoxy cyclic ketones have been well proven in practice[85] and several methods for synthesis of them are known in the literature.[86]

The acetate group can serve as useful protective group for the hydroxy functions in α-hydroxy ketones.[87,88] The α-acetoxy ketones can be prepared via various ways which include the reaction of α-bromo ketones with carboxylate ions,[89] the oxidation of morpholine enamine with thallium(III) triacetate,[90] anodic oxidation of enol acetates in acetic acid,[91] and $Cu(acac)_2$ catalyzed insertion reactions of α-diazo ketones with carboxylic acids.[92] In addition, it has been reported that the solvolytic reaction of α-keto triflate in acetic acid or formic acid can provide corresponding α-acyloxy ketones,[93] but potential difficulties in preparing α-triflyloxy ketone precursors (e.g. α-triflyloxy propiophenone) limits their further synthetic applications. There exist only a few methods that deal with the direct preparation of α-acetoxy ketones from ketones. These involve the oxidation of ketones with lead (IV) tetraacetate,[94] the oxidation of ketones with manganese(III) triacetate in acetic acid,[95] and the oxidation of aromatic ketones with hypervalent

iodine reagent followed by solvolysis in acetic acid in the presence of silver carbonate.[96]

In addition to this, several studies have been directed to the stereoselective synthesis of enantiomerically pure α-hydroxy ketones,[97] since these compounds are important synthons in the asymmetric synthesis of natural products and fine chemicals.[98] A proficient direct method consists in the asymmetric oxidation of enolates.[99-101] Thus, by using enantiomerically pure N-sulfonyloxaziridines, Davis and co-workers[99] were able to achieve good to excellent enantioselectivities in reagent controlled[98d] asymmetric oxidation of prochiral enolates. On the other hand, in the chiral auxiliary approach, the diastereoselective oxidation of chiral enolates has been performed using oxidants such as achiral sulfonyloxaziridines, dibenzyl peroxydicarbonate, and Vedejs' MoOPH reagent.[100] Along these lines, an important addition has been devised by Sharpless and co-workers;[101] indeed, these authors have shown that α-hydroxy ketones in high enantiomeric excess can be obtained by the well-established osmium-catalyzed asymmetric dihydroxylation (AD)[102] of the corresponding enol ethers or silyl enol ethers.[101]

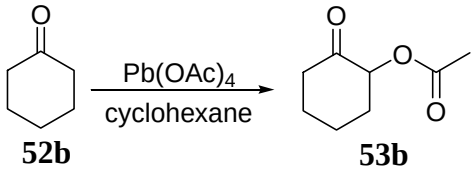
Herein, we tried to describe the synthesis of enantiomerically enriched α′-acetoxy-α,β-unsaturated cyclic ketones via enzymatic resolution of manganese(III) triacetate acetoxylated α,β-unsaturated cyclic ketones and, α-hydroxy and α-acetoxy cyclic ketones via enzymatic hydrolysis of lead(IV) tetraacetate acetoxylated cyclic ketones. The retrosynthetic pathway is shown below:



Scheme 30. Retrosynthetic Pathway for the Work

4.2 Enzymatic Resolution of (±)-2-Acetoxycyclohexanone (53b)

The (±)-2-acetoxycyclohexanone **(53b)** was synthesized from the treatment of cyclohexanone with $Pb(OAc)_4$ in cyclohexane (Scheme 31).



Scheme 31. $Pb(OAc)_4$ Oxidation of Cyclohexanone

The reaction was monitored by TLC (Silica Gel, EtOAc/Hex 1:3) through 12 hours. After the work-up, (±)-2-acetoxycyclohexanone **(53b)** was obtained as the only product in 92% yield and it was a colorless liquid.

The product was identified by NMR spectroscopy. From the ^{1}H-NMR spectrum, we observed a multiplet between 1.48-1.62 ppm, a multiplet between 1.63-1.77 ppm, a multiplet between 1.85-1.95 ppm, a multiplet between 1.98-2.06, a singlet at 2.08 ppm for the hydrogens of the acetoxy group, a multiplet between 2.19-2.26 ppm, a multiplet between 2.27-2.38 ppm, a multiplet between 2.41-2.48 ppm and a multiplet between 5.05-5.14 ppm for the hydrogen of alpha positioned carbon (Figure 26). From the ^{13}C-NMR spectrum, we observed the signals at 204.8 ppm for the carbonyl carbon of the acetoxy group, 170.4 ppm for the C_1, 76.9 ppm for the C_6, 41.1 ppm for the C_2, 33.4 ppm C_5, 27.5 ppm for the C_3, 24.1 ppm for the methyl carbon of the acetoxy group, 21.1 ppm for the C_4 (Figure 27).

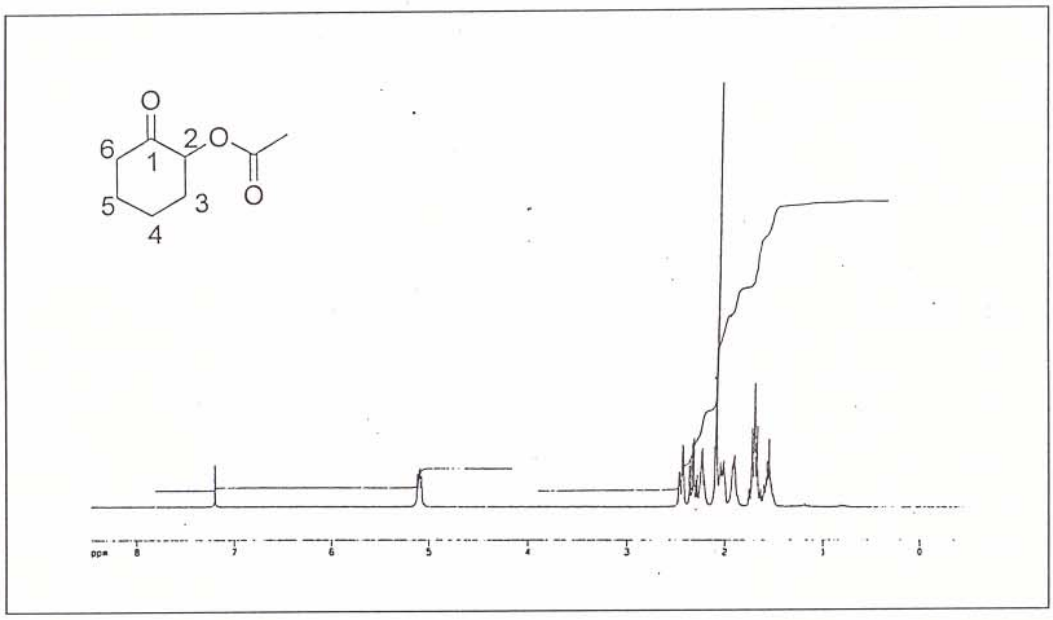


Figure 26. ^{1}H-NMR Spectrum of 2-Acetoxycyclohexanone **(53b)**

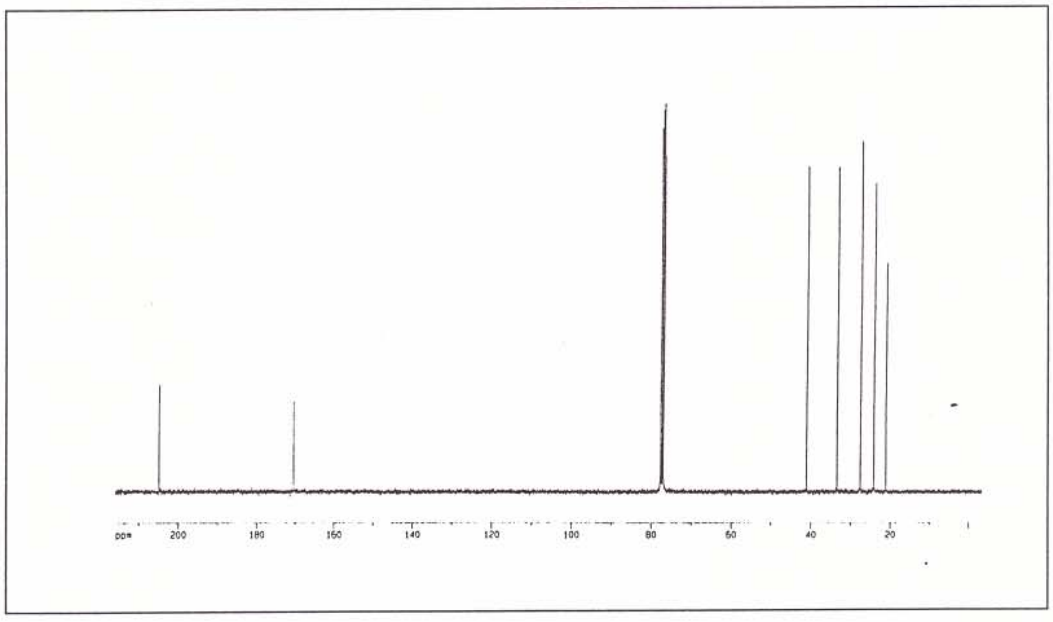
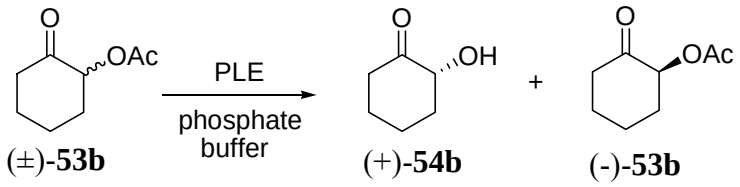


Figure 27. ^{13}C-NMR Spectrum of 2-Acetoxycyclohexanone **(53b)**

During the course of the study on all biotransformations, screening reactions were first completed with various hydrolases (i.e. PLE, CCL, HLE and PPL) using substrate:enzyme ratio from 1:1 to 1:0.5. Among the hydrolases studied, PLE proved suitable for the enantioselective hydrolysis of the substrates. The observed promising preliminary results directed us towards catalytic reactions on the study.

Then, the enzymatic hydrolysis of (±)-2-acetoxycyclohexanone **(53b)** was carrried out with PLE. The reaction was ended in 8 hours when approximately 50% conversion was attained. The crude product was seperated by flash column chromatography to afford the enantiomerically enriched (+)-2-hydroxycyclohexanone **(54b)** and (-)-2-acetoxycyclohexanone **(53b)** (Scheme 32).



Scheme 32. Enzymatic Hydrolysis of (±)-**53b** with PLE

(+)-2-Hydroxycyclohexanone **(54b)** was identified by NMR spectroscopy. From the ^{1}H-NMR spectrum, we observed a multiplet between 1.35-1.88 ppm, a multiplet between 2.01-2.10 ppm, a multiplet between 2.24-2.34 ppm, a multiplet between 2.35-2.45 ppm, a multiplet 2.46-2.55 ppm, a broad singlet at 2.80 ppm for the hydrogen of the hydroxyl group and a multiplet between 4.00-4.15 ppm of the hydrogen of C_6 (Figure 28). From the ^{13}C-NMR spectrum, we observed the signals at 211.7 ppm for the carbonyl carbon, 75.7 ppm for the C_6, 39.8 ppm for the C_2, 37.1 ppm for the C_5, 27.9 ppm for the C_3, 23.8 ppm for the C_4 (Figure 29). According to the chromatogram of (+)-2-hydroxycyclohexanone **(54b)**, it was obtained with 99% enantiomeric excess (Figure 31).

