CHEMOENZYMATIC SYNTHESIS OF CHIRAL A-HYDROXY HETEROCYCLIC KETONES

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Approval of Graduate School of Natural and Applied Science

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

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ABSTRACT

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June, 2003

Chiral α-hydroxy ketones are important starting materials in the syntesis of aureolic acid antibiotics, blowing agents, anti-bacterial cephalosporins, anti-depressive and anti-viral drugs, cardiag drugs, potassium channel openers and HIV protease inhibitors.

In this work, a new synthetic method is developed for the enantioselective biotransformation of acetoxy ketones to chiral α-hydroxy derivatives by using hydrolytic enzymes.

Acetoxy ketones are obtained from aromatic ketones by using manganese(III) acetate oxidation which is an attractive alternative to the other multi-step procedures in the literature.

Keywords: α-Hydroxy ketones, $Mn(OAc)_3$ oxidation, enzymatic resolution, hydrolase type enzymes.

ÖZ

KİMYASAL VE ENZİM YOLUYLA KİRAL α-HİDROKSİ HETEROSİKLİK KETONLARIN SENTEZİ

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Haziran, 2003

Optikçe saf α-hidroksi ketonlar aerolik asit antibiyotiklerin, anti-bacterial, anti-depresyon, anti-virutik, potasyum kanal açıcıları ve çeşitli kalp ilaçlarının sentezlenmesinde önemli yapı taşlarıdır.

Bu çalışmada hidroliz enzimleri kullanılarak asetoksi ketonların kiral α-hidroksi keton türevlerine biyotransformasyonları gerçekleştirilmiştir.

Aromatik ketonlerdan asetoksi ketonlara geçiş mangan(III) asetat yükseltgeyeniyle yapılmakta olup bu yöntem literatürde bulunan pahalı ve karmaşık yöntemlere göre son derece pratiktir.

Anahtar Kelimeler: α-Hidroksi ketonlar, $Mn(OAc)_3$ oksidasyonu, enzimatik ayrıştırma, hidrolaz tipi enzimler.

TO MY FAMILY,

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I wish to express my most sincere thanks to my supervisor Prof. Dr. Ayhan S. Demir for his directing me this interesting study.

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INTRODUCTION

1.1. Bioconversion in Organic Chemistry

An increasing interest in understanding biological processes and the general recognition that chirality plays a crucial role in nature fostered a tremendous effort in enantioselective synthesis.[1] In the course of synthesizing natural products and designing new target compounds, chemists had to acknowledge the fact that enantiopurity is related to biological properties. Opposite enantiomers interact differently within an organism and can display various activities. Some differences are enormous, ranging from distinguishable smells and flavors to teratogenic effects. (Figure1) This has resulted in an increasing need for efficient methods for the industrial synthesis of optically pure products.[2]

There are several ways to obtain enantiomerically pure compounds, of which resolution of the product by means of formation of diastereomeric salts or complexes is still the most frequently applied method in industry. When the synthesis of a complex product consists of several steps, it can be important for practical and economical reasons to introduce the proper stereochemistry in an early stage of the synthesis, since this will reduce the amount of reagents, solvents, and reaction volume. This can be achieved starting from chiral building blocks that are enantiomerically pure and have functionalities that allow them to be transformed in to desired product. Although a wide variety of natural products is

available,currently much effort is also invested in the development of unnatural chiral building blocks.

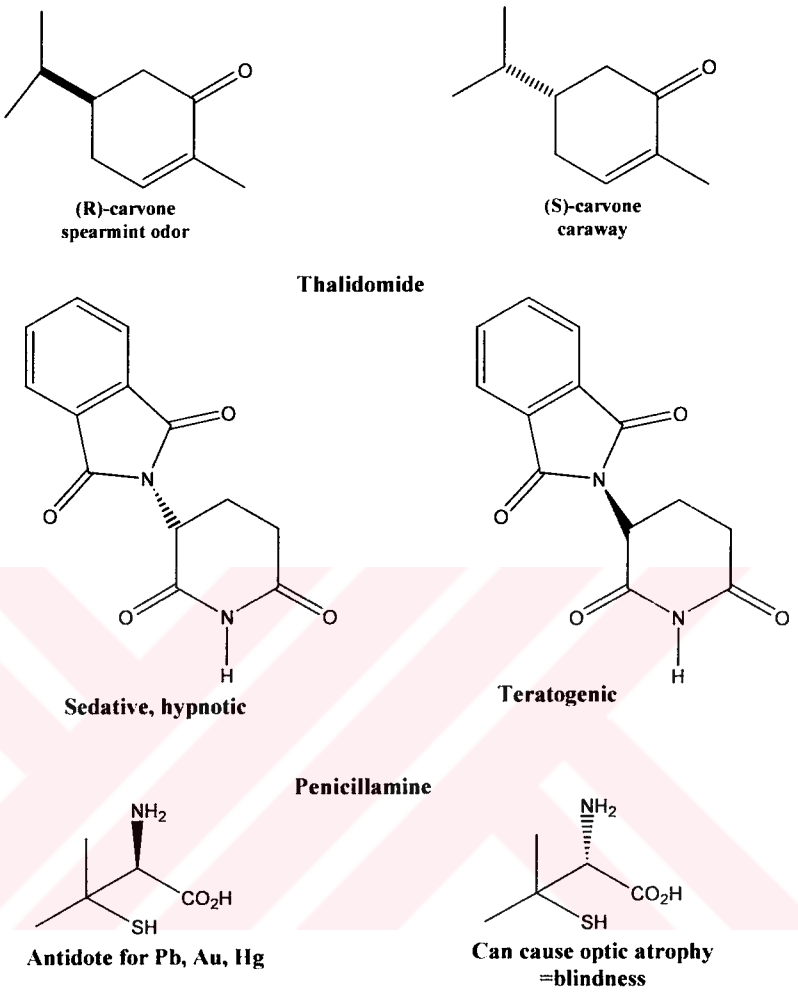


Figure 1. Biological effects of the enantiomers

In figure 1.2 different routes to obtain new enantiomerically pure chiral products are shown schematically. The first approach is to use a natural chiral compound that can be converted into the desired new building block. One can also start with a racemic compound and convert it to a mixture of diastereomers that can be separated. A drawback of this method is that an equimolar amount of a chiral reagent is usually needed. When kinetic resolution is applied to a racemic mixture one enantiomer reacts faster than the other. In the ideal case only one enantiomer reacts to completion within the given time and the desired product or the non-

converted enantiomer of the starting material is obtained enantiomerically pure in 50% yield. The last approach for obtaining enantiopure products is asymmetric synthesis starting from a prochiral compound. In this case it is in principle possible to convert all achiral starting material into enantiomerically pure product.

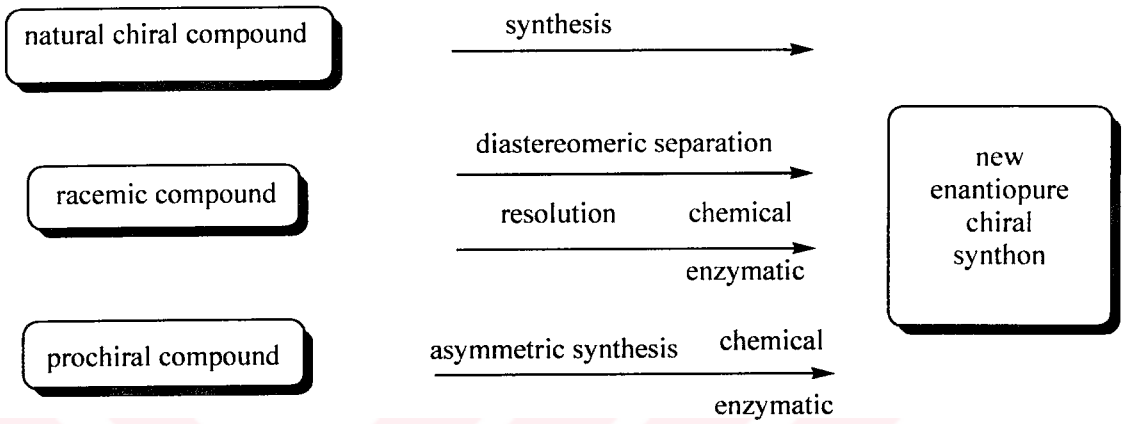


Figure 2. Different routes to new enantiomerically pure chiral synthons

Both kinetic resolution and asymmetric synthesis are most efficient when chiral catalysts are used that cause one enantiomer to be selectively converted or allow only one enantiomer to be formed. Only a small (catalytic) amount of chiral catalyst is needed to produce large amounts of chiral product. These chiral cataysts can roughly be divided into two groups. In the case of chemical catalysts chiral transition metal complexes or organic catalysts are frequently used and there are many examples of very successful asymmetric reactions. During the last decade the application of biocatalysis has become more and more part of the standard methodology of organic synthesis in university and industrial research laboratories and for many of the common organic reactions biocatalytical alternatives have been found.[3]

1.2 Chirality and Asymmetric Synthesis

Carbon atoms carrying four different substituents possess a unique property.[4] The substituents can be arranged in two alternative ways to bring about two forms of the molecule with the same constitution. In figure 1.3', two molecules of the same constitution (CHXYZ) are depicted so that in each case the smallest substituent, hydrogen, lies behind the plane of the paper. The substituent X is drawn in each case in the plane, pointing up, and the other two substituents occupy positions either on the left or right of the central carbon atom. Looking along the bond from the central carbon atom towards the hydrogen at the back, one finds that the two molecules differ in the way the remainig three substituents are arranged in space: in A the substituents X, Y and Z follow a clockwise rotation, whereas in B the rotation is counter-clockwise.

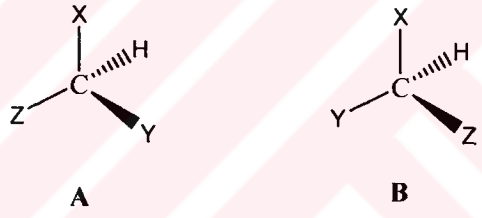


Figure 3. Chirality

The two forms of the molecule are related as hands to each other, being nonsuperimposible mirror images of each other. They are called *chiral* (from Greek cheir= hand), and the central carbon atom is known as the chiral or stereogenic centre. In this case, the whole molecule does not possess any element of symmetry (except identity), and the molecule is also *asymmetric*. However, asymmetry is not a necessary requirement for chirality. Disymmetric molecules which lack one or more elements of symmetry can also be chiral, and the requirement for chirality can be defined as follows: molecules which do not possess rotation-reflection axes (S axes) are chiral or disymmetric.

For instance, the compound trans-2,5-dimethylpiperidine (Figure 4) contains a two-fold rotation axis, belongs to the point group C_2, and is chiral.

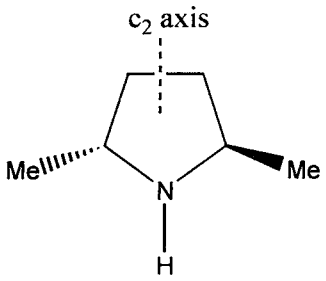


Figure 4. Two-fold rotation axis in trans-2,5-dimethylpiperidine

If the molecule contains more than one chiral centre, there emerges the possibility of another form of stereoisomerism. Stereoisomeric molecules which cannot be superimposed by any symmetry operations are called diastereomers. Thus, for 2-chloro-3-hydroxybutane (Figure 5), one can draw four different structures: two pairs of enantiomeric compounds and two pairs of diastereomeric compounds.

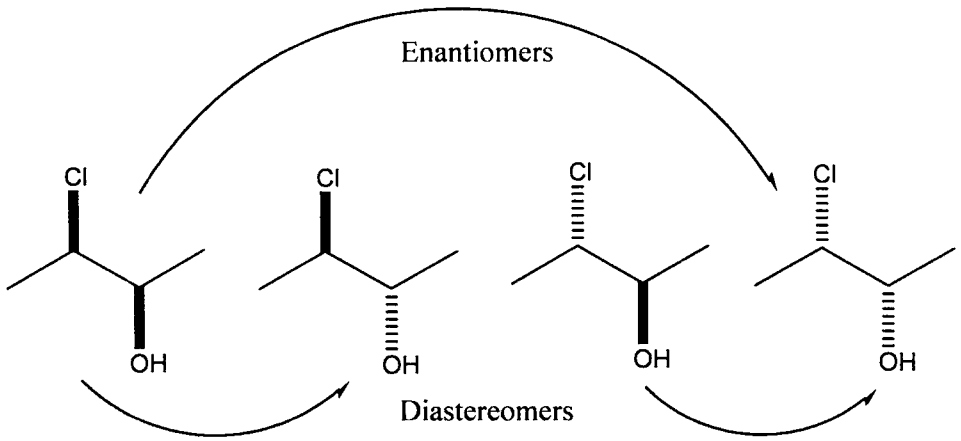
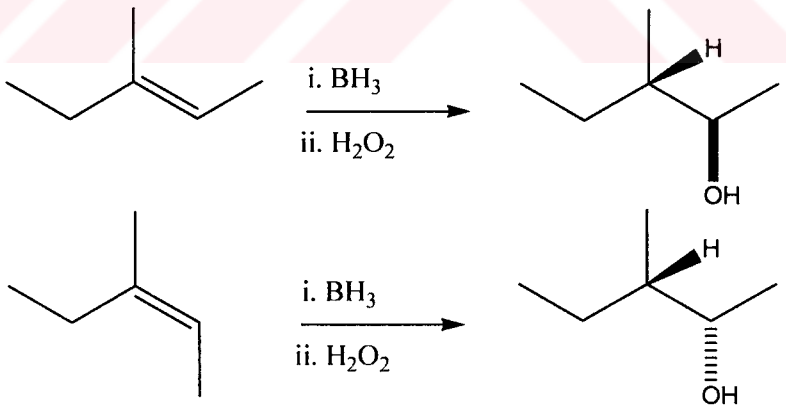


Figure 5. Stereoisomers of 2-chloro-3-hydroxybutane

In reactions involving one or more chiral centres, we are interested in bringing about transformations which produce one stereoisomeric form in abundance over the other possible ones.

