ENZYME-CATALYZED RESOLUTION OF TERTIARY ALCOHOLS

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by
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Approval of the Graduate School of Natural Sciences.

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

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Head of Department

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

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ABSTRACT

ENZYME-CATALYZED RESOLUTION OF TERTIARY ALCOHOLS

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Enantiopure tertiary alcohols are very valuable building blocks containing quaternary carbon centres for the synthesis of many different natural products and pharmaceuticals. In this study, **(±)-**1-methyl-2,3-dihydro-1H-inden-1-ol **54** and (±)-1-methyl-1,2,3,4-tetrahydronaphthalen-1-ol **57** were resolved by using CAL-A-CLEA, CAL-A, Amona PS-C II and CRL. CAL-A-CLEA was the most active biocatalyst in the resolution of **(±)**1-methyl-2,3-dihydro-1H-inden-1-ol **54** and (*R*)-(-)-1-methyl-2,3-dihydro-1*H*-inden-1-yl acetate **53** and (*S*)-(+)-1-methyl-2,3-dihydro-1*H*-inden-1-ol **54** was obtained with 71% ee and 44% ee by 20% conversion, respectively. When (±)-1-methyl-1,2,3,4-tetrahydronaphthalen-1-ol **57** was resolved in the presence CAL-A, (*R*)-(-)-1-methyl-1,2,3,4-tetrahydronaphthalen-1-yl acetate **56** was obtained with 99% ee by 20% conversion while (*S*)-(+)-1-methyl-1,2,3,4-tetrahydronaphthalen-1-ol **57** was obtained with 33% ee by 25% conversion in the presence CAL-A-CLEA.

Keywords: Enzymatic Resolution, Tertiary alcohol, Lipase, Indan, Tetralin.

ÖZET

TERSİYER ALKOLLERİN ENZİM KATALİZÖRLÜĞÜNDE RESOLÜSYONU

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Enantiyomerce saf olan tersiyer alkoller birçok doğal ürünlerin ve ilaçların sentezlerinde kullanılan, kuaterner carbon atomu içeren çok değerli yapı taşlarıdır. Bu çalışmada **(±)-**1-methyl-2,3-dihydro-1H-inden-1-ol **54** and (±**)-**1-methyl-1,2,3,4-tetrahydronaphthalen-1-ol **57** CAL-A-CLEA, CAL-A, Amona PS-C II and CRL enzimleri ile enzimatik resolüsyona tabi tutulmuştur. **(±)-**1-metil-2,3-dihidro-1H-inden-1-ol **54'** un resolüsyonunda en iyi çalışan katalizör CAL-A-CLEA enzimiydi ve %20 lik dönüşüm ile (*R*)-(-)-1-methyl-2,3-dihydro-1*H*-inden-1-yl acetate **53** and (*S*)-(+)-1-methyl-2,3-dihydro-1*H*-inden-1-ol **54** sırasıyla % 71 ve % 44 enantiyomerik zenginlik ile elde edildi. (±**)-**1-methyl-1,2,3,4-tetrahydronaphthalen-1-ol **57** CAL-A-CLEA ile enzimatik resolüsyona tabi tutulduğunda % 25 lik bir dönüşüm ile (*S*)-(+)-1-methyl-1,2,3,4-tetrahydronaphthalen-1-ol **57** % 33 enantiyomerik zenginlik ile elde edilirken CAL-A ile enzimatik resolüsyona tabi tutulduğunda %20 lik dönüşüm ile (*R*)-(-)-1-methyl-1,2,3,4-tetrahydronaphthalen-1-ol **57** % 99 enantiyomerik zenginlik ile elde edildi.

Anahtar Kelimeler: Enzimatik Resolüsyon, Üçüncül alkol, Lipas, Indan, Tetralin.

TO MY PARENTS AND MY ELDER BROTHER

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LIST OF SYMBOLS AND ABBREVIATIONS

CAL-A :Candida antarctica lipase A

CAL-B :Candida antarctica lipase B

CCL :Candida cylindracea lipase

CRL : Candida rugosa

CAL-A-CLEA :Cross-Linked Enzyme Aggregate

Amona PS-C II :Pseudomonas Cepacia on ceramic

PLE : Pig Liver Esterase

Mg : Magnesium

CH_3I : Methyl Iodide

TBAI :Tetrabutyl ammonium iodide

KH : Potassium hydride

THF : Tetrahydrofuran

$CHCl_3$: trichloromethane (chloroform)

CH_2Cl_2 : Dichloromethane

$MgSO_4$: Magnesium sulfate

EtOAc : Ethyl acetate

NMR : Nuclear Magnetic Resonance

IR : Infra Red

HPLC : High Performance Liquid Chromatography

ee : enantiomeric excess

Rf : Retention Factor

M.p. : Melting Point

CHAPTER I

1. INTRODUCTION

1.1. Enzymes

1.1.1. Basic concepts of enzymes

We know that enzymes are necessary biomolecules to continue permanency of the life. They are accepted as biological catalysts which regulate and accelerate chemical reactions producing the enantiomeric compounds used in the pharmaceutical and food industry.

1.1.2. The advantages of enzymes

Enzymes are molecules which are used as biocatalysts. Enzymes have various advantages such as;

- Enzymes are good catalysts. They speed up reactions by reducing the activation energy.
- Enzymes are not denatured at the end of their reactions and they are recycling (immobilize enzymes).
- Enzymes are harmless for natural environment and can be easily disposed of.
- Enzymes act under mild conditions. Most enzymes function optimally at a temperature of 20-40 °C and at pH values which are about 5-8, typically around 7 [1]. The moderate operating temperature range of enzymes minimize any undesired side-reactions. However, every enzyme cannot operate at the same temperature and

pH ranges. Every enzyme shows maximum activity at a certain temperature and pH ranges.

- Enzymes can be reversible. Some catalytic reactions can be run in both directions.
- Enzymes show high selectivity such as chemoselectivity, regioselectivity (or diastereoselectivity) and enantioselectivity.

1.1.2.1. The selectivity of enzymes

a) Chemoselectivity; Enzymes select one of two or more different functional groups in a reaction. The other functionalities which would normally react in a certain amount with chemical catalyst do not changed.

In the study done by Jinwal *et al*. in 2002 (as shown in Figure 1.1), a chemoselective hydrolysis of fatty acid ester (in presence of carbamate of 5-amino-2,4-dihydro-3H-1,2,4-triazole-3 ones) was done by using a lipase isolated from a soil isolate, Pseudomonas mendocina (PK-12CS) at 37 °C and pH 8 [2].

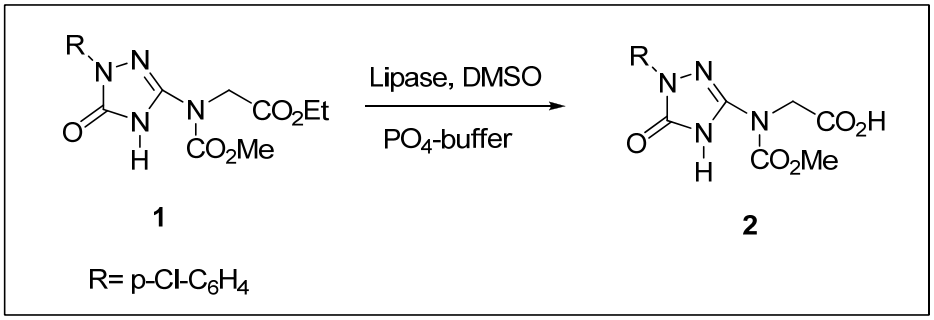
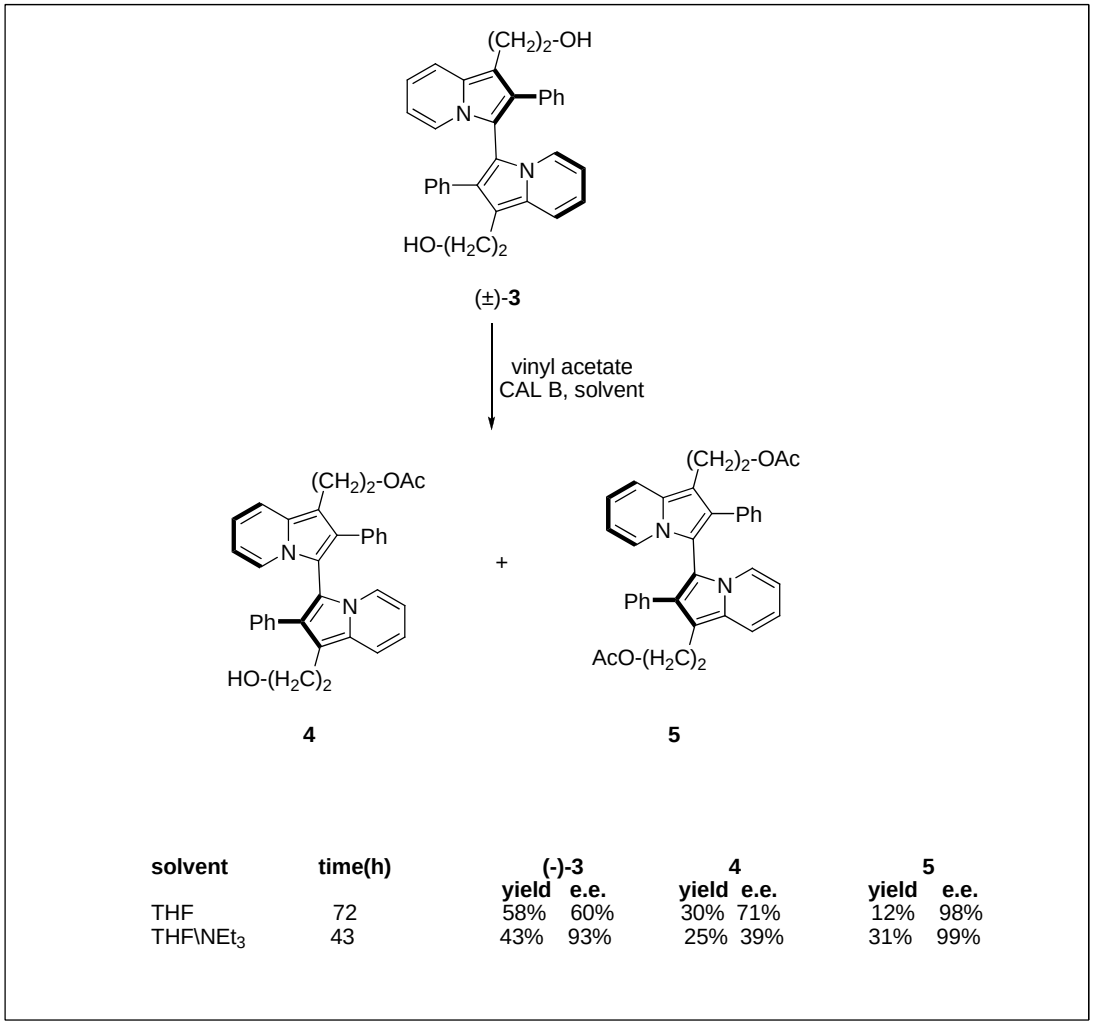


Figure 1.1. Chemoselective Hydrolysis of Fatty Acid Ester.

b) Regioselectivity or Diastereoselectivity; We know that enzymes have complex three dimensional structures. Due to these structure, enzymes may prefer

only one or a predominance of one from constitutional isomers which are chemically situated in different regions of the same substrate molecule.

In 1996, F. Theil and workers established the resolvation of the racemic axially chiral diol **3** by transesterification with vinyl acetate in the presence of lipase B from Candida antarctica (CAL B) in order to prepare non-racemic 3,3'-bi-indolizines (Figure 1.2) [3,4]. This sequential kinetic resolution afforded the unchanged diol (-)-**3** and the diacetate **5** with high enantiomeric excess as well as the corresponding monoacetate **4** with poor e.e.



solvent	time(h)	(-)-3		4		5	
		yield	e.e.	yield	e.e.	yield	e.e.
THF	72	58%	60%	30%	71%	12%	98%
THF\NEt$_3$	43	43%	93%	25%	39%	31%	99%

Figure 1.2. Regioselective acylation of the racemic axially chiral diol **3**.

c) Enantioselectivity; Enzymes are chiral catalysts. As a consequence, any type of chirality present in the substrate molecule is recognized upon the formation of the enzymesubstrate 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 which afford a kinetic resolution.

In 1996, Stead P. and coworkers found that, for the kinetic resolution of trans-2-methoxycyclohexanol with viniyl acetate in the presence of lipase from Candida antarctica (CAL), triethylamine in cyclohexane improves the outcome of the reaction assuming that traces of acid liberated from vinyl acetate are trapped by the base (Figure 1.3). The alcohol (1S,2S)-7 is a precursor for tricyclic β-lactam antibiotics [3,5].

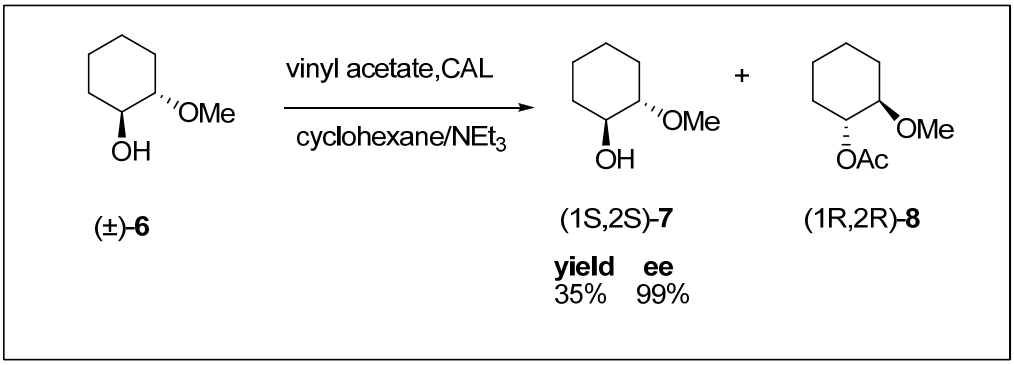


Figure 1.3. Kinetic resolution of trans-2-methoxycyclohexanol with Candida antarctica lipase (CAL).

- An another advantage of enzymes is to being used in several organic reactions.

1.1.2.2. Enzymes which are used in organic reactions

Enzymes which are used in several organic reactions was shown in Table 1.1 and also these reactions are found in Table 1.2.

Tablo 1.1. Enzymes commonly used for organic synthesis

Not requiring cofactors	Not requiring added cofactor	Cofactor requiring
1)Hydroyltic enzymes: Esterases Lipases Amidases Phospholipases Epoxide hydrases Nucleoside phosphorylase SAM synthetase 2)Isomerases and lyases: Glucose isomerase Aspartase Phenylalanine ammonia 3)Aldolases 4)Glycosyl transferases 5)Gycosidases 6)Oxynitrilase	1)Flavoenzymes: Glucose oxidase Amino acid oxidases Diaphorase 2)Pyridoxalphosphate enzymes: Transaminases Tyrosinase Δ-Aminolevulinate Dehydratase Cystathionine synthetase 3)Metalloenzymes: Galactose oxidase Monooxygenases Dioxygenases Peroxidases Enoate Reductases Aldolases Carboxylyases Nitrile Hydrase 4)Thiamin pyrophosphate dependent enzymes: Transketolases Ddecarboxylases 5)Others: SAH hydrolase B_{12}-Dependent enzymes PQQ (Methoxation) enzymes	1)Kinases-ATP 2)Oxidoreductases-NAD(P)(H) 3)Methyl transferases-SAM 4)CoA-Requiring enzymes 5)Sulfurylyases-PAPS

Table 1.2. Chemical reaction types mediated by biocatalysts

Reaction class	Reaction type
Hydrolysis and synthesis	Hydrolysis and synthesis of esters[6], amides[7], lactones[8], lactams[9], ethers, acid anhydrides, epoxides[10,11], nitriles.
Oxidation-reduction	Oxidation-reduction of alkanes, alkenes, aromatics, aldehydes, sulfides[12], sulfoxides, alcohols and ketones [13].
Addition-elimination	Addition-elimination of water, ammonia[14], hydrogen cyanide.
Halogenation and dehalogenation	Alkylation[15], dealkylation, carboxylation, decarboxy-lation, isomerization, acyloin, aldol, Michael-additions

1.1.3. Disadvantages of enzymes

- Enzymes are provided by nature in only one enantiomeric form. Sometimes this can be problem. Enzymes are usually made from L-amino acids. Since there is no general way of creating the mirror images of enzymes from D-amino acids, it is impossible to invert their chiral induction on a reaction by choosing the other enantiomer of the biocatalyst. To be overcome this problem, a strategy which involves a chiral chemical catalyst is possible.

- The advantage of working of enzymes under mild reaction conditions can sometimes turn into a drawback. If a given temperature and pH value cause to slow down the reaction, the scope for alteration is very narrow due to high parameters of temperature and pH cause deactivation of proteins.

- It is know that water has high boiling point and heat of vaporization. Furthermore, the majority of organic compounds are poorly soluble in aqueous media. Since all reasons, water is usually least suitable solvent for most organic reactions and so, organic media would be prefered instead of an aqueous media for enzymatic reaction. However enzymes display highest catalytic activity in water.

This situation cause that the enzymatic reaction in organic synthesis become at some loss activity.

- Enzymes may cause allergic reactions.

1.1.4. Nomenclature and classification of the enzymes

In nomenclature of enzymes, the suffix "ase" is added into their names. To describe each type of enzmes EC (Enzyme Commission) number are required. Every enzymes has four digit numbers EC (A.B.C.D) which are indicated in the follows;

A . denotes the main type of reaction.

B . stands for the subtype , indicating the substrate type or the type of transferred molecule.

C . indicates the nature of the co-substrate.

