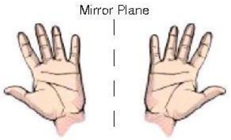
1.1 Introduction of chiral chemistry

Chirality is an important element in nature. The term chirality introduced by Lord Kelvin in 1873 comes from the Greek word *cheir*, which means handedness and define as a property of an object that is non-super imposable with its mirror image [1]. Carbon with four different groups results in a chiral molecule and is referred to as a chiral or asymmetric or stereogenic center. A familiar example is our left and right hands, which possess mirror-image symmetry, but are non-super imposable on each other



The importance of chirality is well recognised, mainly in connection with the fact that nearly all natural products are chiral and their physiological or pharmacological properties depend upon their recognition by chiral receptors, which will interact only with molecules of the proper absolute configuration. Indeed, the use of chiral drugs in enantiopure form is now a standard requirement for every new chemical entity [2]. Life itself depends on chirality, because living systems interact with enantiomers in a different way. Enantiomers are stereoisomers which are nonsuperimposable mirror images of each other.

The preparation of chiral compounds is an important and challenging area of contemporary synthetic organic chemistry. The broad utility of synthetic chiral molecules as a single enantiomer has been recognised by medicinal chemists for the development of targeted chiral molecules. This has made the chiral synthesis a prominent area of investigation [3]. The search for new and efficient methods for the synthesis and resolution of chirally pure compounds has been an active area of research in organic synthesis [4]. Therefore, synthesis of chiral molecule is a significant and key problem in the design and synthesis of many natural products and pharmaceuticals.

1.2 Synthesis of chiral molecules

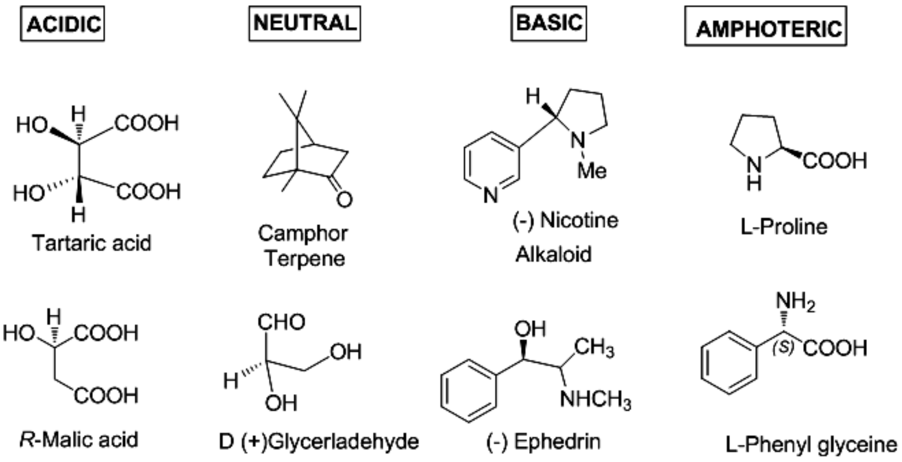
The necessity to produce chiral compounds has been established as a highly demanding goal in various fields because of the different physiological properties of enantiomers. In general, there are three methods of producing optically active compounds [5].

1. Chiral pool synthesis
2. Asymmetric synthesis
3. Chiral resolution

1.2.1 Chiral Pool synthesis

In nature there are different types of chiral molecules with different functionalities. Utilizing them one can design the synthesis accordingly [6].

Scheme 1.1: Structure of natural chiral pool compounds



1.2.2 Asymmetric Synthesis

Asymmetric synthesis is the key process in the preparation of optically pure compounds. It can be defined as synthesis of a compound by method which favors the formation of a specific enantiomer or diastereomer [7].

1.2.3 Chiral resolution

A resolution is defined as separation of the enantiomers from a racemic mixture with recovery of at least one of the enantiomers and can be affected by several means. [8].

During last decade remarkable advances have been made in asymmetric synthesis. Resolution of racemates holds special appeal for their ability to produce large quantities of compound as well as both the isomer [9].

Resolution is still considered as special technique for the synthesis of enantiomerically pure compounds. Chiral resolution is classified in two ways, classical method based on physical separation of diastereomeric forms and other method is based on kinetic resolution. The most elegant way of performing kinetic resolution is use of biocatalyst or enzyme [10].

1.3 Biocatalyst and Biocatalytic transformation

The term bio catalytic process is usually employed to represent transformations of a substrate into a defined target product through one or several enzyme-catalyzed steps. The term bio catalytsis include fermentations, biotransformations and enzyme-mediated reactions.

Fermentation is the transformation of compound (such as sugar, starch) and of defined adducts (such as amino acids, and fatty acids) into more complex target products by means of living system. A well-known example is fermentation of alcohol. This method is still a potential technique for the production of various useful compounds.

The term biotransformation means the conversion of defined reactant or substrates into desired target products using resting cell systems or whole cells. Enzyme catalysis is usually employed when crude extracts, partially purified enzyme or immobilised enzyme on solid support are used to perform the desire transformation. The limits between these two terms are not strictly fixed; generally biotransformations and enzyme catalysis are summarized under the term biocatalysts [11].

1.4 Enzymatic Resolution

In comparison with conventional chemical methods biological transformation has received increasing attention. Reaction catalysed by biocatalyst display far greater specificities than conventional forms of organic reactions [12]. In enzymatic reaction conversion of one molecule (substrate) to another (product) by microorganism. It is a reaction catalysed by particular enzyme or by an enzyme originally produced within the cell. It is not important whether the cell is alive or dormant, the life process of microorganism is required only for the production of enzymes and microorganism itself is simply used as bag of enzymes or catalysts.

In nature, different physiological functions and activities of biological system controls because enzymes, receptors and other natural binding sites recognise substrates with specific chirality [13].

Enzymes are the most refined catalysts in existence. They are usually highly selective, catalysing the reaction of only one compound at a specific reaction site with defined stereochemistry including enantioselectively. Enzymes have been widely used in modern organic synthesis due to their simple processing requirements, high selectivity and mild reaction conditions [14].

Reactions catalysed by enzyme and biological system display far greater specificities than conventional forms of organic reactions.

Research on applications of enzymes as catalysts for asymmetric synthetic transformations is mainly driven by growing demand for enantiomerically pure pharmaceuticals. However, the use of enzymes for this purpose was rather limited until the discovery that enzymes can work in organic solvents. Now bio catalytic processes can be carried out in organic solvents as well as aqueous environments, so that polar organic compounds as well as water-soluble compounds can be modified selectively and efficiently with enzymes or with bio catalytically active cells. Thus, it has been possible to use enzymes for the synthesis of different class of compound with varied substitutions [15].

Enzyme-catalyzed reactions can be divided into six main groups according to the International Union of Biochemistry. These groups are:

1. **Oxido-reductase**
2. **Transferase**
3. **Hydrolase**
4. **Lyases**
5. **Isomerase**
6. **Ligase**

1.5 Advantage of biocatalysts

Enzymes occupy a special position in the field of catalysts because they operate at moderate temperature and mild reaction conditions, which is prime advantage with sensitive reactants and products, thus avoiding the use of more extreme conditions that could cause problems with isomerization, racemization, epimerization, and rearrangements which often plague traditional methodology.