One speaks of stereoselective reactions if the outcome of the reaction is non-statistical, and stereospecific reactions if the product is produced in one enantiomeric form only. All stereospecific reactions are necessarily stereoselective, but selectivity alone is not a sufficient criterion for specificity.

In scheme 1, both reactions are stereospecific because the mechanism of hydroboration necessarily places the hydride and boron on the same face of the double bond (in both reactions both enantiomers are formed). The two faces of the double bond react with equal facility, and thus a racemic mixture is produced. This is obvious, since we are using an achiral reagent, and thus there is no source of asymmetric information in the reaction. But let us consider whether the reaction could be persuaded to give an enhancement in optical activity.



Scheme 1

Most methods used for the preparation of enantiomerically enriched chiral organic molecules can be classified into two distinct strategies.[5]

The first one is asymmetric synthesis- involves the stereocontrolled formation of the new stereogenic center. For example; as the new chiral element is formed it is done so in a non-racemic fashion. This demands that the reactive centers experience some stereo discriminating environment in the transition state. This can originate from an existing stereogenic center in the substrate or in via a chiral reagent catalyst (chemical or enzymatic). The second approach involves resolution: this utilizes a stereoisomeric mixture and does not depend asymmetric induction in the formation of any new chiral element. Thus preparation of a single stereoisomer by resolution of a stereoisomeric mixture may be achieved via a conventional separation procedure or by exploiting the difference in reactivity.

1.2.1 Methods to Achieve Asymmetric Synthesis

Before embarking on a synthesis, careful thought must be given to how a chiral center will be introduced in to a molecule.[6] The three major options are use of a chiral reagent (chemical or biological); use of a chiral environment; and use of a chiral starting material. Chirality can also be introduced in a temporary manner through the use of a chiral auxiliary, although this is a sub-class of chiral substrates. These must be considered on a case by case so that the greatest chance of success arises from the synthetic plan.

Chiral Substrates: The best approach is to have a chiral starting material that can then control the stereoselection of the reaction itself. To achieve this, especially at the beginning of the synthetic sequence, few options are available. Nature produces chiral materials and a number of these are available in quantity. These compounds make up the "chiral pool." This approach is often limited to the amount of the natural product available and its price. Another consideration, sometimes overlooked, is the number of steps necessary to convert natural product into a useful starting material for synthesis. 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.

Chiral Auxiliaries: Since the number of useful natural products available is not large, or the number of steps necessary to convert a cheap, readily available one to a useful intermadiate in a synthesis may require many steps; some of these steps may involve expensive reagents. A number of chiral groups heve been developed that can be attached to an achiral molecule. These groups then induce selectivity through a subsequent chemical reaction to afford diastereoselectivity. Removal of the "chiral auxiliary" then provides the product enriched in one enantiomer. However, this type of approach introduces two extra steps: the attachment and removal of the auxiliary.

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.

Chiral Reagents: In many ways, this is the approach of choice as nature utilizes this methodology through enzymes. The reagent must be selective both in terms of induction and functional group specificity. The need for protection should be carefully considered as this could lead to the introduction of extra steps. The chiral reagent should allow for the expensive cost component to be recycled, if necessary, or have a very high turnover number.

Chiral Environments: It is possible to make the environment of a chemical reaction chiral. The majority of examples in this class utilize chiral solvents or additives. To influence the differentiation of the free energies of the diastereomeric transition states, and hence provide useful induction, these agents must be closely associated with the reaction center. In most cases, this has not been fruitful, as in the use of chiral solvents, but some reactions that use chiral ligands do provide good ee's.

1.2.2 Kinetic Resolution

In this approach a substrate is acted on by a chiral agent to produce one enantiomer or diastereomer of the product at a much faster rate than the other isomer. The transition states have to be of a significant energy difference for this method to be viable. In general the enantiomeric excess of the starting material will increase as the reaction progresses, while the ee of a chiral product will decrease. As this is a resolution, only 50% of the substrate can be converted to the desired product.

There are two main types of kinetic resolution:[7] The chiral agent may be;

i. a catalyst, a medium or a reagent donating or accepting a non-chiral entity,

ii. a reagent which is linked to the substrate in the course of the reaction.

In the first case the chirality of the substrate can either be retained or destroyed.

If it is retained then the pairs of enantiomeric substrates is transformed to a pair of enantiomeric products. When chirality is destroyed both enantiomers give rise to the same achiral product(s).

The product may also be chiral and in the simplest case it is the enantiomer of the substrate itself. This process is called deracemization and can be brought about by equilibration in chiral media or using chiral catalysts.

When the association of a racemic substrate with a chiral agent is not simply transient but leads to the formation of a compound in which the stereogenic elements of both are retained, the product is a mixture of two diastereomers.

Kinetic resolution is an inherently wasteful process for producing optically active compounds and can only compete with conventional resolution when rate differences are extreme. With few exceptions this has so far only been realized with enzymes.

1.3 Asymmetric Synthesis Using Biotechnological Methods

Biocatalysis encompasses catalysis by bacteria, fungi, yeast, or their true catalytic components: enzymes.[8] Enzymes are proteins that are capable of accelerating reactions under mild reaction conditions. Other advantages are the high degrees of substrate-, chemo-, regio- and stereoselectivity and high efficiency. Although these factors make enzymes especially attractive for organic synthesis there are still certain restrictions in their applicability. The use of water as reaction medium is necessary for many enzymes. Sometimes the use of a cofactor is required. The commercial availability of enzymes at a reasonable price can also be a problem and so can be the stability of the enzymes.

1.3.1 Enzyme Mechanism, Kinetics, and Enantioselectivity

The theory advanced by Linus Pauling in1948 holds that an enzyme catalyzes a reaction by stabilizing the transition state of the reaction, which lowers the activation energy that is needed for the reaction to occur and leads to an acceleration of the reaction. For an enzyme to catalyze a reaction selectively, the substrate must bind in a well-defined manner in the active side. Once the substrate is bound, the enzyme exposes it to unique interactions and reactions like hydrolysis, oxidation/reduction, or C-C bond formation will take place. The high selectivity in enzyme catalyzed reaction originates from the close proximity and the precise orientation of the functional groups of the substrate and the enzyme. During a catalytic cycle the substrate is sometimes covalently bound to the enzyme for a short interval. The enzyme then converts the substrate to the product, and after releasing the this newly formed product the enzyme is ready for another cycle. Functionalized amino acids such as serine, cysteine, histidine, lysine, aspartic acid, or glutamate can be present in the active side. Functional groups with nucleophilic character may donate an electron pair to a substrate when it is bound in the enzyme-substrate complex or they act as acids or bases depending on their character.

Stereoselectivity in an enzymatic reaction can be accomplished through kinetic control. This means that one enantiomer reacts faster than the other. The enantioselective performance of enzymes is expressed as the enantiomeric ratio E, which is a measure for the selectivity of an enzyme for one of the enantiomers of a substrate.

Enzymes can be classified according to the reactions they catalyze. Hydrolytic enzymes, such as lipases are able to speed up hydrolytic reactions. This class of enzymes can be divided in to four groups with different catalytic systems. Serine proteases contain a catalytic triad with serine acting as a nucleophile. Examples of serine proteases include trypsin, chymotrypsin, pig liver esterase, and lipases. Figure 6 illustrates a catalytic cycle for serine proteases, which is representative for most lipases.

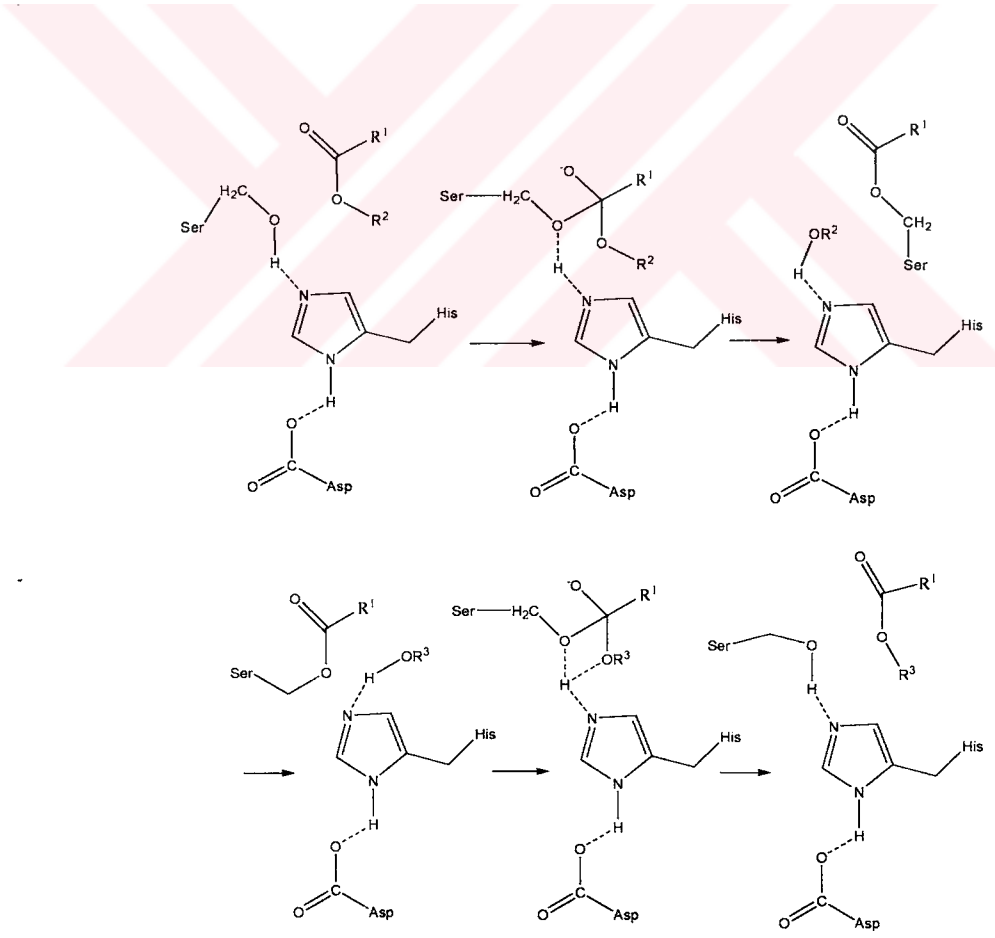


Figure 6. Catalytic cycle for serine proteases

In serine proteases a catalytic triad consisting of the amino acids serine, histidine and aspartic acid are responsible for the catalysis. In figure 1.6 Ser reacts as a nucleophile with a substrate molecule. Here being an ester. During substrate binding a proton is transferred from Ser to His, making Ser more nucleophilic. The positive charge of the protonated imidazole ring is stabilized by interaction with the carboxylate group of Asp. A tetrahedral intermediate is formed in which the enzyme and substrate are covalently linked (enzyme-substrate transition state). The proton on His binds to the alkoxy group that is then eliminated as an alcohol molecule. An acyl enzyme is formed as a covalent intermediate. The highly reactive intermediate formed may react with water (R_3 = H, hydrolysis) or a second alcohol molecule (transesterification) to yield the product of the reaction, being either an acid or an ester.

To avoid trial and error modificaton of the substrate structure and to provide sutable tools to predict the stereochemical outcome of enzymatic reactions on non-natural substrates, useful abstract 'models' for the more commonly used enzymes have been developed.[9] By use of the models, one should be able to redesign a substrate, if the initial results with respect to rate or selectivity of the reaction were not acceptable. One of the most useful model of these is the 'Active side model'. According to this model, instead of developing an ideal substrate structure one can try to picture the structure of the (unknown) active site of the enzyme. Thus substrates of varying size and polarity are used as probes to measure the dimensions of the active side. Such active site models usually resemble an arrangement of assumed 'sites' or 'pockets' which are usually bo or cave shaped. A relatively reliable active-site model for PLE using cubic-space descriptors was based on the evaluation of the results obtained from over 100 substrates.
(Figure 7).