D . is the individual enzyme number

Enzymes have been classified into six categories according to the type of reaction they can catalyze. This is shown in Table 1.3.

Table 1.3. Classification of enzymes [16]

Enzyme Class	Reaction type
1. Oxidoreductases:	Redox reactions: oxygenation of CH, C-C, C=C bonds or removal or addition of H atom equivalents.
2. Transferases :	Transfer of groups: aldehydic, ketonic, acyl, sugar, phosphoryl, methyl
3. Hydrolases:	Hydrolysis formation of esters, amides, lactones, lactams, epoxides, nitriles,anhydrides, glycosides
4. Lyases:	Addition-elimination of small molecules on C=C, C=N, C=O bonds
5. Isomerases	Isomerizations such as racemization, epimerization.
6. Ligases	Formation-cleavage of C-O,C-S, C-N, C-C with concominant triphosphate cleavage.

1.1.5. The structure of enzymes

All enzymes are proteins and made from linear chains of amino acids linked together by peptide bonds to produce a three dimensional folded product. Each unique amino acid sequence produce a specific structure, which has unique properties. Thus, such folded conformation creates an active site that attaches to the substrate.

Every enzyme cannot be only formed from protein part, but they can also contain nonprotein organic and inorganic substances linked together with their proteins.

1.1.5.1. Coenzyme

Many enzymes require the presence of low molecular weight non protein molecules. These small molecules may be bound to the enzyme by a covalent, tight

or noncovalent linkage. These molecules are called as cofactors. The cofactors may be metal ions such as Fe, Ni, Cu, Zn, Mg, or Mn. If this non-protein part is an organic molecule, such as NAD^+, it is called a coenzyme (which are given in the Table 1.4). Coenzymes become chemically changed in the course of the enzymatic reaction. NAD^+ becomes converted to NADH. In some enzymes the cofactor is permanently bound to the enzyme, in which case it is called a prosthetic group.

Table 1.4. The list of the enzymes required for biotransformation.

Coenzyme	Reaction Type
NAD^+/NADH	removal or addition
$NADP^+$/NADPH	of hydrogen
ATP[b]	phosphorylation
SAM	C_1-alkylation
Acetyl-CoA	C_2-alkylation

b= For other triphosphates, such as, GTP,CTP and UTP, the situation is similar.

1.1.6. The working principle of the enzymes

The catalytic properties of enzymes are based on their three dimensional structures and active sites. In order to understand enzyme catalysis, their working princibles are investigated at three different mechanisms.

1.1.6.1. Lock and Key mechanisms

Enzymes are very specific for their reactants (called substrates) and it was suggested by Emil Fischer in 1984 [17]. This assumption supposes that an enzyme (the lock) and its substrate (the key) possess specific complementary geometric

shapes that fit exactly into one another like a lock and key (in Figure 1.4) and it assumes a completely rigid enzyme structure.

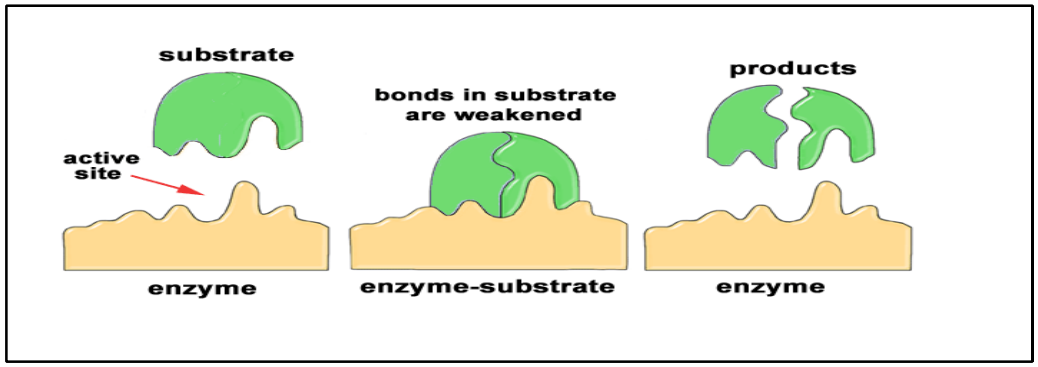


Figure 1.4. The Schematic represantation of the Lock and The Key Mechanism

1.1.6.2. Induced-Fit mechanisms

Induced-fit model was developed by Koshland Jr. In 1960s [18]. This model describes that an enzyme can change its shape to better accommodate the substrate. Since enzymes are rather flexible structures, the active site is reshaped by interactions with the substrate during the formation of the enzyme-substrate complex (Figure 1.5). This phenomenon can be understood with an example of interaction of hand (the substrate) and a glove (the enzyme).

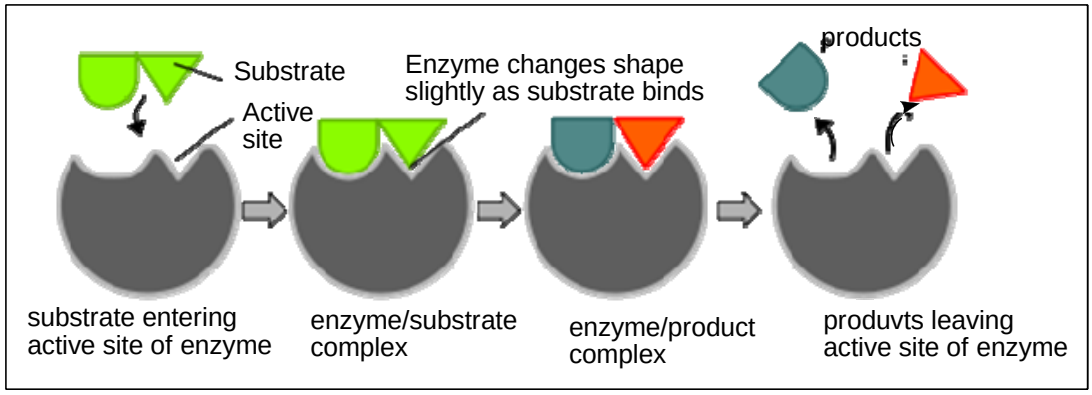


Figure 1.5. Schematic diagram of induced-fit model between an enzyme and its Substrate

1.1.6.3. Three-Point Attachment Rule

A. G. Ogston [19] developed a rationale for the enantioselectivity of enzymes, which states that a substrate must be attached by at least three points on the enzymes for a high enantioselectivity. Figure 1.6 shows the preferred binding of a substrate that will yield just one enantiomer of the product.

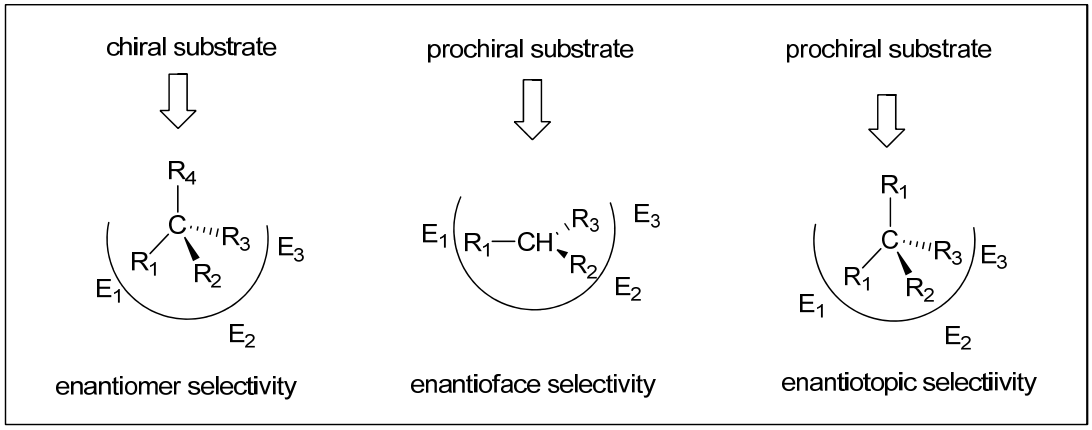


Figure 1.6. Three point attachment rule of enzyme enantioselectivity. The complementary interaction between substituents of the substrate R with enzyme residues E enables catalysis yielding a single enantiomer from a chiral or prochiral substrate

In production of the other enantiomer the complementarity to the enzyme is reduced. In Figure 1.7, case A displays that the structures are perfectly complementary, and the groups of substrate are correctly aligned and fit for binding to these sockets at active site of an enzyme. In case B, however, two of the groups will be misfit to their sockets, which hardly leads a stable binding.

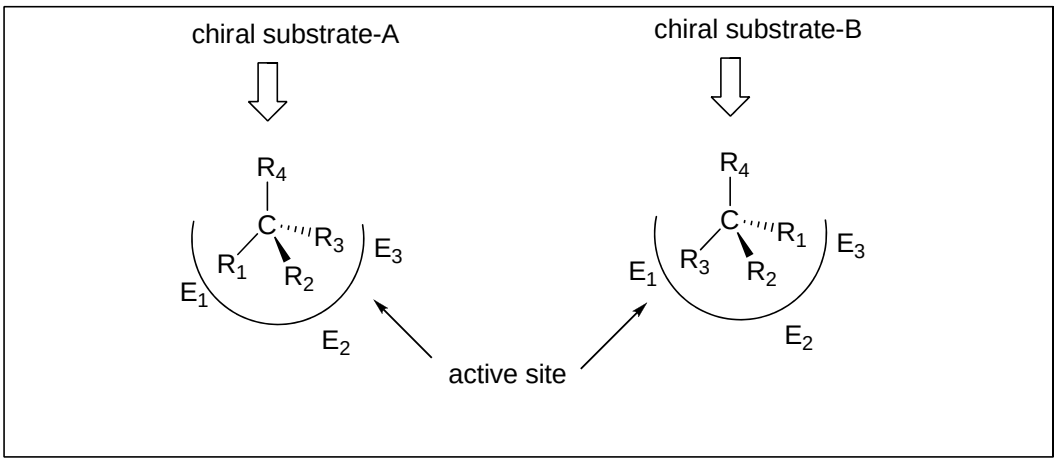


Figure 1.7. Schematic representation of enzymatic enantiomer discrimination.

1.1.7. Factors Affecting The Enzyme Activity

The factors of enzymes might be influenced by changing parameters in organic medium.

1.1.7.1. The Temperature Effect:

Reaction rate of enzymes is directly proportional until a certain temperature point. After the this point, the rate decreases and will cease (as shown in Figure 1.8). This point is called as optimum temperature which shows highest activity in enzyme reactions. While they show little activity at low temperature than their optimum temperatures, enzymes can be denatured in higher temperature than the optimum temperature. It is known that most enzymes function optimally at a temperature of 20-40°C except some enzymes. Some organisms which are called thermophiles live at high temperatures (80 – 110 $^{\circ}$C) sometimes even higher, while some live at extremely cold temperatures termed psychrophiles (0-40 $^{\circ}$C).

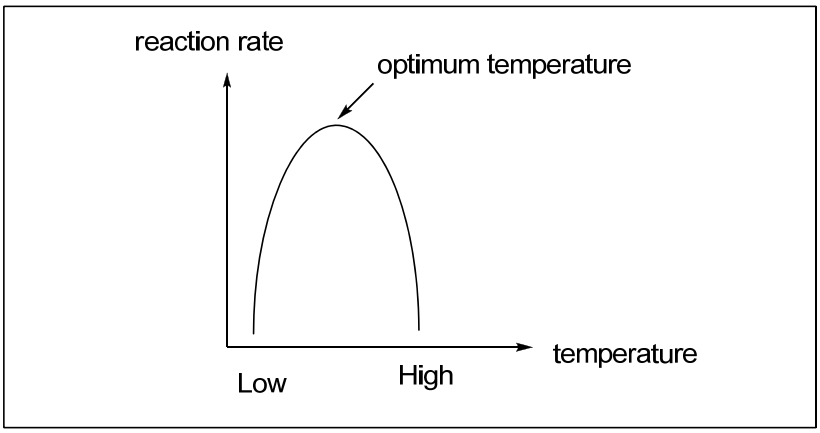


Figure 1.8. The temperature effect in enzyme action

1.1.7.2. The pH Effect:

Most enzymes have mild pH in the range of 5-8. These ranges are not same for all enzymes. Optimum pH value can be different for each enzyme. The activity decreases at lower and higher pH than optimum value. Most high and low pH can cause various problems such as decomposition of enzymes and substrates, seperation of coenzyme from the enzyme and inhibitation of enzyme-substrate complex. However some enzymes can show high activity in low or high pH. For example, the optimal pH is 2 for pepsin while the optimal pH is 8.5 for trypsin (in Figure 1.9).

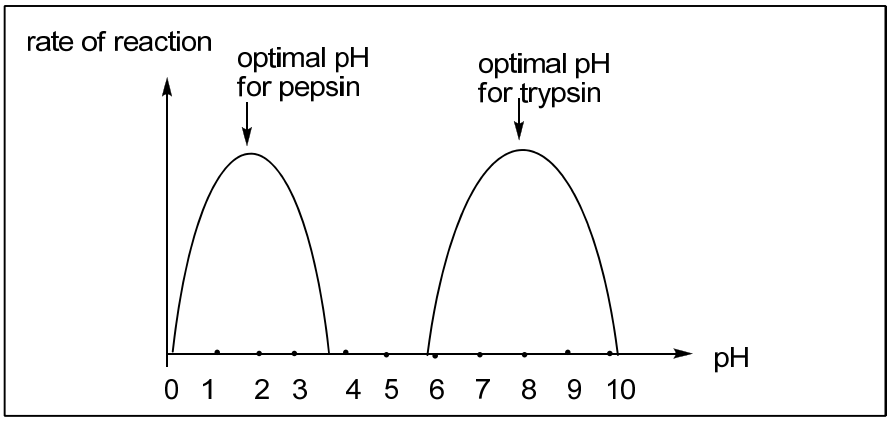


Figure 1.9. The alteration of reaction rate for pepsin and trypsin with different pH values

1.1.7.3. The Concentration of Enzyme and Substrate:

The concentration of enzyme and substrate can affect the speed of reaction. By increasing concentration of both substances the speed of reaction rises. If the substrate concentration incerases while enzyme concentration is constant, the reaction rate of enzyme increases until a certain level and after continutue with constant rate . If enzyme concentration elevates in a medium have certain amounts of substrate, the reaction increases until completely spending of substrate and stop.

1.1.7.4. The Solvent Effect:

Type and amount of solvent is important for enzymatic reactions. It is known that eznymes are highly soluble in water. If the solvent of enzyme changes from water to an organic solvent this causes to slow down of reaction.

1.1.8. Immobilized Enzymes

In several industrial and clinical process enzymes are mixed with their substrate solvents and after the product is occurred their recycling are not possible. However, enzymes are more expensive. These situations cause the high cost for industry. To overcome these problems, immobilization methods are proved. Immobilization is a method that converts solubility of enzymes in water by supporting of an insoluble solid material (or matrix). It is known that the solubility of enzymes in water is extremely high and they gain the insoluble property in water with the help of this material. The immobilization of enzymes in a solid material has been widely investigated and a variety of supporting media has been tested [20].

There are several advantages of immobilize enzymes;

a) These enzymes are easily removed.

b) They are inexpensive and recycling for using expecially in industry.

c) The pH action and thermal stability of immobilize enzymes are increased since these materials preserve the enzyme as physical barrier from deactivity of their.

d) Immobilization provides to be more convenient of enzymes for fermantation.

e) Their control is easyer than enzymes without any supporting material.

f) Multible enzymes can be immobilized in immobilize cells. For this reason they can carry out multible reactions at the same time.

Several methods have investigated for immobilization of enzymes (figure 1.10);

a) enzyme non-covalently adsorbed to an insoluble particle

b) enzyme covalently attached to an insoluble particle

c) enzyme entrapped within an insoluble particle by a cross-linked polymer;

d) enzyme confined within a semipermeable membrane.

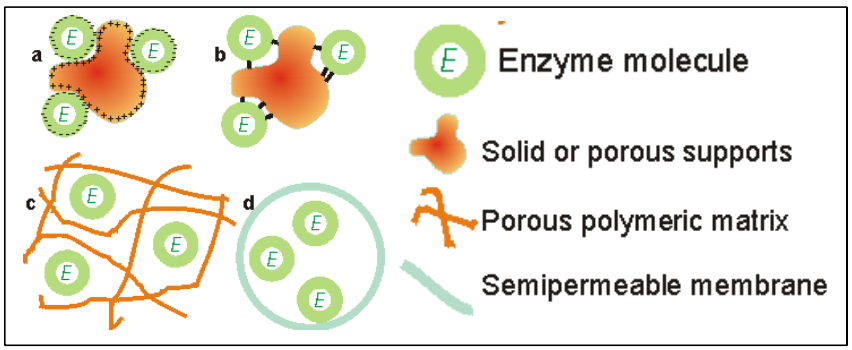


Figure1.10 : Immobilized enzyme systems

1.1.9. Lipase Enzymes

Lipases are enzymes which hydrolyze triglycerides into fatty acids and glycerol [21,22] and occur widely in nature. They are present in several organisms, including animals, plants, fungi and bacteria. They play an important role in biotechnology, not only for food and oil processing but also in organic chemistry because of their enantioselective properties. They are used for the preparation of chiral intermediates [23] and they can be used as valuable chiral catalyst. The lipases catalyze wide range of reactions, including hydrolysis, inter-esterification, alcoholysis, acidolysis, esterification and aminolysis. On the other hand, biocatalysis in aqueous media has emerged as a powerful tool for the production of fine chemicals, pharmaceuticals and food ingredients.

As examples to these lipases can be given CAL-A (Candida antarctica lipase A), CAL-B (Candida antarctica lipase B), CCL (Candida cylindracea lipase and also named as Candida rugosa, CRL), CAL-A-CLEA (Cross-Linked Enzyme Aggregate) and Amona PS-C II (Pseudomonas Cepacia on ceramic).