The main advantage of biocatalysts over traditional chemical synthesis is that the enzymes are much more specific. Enzyme-catalyzed reactions are often highly enantioselective, regioselective and diastereoselective and they can discriminate between functional groups with different chemical environments [16]. A well-known example is amylase, a digestive enzyme which can hydrolyze starch, but not cellulose.

Enzymes can be effective at very low mole fractions of catalyst and enzymes can be immobilized and reused for many cycles which make them cost effective. Besides, biocatalysts are environmentally acceptable, being completely degradedable in the environment. A majority of enzymes display high selectivity for a specific type of reaction while generally tolerating a wide variety of substrates. They can also work in non-aqueous environment.

1.6 Limitation of biocatalyst

However, the use of enzymes is subject to limitations and drawbacks in some cases. For example, enzymes are relatively expensive, and therefore discarding them after a single use is not economical. Another issue is their general instability towards heat, organic solvents, acids or bases, etc. One method to circumvent them is to immobilize the enzyme on a suitable support or medium [17, 18].

The enzymatic transformations generally work well in aqueous medium. Water is poor solvent for most applications in organic transformation, since many organic compounds are unstable in aqueous solution; furthermore its removal is more tedious than that of organic solvents.

1.7 Lipase in organic solvent

It was discussed that one of the main drawbacks for the use of enzymes in aqueous organic synthesis is the poor water solubility of organic compounds. Hydrolases are the most frequently used enzymes due to their broad substrate spectrum and considerable stability in organic solvents. Among the hydrolases, lipases (EC 3.1.1.3) are considered the most popular and useful enzymes for asymmetric synthesis. Lipases are compatible with the range of organic solvents [19]. They are readily available in large quantities, because many of them can be produced in high yields by gene expression in an appropriate microorganism, such as a fungi, yeast or bacteria. Generally, they do not require cofactors nor catalyse side reactions, and remain active in organic solvents. They catalyse hydrolysis or acylation reactions under mild conditions (common organic solvents, room temperature & atmospheric pressure). The handling of lipases is safe for environment, and no specific reaction apparatus is needed.

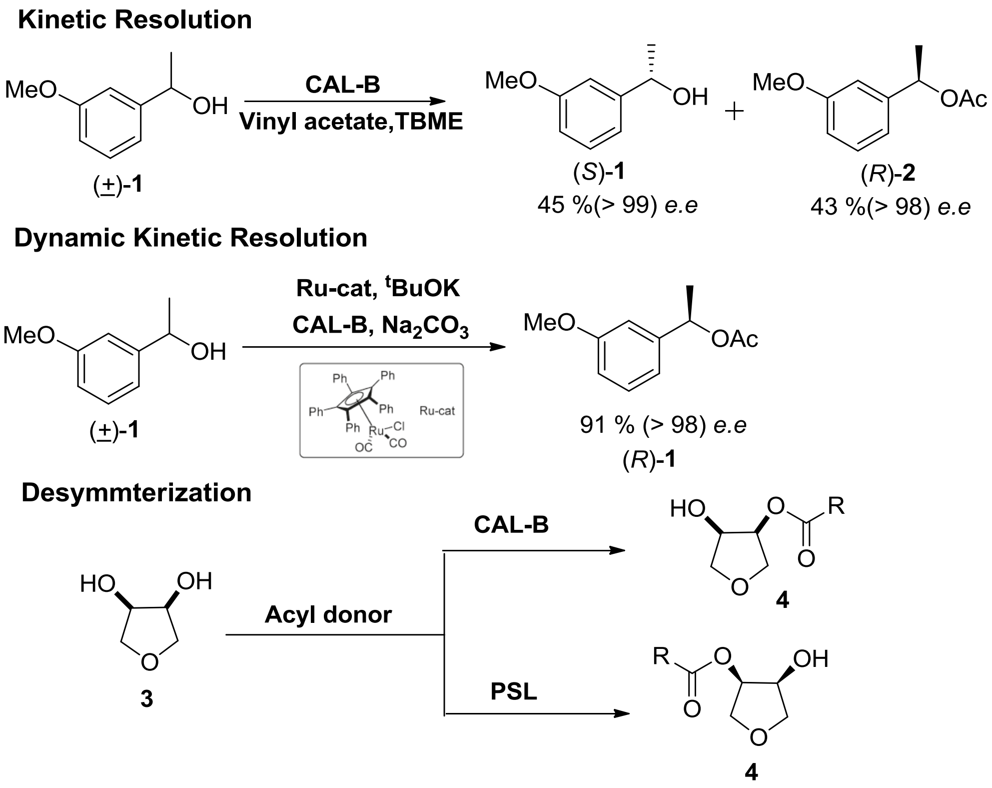
The use of organic solvents presents several advantages including: (a) easier recovery of products with high yields; (b) the possibility of using non-polar substrates; (c) organic solvents avoid many side reactions.

Biocatalysts in non-aqueous media had been widely used for the resolution of alcohols, amines, acids and lactones by enzymatic transesterification reactions using different lipases [20]. The other processes such as the enzymatic acylation of amines also reported and found to be useful in the resolution of amines and the preparation of chiral amides [21].

1.8 Lipase in resolution

The use of enzymes for organic synthesis has become an interesting area for organic and bio organic chemists. Lipases were used in various stereo selective biotransformations such as *kinetic resolutions* (KR) [22], *dynamic kinetic resolutions* (DKR) [23, 24] and *desymmetrisation* [25]. A representative illustration of above transformations by lipase are outlined in scheme 1.2.

Scheme 1.2: Application of enzyme in organic synthesis



1.8.2 Kinetic Resolution: A Kinetic Resolution (KR) is based on a transformation, which subsequently makes easier the separation of the two enantiomers of the racemic substrate. This fact involves a practical difference: in a kinetic resolution only half of the starting material is consumed.

1.8.2 Dynamic Kinetic Resolution: Dynamic Kinetic Resolution (DKR) which consists of carrying out an *in situ* continuous racemization of the substrate, so that, theoretically, all of the racemic starting material can be used for transformation into one enantiomer.

1.8.3 Desymmetrization: It is not necessary that compounds or substrates employed in enzyme-catalyzed resolutions liable to undergo racemization. The desymmetrization of symmetric compounds consists of a modification that eliminates one or more elements of symmetry of the substrate. If the symmetry elements that preclude chirality are eliminated, enantioselectivity can be achieved. Using Enantioselective enzymatic desymmetrizations (EEDs), a maximum yield of 100% can be achieved. For this reason, EEDs constitute interesting alternative to KRs for the preparation of optically active compounds.

In recent years, some reports address the importance and wide application on lipase-catalysed C-C coupling reaction namely aldol, Mannich and Michael reactions [26].

1.9 Application of Lipase in resolution

The acylation of alcohols using lipases is currently the most frequently used process in biocatalysts [16]. In literature there are many examples of the resolution of primary [27] and secondary alcohols [28]. Although the resolution with tertiary alcohols is more difficult and very few examples have been reported [29].