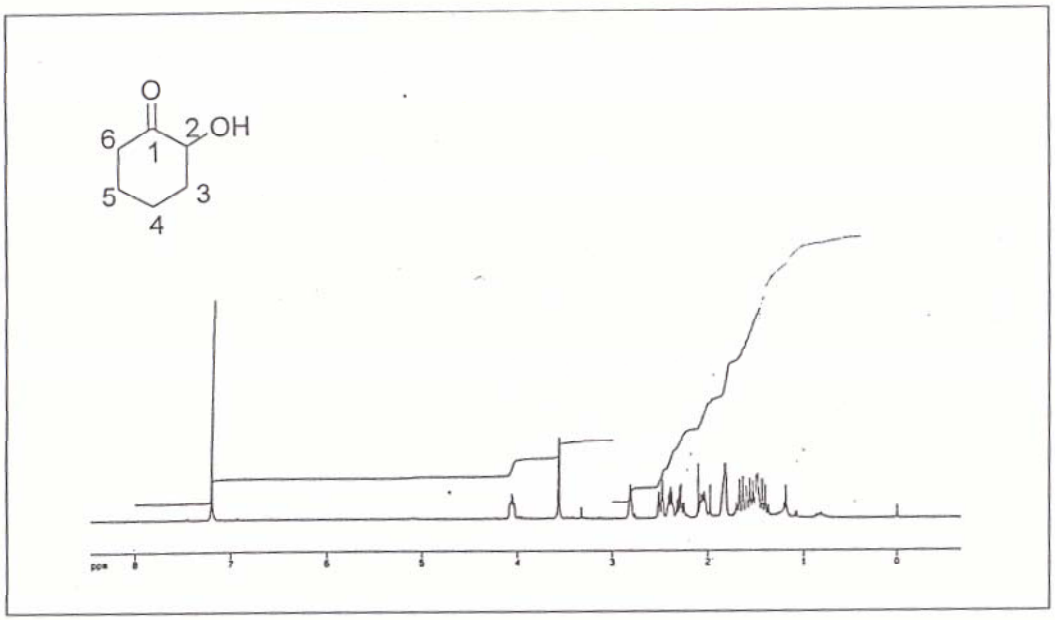


Figure 28. ^{1}H-NMR Spectrum of 2-Hydroxycyclohexanone **(54b)**

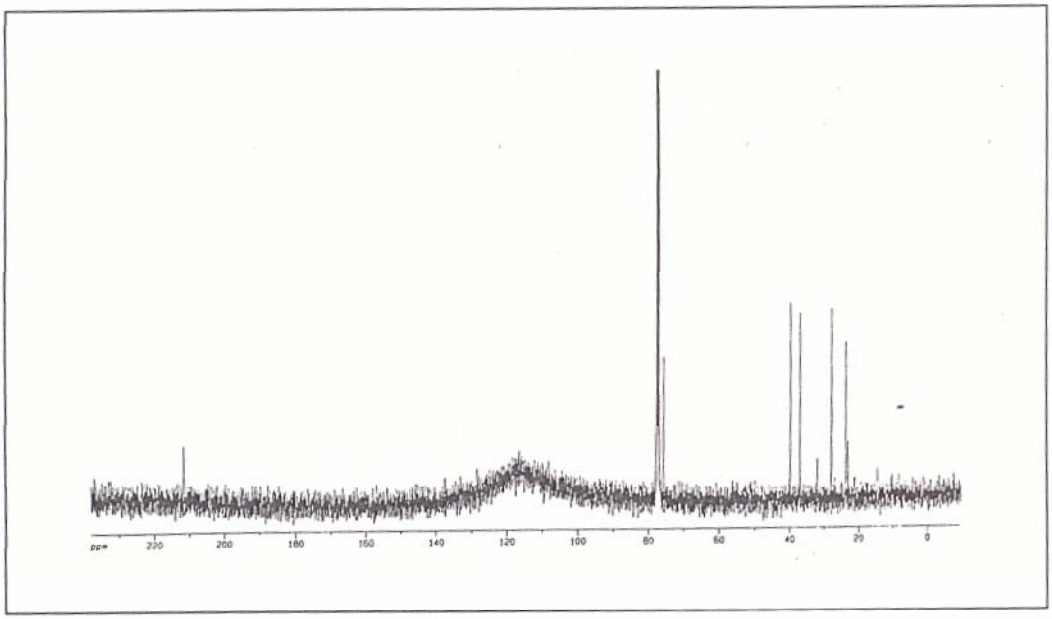


Figure 29. ^{13}C-NMR Spectrum of 2-Hydroxycyclohexanone **(54b)**

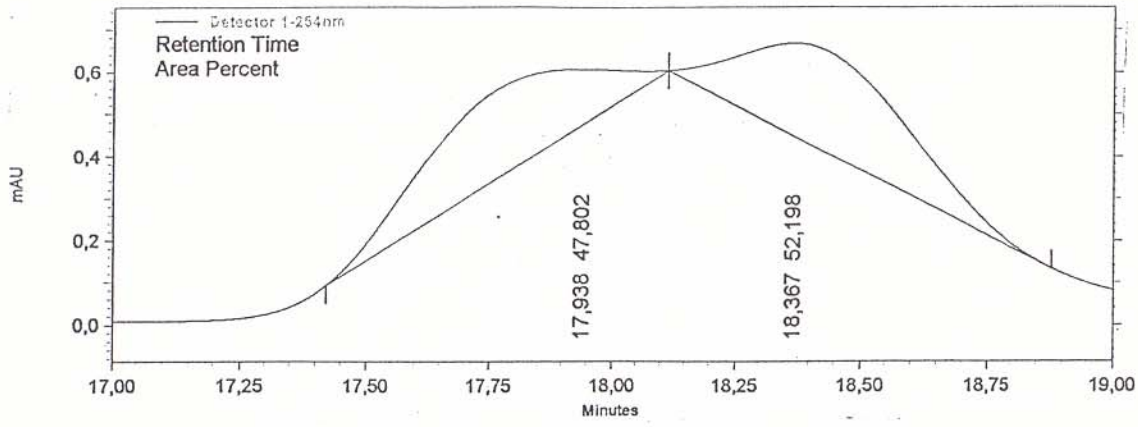


Figure 30. The HPLC-Chromatogram of Racemic 2-Hydroxycyclohexanone **(54b)**

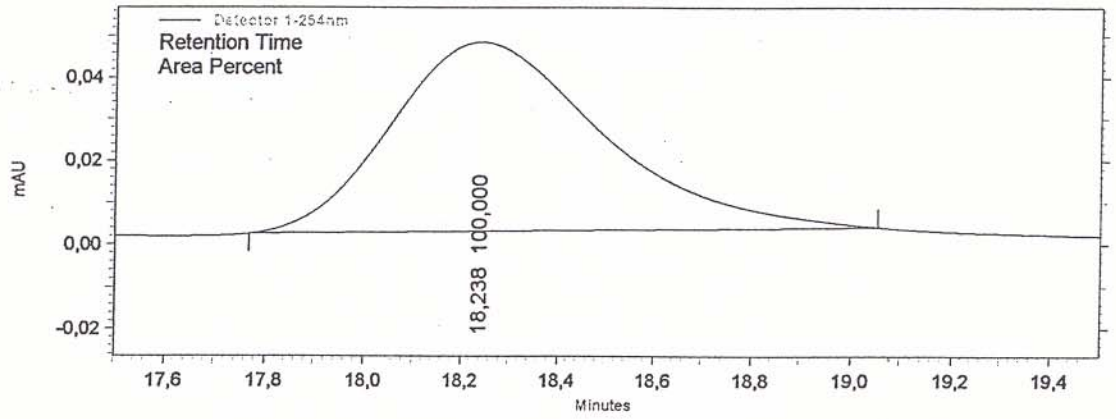
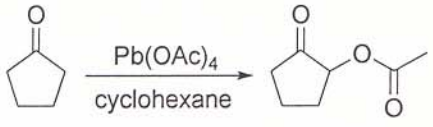


Figure 31. The HPLC-Chromatogram of **(+)-54b**

4.3 Enzymatic Resolution of (±)-2-Acetoxycyclopentanone (53a)

The (±)-2-acetoxycyclopentanone **(53a)** was synthesized from the treatment of cyclopentanone **(52a)** with $Pb(OAc)_4$ in cyclohexane (Scheme 33).



52a (±)-**53a**

Scheme 33. $Pb(OAc)_4$ Oxidation of Cyclopentanone

The reaction was controlled by TLC (Silica Gel, EtOAc/Hex 1:3) through 12 hours. After the work-up and flash column chromatoraphy, (±)-2-acetoxycyclopentanone **(53a)** was obtained in 62% yield as a yellowish liquid.

The isolated product was elucidated by NMR spectroscopy. From the ^{1}H-NMR, we observed a multiplet between 1.69-1.88 ppm, a singlet at 2.01 ppm for the methyl hydrogens of the acetoxy group, a multiplet between 2.07-2.20 ppm, a multiplet between 2.23-2.37 ppm, a triplet (J=10.0 Hz) at 4.99 ppm for the hydrogen of the carbon which the acetoxy group is attached (Figure 32). From the ^{13}C-NMR spectrum, we observed the signals at 212.6 ppm for the carbonyl carbon of the acetoxy group, 170.3 ppm for the C_1, 75.7 ppm for the C_5, 35.1 ppm for the C_2, 28.4 ppm for the methyl carbon of the acetoxy group, 20.2 ppm for the C_4, 17.4 ppm for C_3 (Figure 33).

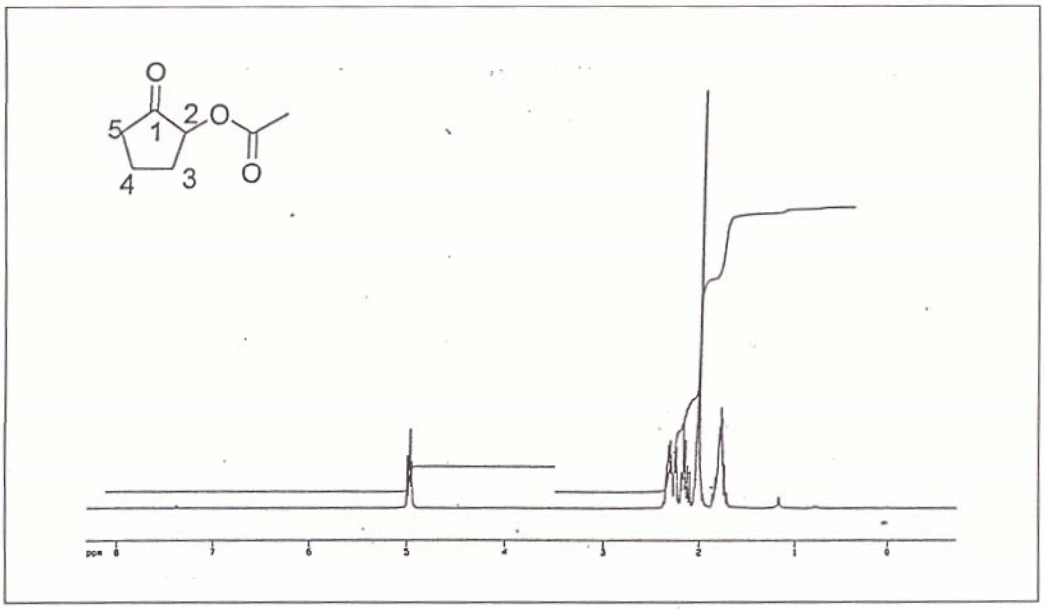


Figure 32. ^{1}H-NMR Spectrum of 2-Acetoxycyclopentanone **(53a)**

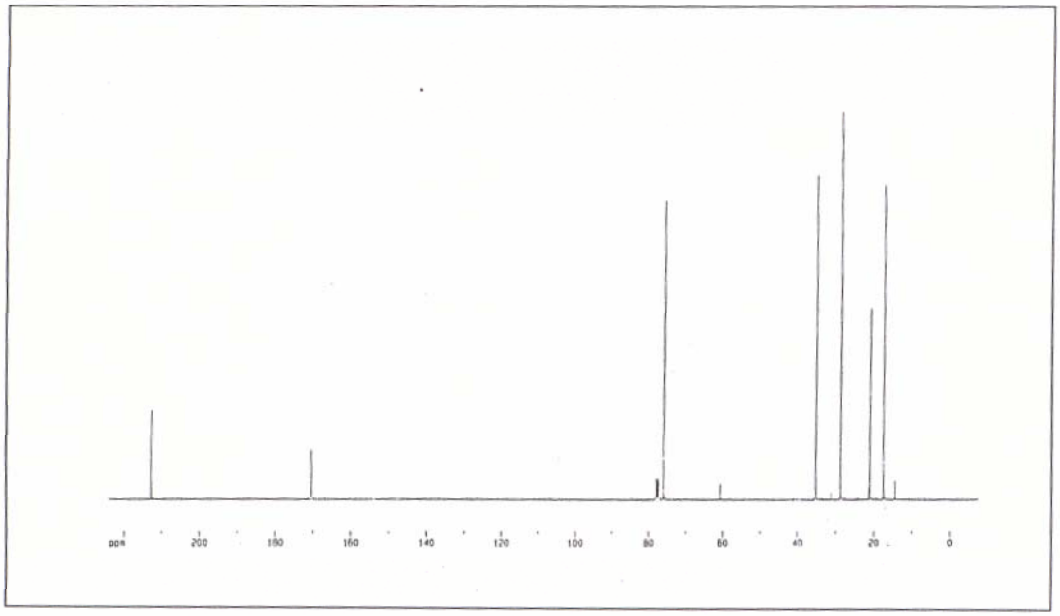
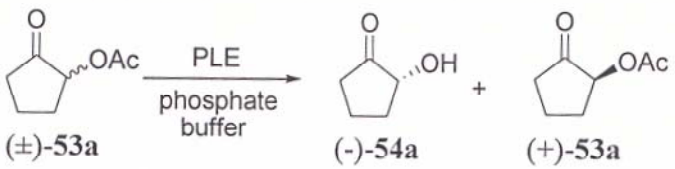


Figure 33. ^{13}C-NMR Spectrum of 2-Acetoxycyclopentanone **(53a)**

Then, the enzymatic hydrolysis of (±)-2-acetoxycyclopentanone **(53a)** was carrried out with PLE. The reaction was ended in 10 hours when approximately 50% conversion was attained. The crude product was seperated by flash column chromatography to afford the enantiomerically enriched (-)-2-hydroxycyclopentanone **(54a)** and (+)-2-acetoxycyclopentanone **(53a)** (Scheme 34).