1.1.9.1. Candida antarctica lipases (CAL-A)

The basidiomyceteous yeast Candida antarctica produces a lipase named as CAL-A [24]. As the name suggests, this enzyme was isolated in Antartica and are most stable at acidic pH. CAL-A is Ca^{+2} dependent and extremely thermostable protein which is deactivated only at 50°C-60°C. This enzyme is able to maintain its hydrolytic activity in organic solvents at higher temperatures and over a broader pH range. Cal-A is highly unusual in that it accepts tertiary alcohols and shows a high selectivity for the N-acylation of β-amino esters.

1.1.9.2. Candida cylindracea lipase (CCL)

Candida cylindracea lipase is formely denoted as Candida rugosa and is produced from the yeast Candida rugosa. This enzyme is used for racemic mixture resolution via both hydrolysis and acylation reaction, in ester synthesis and in the production of fatty acids and glycerol [25].

It is also used for the resolution of menthol in aqueous and oganic solvents and resolution of esters of secondary alcohols. CCL is able to accommodates bulky esters in its active site and its enantioselectivity in hydrophobic organic solvents is better than that in hydrophilic solvents.

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1.1.9.3. Cross linked enzyme aggregate (CAL-A- CLEA)

CLEA is an immobilization method of any enzyme prepared via crosslinking. CAL-A CLEA is immobilized form CAL-A enzyme. They are used in the production of enantiopure alcohols, amines or acids via ester hydrolysis in aqeous media or via direct esterification in organic media. They are penicillin acylase used in antibiotic synthesis [26].

1.1.9.4. Amano PS-C II

Lipase PS–C II is immobilized from lipase PS on ceramic particles chemically modified with methacrylic groups. Lipase PS (Pseudomonas cepacia) immobilized on the ceramics support showed higher reactivity for organic substrates than the free crude enzyme. They can be used in the reaction of catalytic acylation of aldoximes and ketoximes and in kinetic resolution of alcohols.

In the study of Costa et. al. (2004) [27], the Amano PS-C II lipase was used in the kinetic desymmetrization reaction of meso-exo-3,5-dihydroxymethylenetricyclo

[5.2.1.$0^{2.6}$] decane in vinyl acetate at 23-26 $^{\circ}$C (Figure 1.11.). The Amano PS-C II lipase was found as a good catalyst giving the high yield 93%, ee ≥99 % in this reaction.

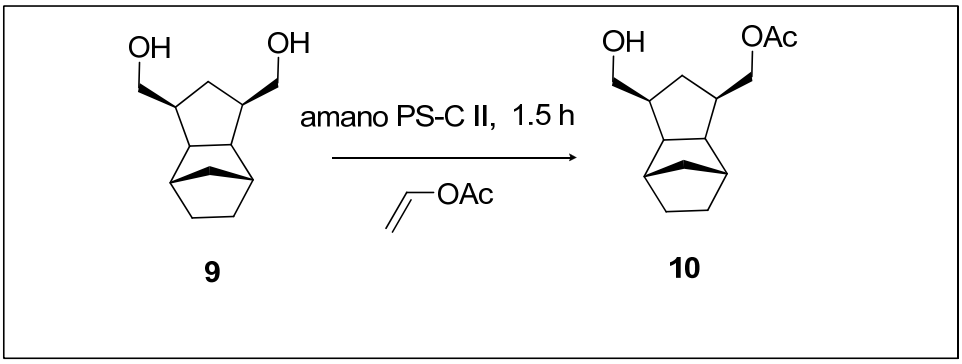

Figure 1.11. Lipase-catalysed acetylation of meso-exo-3,5-dihdroxymethyl enetricyclo [5.2.1.$0^{2.6}$] decane **9**

1.2. Asymmetric Synthesis

1.2.1. Chirality

Chirality is a tremendous importance phenomenon in our daily lives. It plays an enormous role in areas ranging from medicine to agrochemicals and other materials,which possess biological activity. The word chiral cames from the Greek name ‘‘cheir’’, which means hand. Chirality is often referred to as handedness and hands are chiral. Right and left hands cannot be superimposed on each other. If a molecule is nonsuperimposed with its mirror image, this molecule is chiral. Figure 1.12 gives an example of a chiral object.

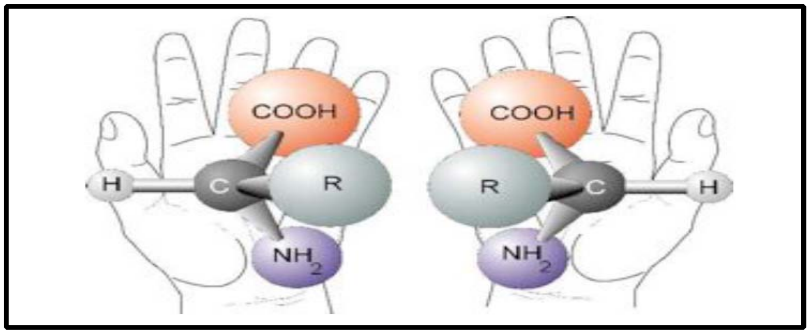

Figure 1.12. Chiral object (generic amino acids)

Chiral molecules have tetrahedral stereogenic carbon atom with four different groups bonded to it. In 1874, the Dutch chemist J.H. van't Hoff [28] and the French chemist J.A.Le Bel [29] , independently of each other, developed an idea that if four different groups are attached to the carbon atom it will result a tetrahedral structure in which two different arrangements could appear. These two forms are called as enantiomers, chiral molecule and its mirror image. To take a simple example related with enantiomers, tartaric acid, developed by Louis Pasteur [30] in 1848, can be obtained in two enantiomers as (+)-tartaric acid and (-)-tartaric acid (Figure 1.13).

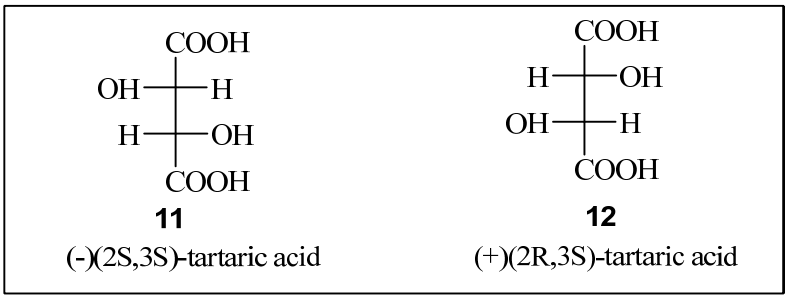

Figure 1.13. The enantiomers of tartaric acid

He not only collected the two different forms of the tartaric acid crystal but also found that the two groups of the crystals polarized the light differently, one to the left and the other to the right. The mirror image isomers, enantiomers, have a

property of rotatable of plane polarized light which is called optical activity, but in opposite directions by the same angle. For example the two common amino acid (*R*)- and (*S*)-alanine can be given (Figure 1.14).

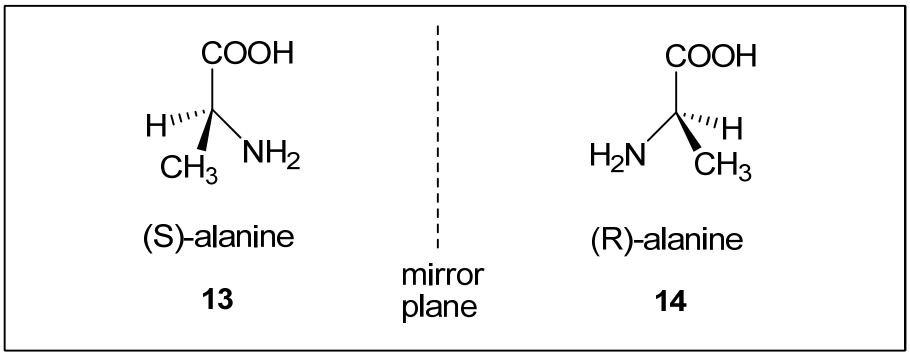

Figure 1.14. The enantiomers of alanine

Pairs of enantiomers have similar physical and chemical properties, such as melting point, boiling point, solubility, chromatographic retention time, IR and NMR spectra. If we want to determine the proportion of the two enantiomers in a mixture, the normal chromatographic and NMR methods must be modified to introduce an external chiral influence. In this conditions the enantiomers behave differently each other and would analysis be possible.

Chirality is also a property which often determines the actions and behavior of molecules. As a good example to this situation, enantiomer pairs of limonene and carvone can be given (Figure 1.15). (R)-limone smells of oranges, while the (S)-limone smells of lemons. Similarly example is carvone which is a ketone found in caraway and spearmint oils. (R) isomer of carvone has a spearmint odor, while (S)-carvone smells like caraway.

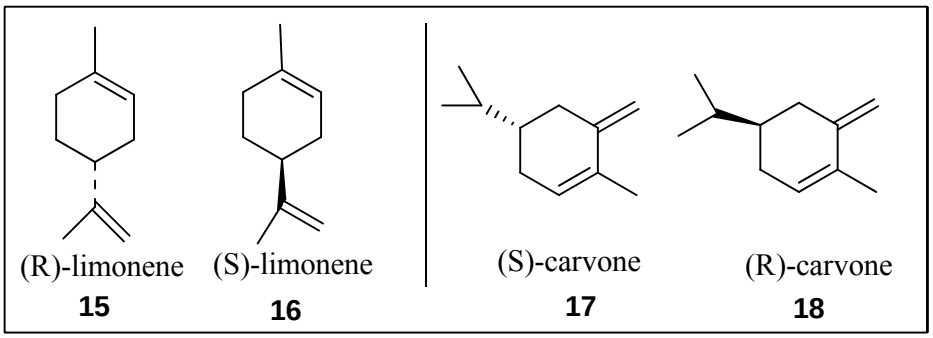

Figure 1.15. The enantiomers of limonene and carvone

To produce only one of these enantiomers or excess of one of these, a reaction which is called asymmetric synthesis is required. Asymmetric synthesis, also called chiral synthesis, enantioselective synthesis or sterioselective synthesis, is organic synthesis which introduces one or more new and desired elements of chirality. Asymmetric synthesis reactions that selectively create one configuration of one or more new stereogenic elements by the action of a chiral reagent or auxiliary.

To obtain enantioselective synthesis, it necessary is to be chiral at least one of the agents in the system. An achiral starting material can be is transformed into a chiral compound by the action of a chiral reagent, a chiral catalayst or a chiral auxiliary under conditions whereby the original chiral group subsequently is removed, leaving behind a chiral product.

1.2.2. Methods of asymmetric synthesis

Asymmetric synthesis consists of two methods as the chemical and biotechnologic.

In biotechnologic methods, biocatalysis can be used to obtain enantiomerically pure products from prochiral substrates and encompasses catalysis by enzymes from bacteria, fungi or yeast [31, 32]. An advantage of these biocatalysis

is the high selectivity and high efficiency. The methods which is used to access enantiomeric compounds can be divided into three categories as the chiral pool, from prochiral substrates and from a racemic mixture.

In chemical methods, for obtaining enantiomerically pure materials, either reagent-controlled conditions (chiral reagent,chiral catalyst) or substrate-controled (chiral substrates,chiral auxiliries) are used;

Chiral reagents

A chiral reagent is used in order to obtain an enantiomerically enriched product. The reagent must be selective both in terms of induction and functional group specificity. Considerable effort and expense can be involved in the preparation of the reagent, and the stoichiometric amounts are required.

Chiral catalysts

Chiral catalysts cause one enantiomer be selectively converted or only one enantiomer to be formed. On using a catalyst, two diastereomeric transition states are involved and an excess of one product will be delivered via the lowest energy transition state. Only catalytic amount of chiral catalyst is needed to produce large amount of chiral product.

Chiral Substrates

The best scenario is to have a chiral starting material that can then control the stereoselection of the reaction itself . Nature produces chiral materials and a number of these are available in quantity [33-34]. 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. 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.

Chiral Auxiliries

Chiral auxiliaries play an important role in asymmetric syntheses of optically active compounds. A chiral auxiliary is a chiral molecule that can be covalently attached to a prochiral substrate as a 'chiral handle' so that the subsequent reaction proceeds with high diastereoselectivity. After the diastereoselective reaction, the auxiliary should be removable and recoverable in good yield and without racemization so that they can be reused.

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. Invariably, a cyclic system is involved. This approach has been used by Seebach and described as the regeneration or self-reproduction of stereogenic center.

1.2.3. Why is the asymmetric synthesis important

Nature produces a wide variety of chiral compounds and asymmetric synthesis is required to prepare nature-identical material. Living organisms are composed of chiral biomolecules such as amino acids, sugars, proteins and nucleic acids. In nature these biomolecules exist in only one of the two possible enantiomeric forms, e.g., amino acids in the L-form and sugars in the D-form. Thus the enzymes in our cells are chiral and play an important part in cell machinery. This means that they prefer to bind to one of the enantiomers. This chiral specificity is the basis of a major industry producing chiral drugs. The two enantiomers of a drug molecule cannot bind equally well to the receptor and therefore cause different biological responses.

The chirality is extremely important in the preparation of therapeutic drugs. If enantiomers of a chiral substance have similar effect in body , the enantiomers do not need to be separated. However, if the pharmacodynamic effects of enantiomers have differences, enantiospecific analysis is urgently needed [35]. One isomer may produce the desired therapeutic activities, while the other may be inactive or produce unwanted effects. For example, (R)-Thalidomide contained the desired therapeutic activity, while its S enantiomer is teratogenic and induces fetal malformations [36]. Penicillamine and methacholine can also be given as examples to chiral drugs which exhibit different pharmacological effects (Figure 1.16).

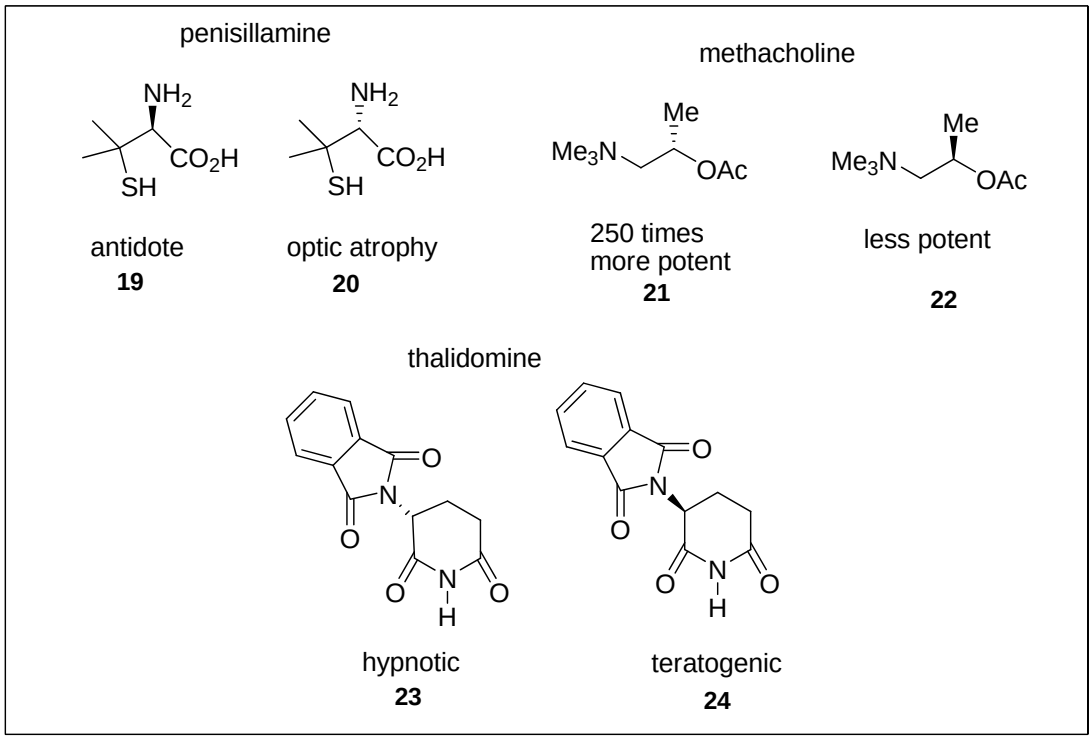

Figure 1.16. The structures and pharmacological effects of the enantiomers of some biologically active chiral molecules

1.3. Indan and tetralin

Aromatic ring fused cyclic compounds, such as tetralin and indan, and alkyl or oxygen-containing derivatives of these compounds are important in at least two different areas of scientific investigation. These compounds have been regarded as biological marker or molecular fossils which have been used to probe the source, maturation, migration, and biodegradation of crude oils. They are also important synthetic intermediates for a varity of chiral and achiral organic compounds [37].

α-Tetralone been used to prepare β-keto esters, heterocyclic spiroazeridines [38], thermorubin, a potent antibiotic substance [39], and a variety of other compounds [40, 41]. There are a large number of important pharmacological compounds that are derivatives of oxygenated aminotetralins [42, 43].

For example, 8-hydroxy-2-(di-n-propylamino)tetralin is a selective serotonin (5-hydroxytryptamine, 5-HT) receptor agonist [41]. Also (R)-7-OH-dipropylamino tetralinseems to be a selective dopamine D_3 agoist [42].

The derivatives of indan and tetralin have been investigated in several studies. There are also several tertiary alcohol synthesis amount of these studies.

1.4. The Resolution Of Tertiary Alcohols By Using Biocatalyst

Enantiopure tertiary alcohols are very valuable building blocks containing quaternary carbon centres for the synthesis of many different natural products and pharmaceuticals. Both chemical and enzymatic routes were tried on resolution of tertiary alcohols. Several enzymatic approaches have been proposed as effective tools for the synthesis of optically active tertiary alcohols.