1.9.1 Transesterification & Hydrolysis reaction

Literature survey revealed that about majority of reported biotransformations could be categorized as hydrolytic transformations involving ester and amide bonds using proteases, esterases or lipases. Other types of application of hydrolase enzymes include the formation or cleavage of epoxides, nitriles and phosphate esters [30]. Recent examples in literature continue to indicate the importance of trans-esterification reactions and hydrolysis [31].

The very first kinetic resolution discovered by Pasteur in 1858, enzyme catalyzed in resolution of tartaric acid by fermenting yeast. An enzymatic KR relies on an enzyme that reacts at substantially higher rate with one enantiomer of racemates than with the other. This phenomenon arises from the fact that diastereomeric transition state structures are formed when the enantiomers of the starting material bind to the enzyme. In ideal case, the difference in reaction rates of the enantiomers is very large and one of the enantiomers is transformed quickly whereas the other one is not converted at all.

In practice, most enzymatic KRs do not show this ideal behaviour. The ratio of rates of conversion of the enantiomers is measurable and to obtain high ee the yield will be lower than 50%. In a KR, the enantiomeric purity of the product and starting material varies as reaction proceeds. Thus, comparing enantiomeric purities for two KRs is meaningful only at the same extent of conversion. Sih *et al*. [32] have developed equation to calculate the inherent enantioselectivity of the biocatalytic KR. This enantioselectivity called the enatomeric ratio (E), measure the ability of the enzyme to distinguish between two isomers. The E value remains constant throughout the reaction and is convenient to easily compare the selectivity of kinetic resolution. For an irreversible enzymatic KR the following three equations may be used to experimentally determine E by measuring two of the three parameters: conversion (c), *ee* of the product and *ee* of the unreacted starting material (equation 1 to 3).

For irreversible reaction and no side-reactions take place, the parameters of kinetic resolution (ee_P, ee_S, Conv. and E) are all interrelated according to equations 3.When both substrate and products are chiral, combining equations (1) and (2) gives equation (3).

Conv. = $ee_S / (ee_S + ee_P)$ …………………………………………………..(Equation-1)

$E = \ln[1 - \text{Conv.}(1 + ee_P)] / \ln[1 - \text{Conv.}(1 - ee_P)]$ ….. ………………....(Equation 2)

and

$E = \ln[(1 - \text{Conv.})(1 - ee_S)] / \ln[(1 - \text{Conv.})(1 + ee_S)]$

$E = \{\ln[ee_P(1 - ee_S) / (ee_S + ee_P)]\} / \{\ln[ee_P(1 + ee_S) / (ee_S + ee_P)]\}$……. (Equation 3)

As a rule of thumb E values below 15 are unacceptable for practical purposes, in the range 15-30 regarded as moderate to good and above these values they are useful. Kinetic resolutions with $E > 200$ are considered highly selective. Values of $E > 200$ are difficult to measure accurately and require advance analytical tools.

1.10 Acyl donor in lipase catalysed acylation

In lipase catalyzed acylation, a carboxylic acid derivative, the acyl donor, donates its acyl group for the substrate while the leaving group of the acyl donor is a co-product of the reaction (scheme 1.3). The co-product may then react further to secondary products. The co-product in acylation reactions are outlined in Table 1.1.

Scheme 1.3: Acyl donor in lipase catalysed transestrification reaction

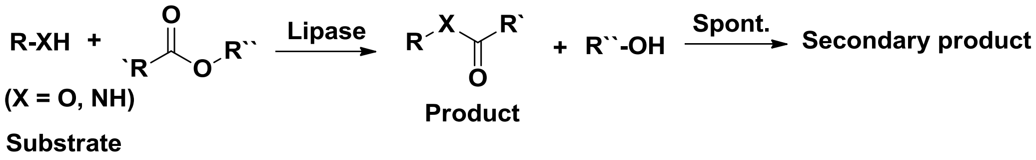
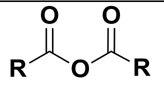
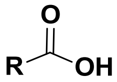
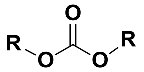
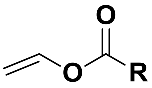
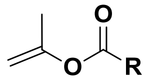
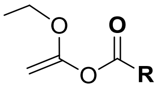


Table 1.1: Structure of different acyl donor and their co product

No	Acyl donor	Co product
1	 **anhydride**	
2	 **Carbonate**	**R-OH**
3	 **Vinyl ester**	$[CH_3CHO]$
4	 **isopropenyl ester**	$[CH_3COCH_3]$
5	 **Ethoxy Vinyl ester**	$[CH_3COOCH_2CH_3]$

In most of the reactions commonly used acyl donors are anhydrides, carbonates and enol esters. Anhydrides are often too reactive for selective *N*-acylation, although succinic anhydride has been used in the kinetic resolution of secondary alcohols [33]. The advantage is that the product now contains a carboxylic acid moiety and can be easily separated from the unreacted substrate. Carbonates are not practical acylating reagents for alcohols although they are suitable for *N*-acylation. The resulting carbamate bond can also be cleaved under milder conditions than amide bonds. By far the most applied acyl donors are enol esters, namely vinyl, iso-propenyl and 1-ethoxyvinyl esters. In case of enol ester back reaction is suppressed due to the tautomerization of the resulting enol alcohol (to acetaldehyde or acetone, depending on whether a vinyl or an iso-propenyl ester act as acyl donors), thereby shifting the equilibrium to the required product [34]. The use of different acyl donor in irreversible acylation reaction catalyzed by lipase has been well documented in literature [35]. 1-Ethoxyvinyl esters are useful alternatives since the co-product ethyl acetate is harmless to lipases [36].

1.11 Enzyme mediated resolution of alcohols

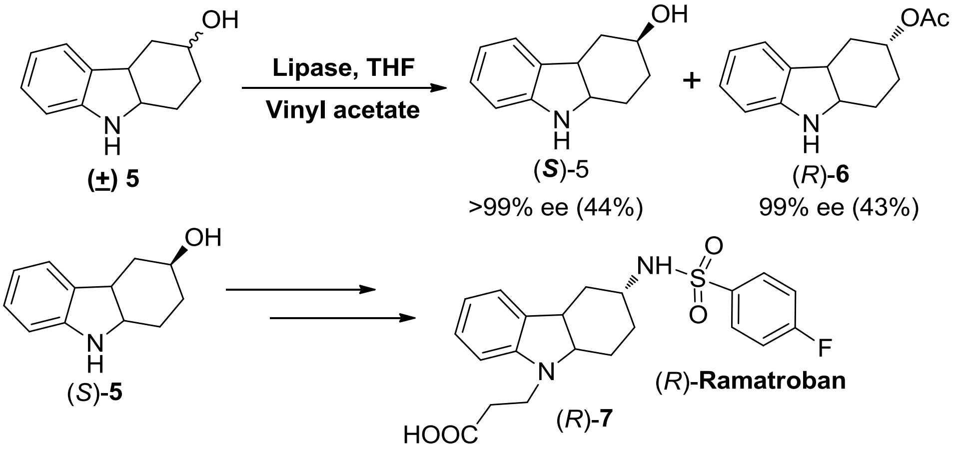
Lipase catalysed kinetic resolution (KR) of racemic alcohols with acyl donor in organic solvents has gained increasing interest as an efficient method for the preparation of optically active compounds.