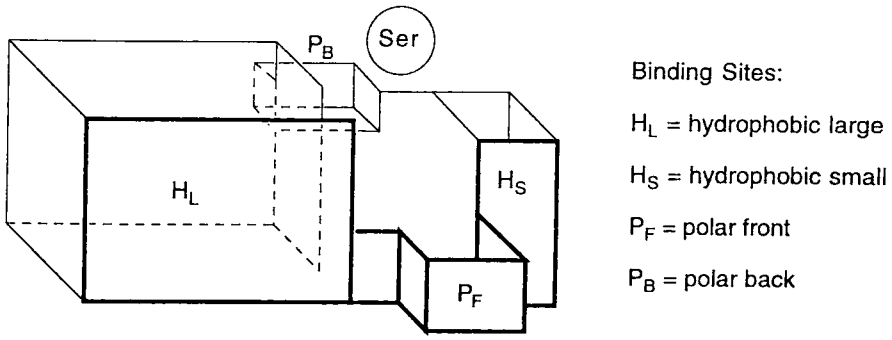


Figure 7. Active-site model for porcine liver esterase.

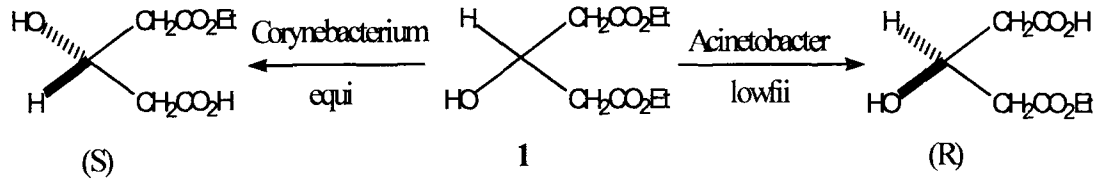
1.3.2 Enzymes as Synthetic Tools

Due to low cost, high stabillity, and substrate tolerance, hydrolases, such as pig liver esterase (PLE), porcine pancreatic lipase (PPL), and α-chymotrypsin, have been frequently used.[10] Lipases are designed by nature to function at a water-lipid interface involving the enzyme in aqueous solution and the insoluble lipid substrate. The use of lipases in organic solvents simply "inverts" this interface; the enzyme and its associated water of hydration is insoluble and the substrate is in solution. It is logical, then, that lipases are particularly useful for transformations in organic solvents.

Oxidoreductases are not as frequently applied because of expensive cofactors that are required and the constraints associated with sensitivity and cofactor regenaration. Baker's yeast is commonly used as a "cocktail" of dehydrogenases and its application is fairly simple, lacking all the technical difficulties that are usually associated with handling oxidoreductases in pure form.[11]

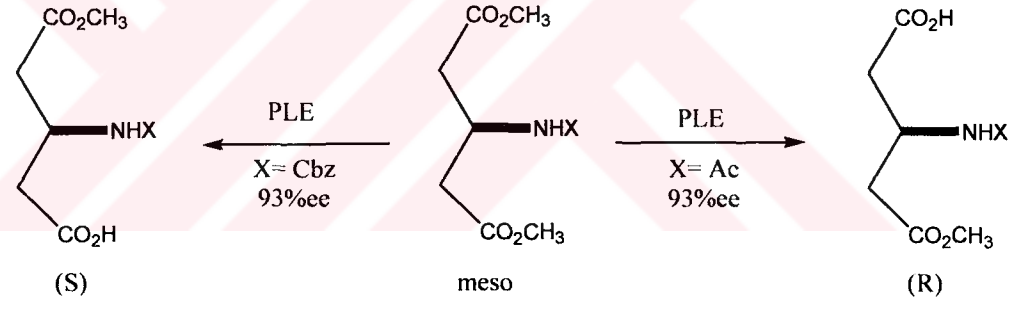
With the increasing availability of biocatalysis, one can take advantage of those that exhibit opposite enantioselectivity.

The prochiral diester **1** was hydrolyzed with different microorganisms; by use of Acinetobacter lowfii the hydrolysis yielded the (R) acid, whereas Corynebacterium equi sp. gave rise to the (S) enantiomer (Scheme 2).[12]



Scheme 2

Certain substrate modifications can cause a reversal of enantioselectivity. The nature of the protection of the amino functionality not only improved the stereoselectivity but also determined the configuration of the product (Scheme 3).[13]



Scheme 3

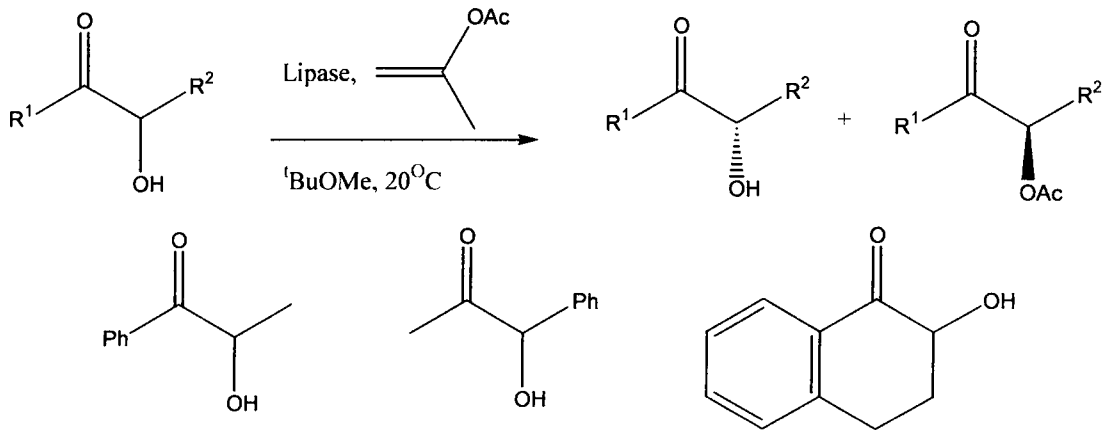
In recent years, enzyme applications in the asymetrization of compounds have become very common, and their importance is reflected by numerous reports in the literature including their use in drug synthesis.[14]

1.4 Lipase Catalyzed Synthesis of Chiral α-Hydroxy and α-Acetoxy Ketones

The synthesis of optically active α-hydroxy carbonyl compounds, in particular ketones, is of significant importance since they are convenient building blocks in the asymmetric synthesis of biologically active compounds.[15] Recently several methods have been developed for their preparation. For example, the stereoselective oxidation of optically active enolates has been reported[16] as an attractive route. On the other hand, prochiral enolates have been oxidized enantioselectively by optically active oxaziridines as electrophilic oxidants.[17]

Alternative to the chemical methods, optically active α-hydroxy ketones can be prepared enzimatically by reduction of the α-diketones with yeast as the biocatalyst.[18] However, this enzymatic method possesses the following disadvantages: further reduction of diketone to the vic-diol, formation of both regioisomeric α-hydroxy ketones and moderate chemical yields.

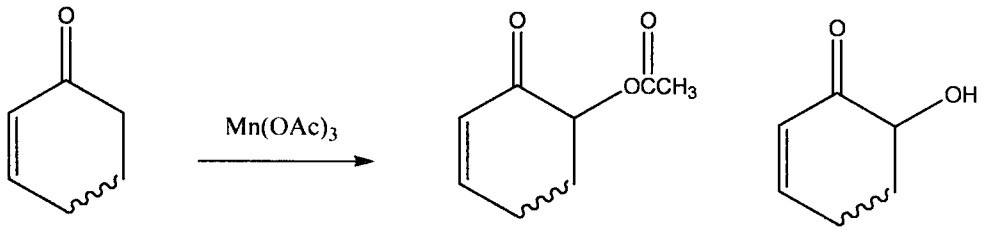
Lipases have been frequently used as convenient and efficient biocatalysts for the asymmetric synthesis of a wide range of organic compounds. They have been widely used for the synthesis of optically active alcohols, carboxylic acids and esters via enantioselective esterification and transesterification in organic solvents. Although numerous α-hydroxy acids and esters have been resolved by lipases, reports on the kinetic resolution of structurally simple α-hydroxy ketones by these readily accessible enzymes are scarce. Recently, Gala et al. have described[19] the resolution of α-hydroxy aryl ketones (precursors of chiral azole antifungal reagents) by lipase catalyzed hydrolysis of the corresponding acetates in phosphate buffer; neverthless, the irreversible transesterification route of this enzymatic reaction appears not to be known. Another report has been presented by Adam et al. that is the kinetic resolution of racemic α-hydroxy ketones by lipase-catalyzed irreversible transesterification with isopropenyl acetate in organic media (Scheme 4).[20]



Scheme 4

1.5 $Mn(OAc)_3$ Mediated Acetoxylation of Enones

Some procedures were developed for the selective oxidation which occupy a central position of common functional groups in the synthesis of complex natural products. Literature methods gave unsatisfactory results for the oxidation of an enone to an α'-acetoxyenone.[21] To overcome this problem, Demir and his coworkers studied on the oxidation of α,β-unsaturated enones using manganese(III) acetate.[22, 23, 24, 25] They got satisfactory result for the preparation of α'-acetoxy enones (Scheme5).

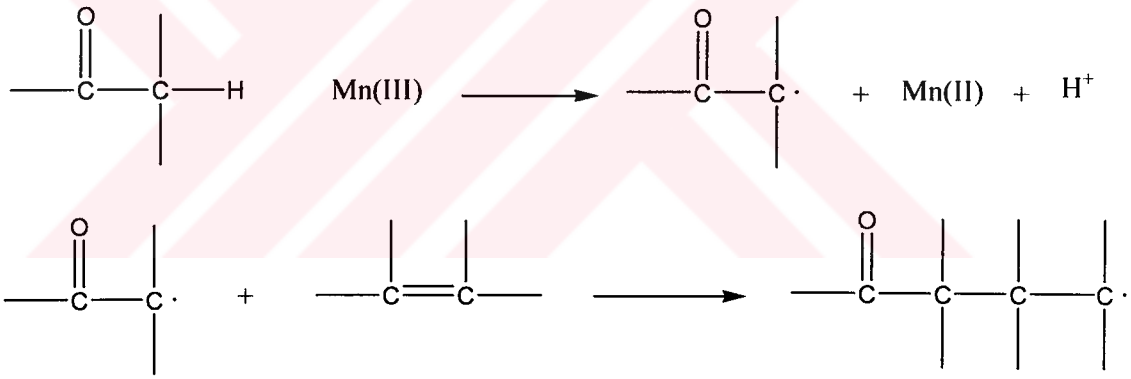


Scheme 5

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

1. Direct Oxidation: Direct inner or outer-sphere one electron oxidation of the substrate; often determines the product is followed by the formation of manganese (III) complex where the subsequent oxidation of the intermediate radical. Numerous examples can be found such as oxidations of alcohols, amino and thio compounds, carboxylic acids and certain aromatics.

2. Indirect Oxidation: Indirect oxidation of the substrate; takes place after the formation of an intermediate adduct free radical which is formed by the interaction of Mn(III) acetate. The result is an enolizable compound or subsequent oxidation/substitution and oxidative addition of enolizable compounds to unsaturated systems. Mn(III) acetate deals with addition reaction of compounds which have α-hydrogen atom to a carbonyl group with olefinic and aromatic unsaturated systems (Scheme 6).



Scheme 6

The fate of the primary radical adduct strongly depends on reaction conditions and the nature of the substrate. Manganese(III) acetate can be used as a free redical generator if substrates are less reactive to common oxidants. The one electron oxidants like Co(III), Ce(IV) and some two electron oxidants like Tl(III) and Pb(IV) also show similar properties as manganese(III) acetate. However, lower reactivity and higher selectivities is obtained with manganese(III) acetate compared with the other oxidizing agents.

Many of these reactions proceed according to the simplified scheme which is shown below;

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

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

Scheme 7

Complications may arise in the presence of water. Water causes disproportionation of trivalent manganese into Mn(IV) and Mn(II) and alternative two-electron oxidants may take place by Mn(IV).

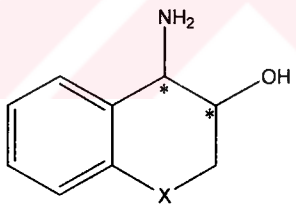
Manganese(III) acetate can be used for initiating the addition of aldehydes to olefinic unsaturated systems, the addition of ketones to olefinic unsaturated systems, the addition of haloalkanes to unsaturated systems, aromatic substitution reactions.

1.6 The Importance of Chromanone and Thiochromanone Structures, Synthesis and Properties of Cromakalim Type Potassium Channel Blockers and HIV Protease Inhibitors

During the last 10 years compounds have been discovered which can activate or block potassium channels.[26] In particular, K channel activators (KCA) have been found to be smooth muscle relaxant with their main utility in hypertension and bronchodilation.

There are at least seven classes of KCA, of which the main four are the benzopyrans, thioformamides, cyanoguanidines and the organic nitrates. Best investigated subgroup is the benzopyrans.

Biologically active cis-amino chromanols **2a** and **2b** are important benzopyran type synthons for the synthesis of anti HIV drugs specially HIV protease inhibitors and KCA's (Scheme 8).