In biocatalytic studies hydrolases, lipase and esterase, are mostly used for the kinetic resolution of tertiary alcohols [44]. Enzymes capable of hydrolysing tertiary structures are very useful, as they display high selectivity towards this functionality without affecting other protected functional groups. For example, an esterase from *Bacillus subtilis* (BS2) and lipase A from *Candida antarctica* (CAL-A) were able to hydrolyse tertiary esters selectively when other amino acid protecting groups were present in the molecules [45, 46] (Figure 1.17).

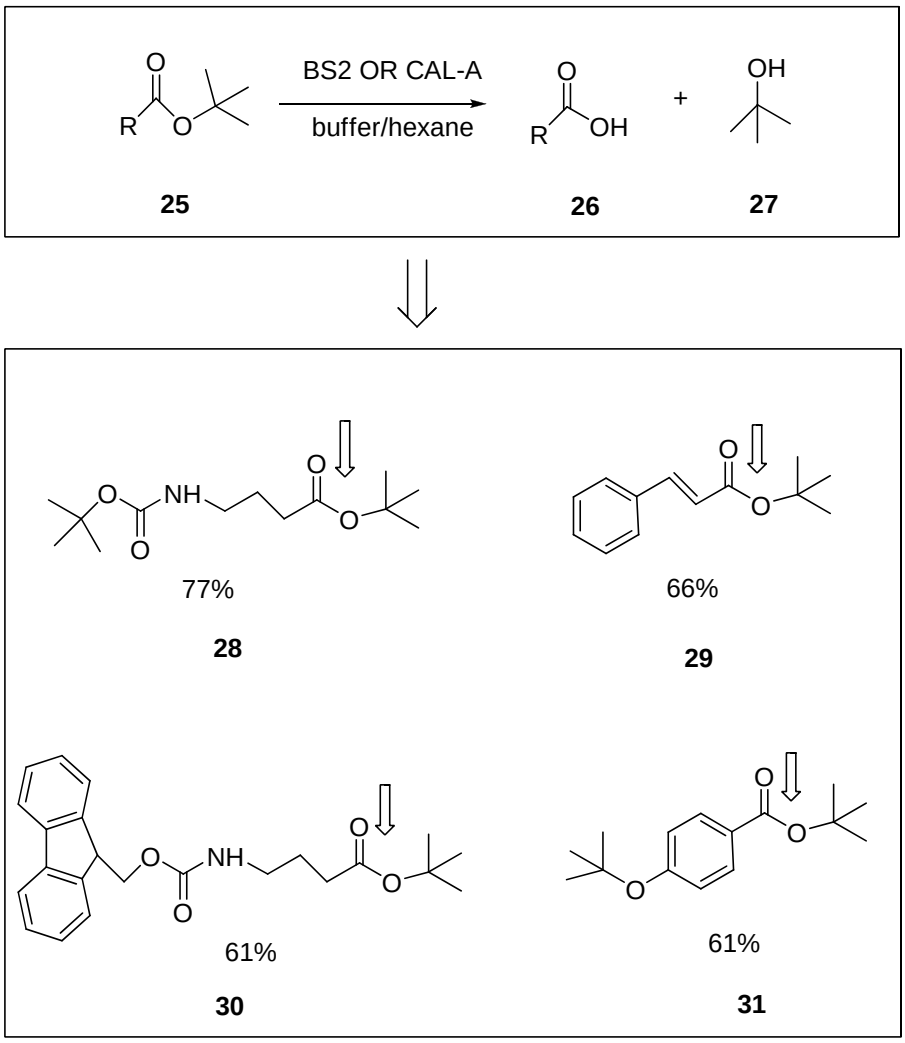

Figure 1.17. Hydrolyse-based removal strategy for tert-buthyl groups.

As an example, CAL-A are also used in the resolution of another tertiary alcohol. CAL-A was found to be an efficient biocatalyst in the resolution of (±)-2-phenylbut-3-yn-2-ol by transesterification with vinyl acetate as acyl donor (Figure 1.18).

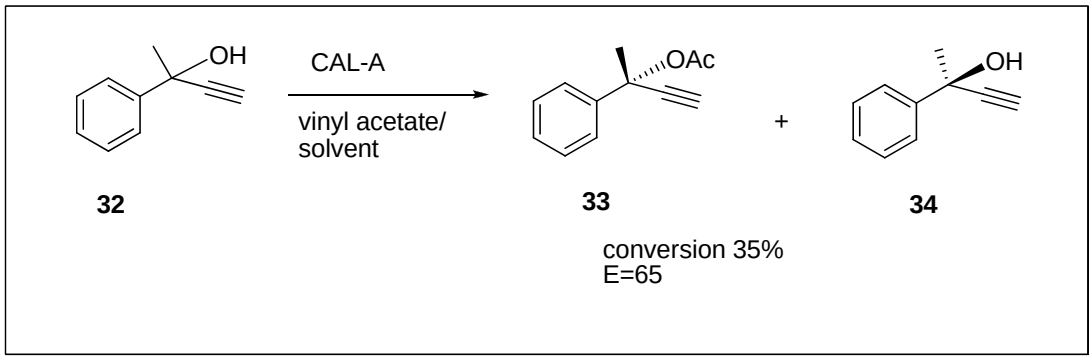

Figure 1.18. CAL-A-catalysed kinetic resolution of (±)-2-phenylbut-3-yn-2-ol with vinyl acetate as acyl donor [47].

Likewise, porcine hydrolases have also been found to accept tertiary alcohols. For instance, porcine pancreatic lipase (PPL) is effective for enantioselective hydrolysis of racemates under aqueous conditions [48] and has also been successfully used in other kinetic resolution to afford tertiary alcohols [49].

In a study, PLE-catalysed enantioselective kinetic resolution of quinuclidine ester was accomplished to produce chiral tertiary esters in acceptable yields [50] (Figure 1.19).

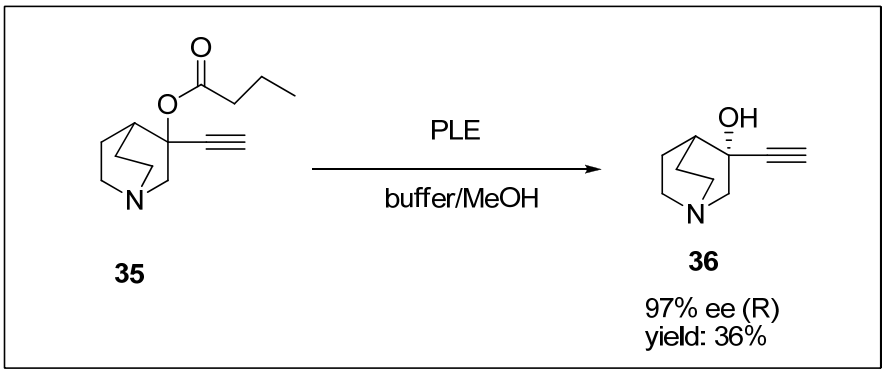

Figure 1.19. PLE-catalysed enantioselective hydrolysis of quinuclidine Esters

Another relevant synthetic application in the field of tertiary alcohols and hydrolases lies in the resolution of racemic cyanohydrins. Some enzymes such as *Pichia miso*, *bacillus coagulans*, protease subtilisin A, *candida rugosa* lipase (CRL) and *Bacillus subtilis* (BS2) have recently been shown to be efficient catalysis in the stereoselective hydrolysis of α,α-disubstituted cyanohydrin acetates. Protease subtilisin A or *candida rugosa* lipase (CRL) can be used in the kinetic resolution of these tertiary esters [51] (Figure 1.20).

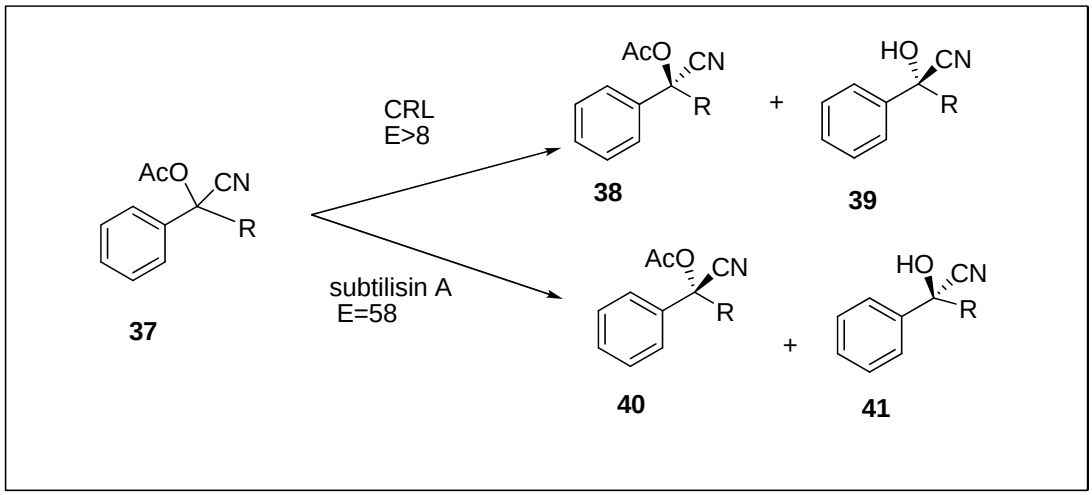

Figure 1.20. Hydrolayse-catalysed resolution of cyanohydrin acetates.

CAL-A and CRL enzyme were used in the hydrolyses of four different tertiary alcohol acetates in a study [52,53] (Figure 1.21).

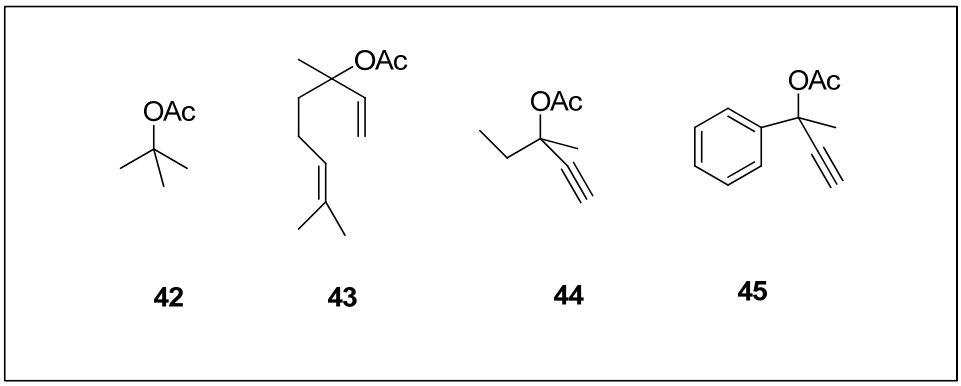

Figure 1.21. Substrates used fort he identification of new enzymes capable of converting tertiary alcohol esters

In another study, G. Jaouen and A. Meyer [54] worked on the preparation of optically pure tertiary alcohol derivatives of 1-methyl-2,3-dihydro-1H-inden-1-ol (1-indanol) and 1-methyl-1,2,3,4-tetrahydronaphthalen-1-ol (1-tetralol) with chiral tricarbonyl chromium complexes (Figure 1.22). These compounds were prepared taking into account the stereospecific attack in the exo position of methylmagnesium iodide on ketonic precursors even when the alicyclic ring was alkyl substituted. The endo alcohol derivative was obtained. After that the optical active tertiary alcohol synthesis was be copmleted by exposure of ether solutions of the compounds which had $Cr(CO)_3$ to sunlight.

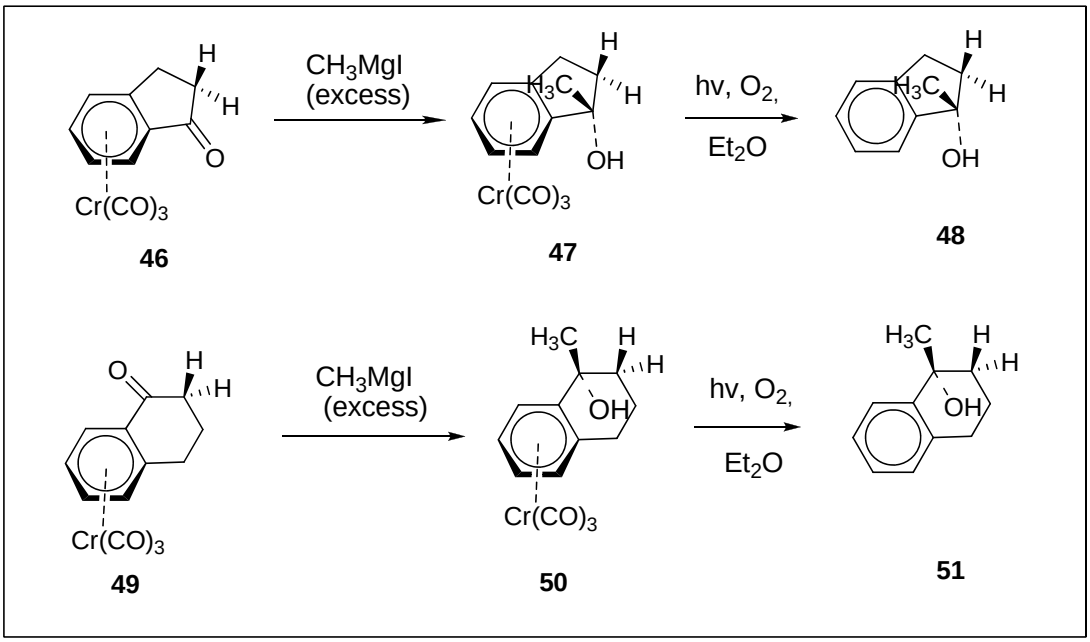

Figure 1.22. The synthesis of optical active tertiary alcohols

Altough significant activity in asymmetric carbonyl addition reactions, a long-standing problem in organic synthesis had been the absence of efficient and highly enantioselective methods to prepare tertiary alcohols. In these cases organozinc reagents are mild and exhibit excellent functional group compatibility although several examples involving organolithium and grignard reagents. Because organolithium and grignard reagents usually require greater than stoichiometric amounts of chiral ligants. In addition to these reagents organotitanium and organoaluminum complexes are tolerant of functional groups and have been applied to the asymmetric addition to aldehydes.

Jeon S. J. and workers investigated in asymmetric additions of alkylzinc to ketones by using titanium-catalyzed. One of their studies contains the peparation of 1-methyl-1-tetranole from α-tetranone by using ZnMe and titanium catalyst. In result of the study, 26% yield and 98% ee were observed [55] (Figure 1.23).

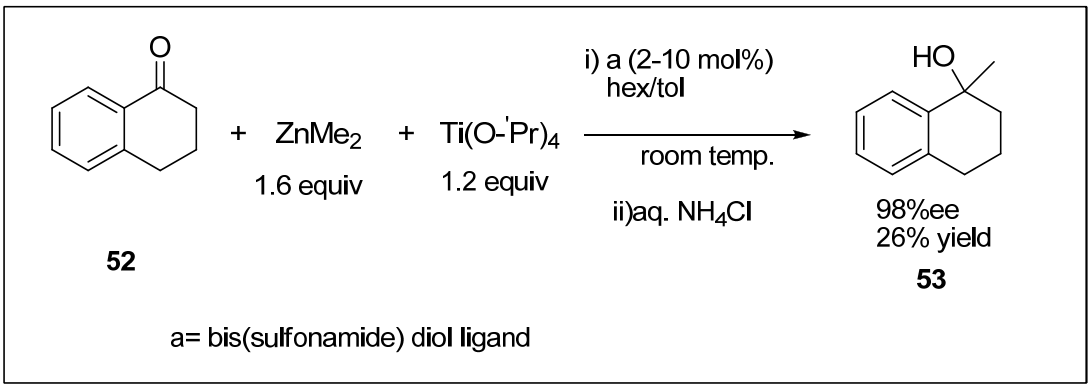

Figure 1.23. Asymmetric additions of alkylzinc to α-tetranone

1.5. Aim of The work

The aim of this work is to resolve (±)-1-methyl-2,3-dihydro-1H-inden-1-ol **54** and (±)-1-methyl-1,2,3,4-tetrahydronaphthalen-1-ol **57** by using several enzymes such as CAL-A, CAL-A-CLEA, CCL and Amona PS-C II and to find the optimum conditions for these enzymes in the resolution steps of these racemic tertiary alcohols. In this study, 2,3-dihydro-1H-inden-1-one **55** and 3,4-dihydronaphthalen-1(2H)-one **58** were chosen as a starting materials.

The aim of this work is shown retrosynthetically in the Figure 1.24.