Some selected examples related to the current work are outlined here.

1.11.1 Enzymatic resolution of tetrahydro carbazole

Gotor and co-worker introduced chemoenzymatic asymmetric route for the preparation of enantiopure (*R*)-ramatroban (*R*)-**7** (Scheme 1.4). The starting substrate (±)-2,3,4,9-tetrahydro-1H-carbazol-3-ol **5** was resolved using lipase, in which both the isomers were obtained in optically pure form [37]. The optically pure alcohol is further converted into drug ramatroban in enationpure form which is used in the treatment of allergic rhinitis and asthma.

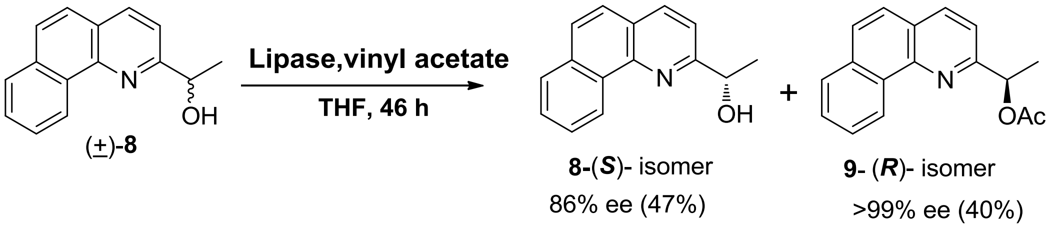
Scheme 1.4: Resolution of tetra hydro-carbazole



1.11.2 Resolution of Pincer type molecules

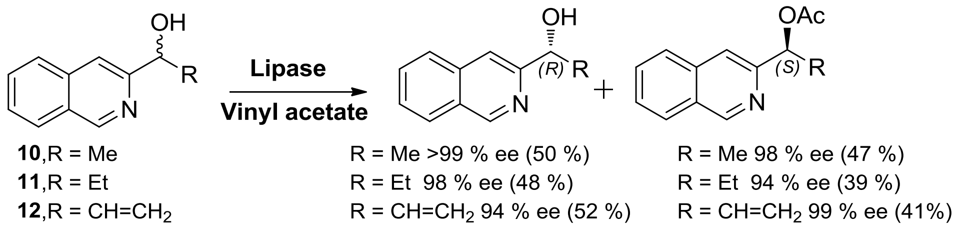
Felluga and co-worker [38] introduced lipase mediated resolution and dynamic kinetic resolution of pincer type alcohol (Scheme 1.5). Their transformation of optical pure alcohol into1-methyl-1-heteroarylethan amines occurred without loss of optical purity, giving access to pincer ligands which were used in asymmetric synthesis.

Scheme1.5: Resolution of pincer type compound



Gaunti *et al* reported synthesis and enzymatic resolution of similar class of compound with good optical purity [39]. They have presented the synthesis and resolution of a series of simple optically pure derivatives of isoquinolines by a chemo enzymatic method (Scheme 1.6).

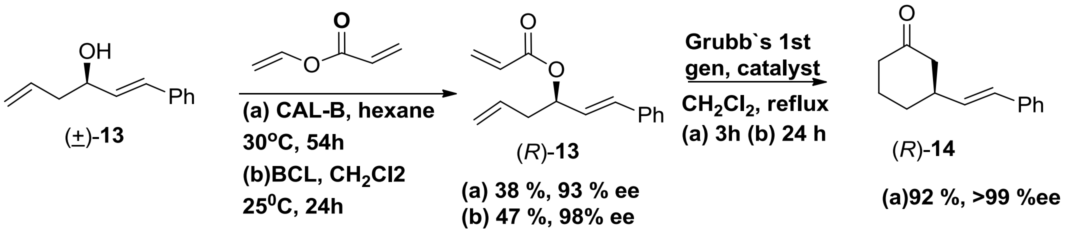
Scheme 1.6: Resolution of isoquinolines compound



1.11.3 Enzymatic resolution followed by metathesis

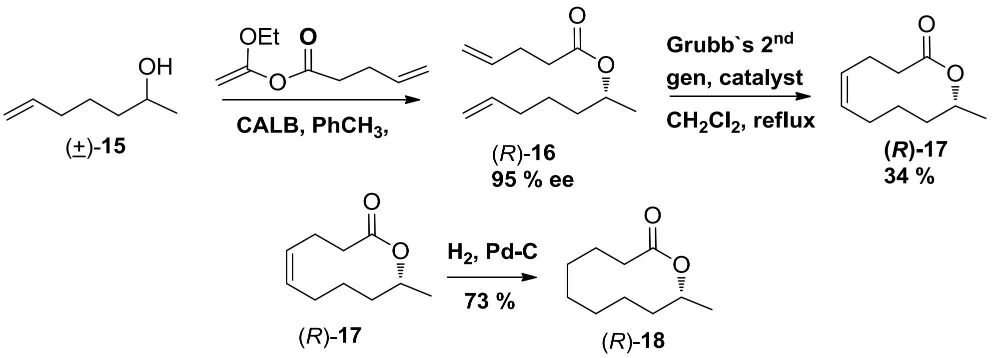
The bioactive natural product (+)-(*R*)-goniothalamin (**14**) has been synthesized by two research groups (routes (a) and (b) in Scheme 1.7) using lipase-catalyzed acylation followed by ring-closing metathesis reaction (RCM) [40 & 41]. The racemic alcohol **13** was prepared via Grignard reaction between allylmagnesiumbromide and cinnamaldehyde. Both CAL-B [40] and BCL [41] were effective lipases for enantioselective acylation with vinyl acrylate. A separate RCM of **13** with Grubbs' first generation ruthenium catalyst then finished the synthesis of (*R*)-**14** in good yields.

Scheme 1.7: Acylation & ring-closing metathesis sequence in the synthesis of goniothalamin (*R*)-**14**



Chenevert *et al* [42] have reported synthesis of phoracantholide natural product using similar sequential domino process (resolution followed by metathesis). Compound **17** and **18** were prepared by first acylating the unsaturated alcohol (±) **15** in a kinetic resolution with CAL-B and ethoxyvinyl ester to give product (*R*)-**16** in 44% yield and 95% *ee* (Scheme 1.8). RCM with Grubbs' second generation catalyst afforded **17** in 34% yield and **18** (73%) was obtained by subsequent hydrogenation.