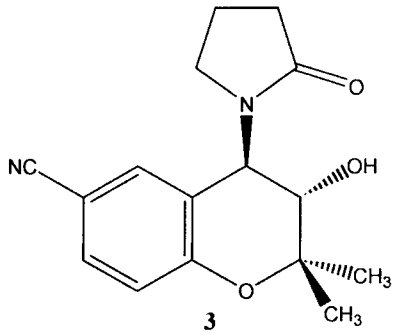


2a: X=O
2b: X=S

Scheme 8

For example, in the syntheis of the HIV protease inhibitor Crixivan, an expedient way of producing cis-aminondanol and related aminoalcohols was established using a Jacobsen epoxidation/Ritter-type reaction sequence.[27] These aminoalcohols have been used successfully in a 'conformational toolbox' of oxazilidine ligands.

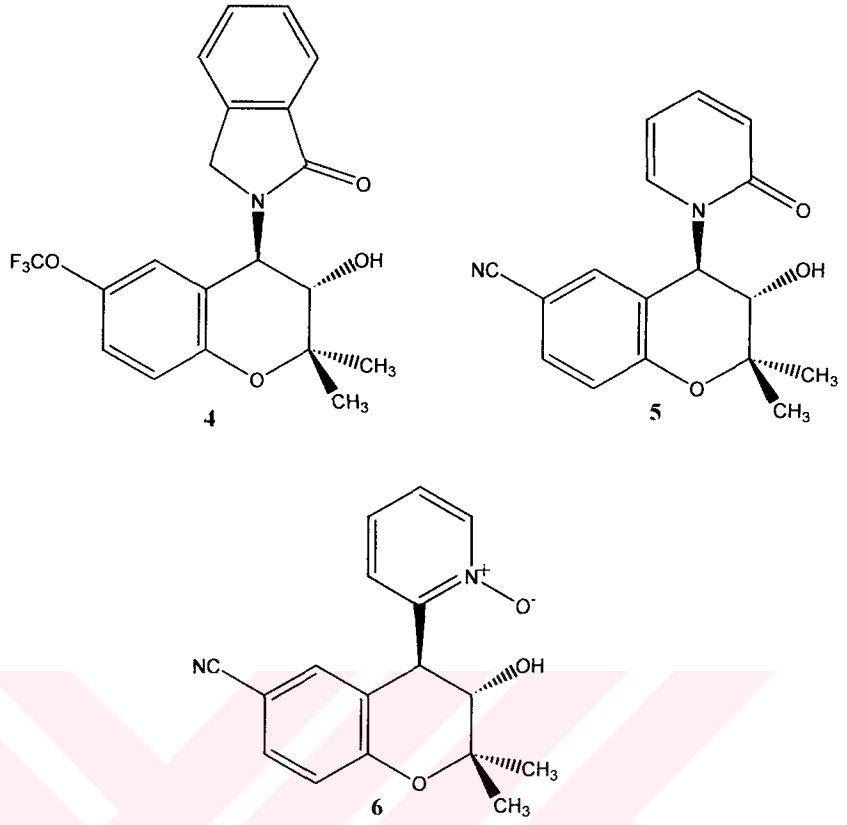
The first compound to be termed as a potassium channel activator (sometimes called potassium channel opener) is the benzopyran based structure cromakalim [(+)-3-hydroxy-2,2-dimethyl-trans-4-(2-oxopyrrolidin-1-yl)-chromane-6-carbonitrile], which is a powerful smooth muscle relaxant with potent antihypertensive and bronchodilator activity.[28]



3

Scheme 9

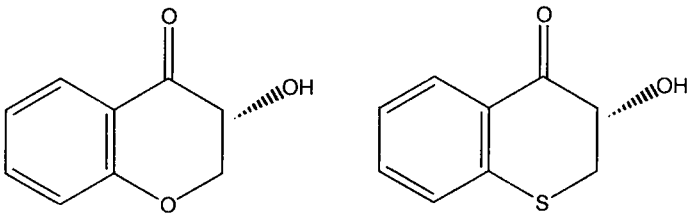
Since the discovery of cromakalim (racemic) in 1985 as a potent hypotensive agent, a large number of benzopyrans have been reported.[29] Among the most well-known of the lead compounds in this group are celikalim **4**, bimakalim **5**, and Ro 31-6930 **6.** All of these benzopyrans exert their hypotensive effect by relaxing smooth muscle via opening of cell membrane ATP-sensitive potassium channels. By virtue of this effect, potassium channel openers may have utility for treatment of hypertension, asthma, incontinence and impotence. In addition, potassium channel openers have been found to be useful for stimulation of hair growth.



Scheme 10

Potassium channels are ubiquitous amongst cells, different channel not only being found amongst different cells, but also within the same cell. With increasingly sophisticated pharmological probes, new substances which selectively modulate the action of potassium channels are continually being found and the whole area is burgeoning.

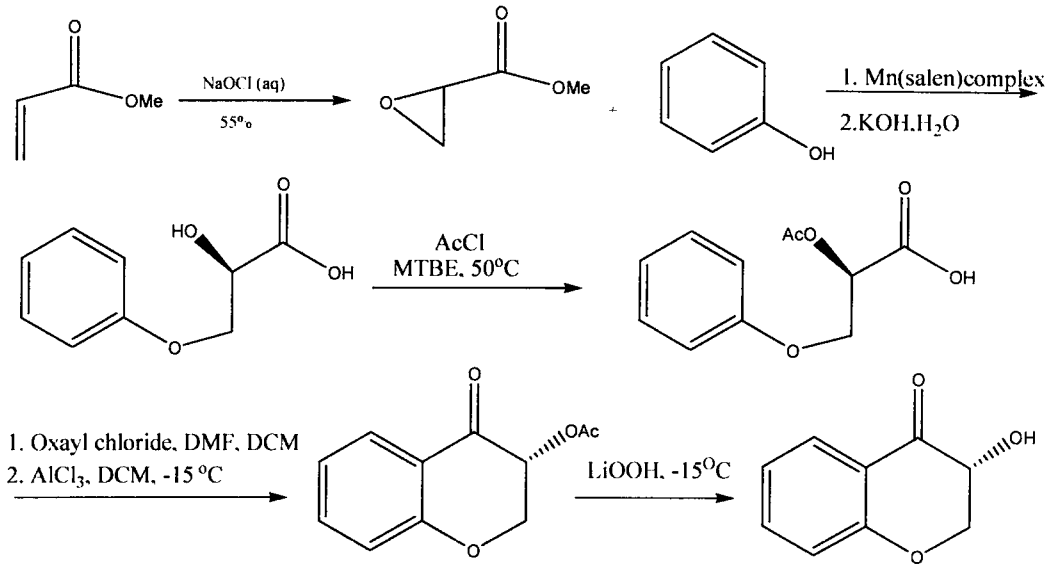
Being the important building block of benzopyran type potassium channel activators, HIV protease inhibitors and also other biologically active cis amino alcohols, α-hydroxy ketones, 3-hydroxy-2,3-dihydro-4H-chromen-one **7a** and 3-hydroxy-2,3-dihydro-4H-thiochromen-one **7b** are of high current interest.



7a **7b**

Scheme 11

Consequently, several studies have been aimed at developing methodology for the synthesis of this structural unit in chiral nonracemic form. Hansen et al. synthesized cis-4-aminochroman-3-ol (**2a**) starting from chiral 3-hydroxy-2,3-dihydro-4H-chromen-4-one (**7a**).[30] It was unsuccessful to obtain cis-4-aminochroman-3-ol (**2a**) starting from chromene via asymmetric epoxidation followed by Ritter reaction. There are several methods in the literature for the synthesis of 3-hydroxy-2,3-dihydro-4H-chromen-4-one, but there are few examples of the enantioselective synthesis of these compounds. Only one method is described for the enantioselective synthesis of **2a** in 6 steps. Hansen et al. applied the catalytic asymmetric synthesis of α-aryloxy alcohols methodology from Jacobsen et al.[31] and synthesized 3-hydroxy-2,3-dihydro-4H-chromen-4-one in 94% ee in four steps starting from methyl glycidate and phenol in large scale. Scheme 12 shows the synthesis of **7a**, which in turn, could be constructed via cyclization of hydroxy acid. The kinetic resolution of methyl glycidate via asymmetric ring opening with phenol, catalyzed by chiral (salen)Co(III) complex, derived α--hydroxy ester in high chemical and optical yield. The stereochemistry is established by using chiral (salen)Co(III) complex.



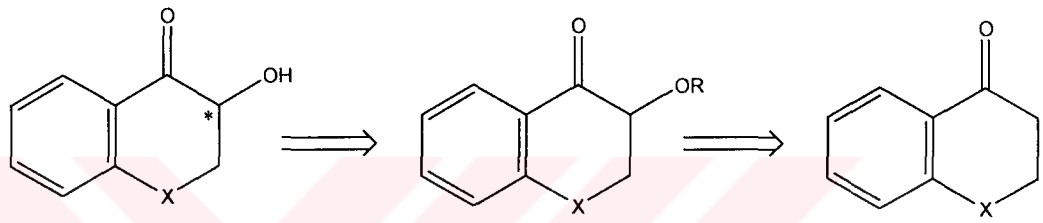
Scheme 12

A drawback of the method shown in Scheme 12 is the tendency of many of the intermediates to epimerize.

This great importance of hydroxy chromanon and thiochromanon moiety, particularly the major class "benzopyrans" led us to explore a new methodology for the synthesis of optically active α-hydroxy ketones, since they are the building blocks of biologically active amino alcohols and potassium channel activators.

1.7. Aim of the work

The major aim of this research is to develop simple and selective method for the synthesis of α-hydroxy chromanone structures which are very important intermediates for the synthesis of K channel activators and HIV protease inhibitor activity compounds. They are also used in the synthesis of anti-bacterial cephalosporins, anti-virutic, anti-depressive and cardiac drugs, antibiotics, blowing agents and steroids. The aim of this work is shown retrosynthetically in scheme 13.



R=H, Ac

Scheme 13

There is no convenient method for the enantioselective synthesis of hydroxy chromanone type compounds in the literature.

Our first approach to enantiopure α-hydroxy ketones was to synthesize the racemic form of corresponding acetates by using manganese(III) acetate, followed by enzymatic bioconversion by lipases.

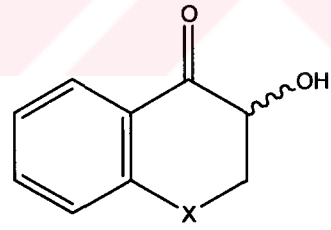
It was also aimed to find the optimum conditions for enzymatic bioconversions for good optical yield. Alternative to the chemical methods, the regioselective one-step α-oxidation of manganese(III) acetate and enantioselective hydrolysis by using lipases provides a low cost production of the α-hydroxy ketones.

CHAPTER 2

RESULTS AND DISCUSSION

2.1 Perspective of the work

α-Hydroxy ketones are versatile chiral synthons for the construction of chiral organic compounds due to reactive functional groups: carbonyl and hydroxyl groups, which can easily be transformed to vic-diols, α-amino ketones, and other functional groups.



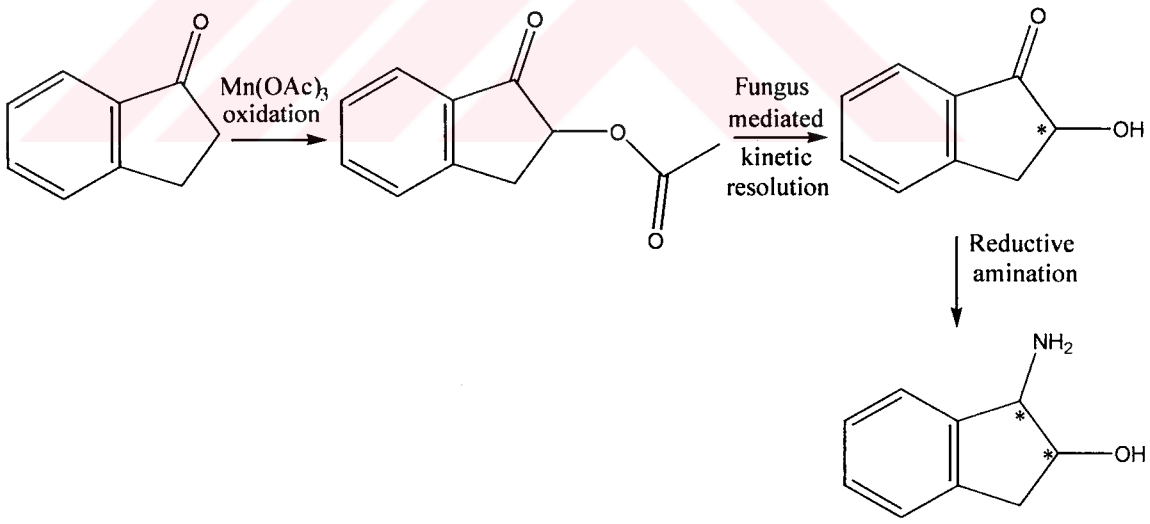
7a: X=O
7b: X=S

Scheme 14

For example the 3-hydroxy-2,3-dihydro-4H-chromen-4-one moiety **7a** (Scheme 14) is found in many natural compounds with different biological activities. Examples of natural products that have been prepared from this synthon are the benzopyran type potassium channel activators, like chromakalim and HIV protease inhibitors.