Scheme 34. Enzymatic Hydrolysis of (±)-**53a** with PLE

(-)-2-Hydroxycyclopentanone **(54a)** was identified by NMR spectroscopy. From the ^{1}H-NMR spectrum, we observed a multiplet between 1.56-1.83 ppm, a multiplet between 1.91-2.05 ppm, a multiplet between 2.06-2.18 ppm, a multiplet

between 2.26-2.40 ppm, a broad singlet between 3.12-3.16 ppm for the hydrogen of the hydroxyl group and a triplet (J=10.0 Hz) at 3.99 ppm for the hygdrogen of the C_5 (Figure 34). From the C-NMR spectrum, we observed the signals at 218.8 ppm for the carbonyl carbon, 76.2 ppm for the C_5, 34.4 ppm for the C_2, 31.0 ppm for the C_4, 16.7 ppm for C_3 (Figure 35). According to the chromatogram of (-)-2-hydroxycyclopentanone **(54a)**, it was obtained with 99% enantiomeric excess (Figure 37).

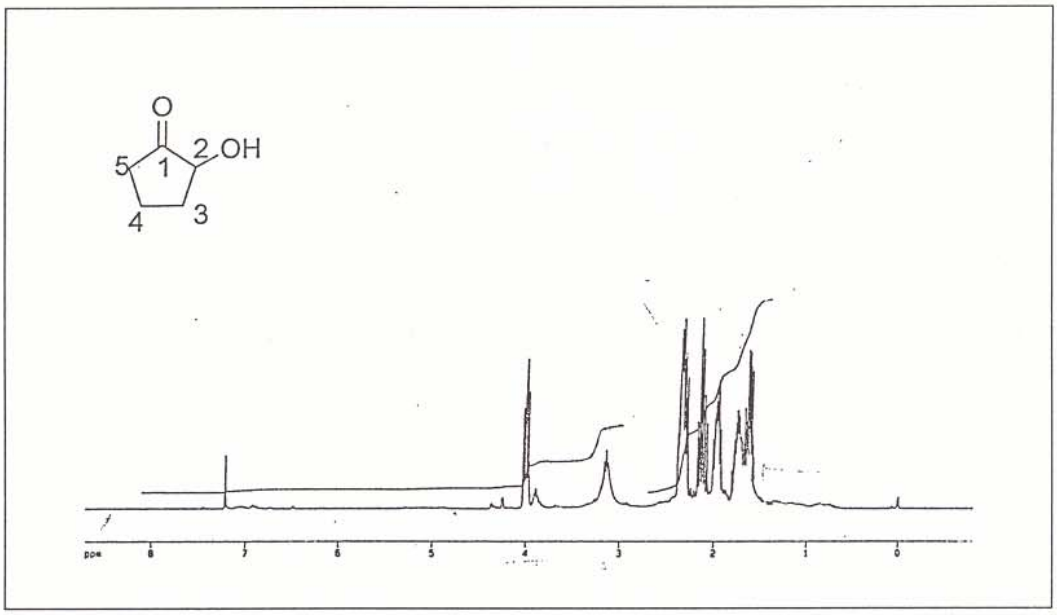


Figure 34. ^{1}H-NMR Spectrum of 2-Hydroxycyclopentanone **(54a)**

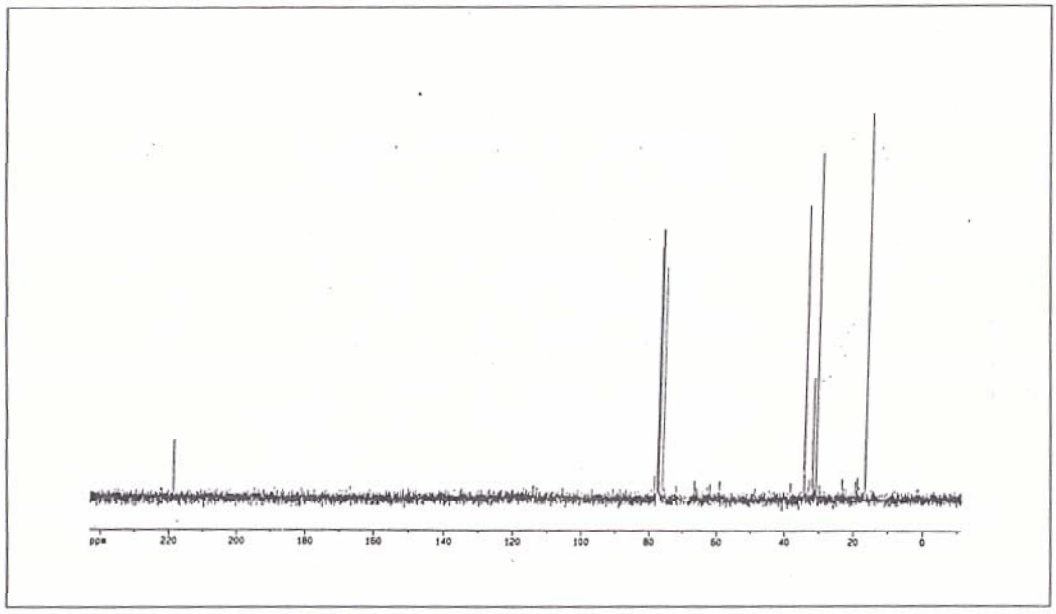


Figure 35. ^{13}C-NMR Spectrum of 2-Hydroxycyclopentanone **(54a)**

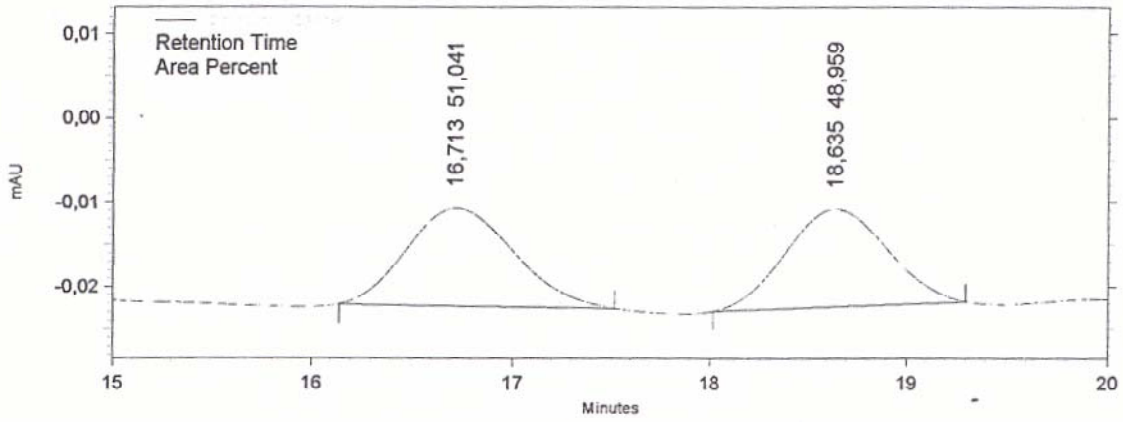


Figure 36. The HPLC-Chromatogram of the Racemic 2-Hydroxycyclopentanone

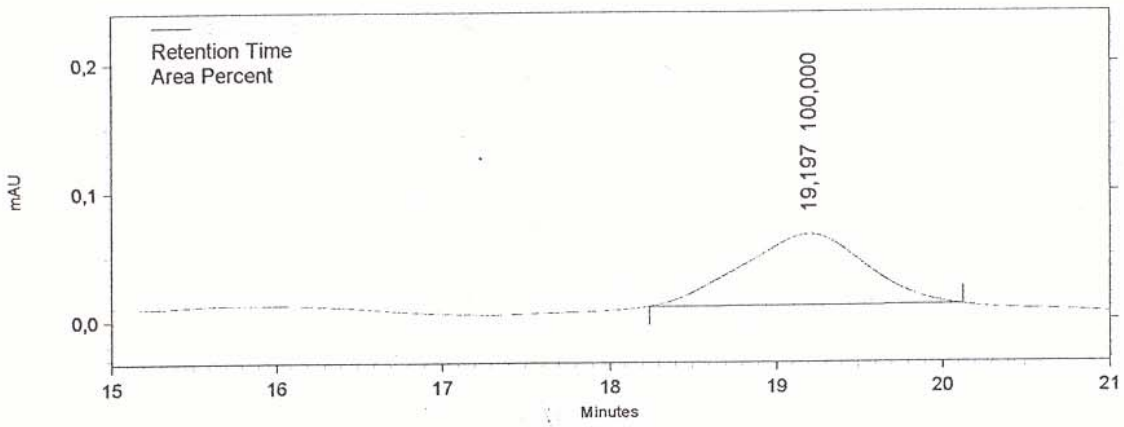
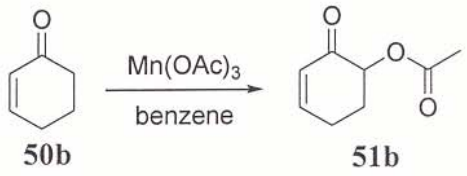


Figure 37. The Chromatogram of (-)-2-Hydroxycyclopentanone **(54a)**

4.4 Enzymatic Resolution of (±)-6-Acetoxy-2-cyclohexenone (51b)

Firstly, (±)-6-acetoxy-2-cyclohexenone **(51b)** was synthesized by manganese (III) triacetate acetoxylation method in benzene (Scheme 35).



Scheme 35. $Mn(OAc)_3$ Oxidation of 2-Cyclohexenone

The reaction was controlled by TLC (Silica Gel, EtOAc/Hex 1:3) through 8 hours. After the work-up and flash column chromatoraphy, (±)-6-acetoxy-2-cyclohexenone **(51b)** was obtained in 72% yield as a yellowish liquid.

The product was identified by NMR spectroscopy. From ^{1}H-NMR spectrum, we observed a multiplet between 2.02-2.09 ppm, a singlet at 2.11 ppm for the methyl hydrogens of the acetoxy group, a multiplet between 2.19-2.23 ppm, a multiplet between 2.47-2.59 ppm, a doublet of doublet (J=5.3 and 8.2 Hz) at 5.30 ppm for the hydrogen of the carbon which acetoxy group is attached, a multiplet between 5.98-

6.02 ppm for the hydrogen of C_2, a multiplet between 6.87-6.92 ppm for the hydrogen of C_3 (Figure 38). From ^{13}C-NMR spectrum, we observed the signals at 194.4 ppm for the carbonyl carbon of the acetoxy group, 170.5 ppm for the C_1, 150.3 ppm for the C_3, 128.9 ppm for the C_2, 73.9 ppm for the C_6, 28.9 ppm for the C_5, 25.9 ppm for the C_4, 21.2 ppm for the methyl carbon of the acetoxy group (Figure 39).