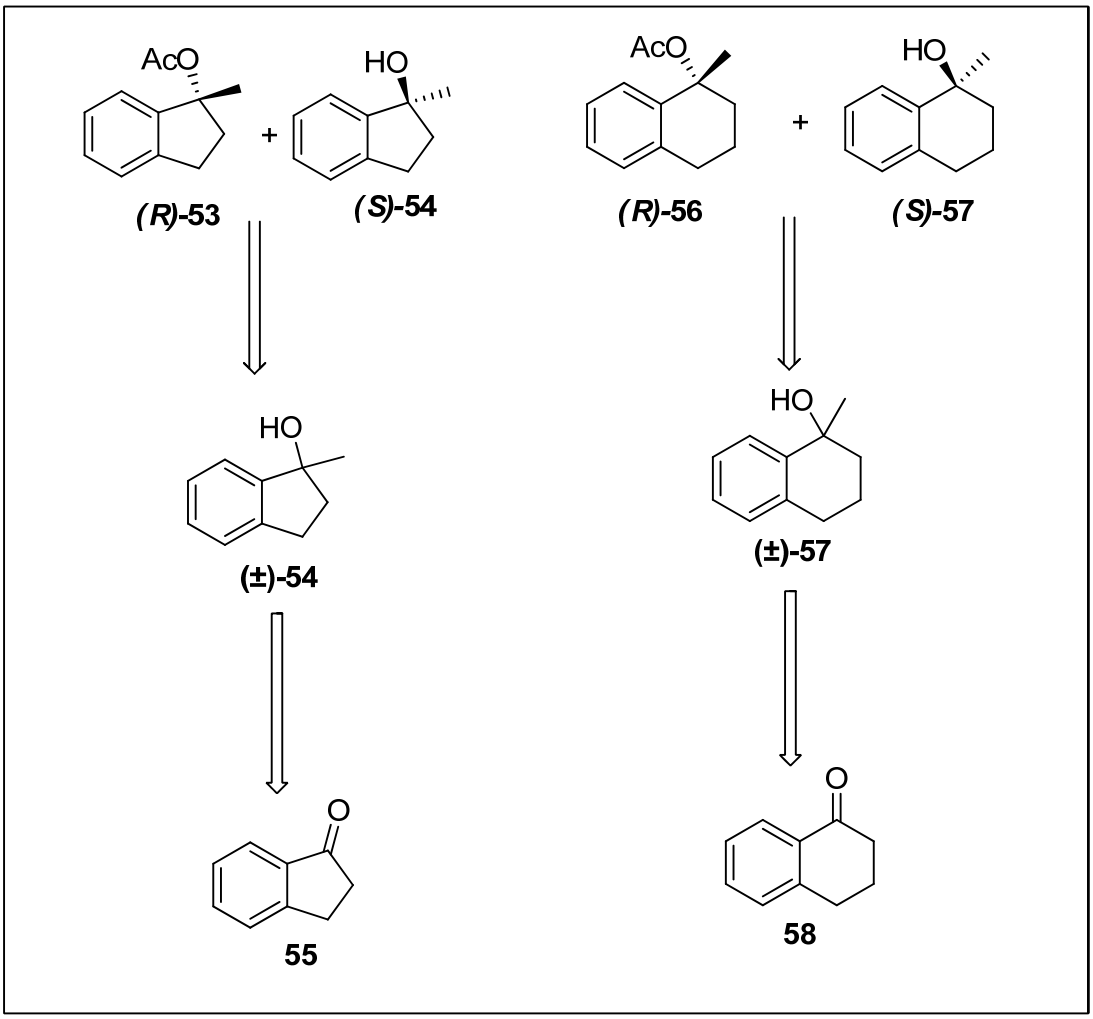

Figure 1.24. Retrosynthesis of the Work

CHAPTER II

2. RESULTS AND DISCUSSION

2.1. The Importance of Tetralin and Indan Derivatives

Nitrogen-, alkyl- and oxygen-containing derivatives of tetralin and indan have been regarded as biological markers (biomarkers) because the molecular structure of these compounds resembles the structural subunits of biological precursors, such as lipids, steroids and porphyrins which occur in the source metarial [56]. They are important synthetic intermediates for a variety of chiral and achiral organic compounds.

An indane ring framework or a tetrahydronaphthalene core is often found in a large number of bioactive and pharmaceutically interesting substances. Molecules that contain a substructure of indanones or tetralones possessing stereogenic centers are even more useful as they can be used as starting materials for the synthesis of biologically active compounds or drugs, such as etoposide, SB209670, and sertraline (Figure 2.1). These products are current interest in the pharmaceutical industry and, therefore, their derivatives have been extensively evaluated for their biological activities. There are only a few approaches to introduce chirality onto the indanone and tetralone ring systems [57].

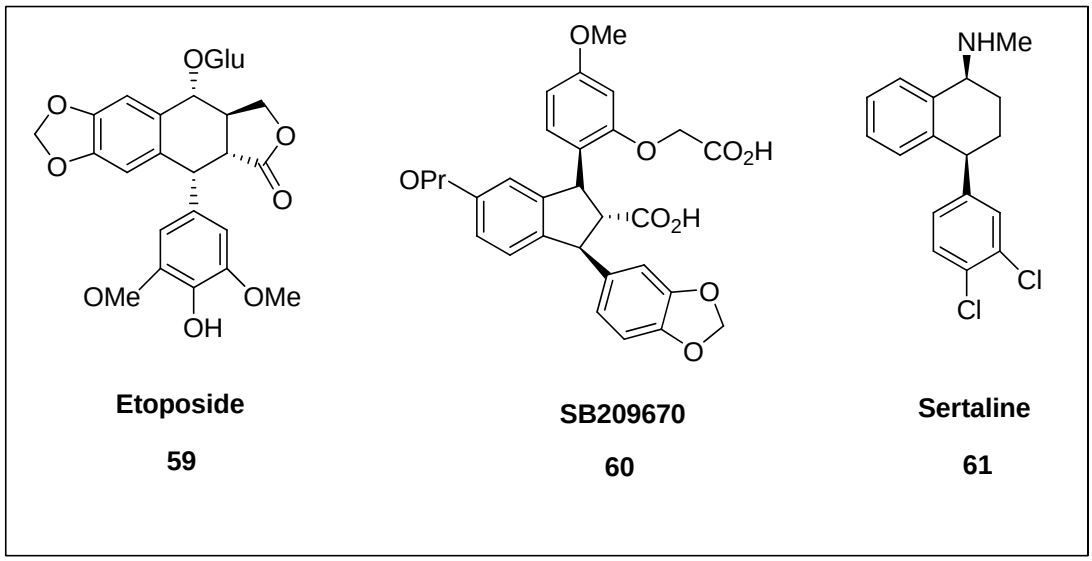

Figure 2.1. Molecules that contain the indanone or tetralone substructure

In addition to tetraline derivatives, 1-methyl-1,2,3,4-tetrahydronaphthalen-1-yl acetate can be given. In 1983, M. T. Reetz and coworkers achieved the preparation of methyl 2-methyl-2-(1-methyl-1,2,3,4-tetrahydronaphthalen-1-yl)propanoate and methyl 2-(1,3-dimethyl-2-vinylcyclohex-2enyl)butanoate from 1-methyl-1,2,3,4-tetrahydronaphthalen-1-yl acetate by using ketene silyl acetals and $ZnCl_2$ [58] (Figure 2.2).

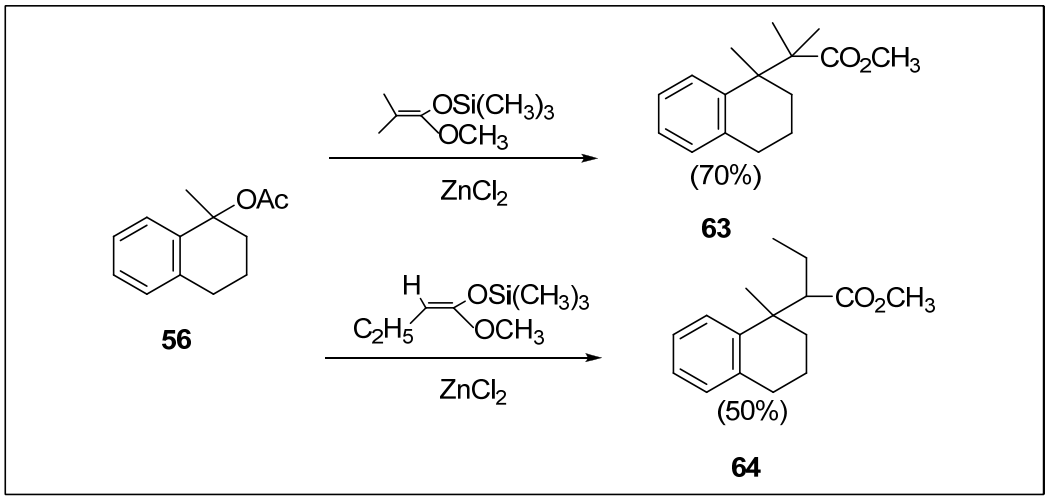

Figure 2.2. The derivatives of 1-methyl-1,2,3,4-tetrahydronaphthalen-1-yl acetate

2.2. Synthesis of 2,3-dihydro-1H-indene (Indan) Derivatives

In this work, 2,3-dihydro-1H-inden-1-one **55** was chosen as a starting material to reach the target optical active substances which are (*S*)-1-methyl-2,3-dihydro-1H-inden-1-ol **54** and (*R*)-1-methyl-2,3-dihydro-1H-inden-1-yl acetate **53**. Racemic the tertiary alcohol was obtained by using grignard reagent from this starting metarial.

2.2.1. The synthesis of (±)-1-methyl-2,3-dihydro-1H-inden-1-ol, 54

The first step of the work were the Grignard type reaction to obtain (±)-1-methyl-2,3-dihydro-1H-inden-1-ol **54** which is a tertiary alcohol by using magnesium metal, methyl iodide and diethyl ether as solvent (Figure 2.3).

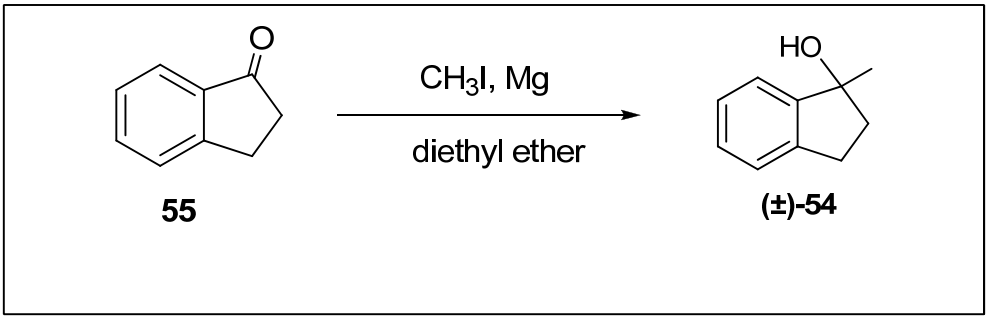

Figure 2.3. The synthesis of (±)-1-methyl-2,3-dihydro-1H-inden-1-ol

2.2.2. The synthesis of (±)-1-methyl-2,3-dihydro-1H-inden-1-yl acetate, 53

(±)-1-methyl-2,3-dihydro-1H-inden-1-ol **54** was converted to the corresponding ester by the reaction with acetic anhydride, tetrabutylammonium iodide and potassium hydride in tetrahydrofuran as shown in figure 2.4.

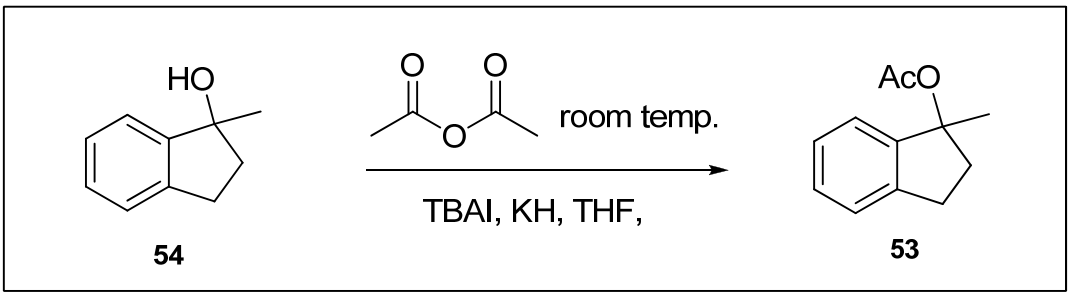

Figure 2.4. The synthesis of (±)-1-methyl-2,3-dihydro-1H-inden-1-yl acetate, **53**

The structure elucidations were done by using ^{1}H-NMR and ^{13}C-NMR spectra.

2.2.3. Characterization of (±)-1-methyl-2,3-dihydro-1H-inden-1-ol, 54

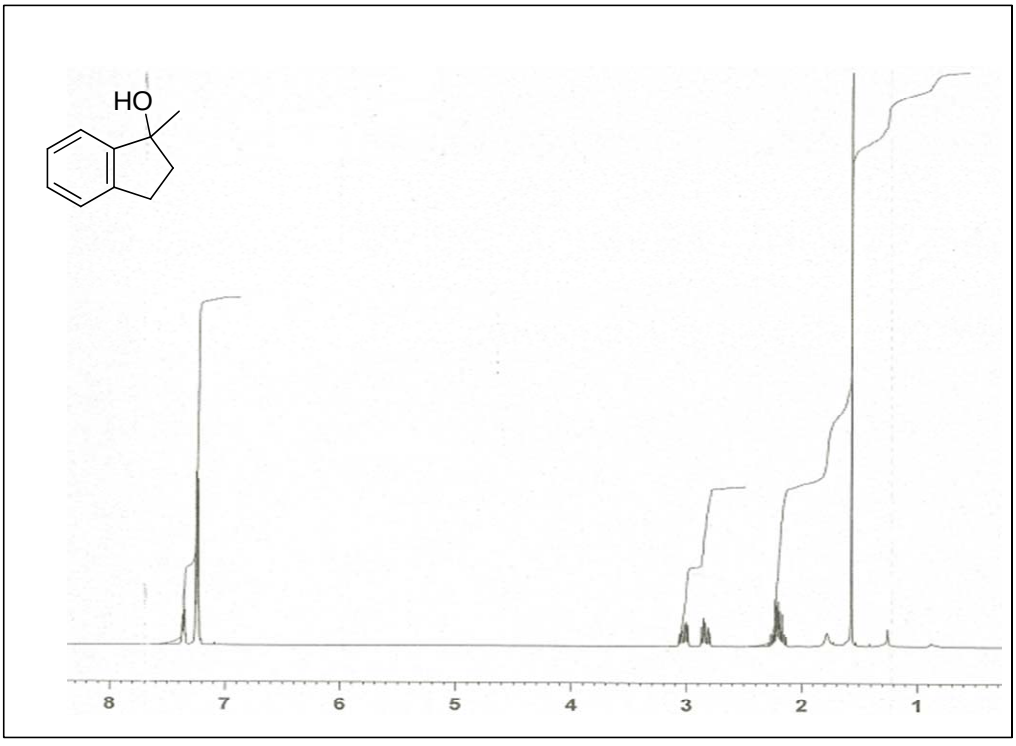

Figure 2.5. ^{1}H-NMR spectrum of (±)-1-methyl-2,3-dihydro-1H-inden-1-ol **54**

^{1}H-NMR spectrum of 1-methyl-2,3-dihydro-1H-inden-1-ol **54** shows a singlet at 1.57 ppm for the methyl protons of the cyclopentane ring. The broad singlet at 1.78 ppm is due to the OH proton. The methylene protons at third position

of cyclopentane ring exhibit the multiplet between 2.17-2.24 ppm. One of the diastereotopic methylene protons show multiplet between 2.80-2.87 ppm and the other diastereotopic methylene proton exhibits multiplet between 2.99-3.02 ppm.

Three methine protons at the forth, fifth and sixth positions of the benzene ring show the multiplet between 7.23-7.25 ppm. Finally the multiplet between 7.35-7.37 ppm is due to the methine proton at the seventh position of the benzene ring.

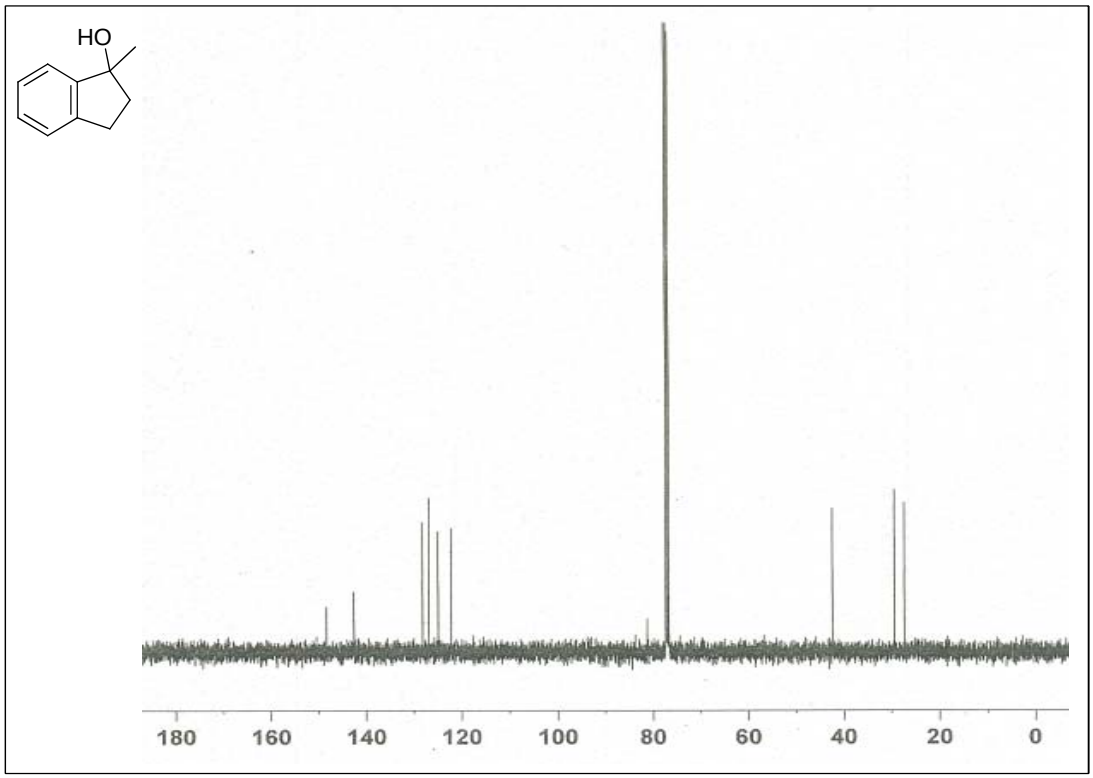

Figure 2.6. ^{13}C-NMR spectrum of (±)-1-methyl-2,3-dihydro-1H-inden-1-ol **54**

^{13}C-NMR spectrum of the compound shows the signals at 27.3 ppm and at 43.4 ppm for the methylene carbons at third position and at second position of cyclopentane ring. The methyl cabon bonded to quaternary carbon shows the signal at 29.4 ppm. The quaternary carbon exhibits a signal at 81.25 ppm.