The related chroman-4-ones have also shown to be very interesting building blocks in several stereoselective reactions. Thiochromanons **7b** have important structural features of natural products such as aminocyclitol antibiotics and amino sugar derivatives eg. acosamine and ristosamine.[32]

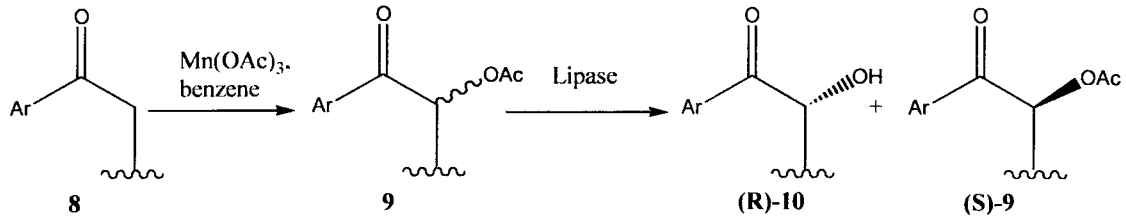
However, up to today there is no general way for the enantioselective synthesis of these chiral α-hydroxy ketones. Our group recently developed the synthesis of (1S, 2R)-1-amino-2-indanol, a key component of HIV protease inhibitor.[33] The synthesis was accomplished in four steps starting from indanone efficiently and with high levels of diastereo- and enantioselectivity. Indanone was converted into 2-acetoxy-1-indanone involving manganese(III) acetate oxidation. The 2-acetoxy ketone is hydrolyzed to 2-hydroxy-1-indanone enantioselectively using *Rhizopus oryzae*. Selective reduction of 2-hydroxy oxime derivative, derived from the 2-hydroxy keton, gives the amino alcohol up to 98% diastereo- and enantioselectivity (Scheme 15).



Scheme 15

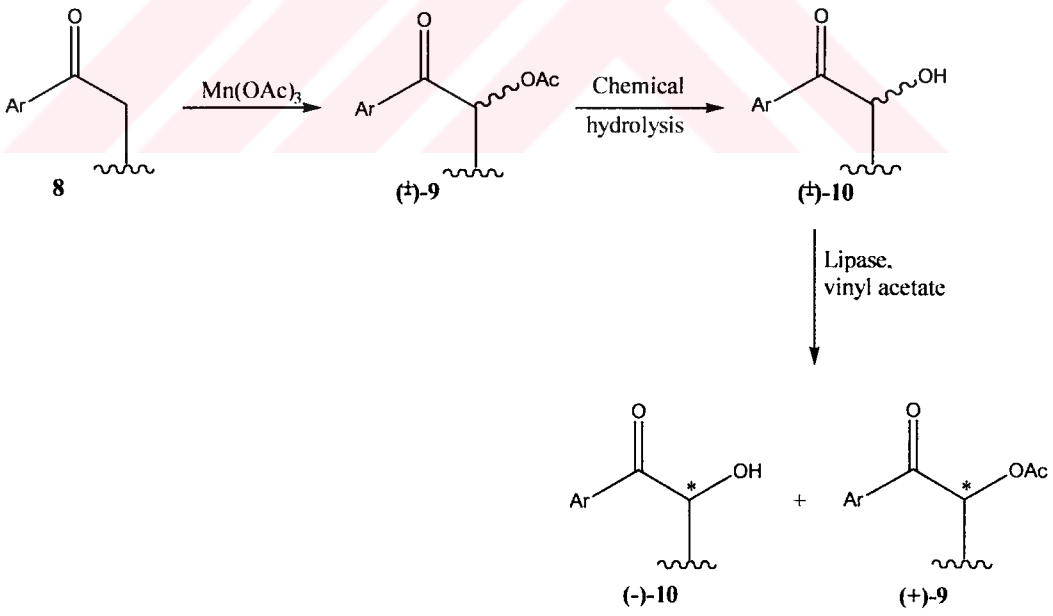
Consequently, alternative to the chemical methods in the literature and on the basis of this preliminary information from the previous work with biocatalyst-mediated reactions, here the chemoenzymatic route for enantioselective synthesis

of α-hydroxy ketones from ketones via $Mn(OAc)_3$ mediated acetoxylation followed enantioselective ester hydrolysis by using lipases is presented (Scheme 16).



Scheme 16

Lipases were chosen for the enantioselective hydrolysis of corresponding esters because of their availability and their reactivity in organic solvents and because of the fact that lipases are also successfully used in the transesterification of the structural related racemic α-hydroxy ketones. As shown in scheme 17, the other alternative was to convert racemic acetoxy ketones in to hydroxy ketones followed by enzyme catalyzed esterification.



Scheme 17

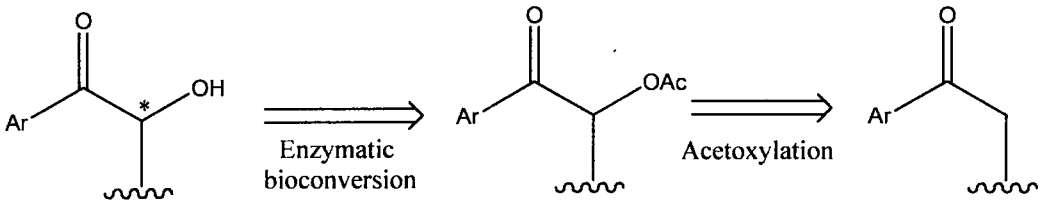
Due to the difficult availability and importance of hydroxy chromanone, chromanon was chosen as the starting material.

To find a suitable experimental setup for the lipase catalyzed resolutions of acetoxy and hydroxy chromanons we have tried to find the reaction conditions which give the best results in the lipase catalyzed hydrolysis and transesterification reactions. Furthermore, it was essential to find an easy and accurate way to determine both conversion of the reaction and enantiomeric excess values of the ester (remaining substrate) and the alcohol (product). For the determination of the e.e of the ester and the alcohol in the kinetic resolutions several chiral HPLC columns were used. Determination of the e.e by this technique was always first performed on the racemate to optimize base-line separation. For the determination of conversion, we have decided to control the reaction with TLC. The stereoselectivity is expressed in the enantiomeric ratio or E-value, which is the ratio of reaction rates of both enantiomers of the starting material. When the difference in reaction rate between the enantiomers is large, E is high, which indicates a better enantioselectivity.

Since only very small amount of samples are needed for HPLC analysis, it was possible to perform the hydrolysis and transesterifications on an analytical scale. In this way the screening of a large number of lipases becomes fast and easy. For screening purposes a library of lipases was available, containing a variety of lipases most often used in literature.

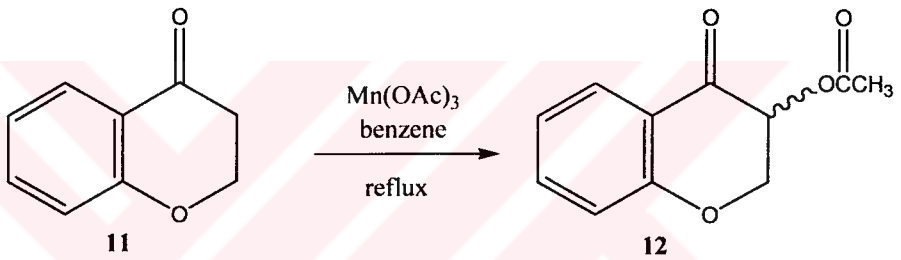
2.2 Synthesis of α-Acetoxy enones

For the synthesis of chiral α-hydroxy ketones first acetoxylation was carried out with ketones. Scheme 18 shows the retro-synthetic way for the synthesis of chiral α-hydroxy ketones.



Scheme 18

4-chromanon **11** was chosen as the starting material and it was allowed to react with 3 equivalent of $Mn(OAc)_3$ in benzene's reflux temperature under a Dean-Stark trap to give the desired acetoxy derivative **12** in racemic form.

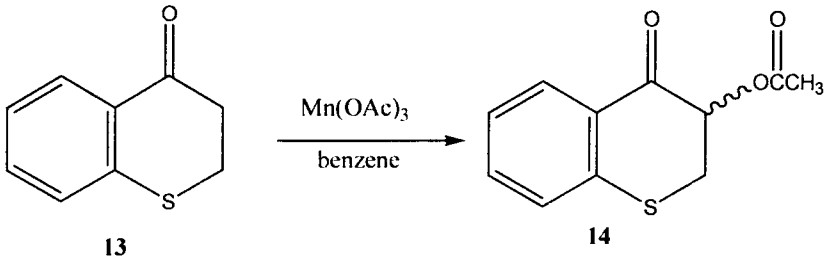


Scheme 19

The reaction is monitored by TLC (Silica gel, EtOAc/Hex 1:10). After the work-up and purification of the crude product by flash column chromatography (EtOAc/Hex 1:10), the desired product, 4-oxo-3,4-dihydro-2-chromen-3-yl acetate **12** was obtained as a colorless oil in 75% yield (Scheme 19).

The product was identified by using NMR spectroscopy. From the ^{1}H-NMR spectrum we observed a singlet at 2.1 ppm from the $-CH_3$ group and dd at 5.6 ppm (J=11.3 and 5.5 Hz) for the α-proton. From the ^{13}C-NMR spectrum we observed a singlet at 20.8 ppm for the CH_3 carbon and a singlet at 169.3 ppm for the O<u>C</u>OCH_3 carbon.

In the next acetoxylation reaction thiochroman-4-one **13** was used as starting material and it was allowed to react with 3 equivalents of $Mn(OAc)_3$ under benzene's reflux temperature to yield corresponding 4-oxo-3,4-dihydro-2-thiochromen-3-yl acetate **14** in 70% yield (Scheme20).

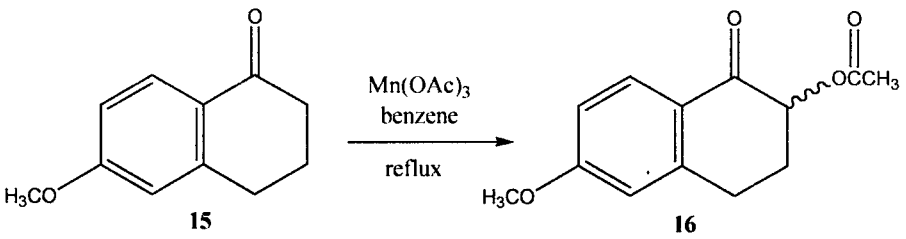


13 **14**

Scheme 20

The product was identified by using NMR spectroscopy. From the ^{1}H-NMR spectrum we observed a singlet at 2.2 ppm from the $-CH_3$ group and dd at 5.7 ppm (J=13.5 and 4.5 Hz) for the α-proton. From the ^{13}C-NMR spectrum we observed a singlet at 20.5 ppm for the CH_3 carbon and a singlet at 169.0 ppm for the O<u>C</u>OCH_3 carbon.

Under similar conditions as described above, 6-methoxytetralone **15**, which is an important structural unit in many natural products, was reacted with 3 equivalents of $Mn(OAc)_3$. The reflux was continued for 48 hours by monitoring with TLC and the product **16** was obtained as an orange colored crystals after flash column chromatography (EtOAc:Hex, 1:4) in 85 % yield (Scheme 21).



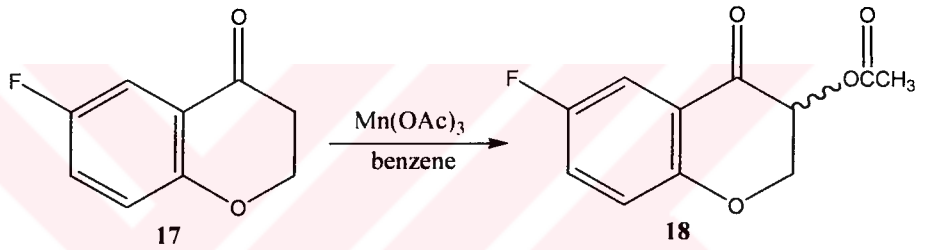
15 **16**

Scheme 21

The ^{1}H-NMR spectrum of **16** showed the formation of the product by appearing as a singlet at 2.10 ppm (CH_3), dd at 5.40 ppm (J=5.1 and 13.1 Hz) for the proton at α- position.

The ^{13}C-NMR spectrum also showed the formation of product by appearing as a singlet at 21.2 ppm for the CH_3- carbon and at 170.4 ppm for the O<u>C</u>OCH_3 carbon.

In the next acetoxylation reaction, another important compound, 6-fluoro-chroman-4-one **17** was reacted with 3 equivalents of $Mn(OAc)_3$ to give the desired acetoxy ketone **18** in 80% yield (Scheme 22).