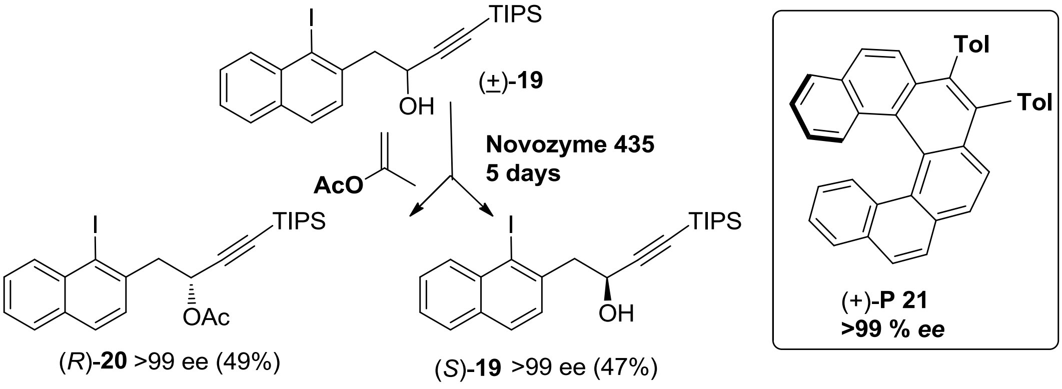
Scheme 1.8: Lipase catalysed acylation for the synthesis of phoracantholide (*R*)-**18**



1.11.4 Enzymatic resolution of precursor of helicene

Selective acylation with lipases can be used to introduce desired structural fragments into complex molecules. Recently Story and co-workers developed a general methodology for the preparation of chiral helicenes [5], [6] and [7] through asymmetric synthesis. Authors have utilized lipase-catalyzed resolution of **19** which has been further used for preparation of *P*-isomer of helicenes such as **21** [43].

Scheme 1.9: Resolution of alcohol (±)-**19**

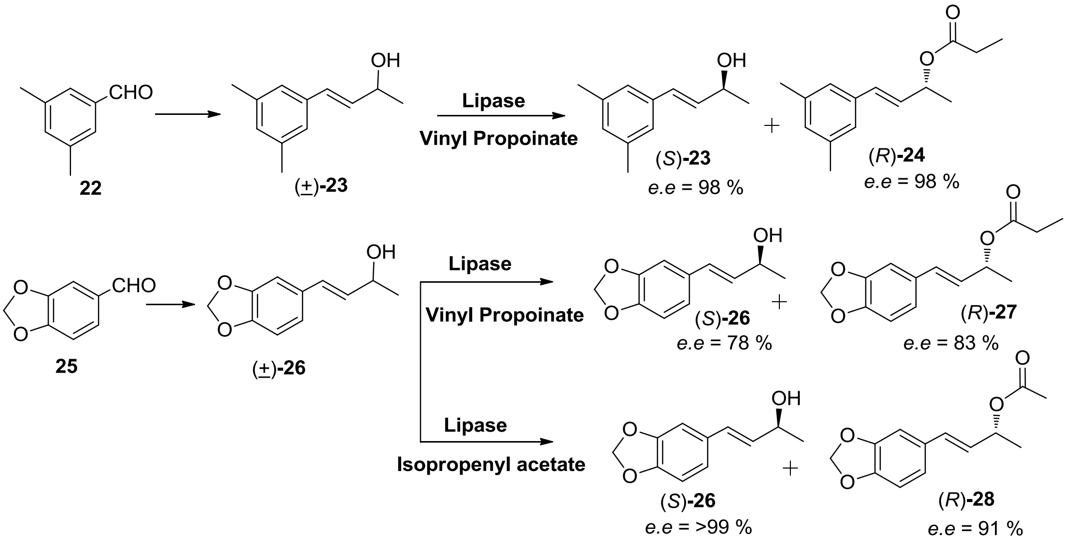


1.11.5 Enzymatic resolution of allyl alcohols

Recently, Gladkowski *et al* reported enzymatic resolution of chiral allyl alcohols by esterification reaction [44]. Chiral allyl alcohols have found wide applications in many branches of industry. Some of them possess valuable odoriferous properties useful in the flavor, food or cosmetic industry and also as valuable building blocks, which can be widely employed in organic synthesis [45]. Both racemic allyl alcohols **23** and **26** were synthesized in a two-step synthesis from 2, 5-dimethylbenzaldehyde

22 and piperonal **25** respectively, by Claisen–Schmidt condensation with acetone and subsequent reduction of the carbonyl group using sodium borohydride (Scheme 1.10). The authors have screened four different types of lipase in which resolution of racemic **23** was achieved with Lipozyme and vinyl propionate as acyl donor in diisopropyl ether as solvent. Similar condition was also applied for resolution of **26**. The application of the same biocatalysts and reaction conditions (solvent diisopropyl ether, vinyl propionate as the acyl donor) for the resolution of racemic **26** (Scheme 1.10) gave significantly lower enantioselectivity, *ee* of unreacted (*S*)-alcohol **26** and (*R*)-propionate **27** (76% and 83% respectively). Further the authors have described effect of the acyl donor and it was observed that in case of iso-propenyl acetate selectivity was highest.

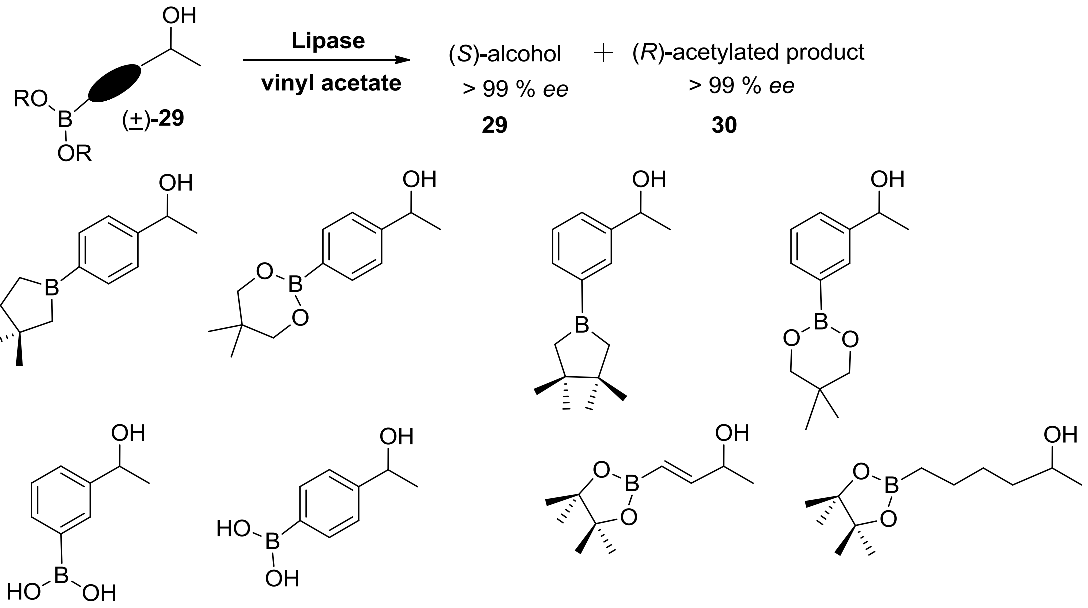
Scheme 1.10: Resolution of chiral allyl alcohol



1.11.7 Enzymatic resolution of boron-containing chiral compounds

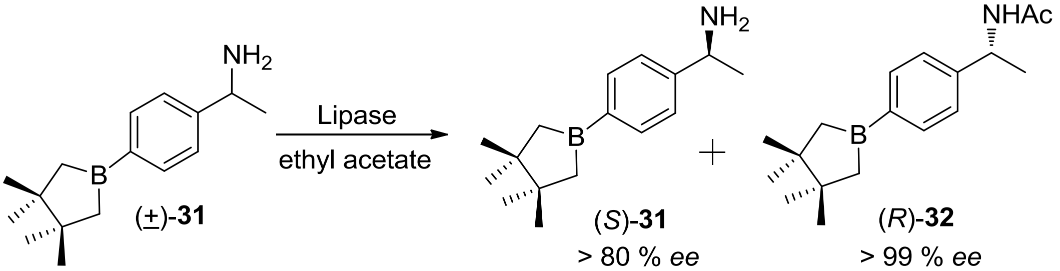
Boron compounds are useful building blocks in organic synthesis and can be prepared by several synthetic methodologies. Andrade *et al* reported kinetic resolution of organoboron compounds [46a]. Aromatic, allylic and aliphatic secondary alcohols containing borane ester or boronic acid groups were resolved via CAL-B enzyme and high enantiomeric excesses of both remaining substrate and acetylated product (Scheme 1.11). In this work, authors had explored the effect of different groups attached at the chiral center and nature of the boron species in the lipase catalyzed enantioselective transesterification.