The signals at 122.2 ppm, 124.4 ppm, 126.9 ppm, 128.2 ppm, 142.6 ppm and 148.3 ppm are for carbons of aromatic part of the compound.

2.2.4. Characterization of (±)-1-methyl-2,3-dihydro-1H-inden-1-yl acetate, 53

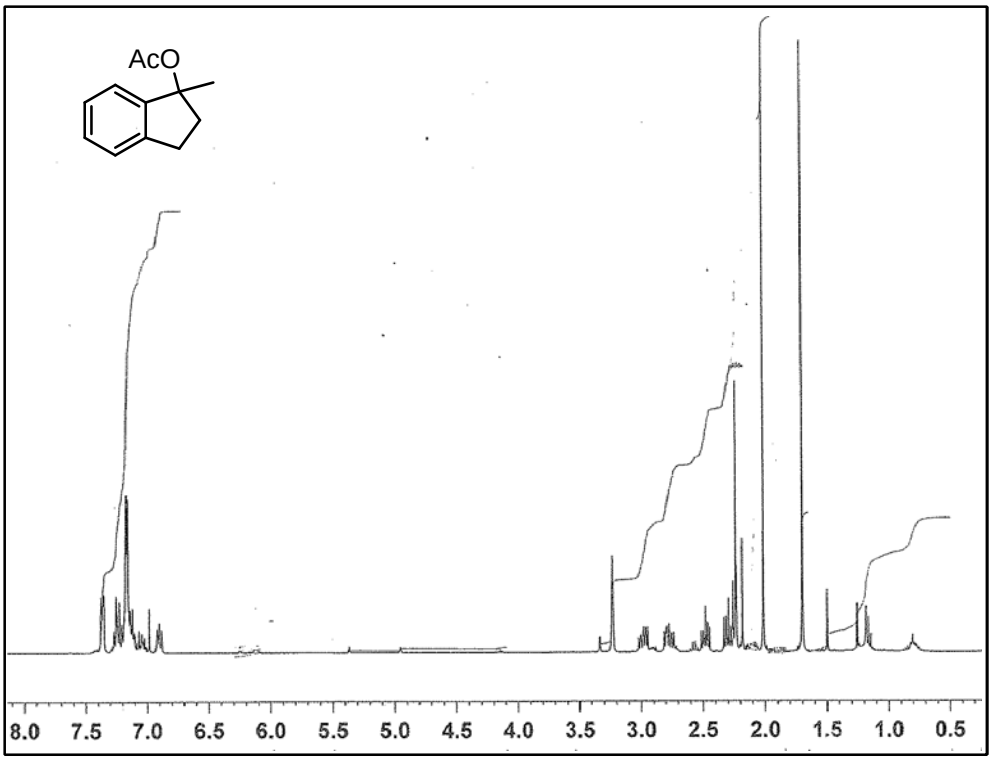

Figure 2.7. ^{1}H-NMR spectrum of (±)-1-methyl-2,3-dihydro-1H-inden-1-yl acetate, **53**

^{1}H-NMR spectrum of the (±)-1-methyl-2,3-dihydro-1H-inden-1-yl acetate **53** shows a strong singlet at 1.70 ppm for methyl protons bonded to the stereogenic center. The methyl protons of acetoxy moiety shows the singlet 2.01 ppm. One of the diastereotopic methylene protons at the third position of cyclopentane ring shows the multiplet between 2.26-2.33 ppm and the other one shows the multiplet between 2.45-2.52 ppm. One of the diastereotopic methylene protons at the second position of

cyclopentane ring shows the multiplet between 2.74-2.82 ppm and the other one shows the multiplet between 2.95-3.02 ppm.

The methine protons at the forth, fifth, sixth position of the benzene ring shows the multiplet between 7.23-7.27 ppm. Finally the multiplet between 7.35-7.37 ppm is due to the methine proton at the seventh position of the benzene ring.

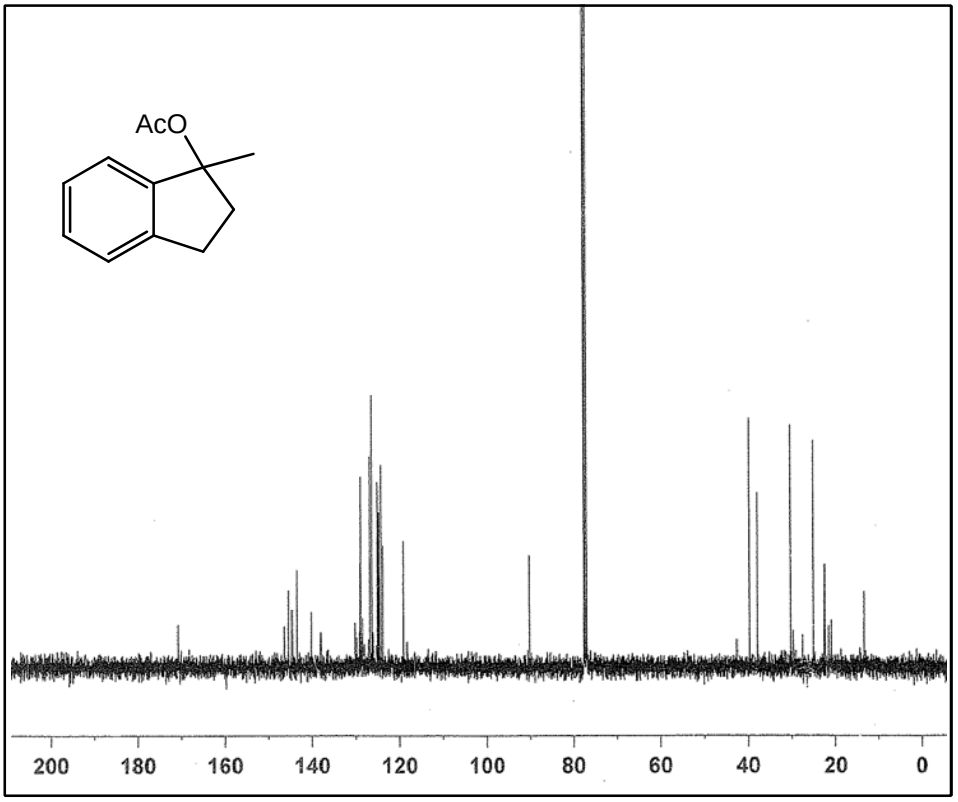

Figure 2.8. ^{13}C-NMR spectrum of 1-methyl-2,3-dihydro-1H-inden-1-yl acetate, **53**

^{13}C-NMR spectrum of the compound gives signal at 22.5 ppm due to the methyl carbon of the acetyl group, the signals at 27.6 ppm and 39.7 ppm for the methylene carbon at the third and second position of the benzene ring, the signal at 37.9 ppm methyl carbon bonded to stereogenic carbon, the signal at 90.2 ppm due to

the stereogenic carbon and the signal at 170.8 ppm for characteristic carbonyl carbon of the acetyl group.

The signals at 119.1 ppm, 123.9 ppm, 124.7 ppm, 128.4 ppm, 143.5 ppm and 145.4 ppm are for aromatic part of the compound.

2.3. Enzyme-Mediated Resolutions of (±)-1-methyl-2,3-dihydro-1H-inden-1-ol, 54

Lipases have been widely used as an asymmetric catalyst for the preparation of enantiomerically pure organic compounds. The enantioselectivity of lipases is known to be usually high in the kinetic resolution of racemic alcohols and esters, but their reactivity and enantioselectivity for various synthetic substrates are not always sufficient.

In this study, the resolution of (±)-1-methyl-2,3-dihydro-1H-inden-1-ol was performed by using CAL-A-CLEA, Amona PS-C II and CRL in vinyl acetate as an organic solvent in 32°C.

The results of the enantiomeric resolution of the (±)-1-methyl-2,3-dihydro-1H-inden-1-ol by using CAL-A-CLEA, Amona PS-C II and CRL were given in Table 2.1.

Table 2.1. The results of the enantiomeric resolution of the **(±)-54** by using CAL-A-CLEA, Amona PS-C II and CRL

Enzyme	**Time (h)**	**Conversion (%)[a]**	**ee_p (%)[b]**	**ee_s (%)[b]**	**$[\alpha]_D^{26}$(prod.)/ Abs.Conf.**	**$[\alpha]_D^{26}$(subs.)/ Abs.Conf**
CAL-A-CLEA	72	20	71	44	$[\alpha]_D^{26}$= -5.0 (*c* 0.25, $CHCl_3$) *R*	$[\alpha]_D^{26}$= +7.9 (*c* 1, $CHCl_3$) *S*
Amano PS-C II	140	7	55	-[c]	$[\alpha]_D^{26}$= +4.6 (*c* 0.25,$CHCl_3$) *S*	-[c]
CRL	168	5	15	-[c]	$[\alpha]_D^{26}$= -1.1 (*c* 0.25, $CHCl_3$) *R*	-[c]

[a] Conversions were determined using HPLC analysis.

[b] Enantiomeric excesses were determined by Daicel Chiralcel OD-H and OJ-H column by HPLC analysis.

[c] Isolated as racemic.

[p] Product

[s] Substract

In the resolution of (±)-1-methyl-2,3-dihydro-1H-inden-1-ol **54** the optimum conditions of these enzymes tried by using substrate:enzyme ratio (w/w).

To find optimum condition in amona PS-C II and CRL the substrate:enzyme ratio (w/w) changed from 1:0.25 to 1:1 at 25-32°C. In the case of 1:1 at 32°C amona PS-C II yielded (*S*)-(+)-1-methyl-2,3-dihydro-1*H*-inden-1-yl acetate **53** with 55% ee by 7% conversion at the end of 140 hours. In the same manner, CRL yielded (*R*)-(-)-1-methyl-2,3-dihydro-1*H*-inden-1-yl acetate **53** with 15% ee by 5% conversion at the end of 168 hours.

To find optimum condition in CAL-A-CLEA the substrate:enzyme ratio (w/w) changed from 1:0.25 to 1:1 at 25-32°C and It was found as 1:0.5 at 32°C. At the end of this reaction catalyzed by CAL-A-CLEA, (*R*)-(-)-1-methyl-2,3-dihydro-1*H*-inden-1-yl acetate **53** and (*S*)-(+)-1-methyl-2,3-dihydro-1*H*-inden-1-ol **54** were obtained with 71% ee and 44% ee by 20% conversion at 72 hours, respectively (Figure 2.9).

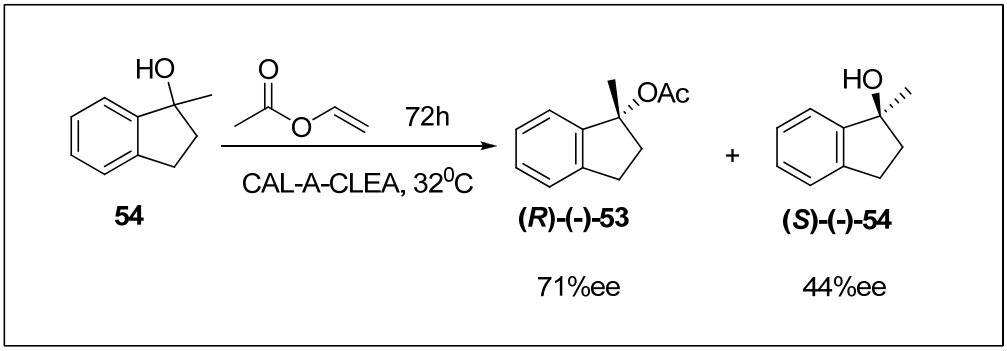

Figure 2.9. The resolution of (±)-1-methyl-2,3-dihydro-1H-inden-1-ol **54** by using CAL-A-CLEA.

After analysis of CAL-A-CLEA, enantiomeric excess values were determined with HPLC (Daicel Chiralcel OD-H, eluent: n-hexane/2-propanal=98:2, flow rate 0.5 mL/ min, λ=214 nm) by using peak area %'s of the enantiomers.

For (*S*)-(+)-1-methyl-2,3-dihydro-1*H*-inden-1-ol **54,** the retention time of the (*S*)-enantiomer is 19.4 min and the other enantiomer is 23.5 min. The optical rotation of **54** is +7.9 for c=1 in $CHCl_3$.

For (*R*)-(-)-1-methyl-2,3-dihydro-1*H*-inden-1-yl acetate **53,** the retention time of the (*S*)-enantiomer is 10.8 min and the other enantiomer is 11.9 min. The optical rotation of **53** is +7.9 for c=1 in $CHCl_3$.

2.4. The synthesis of 3,4-dihydronaphthalen-1(2H)-one (Tetralin) Derivatives

In this work 3,4-dihydronaphthalen-1(2H)-one **58** was chosen as a starting material to reach (*S*)-1-methyl-1,2,3,4-tetrahydronaphthalen-1-ol **57** and (*R*)-1-methyl-1,2,3,4-tetrahydronaphthalen-1-yl acetate **56**. The substrate was obtained by using grignard reagent from this starting metarial.

2.4.1. The synthesis of (±)-1-methyl-1,2,3,4-tetrahydronaphthalen-1-ol

The step in the synthesis of (±)-1-methyl-1,2,3,4-tetrahydronaphthalen-1-ol **57** were the Grignard type reaction by using magnesium metal, methyl iodide and diethyl ether as solvent (figure 2.10).

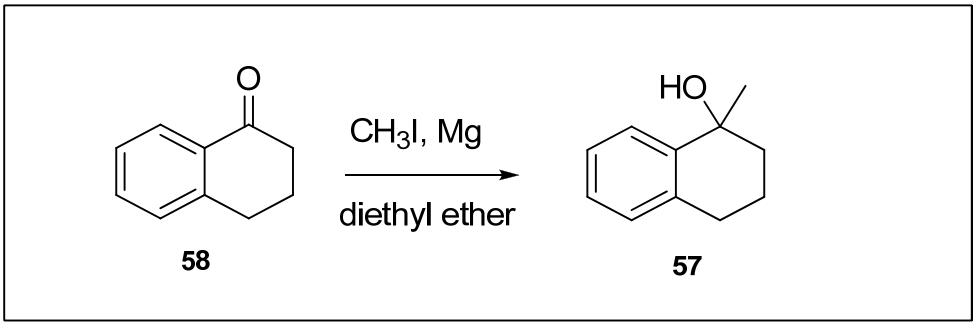

Figure 2.10. The synthesis of (±)-1-methyl-1,2,3,4-tetrahydronaphthalen-1-ol **54** via Grignard type reaction

2.4.2. The synthesis of (±)-1-methyl-1,2,3,4-tetrahydronaphthalen-1-yl acetate

(±)-1-methyl-1,2,3,4-tetrahydronaphthalen-1-ol **57** was converted to the corresponding ester by the reaction with acetic anhydride, tetrabutylammonium iodide and potassium hydride in tetrahydrofuran as shown in figure 2.11.

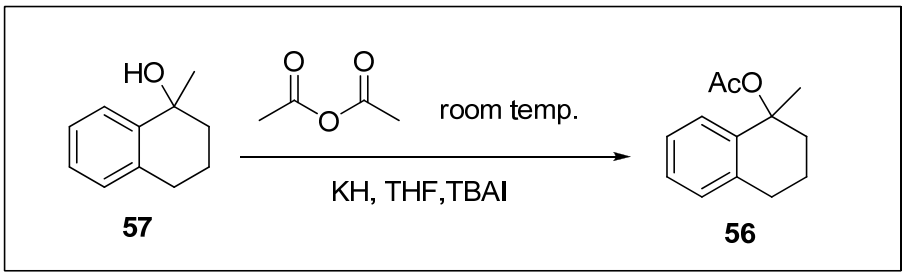

Figure 2.11. The synthesis of (±)-1-methyl-1,2,3,4-tetrahydronaphthalen-1-yl acetate

The products were identified by using NMR spectroscopy. ^{1}H-NMR and ^{13}C-NMR were analyzed.