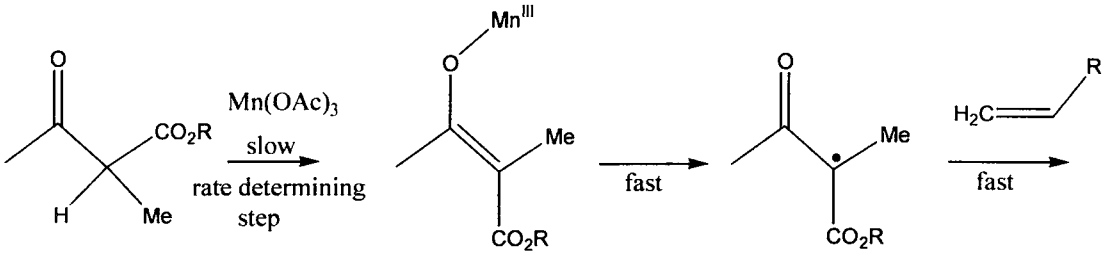
17 18

Scheme 22

The product was isolated as a red wine coloured crystals after flash column chromatography (EtOAc:Hex, 1:10) and identified by NMR spectroscopy. We observed a singlet at 2.15 ppm for CH_3 protons and dd at 5.56 ppm (J=5.5 and 11.4 Hz) for the α-proton. From the ^{13}C-NMR spectra of the product we observed a singlet at 20.8 ppm for the CH_3 carbon and at 169.3 ppm for the O<u>C</u>OCH_3 carbon.

2.2.1 Mechanism of the Oxidation with Manganese(III) acetate

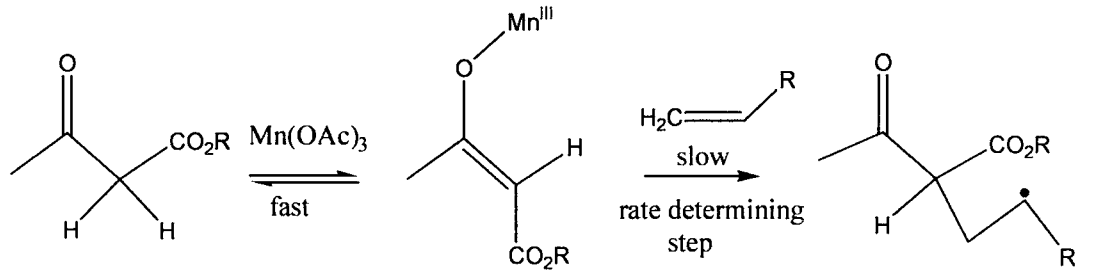
The mechanism of oxidation of monocarbonyl substrates with $Mn(OAc)_3.2H_2O$ has been extensively studied. Snider[35] has found a mechanism which is operative in the oxidation of α-alkyl-β-keto esters (Scheme 23).



19 **20**

Scheme 23

Enolization to give **19** is slow; electron transfer with loss of Mn(II) to give **20** is rapid. The rate of reaction is therefore independent of alkene concentration. This indicates that free radical **20** is involved in the Mn(III)-mediated oxidations. On the other hand, they found that the enolization of α-unsubstituted β-keto esters is fast and reversible, and electron transfer to give the radical is very slow (Scheme 24)

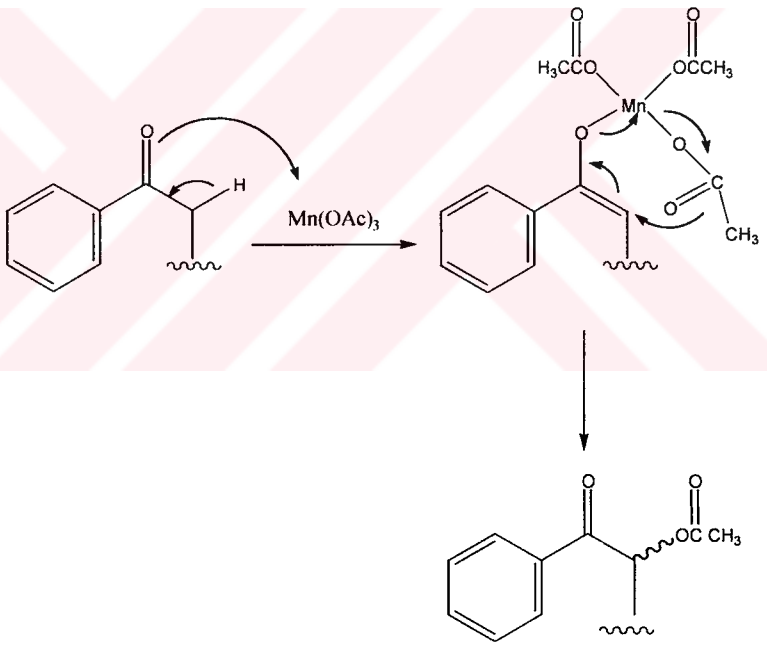


21 **22**

Scheme 24

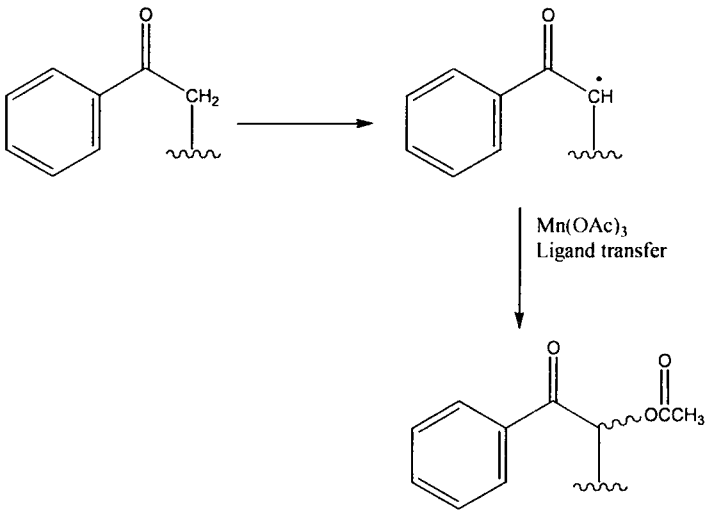
The rate determining step depends on alkene concentration and is presumably the reaction of the Mn(III) enolate **21** with the alkene to give radical **22** with loss of Mn(II). β-Keto ester radicals analogous to **20** do not appear to be intermediates in these reactions. They concluded that the nature of the reaction depend on two variables: the rate of formation of the Mn(III) enolate, which corresponds to the pK_a, and the ease of oxidation of the enolate to give the free radical.

The mechanism of manganese(III) acetate oxidations in benzene remain uncertain, but it seems reasonable based on related oxidations of lead(IV) acetate [36] The interaction of the enol or enolate of aromatic ketone with manganese(III) acetate would result in acetate transfer (Scheme25).



Scheme 25

Another suggested mechanism includes the formation of an α-keto radical resulting from the oxidation of an enol or enolate anion by Mn(III) (Scheme 26)

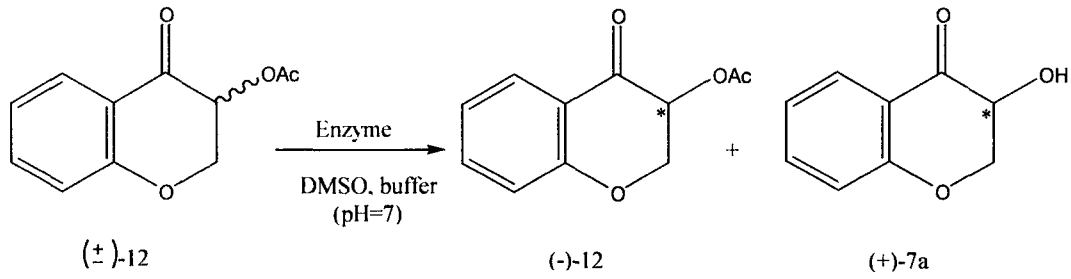


Scheme 26

2.3 Enzyme Mediated Hydrolysis of Acetoxy Ketones

Hydrolytic enzymes are the biocatalysts most commonly used in organic synthesis. Of particular interest among the classes of hydrolytic enzymes are; amidases, proteases, esterases and lipases. These enzymes catalyze the hydrolysis and formation of ester and amide bonds. Among them, lipases (triacylglycerolhydrolases, EC 3.1.1.3) are the most widely employed enzymes not only because they are cheap and readily available from many different sources but because they possess high enantioselectivity for a broad range of substrates and high stability in organic solvents. The enantioselectivity of lipase-catalyzed reactions in aqueous solutions, water-organic solvent mixtures, and in anhydrous organic solvents follows the classical homocompetitive equation. Unlike esterases, which show a normal Michaelis-Menten activity, lipases display little activity in aqueous solutions with soluble substrates.

The first compound used in the enzymatic hydrolysis was 4-oxo-3,4-dihydro-2-chromen-3-yl acetate **12** (Scheme 27). At first it was tried to find the best lipase from all possible enzyme sources for enantioselective hydrolysis of acetoxy chromanon, including Amano PS, PPL, CCL and PLE (Table 1).



Scheme 27

All reactions were carried out in phosphate buffer (pH=7) at room temperature. Because of the poor solubility of the substrate in aqueous medium, a few milliliter's of DMSO was used as an organic solvent. The mixture was stirred at room temperature in the presence of enzyme. The reaction was monitored by TLC and when approximately 50% conversion was attained, the crude product was separated by flash column chromatography to afford (-)-4-oxo-3,4-dihydro-2-chromen-3-yl acetate, (-)-**12,** and (+)-3-hydroxy-2,3-dihydro-4H-chromen-4-one, (+)-**7a**. The products were identified by using NMR spectroscopy. From the ^{1}H-NMR spectrum of (-)-**12,** we observed a singlet at 2.1 ppm from the $-CH_3$ group and dd at 5.6 ppm (J=11.3 and 5.5 Hz) for the α-proton. From the NMR spectrum of (+)-**7a,** we observed a broad singlet at 3.7 ppm from the –OH proton, and multiplet around 4.5 ppm for the α-proton.

Table 1 Enzyme mediated hydrolysis of 12 using several enzymes

Enzyme	Time (min)	e.e%(acetate)	Yield	e.e% (hydroxy)	Yield
Amano *PS*	35	69	38	67	25
PPL	240	57	39	55	17
CCL	30	29	40	51	13
PLE	35	25	34	32	29

From this table, it can be seen that best results were obtained using Amano PS and PPL. Careful monitoring of the reactions with TLC furnished the (-)-**12,** (25-69% ee) and (+)-**7a,** (32-67% ee). Enantiomeric excess values were determined with HPLC (Chiralcel OD column, eluent: hexane/2-propanal=9:1).

Once we found that Amano PS was the best for enantioselective hydrolysis of the substrate 4-oxo-3,4-dihydro-2-chromen-3-yl acetate, the effect of organic solvent in the reaction media for the hydrolytic reaction by Amano PS was studied (Table 2).

Table 2 Amano PS catalyzed hydrolysis of 12.

Solvent	Time (min)	e.e% (acetate)	Yield	e.e% (hydroxy)	Yield
DMSO	35	29	38	57	25
Ether	60	34	41	37	34
Toluene	60	97	36	95	24
Dioxane	75	17	30	49	15
THF	40	32	40	73	10
Acetonitrile	75	21	36	36	19
Benzene	35	67	43	60	38
Xylene	90	75	26	67	35

For the solvent screening purposes, the reactions were tried firstly on analytical scale with 5 mg starting material (12). After finding the suitable conditions, best working reactions were tried on preperative scale with 200 mg starting material.

Under these conditions a strong influence of the organic solvent upon the enantioselectivity of the process was observed. The best result, E=164 was obtained when Amano PS lipase was used in Toluene-phosphate buffer. The enantioselectivity in the hydrolysis of **12** was strongly affected by the nature of the solvent but was independent of the hydrophobicity (logP) or polarity (ε) parameters of the organic solvent in contrast to the well-established correlation. In all solvents tested, the enzyme recognized preferentially the (+) enantiomer of (±)-**12**.

Considering these results, same solvents were tried on PPL catalyzed reactions (Table 3). However, this time, results were not so nice as in Amano PS catalyzed reactions. Xylene was the only solvent which gave high selectivity with the hydroxy compound (+)-**7a.** The ee of the remaining unreacted ester (-)-**12** was

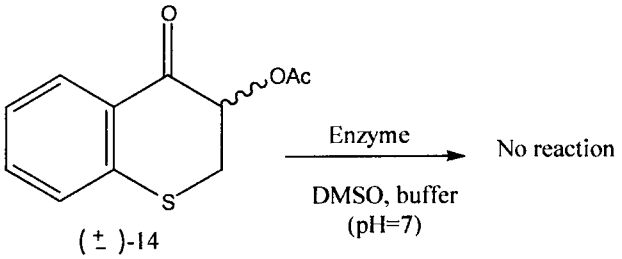
lower than the alcohol. In all solvents tested, PPL also recognized preferentially the (+) enantiomer of (±)-**12**. The best result, E>200, was obtained when PPL was used in Xylene-phosphate buffer (pH7).