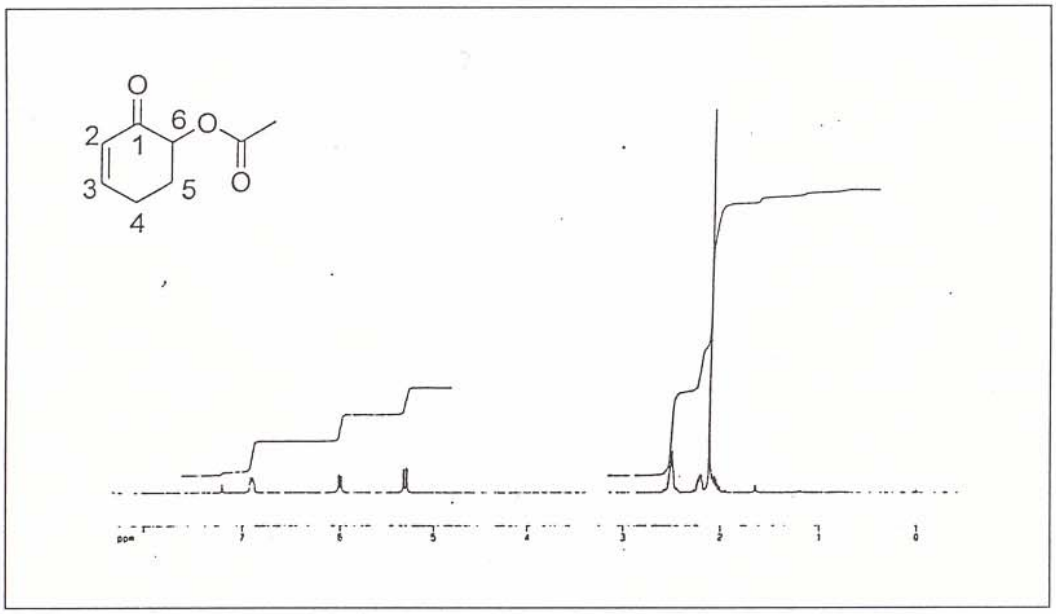


Figure 38. ^{1}H-NMR Spectrum of 6-Acetoxy-2-cyclohexenone **(51b)**

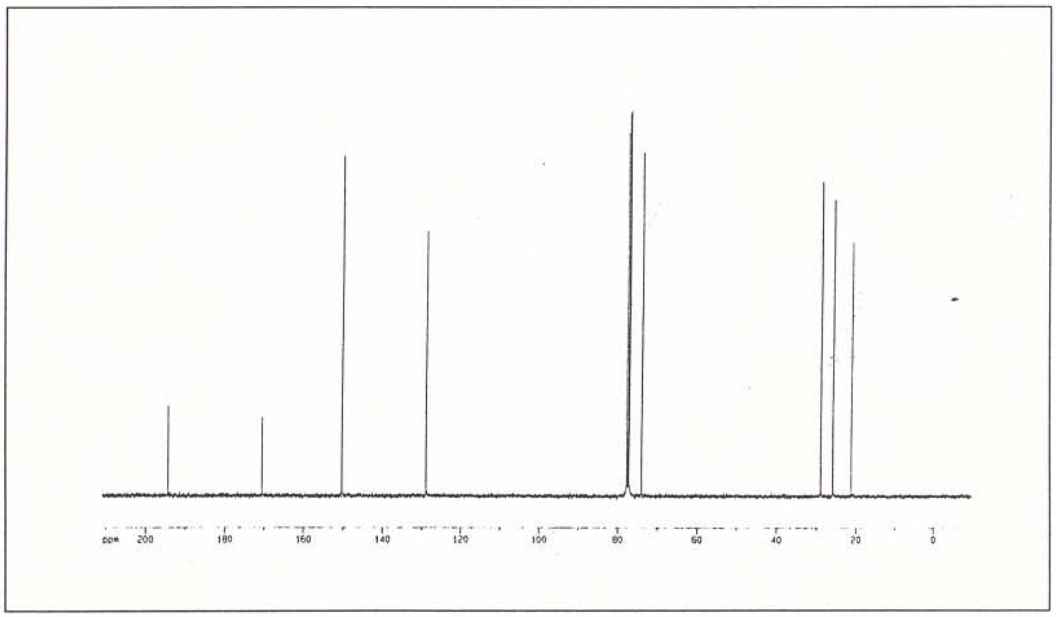
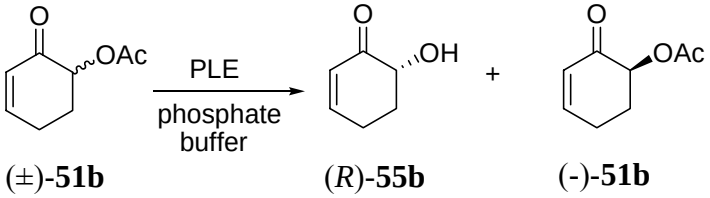


Figure 39. ^{13}C-NMR Spectrum of 6-Acetoxy-2-cyclohexenone **(51b)**

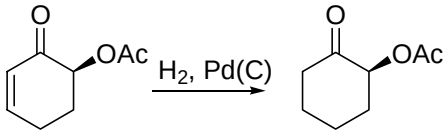
Then, the enzymatic hydrolysis of (±)-6-acetoxy-2-cyclohexenone **(51b)** was carrried out with PLE. The reaction was ended in 7 hours when approximately 50% conversion was attained. The crude product was seperated by flash column chromatography to afford the enantiomerically enriched (*R*)-6-hydroxy-cyclohexenone **(55b)** and (-)-6-acetoxy-cyclohexenone **(51b)** (Scheme 36).



(±)-**51b** (*R*)-**55b** (-)-**51b**

Scheme 36. Enzymatic Hydrolysis of (±)-**51b** with PLE

After the enzymatic hydrolysis of (±)-6-acetoxy-2-cyclohexenone **(51b)**, the hydrogenation of the (-)-6-acetoxy-2-cyclohexenone **(51b)** afforded (-)-2-acetoxycyclohexanone **(53b)** in quantitaive yield (Scheme 37).



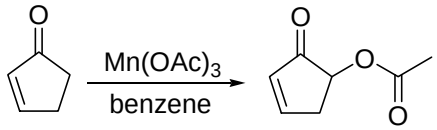
(-)-**51b** (-)-**53b**

Scheme 37. Hydrogenation of (-)-**51b**

6-Hydroxy-2-cyclohexenone **(55b)** were not isolated in enantiomerically enriched forms. This is presumably due to the fast racemization and/or rearrangement of α′-hydroxylated cyclic enones as observed in our previous work.[103]

4.5 Enzymatic Resolution of (±)-5-Acetoxy-2-cyclopentenone (51a)

Firstly, the manganese (III) triacetate acetoxylation method was applied to 2-cyclopentenone **(50a)** (Scheme 38).



50a **51a**

Scheme 38. $Mn(OAc)_3$ Oxidation of 2-Cyclopentenone

The reaction was controlled by TLC (Silica Gel, EtOAc/Hex 1:3) through 8 hours. After the work-up and flash column chromatoraphy, (±)-5-acetoxy-2-cyclopentenone **(51a)** was obtained in 65% yield as a yellowish liquid.

The product was identified by NMR spectroscoopy. From the H-NMR spectrum, we observed a singlet at 1.95 ppm for the methyl hyrogens of the acetoxy group, a multiplet between 2.35-2.2.47 ppm, a multiplet between 2.91-3.04 ppm, a doublet of doublet (J=3.8 Hz) at 4.93 ppm, a multiplet between 6.02-6.11 ppm for the hydrogen of the C_2, a multiplet between 7.44-7.53 ppm for the hydrogen of the C_3 (Figure 40). From the ^{13}C-NMR spectrum, we observed the signals at 203.4 ppm for the carbonyl carbon of the acetoxy group, 170.7 ppm for the C_1, 161.8 ppm for the C_3, 132.9 ppm for C_2, 71.8 ppm for the carbon which acetoxy group is attached, 35.8 ppm for the C_4, 21.0 ppm for the methyl carbon of the acetoxy group (Figure 41).

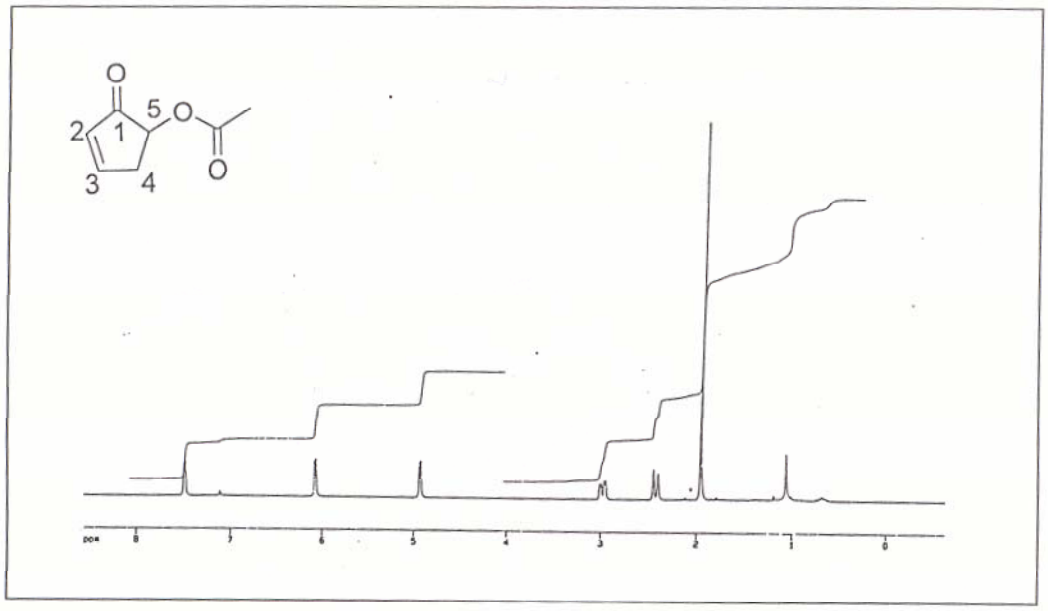


Figure 40. ^{1}H-NMR Spectrum of 5-Acetoxy-2-cyclopentenone **(51a)**

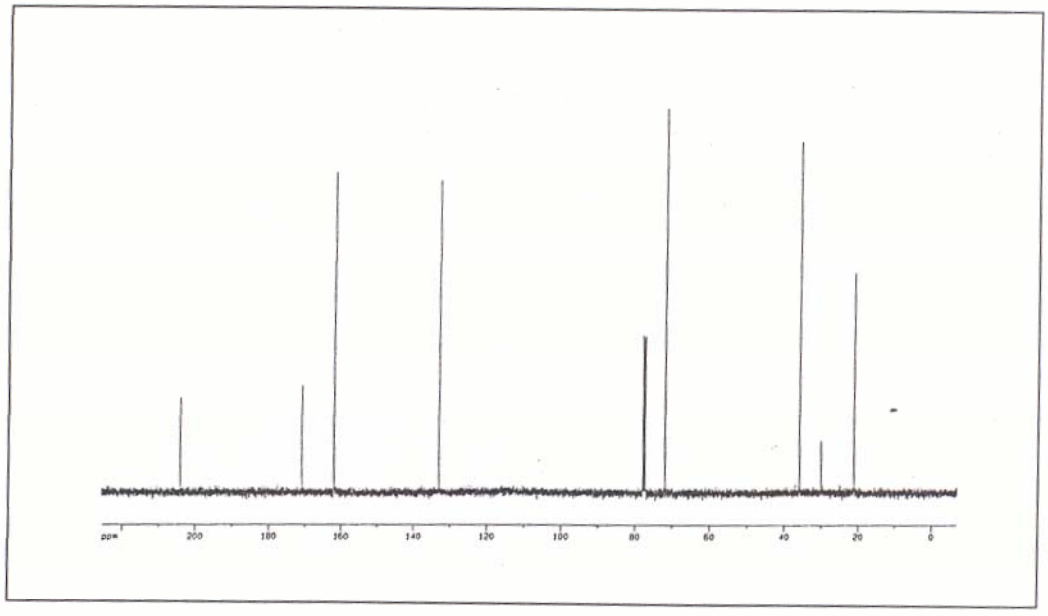
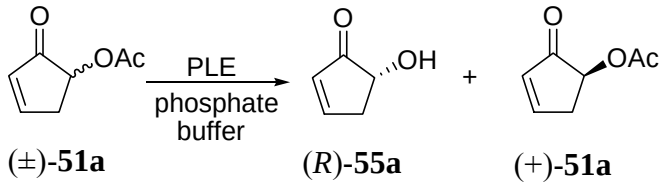


Figure 41. ^{13}C-NMR Spectrum of 5-Acetoxy-2-cyclopentenone **(51a)**

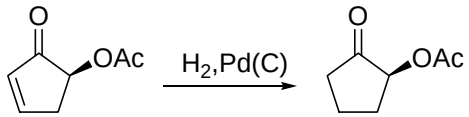
Then, the enzymatic hydrolysis of (±)-5-acetoxy-2-cyclopentenone **(51a)** was carrried out with PLE. The reaction was ended in 10 hours when approximately 50% conversion was attained. The crude product was seperated by flash column chromatography to afford the enantiomerically enriched (*R*)-5-hydroxy-cyclopentenone **(55a)** and (+)-5-acetoxy-cyclopentenone **(51a)** (Scheme 39).