2.4.3. Characterization of (±)-1-methyl-1,2,3,4-tetrahydronaphthalen-1-ol 57

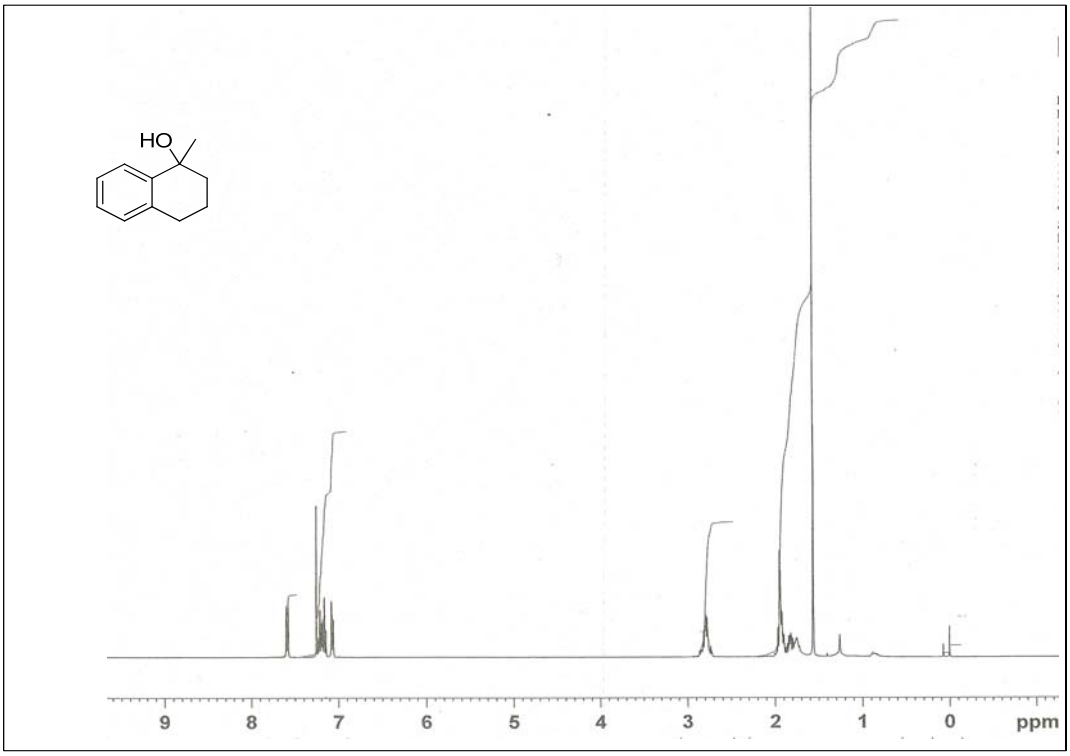

Figure 2.12. ^{1}H-NMR spectrum of (±)-1-methyl-1,2,3,4-tetrahydronaphthalen-1-ol

^{1}H-NMR spectrum of 1-methyl-1,2,3,4-tetrahydronaphthalen-1-ol shows a singlet at 1.56 ppm for methylene protons bonded to the stereogenic center. The

broad singlet at 1.75 ppm is due to the OH proton. The one of the diastereotopic methylene proton in the third position shows the multiplet between 1.77-1.88 ppm and the other one of diastereotopic methylene proton in the third position and the methylene protons at the forth position of cyclohexane rings show the multiplet at 1.90-1.98 ppm. The other diastereotopic protons in second position the multiplet at 2.72-2.86 ppm.

The methine proton in the sixth position shows the doublet at 7.07 ppm with *J*= 7 Hz. The methine proton at the seventh position and fifth position of the benzene ring shows the multiplet between 7.14-7.23 ppm. Finally the doublet of doublet between 7.59 ppm with *J* =1 and 8 Hz is due to the methine proton at the eighth position of the benzene ring.

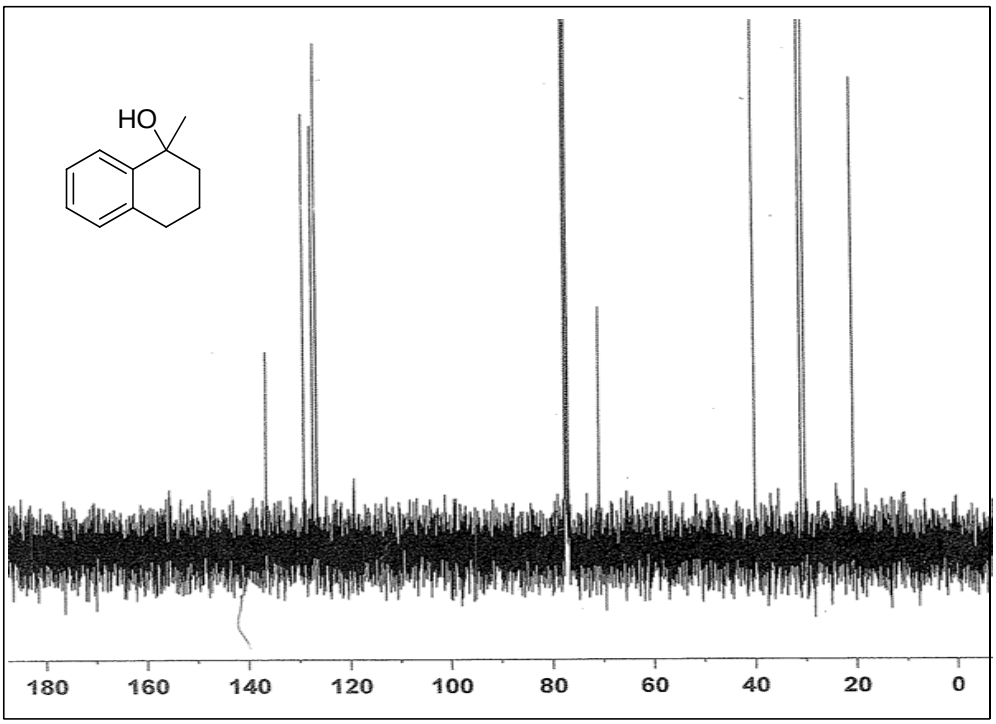

Figure 2.13. ^{13}C-NMR spectrum of (±)-1-methyl-1,2,3,4-tetrahydronaphthalen-1-ol

^{13}C-NMR spectrum exhibits a signal at 29.9 ppm for methyl carbon bonded to quaternary carbon. The diastereotopic carbons at the second, third and forth position of cyclohexane ring show signals at 39.8 ppm, 20.4 ppm and 30.7 ppm. Stereogenic carbon shows at 70.6 ppm.

The carbons in aromatic part of the compound give signals at 126.3 ppm, 126.4 ppm, 127.1 ppm, 128.8 ppm, 136.3 ppm and 142.9 ppm.

2.4.4. Characterization of (±)-1-methyl-1,2,3,4-tetrahydronaphthalen-1-yl acetate, 56

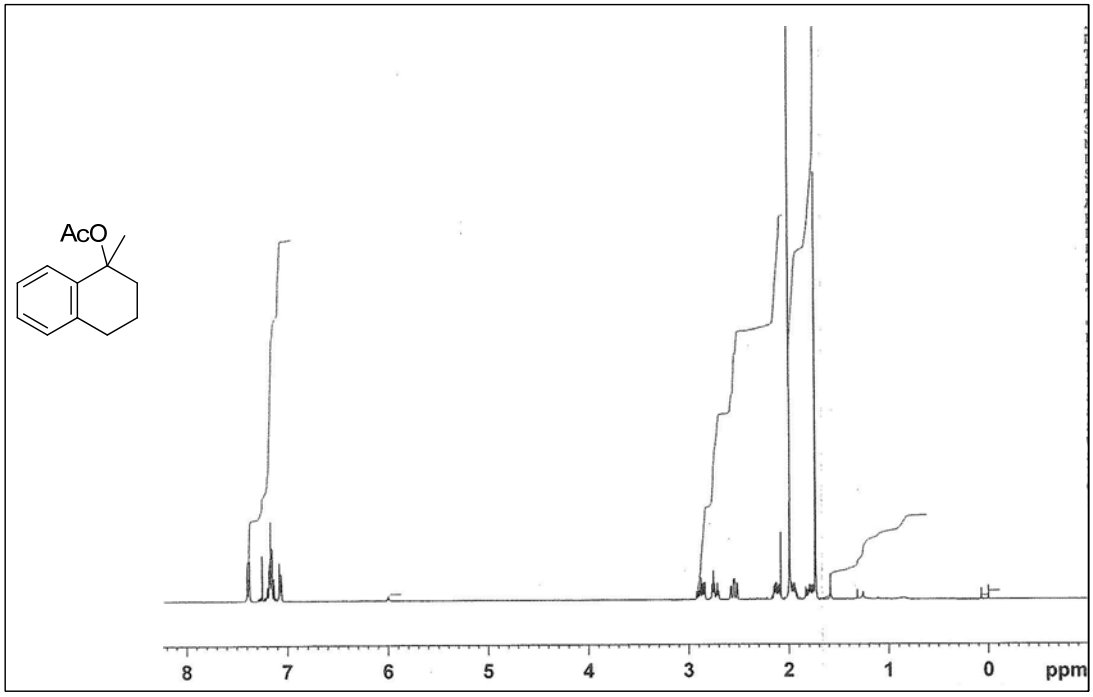

Figure 2.14. ^{1}H-NMR spectrum of (±)-1-methyl-1,2,3,4-tetrahydronaphthalen-1-yl acetate

H-NMR spectrum of (±)-1-methyl-1,2,3,4-tetrahydronaphthalen-1-yl acetate exhibits a singlet at 1.74 ppm for methyl protons bonded to stereogenic carbon. One of the diastereotopic methylene proton at the third position of cyclopentane ring shows the multiplet between 1.76-1.80 ppm and the other one shows the multiplet

between 1.93-1.98 ppm. The methyl protons of acetoxy moiety shows the singlet 1.99 ppm. One of the diastereotopic methylene proton at the forth position of cyclopentane ring shows the multiplet between 2.09-2.15 ppm and the doublet of triplet at 2.55 ppm with J =3 and 12 Hz belongs to the other diastereotopic methylene proton at the forth position of cyclopentane ring. The triplet of doublet between 2.73 ppm with J =5 and 15 Hz is due to one of the diastereotopic methylene proton at the second position and the multiplet between 2.84-2.92 ppm is due to the other diastereotopic methylene protons at the second position of the cyclopentane ring.

The methine proton in the sixth position shows the doublet at 7.06-7.08 ppm. The methine protons at the seventh position and fifth position of the benzene ring shows the multiplet between 7.15-7.18 ppm. Finally the doublet of doublet between 7.38 ppm with J=2 and 8 Hz is due to the methine proton at the eighth position of the benzene ring.

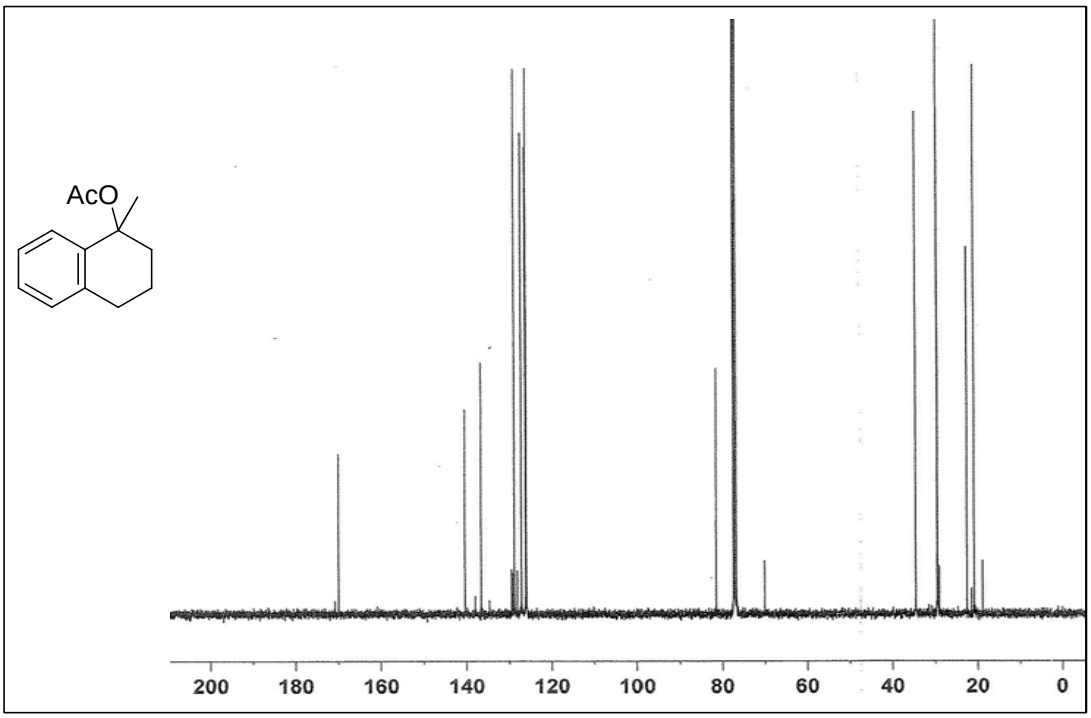

Figure 2.15. ^{13}C-NMR spectrum of (±)-1-methyl-1,2,3,4-tetrahydronaphthalen-1-yl acetate

^{13}C-NMR spectrum of the compound gives a signal at 20.8 ppm due to the methylene carbon at the third position of the cyclohexane ring and at 22.5 ppm due to the methyl carbon of the acetyl group. The methylene carbon at the forth position of the cyclohexane ring shows at 28.9 ppm and the methyl carbon bonded to stereogenic carbon shows at 29.7 ppm. The methylene carbon at the second position of the benzene ring exhibits 34.5 ppm. The signal at 81.4 ppm due to the stereogenic carbon and the signal at 169.9 ppm for the characteristic carbonyl carbon of the acetyl group.

The signals at 125.9 ppm, 126.1 ppm, 127.1 ppm, 128.7 ppm, 136.5 ppm and 140.3 ppm are for aromatic part of the compound.

2.5. Enzyme Mediated Resolutions of (±)1-methyl-1,2,3,4-tetrahydronaphthalen-1-ol

In this study, the resolution of (±)-1-methyl-1,2,3,4-tetrahydronaphthalen-1-ol was performed by using CAL-A-CLEA, CAL-A, Amona PS-C II and CRL in vinyl acetate in 32°C.

The results of the enantiomeric resolution of the (±)-1-methyl-1,2,3,4-tetrahydronaphthalen-1-ol by using CAL-A-CLEA, CAL-A, Amona PS-C II and CRL were given in Table 2.2.

Table 2.2. The results of the enantiomeric resolution of the (±)-**57** by using CAL-A-CLEA, CAL-A, Amona PS-C II and CRL

Enzyme	**Time (h)**	**Conversion (%)[a]**	**ee_p (%)[b]**	**ee_s (%)[b]**	**$[\alpha]_D^{26}$(prod.)/ Abs.Conf.**	**$[\alpha]_D^{26}$(subs.)/ Abs.Conf**
CAL-A-CLEA	48	25	91	33	$[\alpha]_D^{26}$= -5.1 (*c* 0.6, $CHCl_3$) *R*	$[\alpha]_D^{26}$= +7.2 (*c* 1, $CHCl_3$) *S*
CAL-A	144	20	99	20	$[\alpha]_D^{26}$= -14.3 (*c* 0.6, $CHCl_3$) *R*	$[\alpha]_D^{26}$= +4.5 (*c* 1, $CHCl_3$) *S*
Amano PS-C II	96	15	80	15	$[\alpha]_D^{26}$= -4.0 (*c* 1.3, $CHCl_3$) *R*	$[\alpha]_D^{26}$= +2.1 (*c* 1, $CHCl_3$) *S*
CRL	145	10	90	-[c]	$[\alpha]_D^{26}$= -4.5 (*c* 0.25, $CHCl_3$) *R*	-[c]

[a] Conversions were determined using HPLC analysis.
[b] Enantiomeric excesses were determined by Daicel Chiralcel OD-H and OJ-H column HPLC analysis.
[c] Isolated as racemic
[p] Product
[s] Substract

In the resolution of (±)-1-methyl-1,2,3,4-tetrahydronaphthalen-1-ol the optimum conditions of these enzymes tried by using substrate:enzyme ratio (w/w).

To find optimum condition in Amona PS-C II and CRL the substrate:enzyme ratio (w/w) changed from 1:0.25 to 1:1 at 25-32°C and these value for both enzymes was found as 1:1 at 32°C.

Amona PS-C II yielded (*S*)-(+)-1-methyl-2,3-dihydro-1*H*-inden-1-yl acetate, (*S*)-(+)-**56** with 80% ee by 15% conversion in 96 hours. CRL yielded (*R*)-(-)-1-methyl-2,3-dihydro-1*H*-inden-1-yl acetate, (*R*)-(-)-**56** with 90% ee by 10% conversion in 145 hours.

To find optimum condition in CAL-A the substrate:enzyme ratio (weight/weight) changed from 1:0.25 to 1:1 at 25-32°C and it was found as 1:0.25 at 32°C. it can be seen that best results were obtained by using CAL-A among these enzymes. CAL-A gived higest enantiomeric excess (ee) value in formation of the production . At the end of this reaction which was catalyzed by CAL-A, yellow oil (*R*)-(-)-1-methyl-1,2,34-tetrahydronaphthalen-1-yl acetate and white solid (*S*)-(+)-1-methyl-1,2,34-tetrahydronaphthalen-1-ol were obtained with 99% ee and 20% ee, respectively (figure 2.18) by 20% conversion in 144 hours.

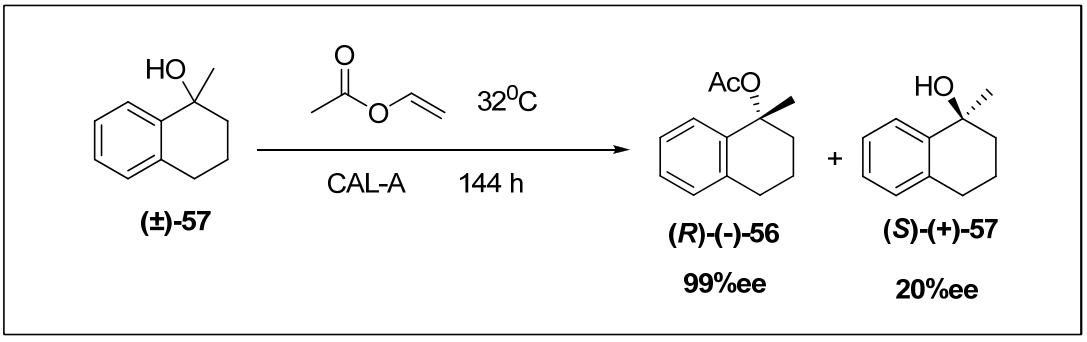

Figure 2.16. The resolution of ±1-methyl-1,2,3,4-tetrahydronaphthalen-1-ol **57** by using CAL-A.