Table 3 PPL catalyzed hydrolysis of 12

Solvent	Time (h)	e.e%(acetate)	Yield	e.e%(hydroxy)	Yield
DMSO	4	27	39	45	17
Ether	6	25	45	35	24
Toluene	24	65	32	75	26
Dioxane	24	7	35	40	30
THF	24	29	38	65	23
Xylene	24	94	35	97	30
Benzene	24	25	45	30	42
Acetonitrile	24	20	35	65	38

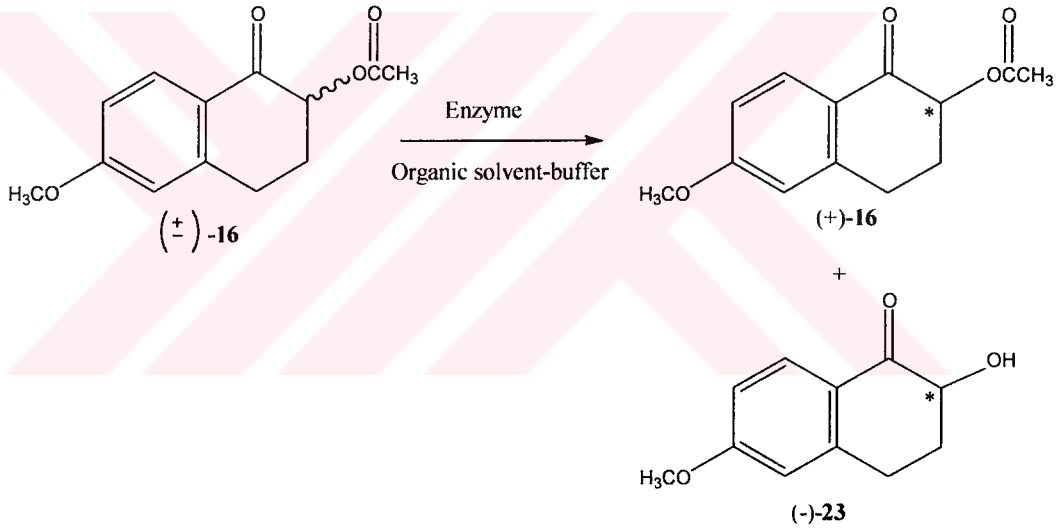
The reaction was monitored by TLC (1:3 EtOAc-Hexane solvent system) and stopped when ~50% of the starting material was converted to the product. The product was isolated by using flash column chromatography and identified by using NMR spectroscopy. From the ^{1}H-NMR spectrum of (+)-**7a** we observed a broad singlet at 3.7 ppm from the –OH proton. The α-proton appeared as a multiplet around 4.1 ppm.

Next, we performed the same reaction according to the optimized conditions with thiochroman-4-one **14** (Scheme 28). The reaction was tried with Amano PS, PPL, CCL and PLE enzymes in different organic solvents. However this time the reaction did not take place. The reason for this enzyme inactivity might be the inhibition of the enzyme through 4-oxo-3,4-dihydro-2-thiochromen-3-yl acetate.



Scheme 28

The same reaction was repeated with 2-acetoxy-6-methoxytetralone **(16)** with the Amano PS and PPL enzymes by using benzene, toluene and xylene as co-solvents (Scheme 29).



Scheme 29

The reaction was monitored by TLC (1:3 EtOAc-Hexane solvent system) and stopped when approximately 50% conversion was achieved. The product was isolated by using flash column chromatography (EtOAc/Hex, 1:4) and identified by using NMR spectroscopy. From the ^{1}H-NMR spectrum of the product, we observed the disappearance of the methyl singlet and α-proton gave dd at 4.2 ppm (J=13.3 and 5.3 Hz). Enantiomeric excess values were determined with HPLC (Chiralcel

OD column, eluent: hexane/2-propanal=9:1 for (+)-**16** and Chiralcel OJ column, eluent: hexane/2-propanal=9:1 for (-)-**23**). This time both enzymes in all solvents, recognized preferentially the (-) enantiomer of (±)-**16.** The results were summarized in Table 4 and 5.

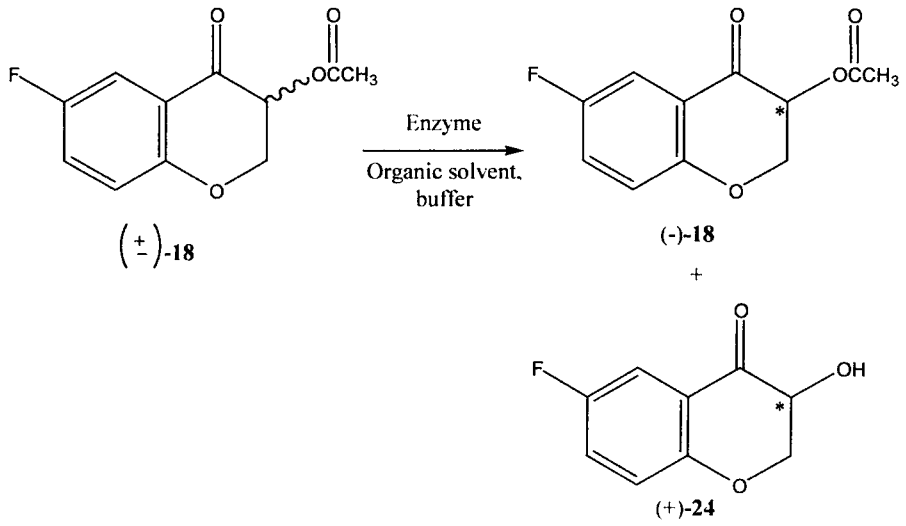
Table 4. Amano PS catalyzed hydrolysis reaction of (±)-**16**

Solvent	Time (h)	e.e% (acetate)	Yield% (acetate)	e.e% (hydroxy)	Yield% (hydroxy)
Benzene	21	55	34	85	47
Toluene	1	40	45	90	40
Xylene	1	30	29	70	30

Table 5. PPL catalyzed hydrolysis of (±)-**16**

Solvent	Time (h)	e.e% (acetate)	Yield% (acetate)	e.e% (hydroxy)	Yield% (hydroxy)
Benzene	28	35	34	65	28
Toluene	24	45	29	80	36
Xylene	21	50	43	75	48

For the next hydrolysis reaction, we tried with the 2-acetoxy-6-fluoro-chroman-4-one **18** with the Amano PS enzyme in benzene toluene and xylene as the co-solvents.



Scheme 30

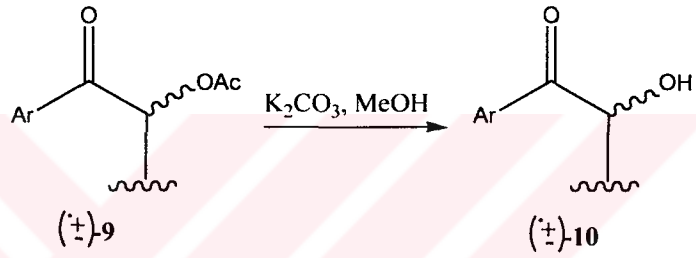
The reaction was monitored by TLC (1:3 EtOAc-Hexane solvent system) and stopped when approximately 50% conversion was achieved. The product was isolated by using flash column chromatography (EtOAc/Hex, 1:4) and identified by using NMR spectroscopy. From the ^{1}H-NMR spectrum of the product (+)-**24**, we observed a broad singlet at 3.42 ppm from the –OH proton and α-proton gave multiplet around 4.54 ppm. Enantiomeric excess values were determined with HPLC (Chiralcel OD column, eluent: hexane/2-propanal=9:1 for (+)-**18** and Chiralcel OJ column, eluent: hexane/2-propanal=99:1 for (-)-**24**). The enzyme in all solvents recognized preferentially the (+) enantiomer of the (±)-**18**. The results were summarized in Table 6.

Table 6. Amano PS catalyzed hydrolysis of (±)-**18.**

Solvent	Time (h)	e.e% (acetate)	Yield% (acetate)	e.e% (hydroxy)	Yield% (hydroxy)
Benzene	2	50	35	60	40
Toluene	3	55	45	53	35
Xylene	3	35	38	57	42

2.4 Enzyme Medited Acetylation of α-Hydroxy Enones

Availability of lipases in transesterification reactions as well as hydrolysis reactions brought us to the idea to make use of this principle in the reverse reaction. If deacetylation was successful to yield racemic α-hydroxy ketones, enantioselective transesterification catalyzed by lipases also could be tried to obtain both optically active acetoxy-ketone as a reaction product and hydroxy ketone as an unreacted substrate. For the synthesis of racemic α-hydroxy ketones, basic hydrolysis was done with K_2CO_3 in MeOH. (Scheme 31).



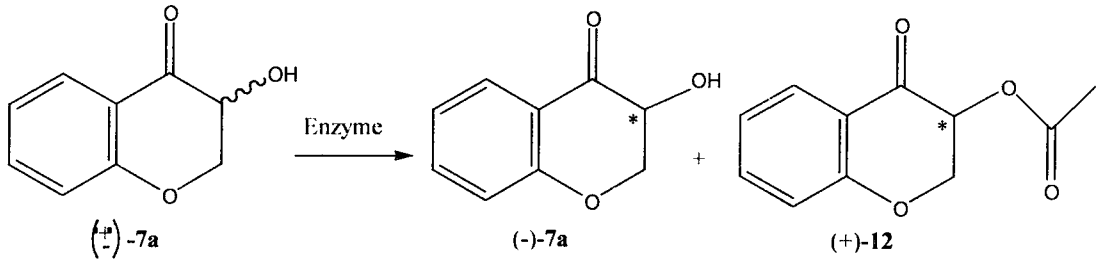
Scheme 31

Because the reaction conditions for an esterification are different from those of hydrolysis (absence of buffer, presence of acyl donor), it was wondered that under the new circumstances the enantioselectivity would be better or not.

In transesterification reactions, the enzyme mediates acyl transfer to the enantiomers at different rates. Acyl donors are needed which provide for a highly desirable irreversible reaction. From the literature it is known that for lipase catalyzed acylations one of the best irreversible acylating agent is vinyl acetate.[37]Therefore, this compound was used in the experiments as a standard acyl donor.

At first, transesterification of (±)-3-hydroxy-2,3-dihydro-4H-chromen-4-one (±)-**7a,** (synthesized from (±)-12 with K_2CO_3/MeOH) with different organic solvents was examined.

As shown in scheme 32 asymmetric transesterification of (±)-**7a** was carried out with Lipase Amano PS, PPL, PLE, and CCL using vinyl acetate in benzene, toluene, and xylene at a molar ratio of vinyl acetate to (±)-**7a** of 2:1 (Scheme 32).



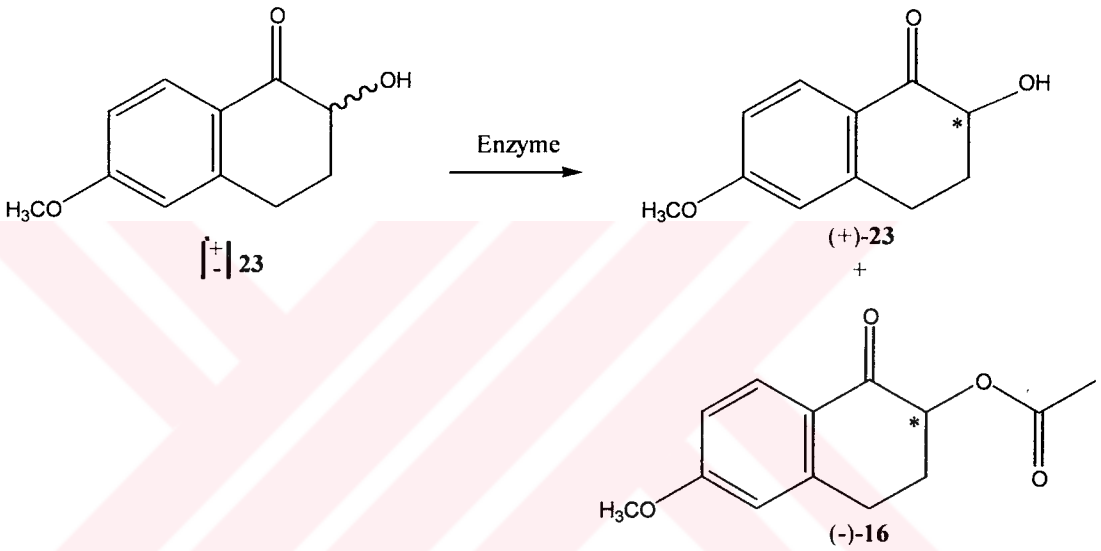
Scheme 32

When approximately 50% conversion was attained, the crude product was separated by flash column chromatography (EtOAc:Hex, 1:4) to give (+)-4-oxo-3,4-dihydro-2-chromen-3-yl acetate, (+)-**12,** and (-)-3-hydroxy-2,3-dihydro-4H-chromen-4-one, (-)-**7a.**

In the transesterification of (±)-**7a** only PPL displayed high enantioselectivity towards **7a**. In regard to the ee of the remaining substrate (-)-**7a** (88%) and that of the product (+)-**12** (94%) as well as the yield of the reaction, PPL was the only promised lipase employed in the transesterification of (±)-**7a** in toluene (c=48; E=94). Other enzymes showed very low to moderate enantioselectivities and yields in the transesterification of (±)-**7a**. The enzyme preferentially transformed the (+)-enantiomer. The results are showing that the enzymatic hydrolysis reaction of the acetate (±)-**12** works better than the transesterification experiments in regard to the ee and yields for the synthesis of (+)-**7a** and (-)-**12**. Compared to the enzymatic hydrolysis of acetate in aqueous-organic medium described above, the transesterification experiments in the non-

aqueous medium proceeded slowly. Enantiomeric excess values were determined with HPLC (Chiralcel OD column, eluent: hexane/2-propanal=9:1).