(±)-**51a** (*R*)-**55a** (+)-**51a**

Scheme 39. Enzymatic Hydrolysis of (±)-**51a** with PLE

After the enzymatic hydrolysis of (±)-5-acetoxy-2-cyclopentenone **(51a)**, the hydrogenation of (+)-5-acetoxy-2-cyclopentenone **(51a)** afforded (+)-2-acetoxycyclopentanone **(53a)** in quantitative yield (Scheme 40).

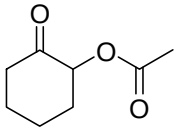
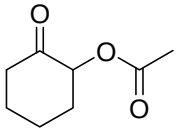
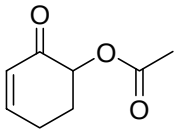
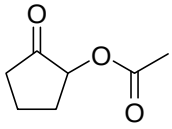
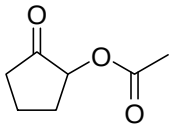
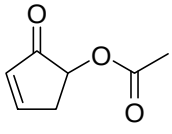


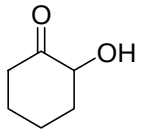
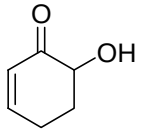
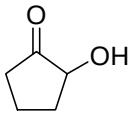
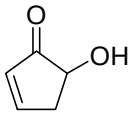
(+)-**51a** (+)-**53a**

Scheme 40. Hydrogenation of (+)-**51a**

5-Hydroxy-2-cyclopentenone **(55a)** was not isolated in enantiomerically enriched forms due to the reasons stated above for 6-hydroxy-2-cyclohexenone **(55b)**.

4.6 Absolute Configuration Determinations

 2-acetoxy-cyclohexanone from $Pb(OAc)_4$ acetoxylation followed by PLE hydrolysis	Observed $[\alpha]_D$= - 87.5 (c 0.19, MeOH) Ee= 96% lit. $[\alpha]_D$= + 89.3 (c 1.0, MeOH)[104a] Absolute configuration: (*S*)
 2-acetoxy-cyclohexanone from $Mn(OAc)_3$ acetoxylation followed by PLE hydrolysis and H_2 reduction	Observed $[\alpha]_D$= - 87.4 (c 0.16, MeOH) Ee=96% Absolute configuration: (*S*)
 6-acetoxy-2-cyclohexenone from $Mn(OAc)_3$ acetoxylation followed by PLE hydrolysis	Observed $[\alpha]_D$= - 88.7 (c 0.54, MeOH) Ee=97% Absolute configuration: (*S*)
 2-acetoxy-cyclopentanone from $Pb(OAc)_4$ acetoxylation followed by PLE hydrolysis	Observed $[\alpha]_D$= + 61.6 (c 1.57, $CHCl_3$) Ee=98% lit. $[\alpha]_D$= + 61.0 (c 2.0, $CHCl_3$)[104b] Absolute configuration: (*S*)
 2-acetoxy-cyclopentanone from $Mn(OAc)_3$ acetoxylation followed by PLE hydrolysis and H_2 reduction	Observed $[\alpha]_D$= + 59.7 (c 0.56, $CHCl_3$) Ee=95% Absolute configuration: (*S*)
 5-acetoxy-2-cyclopentenone from $Mn(OAc)_3$ acetoxylation followed by PLE hydrolysis	Observed $[\alpha]_D$= + 60.3 (c 0.178, $CHCl_3$) Ee=96% Absolute configuration: (*S*)

 2-hydroxy-cyclohexanone from $Pb(OAc)_4$ acetoxylation followed by PLE hydrolysis	Observed $[\alpha]^{20}_D$=+14.1 (*c* 0.5, $CHCl_3$) lit. $[\alpha]^{20}_D$= + 13.4 (c 0.5, $CHCl_3$)[104c] Ee=99%(Chiral HPLC) Absolute configuration: (*R*)
 6-hydroxy-2-cyclohexenone from $Mn(OAc)_3$ acetoxylation followed by PLE hydrolysis	Absolute configuration: (*R*)
 2-hydroxy-cyclopentanone from $Pb(OAc)_4$ acetoxylation followed by PLE hydrolysis	Observed $[\alpha]^{20}_D$=-42.2 (*c* 1.2, $CHCl_3$) lit. $[\alpha]^{20}_D$= -38.4 (c 1.2, $CHCl_3$)[104b] Ee=99%(Chiral HPLC) Absolute configuration: (*R*)
 5-hydroxy-2-cyclopentenone from $Mn(OAc)_3$ acetoxylation followed by PLE hydrolysis	Absolute configuration: (*R*)

The enantiomeric excesses of acetoxylated compounds were determined by the comparsion of optical rotation values with the literature values.

CHAPTER 5

EXPERIMENTAL

In this study, the structure elucidation of the compounds was done with the instruments as written.

^{1}H-NMR and ^{13}C-NMR spectra were recorded in $CDCl_3$ on Bruker Spectrospin Avance DPX 400 spectrometer. Chemical shifts are given in ppm from tetramethylsilane. Spin multiplicities are mentioned as: s (singlet), d (doublet), dd (doublet of doublet), t (triplet), m (multiplet).

IR spectra were obtained from a Perkin-Elmer Model 1600 series FT-IR spectrometer and are reported in cm^{-1}. Optical rotations were measured in solvent solution in a 1 dm cell using a Bellingham & Stanley P20 polarimeter at 20 °C.

Flash column chromatography was performed by using thick-walled glass columns with a flash grade (Merck Silica Gel 60). Reactions were monitored by thin layer chromatography using precoated silica gel plates (Merck Silica Gel PF-254), visualized with UV-light and polymolbyden phosphoric acid in ethanol as appropriate.

All extracts were dried over anhydrous magnesium sulfate ($MgSO_4$) and solutions were concentrated under vacuum by performing rotary evaporator.

5.1 Synthesis of (±)-Methyl 3-Cyclohexene-1-carboxylate (30)

(±)-3-cyclohexene-1-carboxylic acid **(22)**, (3.12 mmol), MeI (3.73 mL, 0.06 mmol), Ag_2O (0.89 g, 3.84 mmol), $CaSO_4$ (0.6 g, 4.4 mmol) were mixed in a 100 mL round bottomed flask, and 0.6 g broken glass were added to catalyze the reaction. The mixture was mixed for 24 hours with the exclusion of moisture by the help of $CaCl_2$ tube. After the mixing period, the residue was diluted with $CHCl_3$. The solution was filtered; washed with $CHCl_3$ and dried over $MgSO_4$. Then, the solvents was evaporated and the product was concentrated under vacuo.

(±)-**30 :** (0.44 g, quantitative) as a yellowish colored oil; R_f (EtOAc/Hexane 1:1) 0.71; ν_{max} (neat) 3027, 2951, 1736 cm^{-1}.

^{1}H-NMR ($CDCl_3$)

δ (ppm): 1.57-1.68 (m, 1H)
1.89-1.98 (m, 1H)
1.98-2.09 (m, 2H)
2.18-2.25 (m, 1H)
2.45-2.56 (m, 2H)
3.61 (s, 3H)
5.61 (t, J=13.2 and 11.7Hz, 2H)

^{13}C-NMR ($CDCl_3$)

δ (ppm): 176.7, 127.0, 125.5, 51.9, 39.6, 27.8, 25.4, 24.8

5.2 The General Procedure For The Enzyme-Catalyzed Hydrolysis of Racemic Esters

The pure substrate (500 mg) was added to the solution of potassium phosphate buffer (pH 7, 50 mL) containing esterase (100 μL). The reaction mixture was stirred at room temperature and monitored by TLC. When maximum conversion

was reached, the reaction was ended by extraction with EtOAc. The unreacted ester and the product were separated by flash column chromatography.

5.2.1 Synthesis of (-)-3-Cyclohexene-1-carboxylic Acid (22)

The unreacted ester (+)-**30**, and the product (-)-**22**, were separated by flash column chromatography (1:3 EtOAc:Hex) after the enzymatic hydrolysis with PLE. The enantiomeric excess of the carboxylic acid were determined by chiral HPLC.

(*R*)-(+)-**30 :** (0.22 g, 44%); $[\alpha]^{20}_{D}$=+83.0 (*c* 1.03, CH_2Cl_2), lit. $[\alpha]^{20}_{D}$=+86.5 (*c* 1.05, CH_2Cl_2).[105]

(*S*)-(-)-**22 :** (0.23 g, 44%); R_f (EtOAc/Hexane 1:1) 0.40; ν_{max} (neat) 3023, 1700 cm^{-1}

HPLC: Phenomenex Chirex (S)-LEU and (R)-NEA, UV detection at 254 nm, eluent: hexane/2-propanol/methanol = 85:15:5, flow 0.5 ml/min.

Table 4. R_f Values for the Enantiomers of **22**

	R_f (min)	
Enzyme	(-)-**22**	(+)-**22**
PLE	7.735	12.053
HLE	7.868	12.122
CCL	8.305	12.280
PPL	8.033	10.383

^{1}H-NMR ($CDCl_3$)

δ (ppm): 1.64-1.71 (m, 1H)
1.91-2.01 (m, 1H)
2.01-2.13 (m, 2H)
2.22 (d, J= 5.9Hz, 2H)
2.39-2.59 (m, 1H)
5.60 (t, J=12 Hz, 2H)
11.4 (br s, 1H)

^{13}C-NMR ($CDCl_3$)

δ (ppm): 182.4, 127.1, 125.4, 39.5, 27.6, 25.2, 24.7

5.3 Synthesis of (-)-2-Iodo-7-oxabicyclo[3.2.1]octan-6-one (21)

A solution of $NaHCO_3$ (1.52 g, 18.1 mmol) in 25 mL of water was added to the (-)-3-cyclohexene-1-carboxylic acid (0.76 g, 6.02 mmol) with ice cooling. After the suspension dissolved, a solution of KI (6.0 g, 36.1 mmol) and I_2 (1.61 g, 6.33 mmol) in 15 mL of water was added. The resulting suspension was treated with $CHCl_3$, and the aqeous layer was extracted with $CHCl_3$. The combined extracts were washed with half-saturated $Na_2S_2O_3$, dried over anhydrous $MgSO_4$ and concentrated under reduced pressure. The product is obtained as white crystalline solid (1.52g, 99%).

(1*S*,2*S*,5*S*)-(-)-**21 :** R_f (EtOAc/Hexane 1:2) 0.68; melting point 132°C (lit. mp 135-136°C)[67] and $[\alpha]^{25}_{D}$= -36.7 (c 1.05 in $CHCl_3$).