The substrate:enzyme ratios (w/w) were tried to find optimum condition in CAL-A-CLEA by changing from 1:0.25 to 1:1 at 25-32°C and the best condition in the substrate:enzyme ratio (w/w) for CAL-A-CLEA was determined as 1:0.5 at 32°C. (*R*)-(-)-1-methyl-1,2,34-tetrahydronaphthalen-1-yl acetate and (*S*)-(+)-1-methyl-1,2,3,4-tetrahydronaphthalen -1-ol were obtained with 91% and 33% ee, respectively by using this enzyme and 20% conversion was determined using HPLC analysis in 48 hours.

As seen as this table, the conversion value for CAL-A-CLEA can be highest while the enantiomeric excess (ee) value of the product which was yielded by CAL-A-CLEA is not higest. While CAL-A-CLEA has the highest conversion, CAL-A gave the product with the highest ee value.

After analysis of CAL-A-CLEA and CAL-A, enantiomeric excess values were determined with HPLC (Daicel Chiralcel OD-H, eluent: n-hexane/2-propanal=98:2, flow rate 0.5 mL/ min, λ=214 nm) by using peak area %'s of the enantiomers.

For (*S*)-(+)-1-methyl-1,2,3,4-tetrahydronaphthalen-1-ol**, 57** the retention time of the (*R*)-enantiomer is 20.2 min and the other enantiomer is 10.5 min. The optical rotation of **57** is +7.2 for c=1 in $CHCl_3$.

For (*R*)-(-)-1-methyl-1,2,34-tetrahydronaphthalen-1-yl acetate**,** (*R*)-(-)-**56** the retention time of the (*R*)-enantiomer is 9.2 min and the other enantiomer is 10.5 min. The optical rotation of **56** is -14.3 for c=0.6 in $CHCl_3$.

CHAPTER III

3. EXPERIMENTAL

In this study, the instruments which are written below for the structure characterization of the compounds were used.

1H-NMR and 13C-NMR spectra were recorded with a Bruker Spectrospin Avance DPX 400 spectrometer, 400 MHz High Performance Digital FT-NMR Spectrometer, and Bruker AC 80 MHz FT-NMR Spectrometer by using $CDCl_3$ as a solvent and tetramethylsilane (TMS) as an internal standard. Chemical shifts are given in parts per million (δ) from internal standard tetramethylsilane. Spin multiplicities are mentioned as: s (singlet), ss (strong singlet), bs (broad singlet), d (doublet), dd(doublet of doublet), dt (doublet of triplet), t (triplet), td (triplet of doublet), m (multiplet).

IR spectra were recorded on a SHIMADZU FTIR 8400-S instrument (KBr pellet). Bond positions were reported in reciprocal centimeters (cm^{-1}).

Optical rotations were measured in $CHCl_3$ in a 1 dm cell using a Bellingham & Stanley P20 polarimeter.

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 the solutions were concentrated under vacuum by performing rotary evaporator.

3.1. General procedure for the synthesis of (±)-1-methyl-2,3-dihydro-1H-inden-1-ol, 54

First part was the preparation of the Grignard reagent. To a stirred solution of Mg turnings (0.36 g, 15 mmol) and iodine (2 pieces) in dry diethyl ether (7 mL) at 25 °C equipped with a reflux condenser was added dropwise a mixture of iodomethane (0.69 mL, 11 mmol) in anhydrous diethyl ether (5 mL) with an injection syringe until the reaction begins. The mixture was allowed to reflux for 1 hour at room temperature. The mixture was cooled down to 0°C and the corresponding 2,3-dihydro-1H-inden-1-one (1.32 g, 10 mmol) in diethyl ether (3 mL) was added dropwise. The resultant mixture was stirred for 16 hours. The reaction mixture was hydrolyzed with saturated ammonium chloride solution (10 mL) and with 1N HCl (2 mL). The resultant mixture was extracted with diethyl ether (3 x 30 mL) and dried over anhydrous $MgSO_4$ and evaporated in vacuo. The crude product was purified by flash column chromatography by using a mixture solvent of EtOAc/hexane at the ratio of 1:10 as the eluent to obtain the corresponding (±)-**54**. Yield 83%.

R*f*=0.66 (silica gel – (EtOAc – Hexane), (1:10)

IR (Neat) : 3327 cm^{-1}, 2964 cm^{-1}, 2378 cm^{-1}, 1485 cm^{-1}, 1638 cm^{-1}, 1365 cm^{-1}.

^{1}H-NMR ($CDCl_3$) δ ppm

1.57 (s, 3H, for the methyl protons of the cyclopentane ring)

1.78 (bs,1H, for the OH of the cyclopentane ring)

2.17-2.24 (m, 2H, for the methylene protons at the third position of the cyclopentane ring)

2.80-2.87 and 2.99-3.02 (two ms, 2H, for the diastereotopic methylene protons of the cyclopentane ring)

7.23-7.25 (m, 3H, for the methine protons at the forth, fifth and sixth positions of the phenyl ring)

7.35-7.37 (m, 1H, for the methine protons at the seven position of the phenyl ring)

^{13}C-NMR ($CDCl_3$) δ ppm

27.3 (for the methylene carbons at third position of cyclopentane ring)

29.4 (for the methyl cabon bonded to quaternary carbon)

43.4 (for the methylene carbons at second position of cyclopentane ring)

81.25 (for the quaternary carbon)

122.2-148.3 (for the carbons of the phenyl ring).

3.2. General procedure for the synthesis of 1-methyl-2,3-dihydro-1H-inden-1-yl acetate

A 30 % suspension of KH in mineral oil (0.63 g, equivalent to ca. 4.86 mmol of active hydride) was washed with dry hexane three times. Dry THF (6 mL) was then added, followed by a solution of 1-methyl-2,3-dihydro-1H-inden-1-ol (0.35 g, 2.43 mmol) in dry THF (6 mL). The solution was stirred at room temperature for 1 hour. Acetic anhydride (4.86 mmol) was then added dropwise, followed by

tetrabutylammonium iodide (0.087 g, 0.24 mmol). The reaction mixture was then heated overnight at reflux about 2 hours. Work-up was done by adding CH_2Cl_2 and it was dried over anhydrous $MgSO_4$ and the product was purified by flash column chromatography by using EtOAc/hexane (1:12) as the eluent to obtain the corresponding alcohol. Yield 83%.

R*f*=0.5 (silica gel – (EtOAc – Hexane), (1:12)

IR (Neat) : 2957 cm^{-1}, 2860 cm^{-1}, 1746 cm^{-1}, 1452 cm^{-1}, 1238 cm^{-1}

^{1}H-NMR ($CDCl_3$) δ ppm

1.70 (s, 3H, for the methyl protons bonded to the stereogenic center)

2.01(s, 3H, for the methyl protons of acetoxy moiety)

2.26-2.33 and 2.45-2.52 (m, 2H, for the methylene protons at the third position of cyclopentane ring)

2.74-2.82 and 2.95-3.02 (m, 2H, for the diastereotopic methylene protons at the second position of cyclopentane ring)

7.23-7.27 (m, 3H, for the methine protons of the phenyl rings)

7.35-7.37 (m, 1H, for the methine proton at the seventh position of the phenyl rings)

^{13}C-NMR ($CDCl_3$) δ ppm

22.5 (for methyl carbon of the acetyl group)

27.6 (for the methylene carbon at third position of cyclopentane ring)

37.9 (for the methyl carbon bonded to stereogenic carbon)

39.7 (for the methyl cabon at second position of cyclopentane ring)

90.2 (for the stereogenic carbon)

170.8 (for the carbonyl carbon of the acetyl group)

119.1-145.4 (for the carbons of the phenyl ring)

3.3. General procedure for the synthesis of ± 1-methyl-1,2,3,4-tetrahydro naphthalen-1-ol, 57

To a stirred solution of Mg turnings (O.36 g, 15 mmol) and iodine (2 pieces) in dry diethyl ether (7 mL) at 25 °C equipped with a reflux condenser was added dropwise a mixture of iodomethane (0.69 mL, 11 mmol) in anhydrous diethyl ether (5 mL) with an injection syringe until the reaction begins. The mixture was allowed to reflux for 1 hour at room temperature. The mixture was cooled down to 0°C and the corresponding 3,4-dihydro naphthalen-1-(2H)-one (1.46 g, 10 mmol) in diethyl ether (3 mL) was added dropwise. The resultant mixture was stirred for 16 hours. The reaction mixture was hydrolyzed with saturated ammonium chloride solution (10 mL) and with 1N HCl (2 mL). The resultant mixture was extracted with diethyl ether (3 x 30 mL) and dried over anhydrous $MgSO_4$ and evaporated in vacuo. The crude

product was purified by flash column chromatography by using a mixture solvent of EtOAc/hexane at the ratio of 1:10 as the eluent to obtain the corresponding (±)-**54**. Yield 83%.

R*f*=0.54 (silica gel – (EtOAc – Hexane), (1:12)

IR (Neat):3323 cm^{-1}, 2931 cm^{-1}, 2314 cm^{-1}, 1441 cm^{-1}.

^{1}H-NMR ($CDCl_3$) δ ppm

1.56 (s, 3H, for the methyl protons bonded to the stereogenic center)

1.75 (bs,1H, for the OH of the cyclopentane ring)

1.77-1.88 (m, 1H, for the one of the diastreotopic methylene protons at the third position of the cyclohexene ring)

1.90-1.98 (m, 3H, for the other three diastreotopic methylene protons at the third and forth position of the cyclohexene ring)

2.72-2.86 (m, 2H, for the diastreotopic methylene protons at the second position of the cyclohexene ring)

7.07 (d,1H, *J*=7 Hz, for the methine proton at the fifth position of the phenyl ring)

7.14-7.23 (m, 2H, for the methine protons at the sixth and seventh position of the phenyl ring)

7.59 (dd, 1H, *J*= 1 and 8 Hz, for the methine proton at the eighth position of the phenyl ring)

^{13}C-NMR ($CDCl_3$) δ ppm

20.4 (for the diastereotopic carbon in third position of cyclohexane ring)

29.9 (for methyl carbon bonded to quaternary carbon)

30.7 (for the diastereotopic carbon in forth position of cyclohexane ring)

39.8 (for the diastereotopic carbon in second position of cyclohexane ring)

70.6 (for the quaternary carbon)

126.3-142.9 (for the carbons of the phenyl ring).

3.4. General procedure for the synthesis of the synthesis of 1-methyl-1,2,3,4-tetrahydronaphthalen-1-yl acetate

A 30 % suspension of KH in mineral oil (0.32 g, equivalent to ca. 2.35 mmol of active hydride) was washed with dry hexane three times. Dry THF (6 mL) was then added, followed by a solution of 1-methyl-1,2,3,4-tetrahydronaphthalen-1-ol (0.19 g, 1.17 mmol) in dry THF (6 mL). The solution was stirred at room temperature for 40 min. Acetic anhydride (0.22 ml, 2.35 mmol) was then added dropwise, followed by tetrabutylammonium iodide (0.044 g, 0.117 mmol). The reaction mixture was then heated overnight at reflux about 2 hours. Work-up was done by adding CH_2Cl_2 and it was dried over anhydrous $MgSO_4$ and the product was purified by flash column chromatography by using EtOAc/hexane (1:12) as the eluent to obtain the corresponding alcohol. Yield 83%.

R*f*= 0.4 (silica gel – (EtOAc – Hexane), (1:12)

IR (Neat): 2924 cm^{-1}, 2850 cm^{-1},2384 cm^{-1},1730 cm^{-1}, 1475 cm^{-1}, 1435 cm^{-1}, 1370 cm^{-1}

^{1}H-NMR ($CDCl_3$) δ ppm

1.74 (s, 3H, for methyl protons bonded to stereogenic carbon)

1.75-1.80 (m, 1H, the one of the diastereotopic methylene proton at the third position of cyclohexane ring)

1.93-1.98 (m, 1H, the other one of the diastereotopic methylene proton at the third position of cyclohexane ring)

1.99 (s, 3H, for the methyl protons of acetoxy moiety)

2.09-2.15 (m, 1H, the one of the diastereotopic methylene protons at the forth position of cyclohexane ring)

2.55 (dt, 1H, *J*= 3 and 12 Hz, the other diastereotopic methylene protons at the forth position of cyclohexane ring)

2.73 (td, 1H, *J*= 5 and 15 Hz, for the one of the diastereotopic methylene protons at the second position of the cyclopentane ring)

2.84-2.92 (m, 1H, for the other one of the diastereotopic methylene protons at the second position of the cyclopentane ring)

7.06-7.08 (m, 1H, for the methine proton at the fifth, position of the phenyl ring)

7.15-7.18 (m, 2H, for the methine proton at the sixth, seventh position of the phenyl ring)

7.38 (dd, 1H, *J*= 2 and 7 Hz, for the methine proton at the eighth position of the phenyl ring)

^{13}C-NMR ($CDCl_3$) δ ppm

20.8 (for the methylene carbon at the third position of the cyclohexane ring)

29.6 (for the methylene carbon at the forth position of the cyclohexane ring

29.7 (for the methyl carbon bonded to stereogenic carbon)

32.5 (for the methyl carbon of the acetyl group)

34.5 (for the methylene carbon at the second position of the cyclohexane ring)

81.4 (for the stereogenic carbon)

125.9-140.3 (the carbons of the phenyl ring).

169.9 (for the characteristic carbonyl carbon of the acetyl group).

3.5. General procedure for the transesterification of reaction of (±)-1-methyl-2,3-dihydro-1H-inden-1-ol, 54 and ± 1-methyl-1,2,3,4-tetrahydronaphthalen-1-ol, 57

This racemic tertiary alcohol was mixed with vinyl acetate by using CAL-A-CLEA, Amano PS-C II and CRL (CCL) as the catalyst**,** respectively.

To 100 mg of (±)-1-methyl-2,3-dihydro-1H-inden-1-ol, **54** or (±)-1-methyl-1,2,3,4-tetrahydronaphthalen-1-ol, **57** and 1.5 mL (16 mmol) of vinyl acetate corresponding enzyme (i.e. 25 mg of CAL-A, 50 mg of CLEA, 100 mg of Amano PS-C II, 100 mg of CRL) was added, followed by shaking at 32 °C. It was monitored by TLC. When the reaction was completed the mixture was filtered and the filtrate cocentrated *in vacuo*. Purification was done by flash column chromatography using ethyl acetate/hexane (1:10) as eluent.

3.5.1. (*S*)-(+)-1-methyl-2,3-dihydro-1*H*-inden-1-ol, 54

The product was isolated as a yellow solid with 44% yield and its mp is between 65-68 °C. The enantiomeric excess of the product was determined by Daicel chiralcel OD-H and OJ-H column by HPLC analysis, UV detection at 214 nm, eluent: hexane/2-propanol= 98:2.

Flow rate= 0.5 mL/ min, λ=214 nm .

$[\alpha]_D^{26}$ = +7.9 (c=1, $CHCl_3$), $t_{1\,(S)}$= 19.4 min, $t_{2\,(R)}$= 23.5 min.

3.5.2. (*R*)-(-)-1-methyl-2,3-dihydro-1*H*-inden-1-yl acetate, 53

The product was obtained as yellow oil with 71% ee. The enantiomeric excess of the product was determined by Daicel chiralcel OD-H and OJ-H column by HPLC analysis, UV detection at 214 nm, eluent: hexane/2-propanol= 98:2

Flow rate= 0.5 mL/ min, λ=214 nm.

$[\alpha]_D^{26}$ = -5 (c=0.25, $CHCl_3$), $t_{1\,(S)}$= 10.8 min, $t_{2\,(R)}$= 11.9 min.

3.5.3. (*S*)-(+)-1-methyl-1,2,3,4-tetrahydronaphthalen-1-ol, 57

The product was isolated as a white solid with 33% ee. The enantiomeric excess of the product was determined by Daicel chiralcel OD-H and OJ-H column by HPLC analysis, UV detection at 214 nm, eluent: hexane/2-propanol= 98:2

Flow rate= 0.5 mL/ min, λ=214 nm, 32°C.

$[\alpha]_D^{26}$ = +7.2 (c=1, $CHCl_3$), $t_{1\,(R)}$= 20.2 min, $t_{2\,(S)}$= 22.2 min.

3.5.4. (*R*)-(-)-1-methyl-1,2,3,4-tetrahydronaphthalen-1-yl acetate, 56

The product was obtained as yellow oil with 99% ee. The enantiomeric excess of the product was determined by Daicel chiralcel OD-H and OJ-H column by HPLC analysis, UV detection at 214 nm, eluent: hexane/2-propanol= 98:2.

Flow rate= 0.5 mL/ min, λ=214 nm, 32°C.

$[\alpha]_D^{26}$ = -14.3 (c=0.6, $CHCl_3$), $t_{1\,(R)}$= 9.2 min, $t_{2\,(S)}$= 10.5 min.

CHAPTER IV

4. CONCLUSION

In this study racemic tertiary alcohols was resolved by using several enzymes such as CAL-A, PS-C II, CRL and CAL-A-CLEA.

In the resolution of (±)-1-methyl-2,3-dihydro-1*H*-inden-1-ol, CAL-A-CLEA and CRL favor (*S*)-enantiomer while Amano PS-C II favor the (*R*)-enantiomer. According to the result CAL-A-CLEA yielded (*R*)-(-)-1-methyl-2,3-dihydro-1*H*-inden-1-yl acetate, **53** and (*S*)-(+)-1-methyl -2,3-dihydro-1*H*-inden-1-ol, **54** with 71% ee and 44% ee by 20% conversion, respectively. In the resolution of (±) 1-methyl-1,2,3,4-tetrahydronaphthalen-1-ol, CAL-A yielded (*R*)-(-)-1-methyl-1,2,3,4-tetra hydronaphthalen-1-yl acetate **56** with 99% ee by 20% conversion while CAL-A-CLEA yielded (*S*)-(+)-1-methyl-1,2,3,4- tetrahydronaphthalen-1-ol **57** with 33% ee by 25% conversion.

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