Scheme 1.11: Enzymatic resolution of boron alcohols



Same authors have also studied several reaction conditions to achieve the kinetic resolution of boron-containing amines via enantioselective acylation using *CAL-B* as enzyme [46b].

Scheme 1.11: Enzymatic resolution of boron containing amine

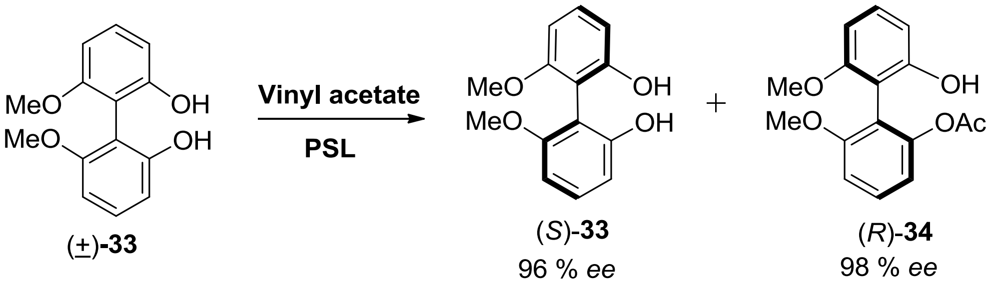


1.10.7 Enzymatic resolution of axially chiral compounds

Lipases are also valuable tools for the resolution of biaryl derivatives with axial chirality. *Pseudomonas cepacia* lipase (PCL) has been used for the transesterification of 2,2-dihydroxy-6,6-dimethoxy-1,1-biphenyl in a reaction using vinyl acetate as the acyl donor and *tert*-butyl methyl ether as the solvent. Absolute configuration of product of enzymatic reaction was found to be (*R*) and obtained with >98% *ee* while the unreacted alcohol (*S*)-substrate was recovered with >96% *ee* (Scheme 1.12) [47].

These types of compounds and their derivatives have various applications in asymmetric organic synthesis [48].

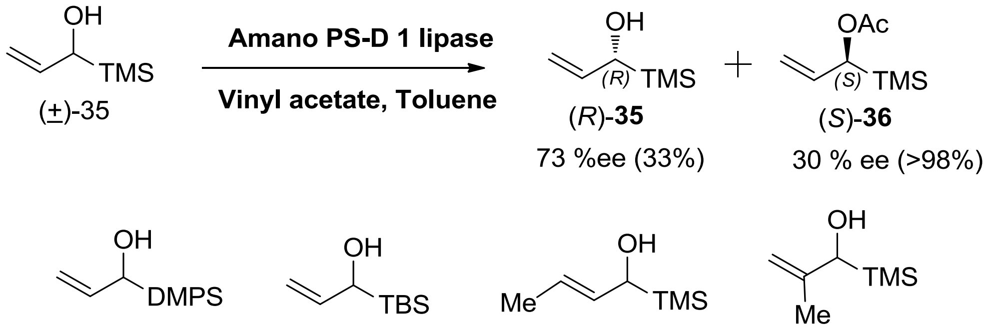
Scheme 1.12: Enzymatic resolution of axially chiral compound **33**



1.11.8 Enzymatic resolution of α–hydroxysilane

Maleczka *et al* explored kinetic resolution of α–hydroxysilane where silicon bears a variety of substituent. The resulting optically active α-hydroxysilane and their corresponding acetates were obtained in varying yields and ee [49].

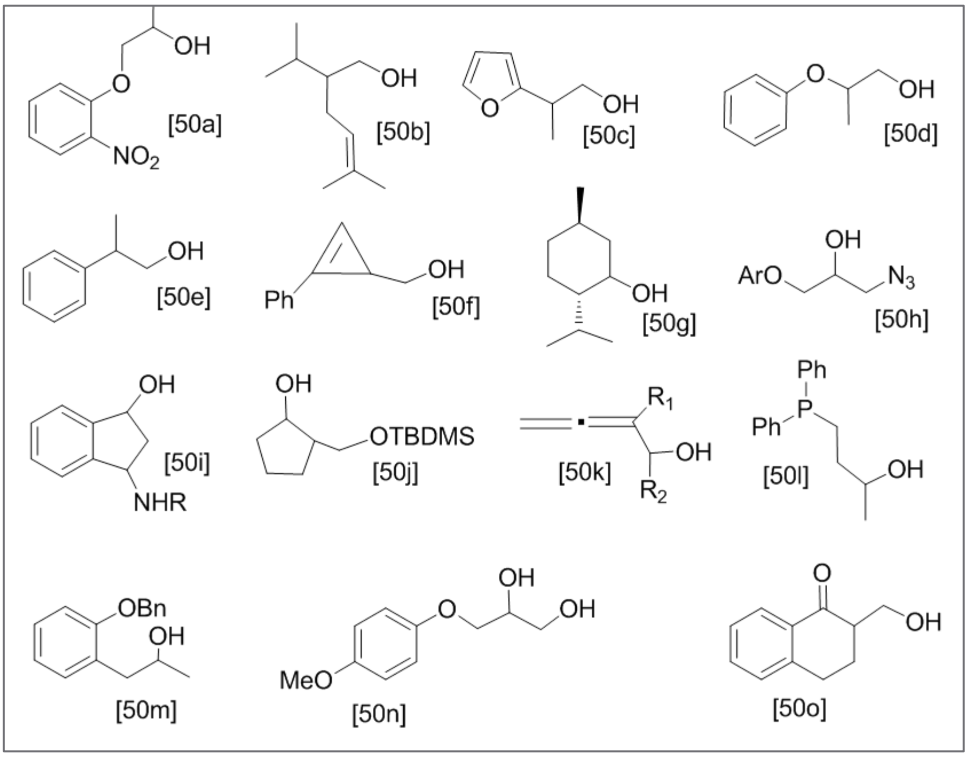
Scheme 1.12: Enzymatic resolution of hydroxysilane



1.11.9 Literature example of resolution of alcohol with lipase

In literature various types of alcohols with different substituent were resolved using enzymatic acylation [50a to 50o]. Some representative examples of various alcohols and diols, resolved successfully using lipase are presented in scheme 1.13.