^{1}H-NMR ($CDCl_3$)

δ (ppm): 1.72-1.79 (m, 1H)
1.79-1.89 (m, 1H)
2.05 (dd, J=16.4 and 5.1Hz, 1H)
2.28-2.44 (m, 2H)
2.60 (broad s, 1H)
2.73 (d, J=12.3Hz, 1H)
4.44 (br t, J=4.5Hz, 1H)
4.76 (br t, J=4.6Hz and 5.4Hz, 1H)

^{13}C-NMR ($CDCl_3$)

δ (ppm): 180.1, 82.5, 40.9, 36.8, 32.1, 26.2, 25.5

5.4 Synthesis of (-)-7-Oxabicyclo[3.2.1]oct-2-en-6-one (20)

To a solution of (-)-2-iodo-7-oxabicyclo[3.2.1]octan-6-one **(21)** (1.01 g, 4.01 mmol) in 28 mL THF was added was added DBU (0.90 mL, 6.0 mmol), and the mixture was stirred at reflux for 8h. Upon being cooled to room temperature, the mixture was poured into 0.5 N HCl and extracted three times with with Et_2O. The combined extracts were washed with brine, dried over anhydrous $MgSO_4$, concentrated under reduced pressure, and chromatographed on silica gel. Elution with 75% EtOAc-Hexane afforded 452 mg (91%) of olefin as a colorless liquid.

(1*S*,5*S*)-(-)-**20 :** R_f (EtOAc/Hexane 1:3) 0.26; ν_{max} (neat) 1770,1634 cm^{-1}; $[\alpha]^{24}_{D}$= -180.3 (c=3.2 in $CHCl_3$).

^{1}H-NMR ($CDCl_3$)

δ (ppm): 2.03 (d, J=11.2Hz, 1H)
2.34-2.51 (m, 3H)
2.85 (broad s, 1H)
4.69 (t, J=5.3Hz, 1H)
5.78 (m, 1H)
6.12 (m, 1H)

^{13}C-NMR ($CDCl_3$)

δ (ppm): 179.8, 130.6, 129.7, 73.7, 38.4, 34.8, 29.5

5.5 Synthesis of (-)-5-(Hydroxymethyl)-2-cyclohexen-1-ol (19)

(-)-7-Oxabicyclo[3.2.1]oct-2-en-6-one **(20)** (4.02 g, 0.0324 mol) was dissolved in THF (10 mL) and added dropwise to $LiAlH_4$ (32.4 mL, 0.0324 mol, 1 eq, 1M/ether) dissolved in THF (70 mL) at 0^{o}C. A white precipitate formed. After 1 h, TLC (75% EtOAc-Hexane) showed no starting material. Water (1.24 mL), 15% aqeous NaOH (1.24 mL), and water (3.67 mL) were added sequentially, and the mixture was allowed to warm to room temperature with stirring over several hours. The salts were removed by filtration, and the solution was dried over $MgSO_4$ and concentrated at room temperature to give as a colorless semi-solid with 81% yield.

(1*S*,5*S*)-(-)-**19 :** ν_{max} (neat) 3590, 3040, 1390, 1090; $[\alpha]^{23}_{D}$= -20.1 (c=1.2 in MeOH).

^{1}H-NMR ($CDCl_3$)

δ (ppm): 1.15-1.27 (m, 1H)
1.65-1.77 (m, 1H)
1.77-1.88 (m, 1H)
1.96-2.11 (m, 2H)
2.89 (broad s, 2H)
3.40-3.53 (m, 2H)
4.29-4.39 (m, 1H)
5.58-5.64 (br d, J=10.2Hz, 1H)
5.64-5.73 (m, 1H)

^{13}C-NMR ($CDCl_3$)

δ (ppm): 131.4, 128.5, 67.4, 67.5, 35.56, 35.53, 28.4

5.6 Synthesis of (±)-2-Acetoxycyclohexanone (53b)

A mixture of $Pb(OAc)_4$ (5 g, 11 mmol) in cyclohexane (50 mL) with cyclohexanone **(52b)** (1.08 g, 11 mmol) was refluxed for several hours and monitored by TLC. Then the mixture was cooled, washed once with water, saturated NaCl, saturated $NaHCO_3$, and saturated NaCl. After the extraction, the organic layer was dried with $MgSO_4$ and evaporated in vacuo. The crude product was seperated by flash column chromatography using ethyl acetate/hexane(1:3) as eluent to afford the (±)-2-acetoxycyclohexanone **(53b)** (1.58 g, 92%).

(±)-**53b :** Colourless oil; *Rf* (EtOAc/Hexane 1:2) 0.51; ν_{max} (neat) 1750, 1720, 1230 cm^{-1}

^{1}H-NMR ($CDCl_3$)

δ (ppm): 1.48-1.62 (m, 1H)
1.63-1.77 (m, 2H)
1.85-1.95 (m, 1H)
1.98-2.06 (m, 1H)
2.08 (s, 3H)
2.19-2.26 (m, 1H)
2.27-2.38 (m, 1H)
2.41-2.48 (m, 1H)
5.05-5.14 (m, 1H)

^{13}C-NMR ($CDCl_3$)

δ (ppm): 204.8, 170.4, 76.9, 41.1, 33.4, 27.5, 24.1, 21.1

5.7 Enzymatic Resolution of (±)-2-Acetoxycyclohexanone (53b)

The (±)-2-acetoxy-cyclohexanone **(53b)** (500 mg) was added to the solution of potassium phosphate buffer (pH 7, 50 mL) containing esterase (100 μL). The reaction mixture was stirred at room temperature and monitored by TLC. When maximum conversion was reached, the reaction was ended by extraction with EtOAc. The unreacted (*S*)-(-)-2-acetoxycyclohexanone **(53b)** (0.23 g, 45%) and (*R*)-(+)-2-hydroxycyclohexanone **(54b)** (0.14 g, 39%) were separated by flash column chromatography.

(*S*)-(-)-**53b :** Colourless oil; 96% ee $[\alpha]^{20}_{D}$=-87.5 (*c* 0.2, MeOH), lit.[104a] for (*R*)-(+)-**53b** $[\alpha]^{20}_{D}$=+89.3 (*c* 1.0, MeOH).

(*R*)-(+)-**54b :** Colourless oil; 99% ee $[\alpha]^{20}_{D}$=+14.1 (*c* 0.5, $CHCl_3$), lit.[104c] for (*R*)-(+)-**54b** $[\alpha]^{20}_{D}$=+13.4 (*c* 0.5, $CHCl_3$); ν_{max} (neat) 3146, 1790, 1716 cm^{-1}

HPLC: Phenomenex Chirex (S)-LEU and (R)-NEA, UV detection at 254 nm, eluent: hexane/2-propanol = 90:10, flow 0.5 ml/min. R_f values for (+)-**54b**: 17.938 min and (-)-**54b**: 18.367 min.

^{1}H-NMR ($CDCl_3$)

δ (ppm): 1.35-1.88 (m, 4H)
2.01-2.10 (m, 1H)
2.24-2.34 (m, 1H)
2.35-2.45 (m, 1H)
2.46-2.55 (m, 1H)
2.8 (br s, OH)
4.00-4.15 (m, 1H)

^{13}C-NMR ($CDCl_3$)

δ (ppm): 211.7, 75.7, 39.8, 37.1, 27.9, 23.8

5.8 Synthesis of (±)-2-Acetoxycyclopentanone (53a)

A mixture of $Pb(OAc)_4$ (5 g, 11 mmol) in cyclohexane (50 mL) with cyclopentanone **(52a)** (0.951 g, 11 mmol) was refluxed for several hours and monitored by TLC. Then the mixture was cooled, washed once with water, saturated NaCl, saturated $NaHCO_3$, and saturated NaCl. After the extraction, the organic layer was dried with $MgSO_4$ and evaporated in vacuo. The crude product was seperated by flash column chromatography using ethyl acetate/hexane(1:3) as eluent to afford the (±)-2-acetoxycyclopentanone **(53a)** (0.96 g, 62%).

(±)-**53a :** Colourless oil; R_f (EtOAc/Hexane 1:2) 0.47; ν_{max} (neat) 1753, 1744 cm^{-1}

^{1}H-NMR ($CDCl_3$)

δ (ppm): 1.69-1.88 (m, 2H)
2.01 (s, 3H)
2.07-2.20 (m, 2H)
2.23-2.37 (m, 2H)
4.99 (t, J=10.0Hz, 1H)

^{13}C-NMR ($CDCl_3$)

δ (ppm): 212.6, 170.3, 75.7, 35.1, 28.4, 20.2, 17.4

5.9 Enzymatic Resolution of (±)-2-Acetoxycyclopentanone (53a)

The (±)-2-acetoxy-cyclopentanone **(53a)** (500 mg) was added to the solution of potassium phosphate buffer (pH 7, 50 mL) containing esterase (100 μL). The reaction mixture was stirred at room temperature and monitored by TLC. When maximum conversion was reached, the reaction was ended by extraction with EtOAc. The unreacted (*S*)-(+)-2-acetoxycyclopentanone **(53a)** (0.24 g, 47%) and (*R*)-(-)-2-hydroxycyclopentanone **(54a)** (0.15 g, 44%) were separated by flash column chromatography.

(*S*)-(+)-**53a :** Colourless oil; 98% ee $[\alpha]^{20}_{D}$=+61.6 (*c* 1.57, $CHCl_3$), lit.[104b] $[\alpha]^{20}_{D}$=+61.0 (*c* 2.0, $CHCl_3$).

(*R*)-(-)-**54a :** Colourless oil; 99% ee $[\alpha]^{20}_{D}$=-42.2 (*c* 1.2, $CHCl_3$), lit.[104b] $[\alpha]^{20}_{D}$= -38.4 (*c* 1.2, $CHCl_3$); ν_{max} (neat) 3153, 1790, 1721 cm^{-1}

HPLC: Phenomenex Chirex (S)-LEU and (R)-NEA, UV detection at 254 nm, eluent: hexane/2-propanol = 90:10, flow 0.5 ml/min. R_f values for (+)-**54a**: 16.713 min and (-)-**54a**: 18.635 min.

^{1}H-NMR ($CDCl_3$)

δ (ppm): 1.56-1.83 (m, 2H)
1.91-2.05 (m, 1H)
2.06-2.18 (m, 1H)
2.26-2.40 (m, 2H)
3.12-3.16 (br s, 1H)
3.99 (t, J=10Hz, 1H)

^{13}C-NMR ($CDCl_3$)

δ (ppm): 218.8, 76.2, 34.4, 31.0, 16.7

5.10 Synthesis of (±)-6-Acetoxy-2-cyclohexenone (51b)

A mixture of $Mn(OAc)_3$ (3.25 g, 14.0 mmol) in benzene (150 mL) was refluxed for 45 min using a Dean-Stark trap. Then the mixture was cooled to room temperature and 2-cyclohexenone **(50b)** (0.67 g, 7.0 mmol) was gradually added. The mixture was allowed to reflux until the dark brown color disappeared and also monitored by TLC. The reaction mixture was diluted with an equal amount of ethyl acetate and the organic phase was washed with 1N HCl followed by saturated $NaHCO_3$ and brine. The organic phase was dried over $MgSO_4$ and evaporated in vacuo. The crude product was seperated by flash column chromatography using ethyl acetate/hexane(1:3) as eluent to afford the (±)-6-acetoxy-2-cyclohexenone **(51b)** (0.77 g, 72%).