Scheme 1.13: List of different alcohols and diols

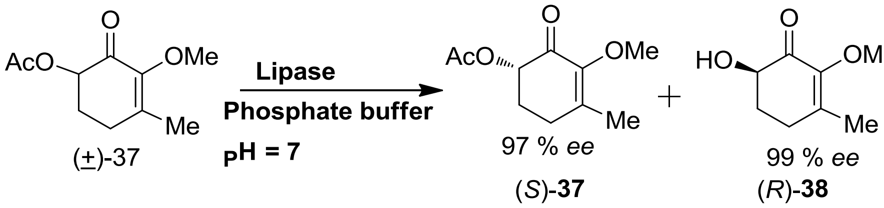


1.12 Hydrolysis reaction

Nowadays process of enzymatic hydrolysis is widely used process that is widely used for the preparation of chiral compounds. However, in enzymatic hydrolysis, the natural reaction of lipase, is also very useful for the resolution of racemic mixture or the desymmetrisation of prochiral compounds. Hydrolysis and transestrification can be complementary process for the resolution of alcohol.

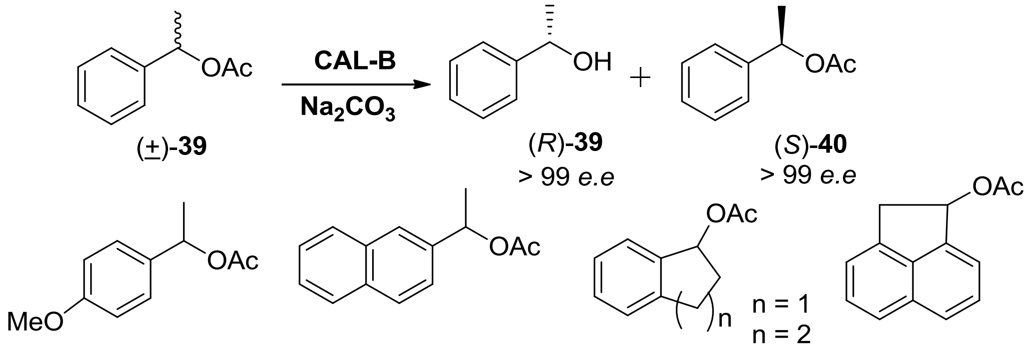
Bicera *et al* describes that enzyme-mediated hydrolysis of the acetoxy group provides hydroxy enones, and acetoxy enones with high enantiomeric excess (up to 99%) and good chemical yields. This method provides a simple route for the synthesis of cyclic chiral hydroxy enones, which are important precursors for pharmacological compounds [51].

Scheme 1.14: Enzymatic hydrolysis



Riant *et al* reported a novel methodology for kinetic resolution via enzymatic hydrolysis in non-conventional media via carbonate salts. Enzymatic hydrolysis was carried out under hydrophobic organic media, in the presence of both sodium carbonate and CAL-B lipase. The effect of the carbonate salts was investigated, and proven to have a significant influence on the enzymatic reactivity, without any perturbation on its selectivity.

Scheme 1.15: Enzymatic hydrolysis in presence of sodium carbonate



In this work it has been described that an equimolar amount of Na_2CO_3 must be present in order to achieve the optimal conversion. Using sodium carbonate as base the author had performed this reaction with different substrates which were outlined in scheme 1.15 [52].

1.13 Enzymatic Desymmetrisation

Enzymes are nowadays widely recognized among the most active and selective catalysts for the preparation of optically active compounds. Some of the factors that account for this popularity were discussed previously. Because of the mild conditions under which they operate, enzymatic reactions are affected to a lesser extent by side reactions (viz. isomerization, racemization, epimerization, and rearrangement of molecules) as compared to non-enzymatic processes.

Desymmetrization of *meso* or *prochiral* compounds also offer routes into single enantiomers with more than 50 % yield. The former type has one or more asymmetric centers, but contains a plane of symmetry and is therefore achiral. Modification of one of the symmetrically positioned groups in the molecule but not the other will result in a chiral product. This process is called desymmetrization. Mechanistically it is identical to kinetic resolution, the only difference being that the molecular symmetry allows the process to overcome limitation of the 50% yield [53].

Enzymatic desymmetrisation of prochiral or *meso* compounds to yield enantiomerically enriched products has proved to be valuable tools in asymmetric synthesis. In contrast to the classical kinetic resolution of racemic mixtures, the theoretical maximum yield of these transformations is 100% [54].

Basically molecules with two identical functional groups are subjected to selectively convert one of them to different functionality, theoretically giving quantitative yield of the product. Such desymmetrization has been known to be achieved by metal catalytic or non-enzymatic reactions or by biocatalyzed conversions [50].

In general, arrangements of *meso*-diol **A** to **D** which can be subjected to desymmetrization are presented in Figure 1.1.

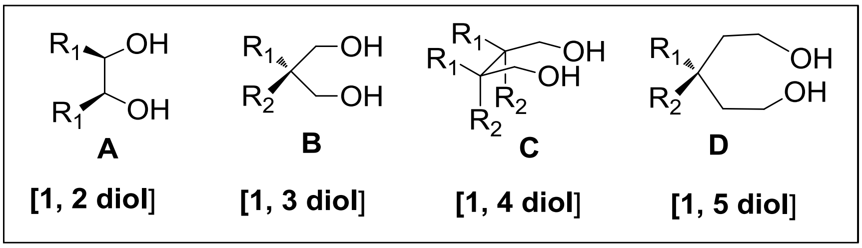
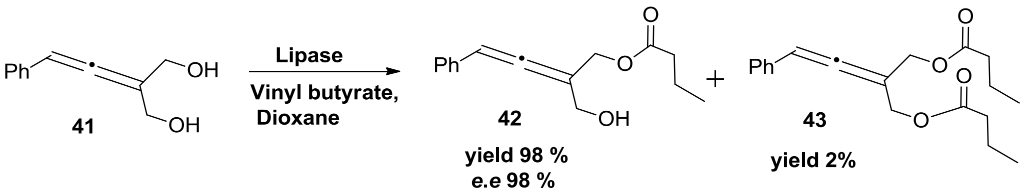


Figure 1.1: Basic types of *meso* diols.

1.13.1 Example of enzymatic desymmetrisation

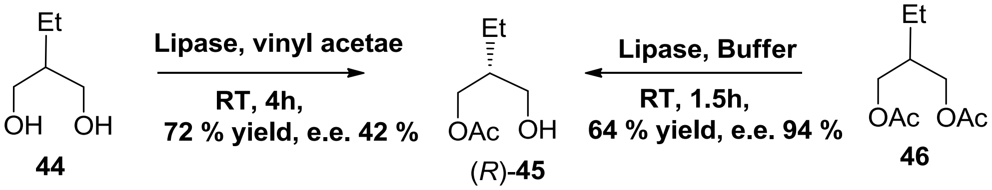
Deska *et al* reported enzymatic desymmetrisation of prochiral allene diol where 98 % yield was obtained with 98 % optical purity (scheme 1.16). The product obtained in this process has structure similar to core structure of hyperiones [55].