(±)-**51b :** Colourless oil; R_f (EtOAc/Hexane 1:2) 0.26; ν_{max} (neat) 1732, 1677, 1608 cm^{-1}

^{1}H-NMR ($CDCl_3$)

δ (ppm): 2.02-2.09 (m, 1H)
2.11 (s, 3H)
2.19-2.23 (m, 1H)
2.47-2.51 (m, 2H)
5.30 (dd, J=5.3 and 8.2 Hz, 1H)
5.98-6.02 (m, 1H)
6.87-6.92 (m, 1H)

^{13}C-NMR ($CDCl_3$)

δ (ppm): 194.4, 170.5, 150.3, 128.9, 73.9, 28.9, 25.9, 21.2

5.11 Enzymatic Resolution of (±)-6-Acetoxy-2-cyclohexenone (51b)

The (±)-6-acetoxy-2-cyclohexenone **(51b)** (500 mg) was added to the solution of potassium phosphate buffer (pH 7, 50 mL) containing esterase (100 μL). The reaction mixture was stirred at room temperature and monitored by TLC. When maximum conversion was reached, the reaction was ended by extraction with EtOAc. The unreacted (*S*)-(-)-6-acetoxy-2-cyclohexenone **(51b)** (0.23 g, 46%) and (*R*)-6-hydroxy-2-cyclohexenone **(55b)** were separated by flash column chromatography.

(*S*)-(-)-**51b :** Colourless oil; 97% ee $[\alpha]^{20}_{D}$=-88.7 (*c* 0.5, MeOH)

5.12 Synthesis of (±)-5-Acetoxy-2-cyclopentenone (51a)

A mixture of $Mn(OAc)_3$ (3.25 g, 14.0 mmol) in benzene (150 mL) was refluxed for 45 min using a Dean-Stark trap. Then the mixture was cooled to room temperature and the 2-cyclopentenone **(50a)** (0.57 g, 7.0 mmol) was gradually added. The mixture was allowed to reflux until the dark brown color disappeared and also monitored by TLC. The reaction mixture was diluted with an equal amount of ethyl acetate and the organic phase was washed with 1N HCl followed by saturated

$NaHCO_3$ and brine. The organic phase was dried over $MgSO_4$ and evaporated in vacuo. The crude product was seperated by flash column chromatography using ethyl acetate/hexane(1:3) as eluent to afford the (±)-5-acetoxy-2-cyclopentenone **(51a)** (0.64 g, 65%).

(±)-**51a :** Colourless oil; R_f (EtOAc/Hexane 1:3) 0.38; ν_{max} (neat) 1743, 1635 cm^{-1}

^{1}H-NMR ($CDCl_3$)

δ (ppm): 1.95 (s, 3H)
2.35-2.47 (m, 1H)
2.91-3.04 (m, 1H)
4.93 (dd, 3.8Hz, 1H)
6.02-6.11 (m, 1H)
7.44-7.53 (m,1H)

^{13}C-NMR ($CDCl_3$)

δ (ppm): 203.4, 170.7, 161.8, 132.9, 71.8, 35.8, 21.0

5.13 Enzymatic Resolution of (±)-5-Acetoxy-2-cyclopentenone (51a)

The (±)-6-acetoxy-2-cyclopentenone **(51a)** (500 mg) was added to the solution of potassium phosphate buffer (pH 7, 50 mL) containing esterase (100 μL). The reaction mixture was stirred at room temperature and monitored by TLC. When maximum conversion was reached, the reaction was ended by extraction with EtOAc. The unreacted (*S*)-(+)-5-acetoxy-2-cyclopentenone **(51a)** (0.23 g, 45%) and (*R*)-5-hydroxy-2-cyclopentenone **(55a)** were separated by flash column chromatography.

(*S*)-(+)-**51a :** Colourless oil; 96% ee $[\alpha]^{20}_D$=+60.3 (*c* 0.2, $CHCl_3$)

5.14 Hydrogenation of (*S*)-51a and (*S*)-51b

To a stirred solution of (*S*)-**51a** (0.20 g) in EtOAc (20 mL), Pd(C) (10 mg) was added and stirred at room temperature under hydrogen atmosphere for 3 h. The filtration of the mixture followed by evaporation of solvent in vacuo afforded quantitatively (*S*)-**53a**. The same procedure was applied for the transformation of (*S*)-**51b** into (*S*)-**53b**. All spectroscopic data of the products are in accordance with (*S*)-**53a** and (*S*)-**53b**, respectively.

CHAPTER 6

CONCLUSIONS

In this study, the enantiomerically enriched form of 3-cyclohexene-1-carboxylic acid **(22)**, starting material for the synthesis of cylohexenyl and cyclohexanyl nucleosides, was synthesized via enzymatic hydrolysis with high stereoselectivity. According to the results, PLE and HLE catalyzed reactions afforded (*S*)-configurated carboxylic acids with 95% ee and 94% ee, respectively. In contrast to this, PPL yielded (*R*)-configurated carboxylic acid with 72% ee. Thus, enzyme type can be changed according to the desired configuration.

Also, the direct regioselective oxidations of α,β-unsatuared and saturated cyclic ketones was improved using $Mn(OAc)_3$ and $Pb(OAc)_4$, respectively, in good yields and have shown that enantiomerically enriched forms of (*S*)-**51a-b**, (*S*)-**53a-b** and (*R*)-**54a-b** was obtained through enzymatic hydrolyses of racemic substrates **51a-b** and **53a-b**. Among the enzymes used in hydrolysis conditions, PLE showed the best enantioselectivity. The absolute configuration of both **51a** and **51b** were determined by transforming them into the corresponding saturated derivatives **53a** and **53b**, respectively, with H_2, Pd(C) in EtOAc.

REFERENCES

1. Gawley R. E., Aube J. *Tetrahedron Organic Chemistry Series Volume 14, Principles of Asymmetric Synthesis*, Elsevier Science Ltd., pp xi

2. Gawley R. E., Aube J. *Tetrahedron Organic Chemistry Series Volume 14, Principles of Asymmetric Synthesis* **1996**, Elsevier Science Ltd., pp xiii

3. Blashke G., Kraft H. P., Fickenscher K., Kohler F. *Arzniem-Forsch/Drud Res.* **1979**, 29,10

4. Blashke G., Kraft H. P:, Fickenscher K., Kohler F. *Arzniem-Forsch/Drud Res.* **1979**, 29, 1140

5. Powell J. R., Ambre J. J., Ruo T. I. In *Drug Stereochemistry*; I. W. Wainer and D. E. Drager, Eds.; Marcel Dekker: New York, **1988**; pp 245

6. Ariens E. J., Soudijn W., Timmermas P. B. M. W. M. *Stereochemistry on Biological Activity Of Drugs* Blackwell Sciencetific: Palo Alto, **1983**

7. Stinson S.C. *Chem. Eng. News.* **1993**, September 27, 38.

8. Williams K., Lee E. *Drugs* **1985**, 30, 333

9. Richards A. *Chiral'93* USA, **1993**

10. Ager D. J., East M. B. *Asymmetric Synthetic Methodology* CRC Press, USA, **1996**, pp 7

11. Scott J.S., In *Asymmetric Synthesis*; J. D. Morrison, Ed.; Academic Press: Orlando, **1984**; Vol 4; pp 1

12. Blaser H. –V. *Chem. Rev.* **1992**, 92, 935

13. Gringore O. H., Rouessac F. P. *Org. Synth.* **1984**, 63, 121

14. Ager D. J., East M. B. *Asymmetric Synthetic Methodology* CRC Press, USA, **1996**, pp 12

15. Eliel E. L. *Stereochemistry of Carbon Compounds* McGraw-Hill Kogakusha: Tokyo, **1962**

16. Secor R. *Chem. Rev.* **1963**, 63, 297

17. Boyle P. H. *Quart. Rev.* **1971**, 25, 323

18. Mazon A., Najera C., Yus M., Heumann A. *Tetrahedron:Asymmetry* **1992**, 3,1455

19. Leusen F. J. J., Noordik J. H., Karfunkel H. R. *Tetrahedron* **1993**, 49, 5377

20. Ager D. J., East M. B. *Asymmetric Synthetic Methodology* CRC Press, USA, **1996**, pp 17

21. Leuenberger H. G. W. *Pure Appl. Chem.* **1990**, 62, 753

22. Leuenberger H. G. W. In *Biotechnology* H. J: Rehm and G. Reed, Ed.; Verlag Chemie: Weinheim, **1984**; Vol. 6a; pp 5

23. Zhang D., Poulter C. D. *J. Am. Chem.Soc.* **1993**, 115, 1270

24. Only proteases are exceptions to this role for obvious reasons.

25. Jinwal U. K., Roy U., Chowdhury A. R., Bhaduri A. P., Roy P. K. *Bioorganic and Medicinal Chemistry* **2003**, 11, 1041

26. Sweers H. M., Wong C. –H. *J. Am. Chem. Soc.* **1986**, 108, 6421

27. Bashir N. B., Phythian S. J., Reason A. J., Roberts S. M. *J. Chem. Soc. Perkin Trans. 1* **1995**, 2203

28. Fujisawa T., Itoh T., Sato T. *Tetrahedron Lett.* **1984**, 25, 5083

29. Buisson D., Henrot S., Larcheveque M., Azerad R. *Tetrahedron Lett.* **1987**, 28, 5033

30. For a convenient method for controlling the substrate concentration see: Tetrahedron, 54, 15017

31. Fischer E. *Ber. Dtsch. Chem. Ges.* **1894**, 27, 2985

32. Koschland D. E., Neet K. E. *Ann. Rev. Biochem.* **1968**, 37, 359

33. Dewar M. J. S. *Enzyme* **1986**, 36, 8

34. A record of rate acceleration factor of 10^{14} has been reported. See: Lipscomb W. N., *Acc. Chem. Res.* **1982**, 15, 232

35. Ogston A. G. *Nature* **1948**, 162, 963

36. Eyring H. *J. Chem. Phys.* **1935**, 3, 107

37. Kraut J. *Science* **1988**, 242, 533

38. Wong C. –H. *Science* **1989**, 244, 1145

39. a) Sabbioni G., Jones J. B. *J. Org. Chem.* **1987**, 52, 4565 b) Adachi K., Kobayashi S., Ohno M. *Chimia* **1986**, 40, 311 c) Bjorkling F., Boutelje J., Gatenbeck S., Hult K., Norin T., Szmulik P. *Tetrahedron* **1985**, 41, 1347 d) Boutelje J., Hjalmarsson M., Szmulik P., Norin T., Hult K. In *Biocatalysis in Organic Media*; Laane C, Tramper J., Lilly M. D., Eds.; Elsevier: Amsterdam, **1987**

40. Toone E. J., Werth M. J., Jones J. B. *J. Am. Chem. Soc.* **1990**, 112, 4946

41. a) Jones J. B., Jakovac I. J. *Can. J. Chem.* **1982**, 60, 19 b) Sih C. J., Rosazza J. P. *Tech. Chem.* **1976**, 10, 69 c) Johnson R. A. In *Oxidation in Organic Chemistry, Part C*; Trahnanovsky, W. S., Ed.; Academic: New York, **1978** d) Nazazaki M., Chikamatsu H., Naemura K., Asao M. *J. Org. Chem.* **1980**, 45, 4432 e) Nakazaki M., Chikamatsu H., Naemura K., Sasaki Y., Fugii T. *J. Chem. Soc. Chem. Commun.* **1980**, 626

42. Prelog V. *Pure Appl. Chem.* **1964**, 9, 119

43. Lam L. K. P., Browne C. M., DeJeso B., Lym L., Toone E. J., Jones J. B. *J. Am. Chem. Soc.* **1988**, 110, 4409

44. a) Gais H. –J., Buelow G., Zatorski A., Jentsch M., Maidonis P., Hemmerle H. *J. Org. Chem.* **1989**, 54, 5115 b) Nagao Y., Kume M., Wakabayashi R. C., Nakamura T., Ochiai M. *Chem. Lett.* **1989**, 239 c) Kuhn T., Tamm C., Riesen A., Zehnder A. *Tetrahedron Lett.* **1989**, 30, 693 d) Kaga H., Kobayashi S., Ohno M. *Tetrahedron Lett.* **1989**, 30, 113

45. a) Mohr P., Roesslein L., Tamm C. *Tetrahedron Lett.* **1989**, 30, 2513 b) Mori K., Ogoche J. I. J. *Liebigs Ann. Chem.* **1988**, 903 c) Klunder A. J. H., van Gastel F. J. C., Zwanenburg B. *Tetrahedron Lett.* **1988**, 29, 2697 d) Sicsic S., Ikbal M., le Goffic F. *Tetrahedron Lett.* **1987**, 28, 1887 e) Whitesell J. K., Lawrence R. M. *Chimia* **1986**, 40, 318

46. Brockerhoff H. *Biochim. Biophys. Acta* **1968**, 159,296

47. Brockmann H. L. *Methods Enzymology* **1981**, 71, 619

48. Desnuelle P. *Adv. Enzymol.* **1961**, 23, 129

49. Hultin P. G., Jones J. B. *Tetrahedron Lett.* **1992**, 33, 1399

50. Wimmer Z. *Tetrahedron* **1992**, 48, 8431

51. Shaw J. F., Klibanov A. M. *Biotechnol. Bioeng.* **1987**, 29, 648

52. Sweers H. M., Wong C. –H. *J. Am.Chem. Soc.* **1986**, 108, 6421

53. Hennen W. J., Sweers H. M., Wang Y. –F., Wong C. –H. *J. Org. Chem.* **1988**, 53, 4939

54. Ballesteros A., Bernabe M., Cruzado C., Martin Lomas M., Otero C. *Tetrahedron* **1989**, 45, 7077

55. Barral K., Halfon P., Pepe G., Camplo M. *Tetrahedron Lett.* **2002**, 43, 81

56. a) Agrofoglia L., suhas E., Farese A., Condom R., Challand S. R., Earl R. A., Guedj R. *Tetrahedron* **1994**, 50, 10611 b) Cheng C. M., Shimo T., Somekawa K., Baba M. *Tetrahedron* **1998**, 54, 2031

57. Nair V., Buenger G. S. *J. Org. Chem.* **1990**, 55, 3695

58. Fauci A. S. *Science* **1988**, 239, 617

59. Herdewjin P., Pauwels R., Baba M., Balzarini J., Declerq E. *J. Med. Chem.* **1987**, 30, 2131

60. Broder S., Ed. *AIDS*, Dekkar: New York, **1987**

61. Dahberg J. E., Mitsuya H., Blam S. B. Broder S., Aaronson S. A. *Proc. Natl. Acad. Sci. USA* **1987**, 84, 2469

62. Herdewjin P., De Clerq E. *Bio. Med. Chem. Lett.* **2001**, 11, 1591

63. a) Ceder O., Hansson B. *Acta Chem. Scand.* **1970**, 24, 2693 b) Schwartz H. M., Wu W. –S., Marr P. W., Jones J. B. *J. Am.Chem. Soc.* **1978**, 100, 5199

64. Martin S. F., Dappen M. S., Dupre B., Murphy C. J., Colapnet J. A. *J. Org. Chem.* **1989**, 54, 2209

65. Smith A. B., Hale K. J., Laokso L. M., Chen K., Riera A. *Tetrahedron Lett.* **1989**, 30, 6963

66. Poll T., Sobczak a., Hortman H., Helmchen G. *Tetrahedron Lett.* **1985**, 26, 3095

67. Marshall J. A., Shiping X. *J. Org. Chem.* **1995**, 60, 7230

68. Trost B. M., Kondo Y. Tetrahedron Lett. **1991**, 32, 1613

69. Lind II R. G., Egbertson M., Coleman R. S., Jones A. B., Danishefsky S. J. *J. Org. Chem.* **1990**, 55, 2771

70. Klein W. J. *Recl. Traw. Chim. Pays-Bas* **1997**, 96, 22

71. Heiba E. I., Dessau R. *J. Am. Chem. Soc.* **1972**, 94, 2888

72. Vinagradov M., Verenehikov S., Fedorova T. *SSSR Ser. Khim.* **1975**, 11, 947

73. Okanu M., Aratoni T. *Bull. Chem. Soc. Jpn.* **1976**, 49, 2811

74. Vinagradov M., Duri P., Nikishin G. *Zh. Org. Khim.* **1976**, 12, 527

75. Evans E. A., Huston J. L., Horris T. H. *J. Am. Chem. Soc.* **1952**, 74, 4985

76. Davidson A. W., Lanning W.C., Zeller M. M. *J. Am. Chem. Soc.* **1942**, 55, 2107

77. Benson D., Sutcliffe H. *Trans. Faraday Soc.* **1959**, 55, 2107

78. Mosher W. A., Hehr C. L. *J. Am. Chem. Soc.* **1953**, 75, 3172

79. Finch N., Gemenden C. W., Hsu J. H., Taylor W. J. *J. Am. Chem. Soc.* **1963**, 85, 1520

80. Levitt L. S. *J. Org. Chem.* **1955**, 20, 1297

81. Barron H. E., Cavill G. W. K., Cole E. R.,Gilham P.T., Solomon D. H. In *Chem. And Ind.* London, **1954**, pp 76

82. Fuson R. C., Maynert E. W., Tan T., Trumbull R. E., Wassmundt F. W. *J. Am. Chem. Soc.* **1957**, 79, 1938

83. Ichikawa K., Yamaguchi Y. *Nippon Kagaku Zassi* **1952**, 73, 415; *Chem. Zentr.* **1956**,, pp 4095

84. Henbest H. B., Jones d. N., Slater G. P. *J. Chem. Soc.* **1961**, pp 4472

85. a) Brown M. J., Harrison T., Herrinton P. M., Hopkins M. H., Hutchinson K. D., Overman L. E, Mishra P. J. *J. Am. Chem. Soc.* **1991**, 113, 5365 b) ohno M., Oguri I., Eguchi S. *J. Org. Chem.* **1999**, 64, 8995 c) Gatling S. C., Jackson J. E. *J. Am. Chem. Soc.* **1999**, 121, 8655 d) Hoffmann T., Zhong G., List B., Shabat D., Anderson J., Gramatikova S., Lerner R. A., Barbas III C. F. *J. Am. Chem. Soc.* **1998**, 120, 2768

86. a) Yamamoto H., Tsuda M., Sakaguchi S., Ishii Y. *J. Org. Chem.* **1997**, 62, 7174 b) Reddy D. R., Thornton E. R., *J. Chem. Soc., Chem. Commun.* **1992**, 172 c) Moriarty R. M., Hou K. –C. *Tetrahedron Lett.* **1984**, 25, 691 d) Cookson R. C., Lane R. M. *J. Chem Soc., Chem. Commun.* **1976**, 804 e) Shinada T., Kawakami T., Sakai H., Takada I., Ohfune Y. *Tetrahedron Lett.* **1998**, 39, 3757 f) Cummins C. H., Coates R. M. *J. Org. Chem.* **1983**, 48, 2070 g) Rubottom G. M., Mott R. C., Juve H. D. Jr. *J. Org.Chem.* **1981**, 46, 2717 h) Fry A. J., Donaldson W. A., Ginsburg G. S. *J. Org. Chem.* **1979**, 44, 349 i) Shono T., Okawa M., Nishiguchi I. *J. Am. Chem. Soc.* **1975**, 97, 6144 j) Shono T., Matsumura Y., Nakagawa Y. *J. Am. Chem. Soc.* **1974**, 96, 3532

87. a) Wolfrom M.L., Thompson A., Evans E. F. *J. Am. Chem. Soc.* **1945**, 67, 1793 b) Fenselau C. *Steroid Reactions*, ed. Djerassi C, Holden-Day, San Francisco, **1963**, pp. 537-591

88. a) Green T. W., Wuts P.G. M. Protective Groups In *Organic Synthesis*, Wiley, New York, **1991**, pp. 87-104 b) Kocienski P. J. *Protecting Groups*, Thieme, Stuttgart, **1994**, pp. 22-29

89. a) Levine P. A., Walti A. *Org. Synth.*, Coll. Vol. II, **1943**, 4843 b) Reid e. B., Fortenbauch R. B., Patterson H. R. *J. Org. Chem.* **1950**, 15, 579

90. Kuehne M. E., Giacobbe T. J. *J. Org. Chem.* **1968**, 33, 3757

91. Shono T., Matsumura Y., Nakagawa Y. *J. Am. Chem. Soc.* **1975**, 97, 6144

92. Shinada T., Kawakami T., Sakai H., Takada I., Ohfune Y. *Tetrahedron Lett.* **1998**, 39, 3757

93. Creary X. *J. Am. Chem. Soc.* **1984**, 106, 5568

94. Rawilson D. J., Sosnovsky G. *Synthesis* **1973**, 567

95. Demir A. S., Camkerten N., Akgun H., Tanyeli C., Mahasneh A.S., Watt D. S. *Synth. Commun.* **1990**, 20, 2279

96. Lee J. C., Hand T. *Synth. Commun.* **1997**, 27, 4085

97. a) Davis F. A., Chen B. –C. *Chem. Rev.* **1992**, 92, 919 b) Lohnay B. B., Enders D. *Helv. Chim. Acta* **1989**, 72, 980

98. Hannessian S. *Total Synthesis of Natural: The Chiron Approach*; Pergamon Press: New York, **1983**, Chapter 2 b) Oppolzer W. *Angew. Chem., Int. Ed. Engl.* **1984**, 23, 876 c) Fujita M., Hiyama T. *J. Am. Chem. Soc.* **1984**, 106, 4629 d) Reez M. T. *Angew. Chem., Int. Ed. Engl.* **1984**, 23, 556 e) Masamune S., Choy W., Petersen, J. S., Sita, L. R. *Angew. Chem., Znt. Ed. Engl.* **1985**, 24, l f) Evans D. A., Bender, S. L. *Tetrahedron Lett.* **1986**, 27, 799 g) Samuels W. D., Nelson D. A., Hallen R. T. *Tetrahedron Lett.* **1986**, 27, 3091

99. a) Davis F. A., Weismiller M. C., Murphy C. K., Reddy R. T., Chen, B. -C. *J. Org. Chem.* **1992**, 57, 7274 b) Davis F. A., Sheppard A. C., Chen B.-C., Haque M. S. *J. Am. Chem. Soc.* **1990**, 112, 6679.

100. a) Davis F. A., Vishwakarama L. C. *Tetrahedron Lett.* **1985**, 26, 3539 b) Evans D. A., Morrissey M. M., Dorrow R. L. *J. Am. Chem. Soc.* **1985**, 107, 4346 c) Enders D., Bhushan V. *Tetrahedron Lett.* **1988**, 29, 2437 d) Taschner M. J., Aminbhavi A. S. *Tetrahedron Lett.* **1989**, 30, 1029 e) Gore M. P., Vederas J. C. *J. Org. Chem.* **1986**, 51, 3700 f) Aamboni R., Tamm C. *Helu. Chim. Acta* **1986**, 69, 615

101. Hashiyama T., Morikawa K., Sharpless K. B. *J. Org. Chem.* **1992**, 57, 5067

102. a) Xu D., Crispino G. A., Sharpless K. B. *J. Am. Chem. Soc.* **1992**, 114, 7570 b) Sharpless K.B., Amberg W., Bennani Y.L., Crispino G. A., Hartung J., Jeong K., Kwong H., Morikawa K., Wang, Z. *J. Org. Chem.* **1992**, 57, 2768 c) Wang L., Sharpless K. B. *J. Am. Chem. Soc.* **1992**, 114, 7568 d) Sharpless K. B., Amberg W., Beller M., Chen H., Hartung J., Kawanami

Y.,Lübben D., Manoury E., Ogino Y., Shibata T., Ukita T. *J. Org. Chem.* **1991**, 56, 4585 e) Wai, J. S. M., Marko I., Svendsen J. S., Finn M. G., Jacobsen E. N., Sharpless K. B. *J. Am. Chem. Soc.* **1989**, 111, 1123

103. Ülkü D., Tahir M. N., Tanyeli C., Demir A. S., Dikici E. *Acta. Cryst*. **1997**, C53, 1998

104. a) Sugimura T., Iguchi H., Tsuchida R., Tai A., Nishiyama N., Hakushi T. *Tetrahedron:Asymmetry* **1998**, 9, 1007 b) Easwar S., Deasi S. B., Argade N. P., Ganesh K. N. *Tetrahedron:Asymmetry* **2002**, 13, 1367 c) D'Accolti L., Detomaso A., Fusco C., Rosa A., Curci R. *J. Org. Chem.* **1993**, 58, 3600

105. Karig G., Fuchs A., Büsing A., Brandstetter T., Scherer S., Bats J. W., Eschenmoser A., Quinkert G. *Helu. Chim. Acta* **2000**, 83, 1049