Scheme 1.16: Desymmetrisation of allene diol **41**



Izquierdo and co-workers have desymmetrized 2-ethylpropane- 1,3-diol **44** and its di-*O*-acetate **46** through PFL-catalyzed transesterification and hydrolysis, respectively [53] (Scheme 3). Both processes led to the monoacetate of (*R*)-configuration, the enzymatic enantioselectivity being higher for the hydrolytic transformation, from which (*R*)-**45** was isolated in high yield and *ee* [56].

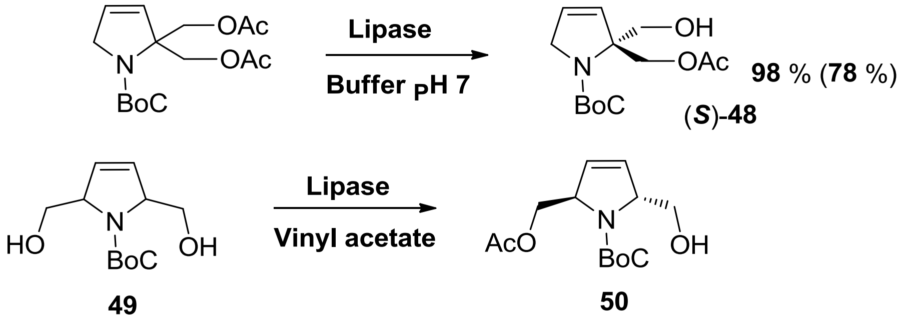
Scheme1.17: Desymmetrisation of diol **44**



This chiral building block was further used for the preparation of a dioxaspiro compound that closely resembles to skeleton of talaromycins.

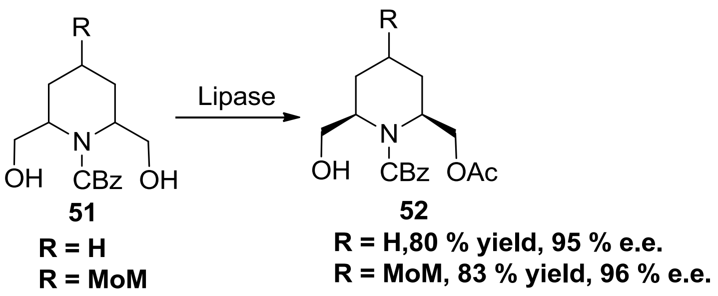
The enzymatic desymmetrization of 2,2- and 2,5-disubstituted pyrrolizidine containing diols catalyzed by a lipoprotein lipase from *Pseudomonas* sp. In particular, the hydrolysis of diacetate **47** and acylation of diol **49** proceeded in excellent enantioselectivity and yields [57].

Scheme 1.18: Desymmetrisation of pyrrolizidine contain compounds



The piperidine ring is a widespread structural fragment of biologically active compounds. In this context, enantiomer *cis*-2,6- and substituted piperidine have been obtained through desymmetrization strategies developed by Chenevert and co-workers [58] (Scheme 1.19).

Scheme 1.19: Hydrolysis of piperidine diol



1.14 Aim of the Thesis

The focus of this thesis is to study the enzymatic kinetic resolution of alcohol and diol which have different shape. The study is focused on the development of efficient condition for kinetic resolution of roof shape alcohol, and diol, along with enzymatic desymmetrisation of roof shape *meso* diol is also studied. The resolved molecules are converted into chiral amine, diamines and amides. The synthesised amine diamines and amide are screened as chiral solvating agents in NMR. We also present our efforts to scan roof shape alcohol as possible chiral auxiliary.

The work is arranged in four chapters, with an introduction to biocatalyst in Chapter **1**.

Chapter 2 deals with the application of lipase for the preparation of useful and unusual chiral molecules. Enzymatic resolution is main focus of chapter 1, which is further divided into two sections. Chapter 2 begins with the discussion on molecules with different shape and size and particular property due to their structure. We are interested in resolution of molecules with unusual shape and size of alcohol and diol. The basic introduction for the synthesis of roof shape molecules will be discussed. We prepared three different types of roof shape molecules using Diels-Alder reaction with anthracene with different dienophiles. The roof shape alcohol and *trans*-diol are subjected for lipase mediated kinetic resolution. Absolute configuration of roof shape alcohol was confirmed by single crystal X-ray analysis. The roof shape alcohol and diol are converted into amine and diamines by simple chemical transformations. Along with enzymatic resolution, enzymatic desymmetrisation of *meso* diol is also studied. The product obtained after desymmetrisation shows *P*-helical motif due to the extended intermolecular hydrogen bonding. The *cis*-isomers undergo loss of its optical purity due to the internal acyl transfer process. To prevent isomerization of *cis* isomer a protocol of *in situ* conversion to stable isomer using Mitsunobu reaction was applied.

In section II enzymatic resolution of fluorinated alcohol is also studied. One of the limitations of kinetic resolution is maximum 50 % yield.

To overcome this difficulty a strategy of combination of enzymatic resolution followed by Mitsunobu reaction has been applied. With the aid of Mitsunobu protocol greater than 50 % yields are achieved. The resolved fluorinated alcohol is further converted into chiral amides.

Chapter 3 focuses on the chiral discrimination and the measurement of enantiomeric excess. The NMR approach to discriminate enantiomers using chiral auxiliaries such as, solvating agents, derivatizing agents, lanthanide shift reagents, the choice of such auxiliaries and the limitations are discussed in detail. The main focus is utilization of the synthesized amine, diamine and amide for possible application as chiral solvating agents for various functional groups. The chapter is accordingly divided into four sections. In the first section, application of various roof shape amine and diamine as chiral solvating agents is discussed for α functionalized acids. The present protocol of roof shape amines as CSA is also tested for hetro nuclei NMR in section II. In section-III roof shape anchored crown ether as possible CSA for ammonium cation are tested. In section IV hydrogen bond interactions are probed for different functionality such as sulphoxide, amide, keto alcohol and acids to test discrimination in ^{1}H NMR. The study revealed that introduction of electron withdrawing group enhances chiral discrimination property. The CSA has been scanned for few chiral drugs and drug intermediates with good separations. Few examples of the separation of signals in ^{19}F NMR are also studied.

Chapter 4 is about application of roof shape alcohol as chiral auxiliary for preparation of halo acids. Esterification reaction of roof shape chiral alcohol and racemic α-halo acids in presence of DCC, DMAP furnished diastereomers of ester in non-racemic form.

The detailed discussion of each of these studies is contained in the appropriate chapters and only a brief discussion on the concepts of kinetic resolution and desymmetrisation is given in chapter 1 as introductory note.

The enzymatic work presented in this thesis has been performed with the use of steapsin lipase purchased from Sisco Research Laboratories, Mumbai and CAL-B as Novozyme-435, manufactured by Novozyme (Denmark). This enzyme immobilized on macro porous acrylic resin. Novozyme commercial manufacturing of Novozyme 435 (*Candida antarctica Lipase*-B [CAL-B]) is done by gene expression in an Aspergilius microorganism. As indicated by its name the yeast was isolated in *Antarctica* with the aim of finding enzyme. *Candidia rugasa* lipase (CRL) was obtained from Sigma-Aldrich. This steapsin and *CRL* lipase were purchased and used as such, while Novozyme-435 was a generous gift from Novozyme, Bangalore, India.

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