The lipase catalyzed transesterification was then applied to 2-hydroxy-6-methoxy-tetralone (synthesized from (±)-**16** with K_2CO_3/MeOH) using Amano PS and PPL in benzene, toluene, and xylene at a molar ratio of vinyl acetate to (±)-23 of 2:1 (Scheme 33).



Scheme 33

When approximately 50% conversion was attained, the crude product was separated by flash column chromatography (EtOAc:Hex, 1:4) to give (-)-2-acetoxy-6-methoxy tetralone, (-)-**16,** and (+)-2-hydroxy-6-methoxy tetralone, (+)-**23.**

This time the best result was obtained with the Amano PS enzyme in toluene to yield 2-hydroxy-6-methoxy-tetralone (+)-**23** with 85% ee at 48% conversion and 2-acetoxy-6-methoxy-tetralone (-)-**16** with 90% ee at 45% conversion after 1 hr of the reaction time. This time the results are showing that the enzymatic transesterification reaction of the (±)-**23** works better than the hydrolysis

experiments in regard to the ee of (-)-**16.** Enantiomeric excess values were determined with HPLC (Chiralcel OD column, eluent: hexane/2-propanal=9:1 for (-)-**16** and Chiralcel OJ column, eluent: hexane/2-propanal=9:1 for (+)-**23**).

As a future work, we are going to try the transesterification experiments with the 2-hydroxy-6-fluoro chroman-4-one.

2.5 Summary of Enzyme Catalyzed Synthesis of α-Hydroxy and α-Acetoxy Chromanone Systems

In summary, it was described here, the first efficient synthesis of both enantiomers of 3-hydroxy-2,3-dihydro-4H-chromen-4-one, and 4-oxo-3,4-dihydro-2-chromen-3-yl acetate via enzymatic kinetic resolution. The enzymatic hydrolysis of the acetate, (±)-**12**, in aqueous-organic medium and the transesterification experiments with (±)-**7a** in nonaqueous solvents by using vinyl acetate furnished **12** and **7** in high ee. By the enzymatic kinetic resolution of (±)-4-oxo-3,4-dihydro-2-chromen-3-yl acetate, (±)-**12,** high enantioselectivities can be achieved by an appropriate choice of the solvents. The best results in the enzymatic hydrolysis of the (±)-**12** were obtained with the AmanoPS enzyme in toluene (E:164, 97% for (-)-12 and 95% for (+)-7a) and with PPL enzyme in xylene (E>200, 94% for (-)-12 and 97% for (+)-7a). Moreower, the best result in the enzymatic transesterification of the (±)-**7a** was obtained with the PPL enzyme in toluene (E:94, 88% for (+)-12 and 94% for (+)-12). Hydrolysis of 4-oxo-3,4-dihydro-2-thiochromen-3-yl acetate did not work under these conditions.

According to these results, same hydrolysis and transesterification experiments were applied to the derivatives of chromanone to obtain high e.e's of corresponding acetoxy and hydroxy-ketones.

Again, both enantiomers of 2-acetoxy-6-methoxy tetralone (±)-**16,** and 2-hydroxy-6-methoxy tetralone (±)-**23** were obtained in high ee by appropriate choice of the organic solvents. However the results were not so good this time as in the

chroman-4-one case. The best result was obtained with the Amano PS enzyme in toluene (40% for (+)-16 and 90% for (-)-23) for the hydrolysis reaction and again with Amono PS enzyme in toluene (85% for (-)-16 and 90% for (+)-23) for the transesterification reaction.

The other compound that was tried for the enzymatic hydrolysis was 2-acetoxy-6-fluoro chroman-4-one, (±)-**18** (synthesized from 6-fluoro chroman-4-one with $Mn(OAc)_3$ in good yield). The hydrolysis reactions afforded (-)-2-acetoxy-6-fluoro chroman-4-one, (-)-**18**, (35-50% ee) and (+)-2-hydroxy-6-fluoro chroman-4-one, (+)-**24**, (53-60% ee). The transesterification reaction of the (±)-**24** will also be tried.

According to results up to now, we can safely conclude that the method we introduced for the chemoenzymatic synthesis of important building blocks of the drugs is indeed a very valuable one for the chiral synthesis of benzopyran type building blocks.

CHAPTER 3

EXPERIMENTAL

3.1 Materials and Methods

In this study all compounds were identified by using Nuclear Magnetic Resonance Spectra (NMR) (Bruker DPX 400 MHz) by using tetramethylsilane (TMS) as an internal standard and deutereo chloroform as solvent.

Flash column chromatography was done for purifying the products by using silica gel 60 (mesh size 40-63 $^{\mu}$m).

Optical rotations were measured with a Bellingham-Stanley P20 polarimeter or Autopol IV automatic polarimeter. Enantiomeric excesses were determined by HPLC analysis using a Thermo Quest (TSP) GC-LC-MS equipped with an appropriate optically active column.

3.2 General Procedures

3.2.1 General Procedure For $Mn(OAc)_3$ Oxidation

A solution of **7a** or **7b, 15** and **17** (22.3 mmol), $Mn(OAc)_3$ (17.2 g, 66.9 mmol) and cyclohexane or benzene (200 mL) were heated under reflux for 45-54 h.

The reaction was monitored by TLC. After cooling, the reaction mixture was filtered then washed with saturated $NaHCO_3$ solution. The solution was then dried over $MgSO_4$, concentrated and purified by flash column chromatography to yield desired racemic acetoxy ketones.

3.2.1.1 Synthesis of 4-oxo-3,4-dihydro-2-chromen-3-yl acetate, (±)-12

The product was isolated as a colorless solide after flash column chromatography (1:10 EtOAc:Hexane) with 75% yield.

^{1}H-NMR ($CDCl_3$+CCl_4):

δ (ppm): 2.1 (s, 3H)
4.3 (dd, J=11.2 and 11.3 Hz)
4.5 (dd, J=11.2 and 5.5 Hz)
5.6 (dd, J=11.3 and 5.5 Hz)
6.8 (d, J=8.4 Hz)
6.9 (dd, J=7.5 and 7.4 Hz)
7.4 (dd, J=8.4 and 7.1 Hz)
7.8 (d, 7.8 Hz)

^{13}C-NMR ($CDCl_3$+CCl_4)

δ (ppm): 187.8, 169.3, 161.6, 136.6, 128.0, 122.3, 120.3, 118.1, 69.7, 68.6, 20.8.

3.2.1.2. Synthesis of 4-oxo-3,4-dihydro-2-thiochromen-3-yl acetate, (±)-7a

The product was isolated as an orange colored solid; (mp 79-80 °C) after flash column chromatography (1:10 EtOAc:Hexane) with 70% yield.

^{1}H-NMR ($CDCl_3$+CCl_4):

δ (ppm): 2.2 (s, 3H)

3.1 (dd, J=12.6 and 4.5 Hz)

3.5 (dd, J=13.2 and 12.9 Hz)

5.7 (dd, J=13.5 and 4.5 Hz)

7.2 (m, 2H)

7.4 (dd, J=7.9 and 7.3 Hz)

8.0 (d, J=7.9)

^{13}C-NMR ($CDCl_3$+CCl_4)

δ (ppm): 188.6, 169.0, 140.5, 133.5, 130.4, 129.9, 126.9, 125.1, 73.1, 30.1, 20.5.

3.2.1.3. Synthesis of 2-acetoxy-6-methoxy tetralone

The product was isolated as an orange coloured crystals after flash column chromatography (1:4 EtOAc:Hex) with 85% yield.

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

δ (ppm): 2.10 (s, 3H)

2.20-2.40(m, 2H,CH_2)

2.90-3.20 (m, 2H, CH_2)

3.79 (s, 3H)

5.40 (dd, J=5.1 and 13.1Hz)

6.60 (d, J=1.7 Hz)

6.76 (dd, J=2.2 and 8.75 Hz)

7.92 (d, J=8.75 Hz)

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

δ (ppm): 191.9, 170.4, 164.3, 146.0, 130.4, 125.3, 113.9, 112.8, 74.6, 55.8, 29.4, 28.5, 21.2

3.2.1.4 Synthesis of 2-acetoxy-6-fluoro-chroman-4-one

After the purification by column chromatography (1:10 EtOAc:Hex), the product was isolated as red wine coloured crystals with 80% yield.

^{1}H-NMR ($CDCl_3$+CCl_4):

δ (ppm): 2.15 (s, 3H)
4.32 (dd, J=11.3 Hz)
4.48 (dd, J=5.5 and 11.1 Hz)
5.56 (dd, J=5.5 and 11.4 Hz)
6.91 (dd, J=4.1 and 9.1 Hz)
7.20 (m, 1H)
7.47 (dd, J=3.1 and 7.9 Hz)

^{13}C-NMR ($CDCl_3$+CCl_4)

δ (ppm): 187.1, 169.3, 158.0 (d, $^{1}J_{CF}$=241.9 Hz), 157.8, 124.2 (d, $^{2}J_{CF}$=24.6 Hz), 120.8 (d, $^{3}J_{CF}$=6.6 Hz), 119.8 (d, $^{3}J_{CF}$=7.2 Hz), 113.0 (d,$^{2}J_{CF}$=23.4 Hz), 69.6, 68.9, 20.8

3.2.2 General Procedure for the Synthesis of Racemic α-Hydroxy Ketones

A solution of **7a** or **7b, 15** and **17** (1.73 mmol), anhydrous K_2CO_3 (1.73 mmol), and methanol (20 mL) were stirred at room temperature for 24 hrs. Then the mixture is diluted with EtOAc, washed with 1N HCl and brine solution and

dried over $MgSO_4$. The crude product was separated by flash column chromatography to afford corresponding racemic α-Hydroxy ketones.

3.2.2.1. Synthesis of (±)-3-hydroxy-2,3-dihydro-4H-chromen-one

The product was isolated as a yellow solid after flash column chromatography (1:4 EtOAc:Hex) in 68%yield.

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

δ (ppm): 3.7 (s, 3H)
4.1 (m, 1H)
4.5 (m, 2H)
6.8 (d, J=8.3 Hz)
6.9 (dd, J=7.2 and 7.3 Hz)
7.4 (dd, J=7.2 and 7.1 Hz)
7.8 (d, J=7.2 Hz)

^{13}C-NMR ($CDCl_3$+CCl_4)

δ (ppm): 194.5, 162.5, 136.8,127.7, 122.1, 119.2, 118.2, 70.9, 69.4.

3.2.2.2. Synthesis of (±) 2-hydroxy-6-methoxy-tetralone

The crude product was separated by flash column chromatography (1:4 EtOAc:Hex) in 72% yield.

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

δ (ppm): 1.92 (ddd, J=4.6 Hz)
2.42 (m, 1H)
2.97 (m, 2H)

3.79 (s, 3H)

4.20 (dd, J=13.3 and 5.3 Hz)

6.60 (s, 1H)

6.76 (dd, J=8.7 and 2.2 Hz)

7.91 (d, J=8.7 Hz)

^{13}C-NMR ($CDCl_3$+CCl_4)

δ (ppm): 198.5, 164.6, 147.3, 130.4, 124.2, 114.0, 113.1, 73.9, 55.9, 32.2, 28.5

3.2.2.3 Synthesis of (±)-2-hydroxy-6-fluoro-chroman-4-one

The crude product was separated by flash column chromatography (1:4 EtOAc:Hex) in 50% yield.

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

δ (ppm): 3.42 (s, 1H)

4.03 (dd, J=10.2 and 12.8 Hz)

4.54 (m, 2H)

6.89 (dd, J=4.1 and 9.1 Hz)

7.16 (m, 1H)

7.45 (dd, J=3.1 and 7.8 Hz)

^{13}C-NMR ($CDCl_3$+CCl_4)

δ (ppm): 193.7, 157.8 (d, $^{1}J_{CF}$=242.4 Hz), 158.8, 124.4 (d, $^{2}J_{CF}$=24.2 Hz), 119.8 (d, $^{3}J_{CF}$=7.1 Hz), 119.6 (d, $^{3}J_{CF}$=6.6 Hz), 112.6 (d, $^{2}J_{CF}$=23.2 Hz), 71.1, 69.5

3.2.3 General Procedure for the Lipase-Catalyzed Hydrolysis of Racemic α-Acetoxy Ketones

Lipase (200-300 mg) was dissolved in potassium phosphate buffer (20mM, pH7, 50 ml) and added to a solution of the pure substrate (200 mg) in organic solvent (10ml) and the reaction mixture was stirred at RT. The reaction was monitored by TLC and when maximum conversion was reached, the reaction was terminated by filtration. The unreacted acetate and the product were separated by flash column chromatography.

3.2.3.1 Synthesis of (+)-3-hydroxy-2,3-dihydro-4H-chromen-one, (+)-7a

The unreacted acetate (-)-**12** and the product (+)-**7a** were separated by flash column chromatography (1:5 EtOAc:Hex). The ee's of the ester and the alcohol were determined by chiral HPLC.

HPLC: Chiralcel OD column, UV detection at 254 nm, eluent: hexane/2-propanol=9:1, flow 0.8 ml min^{-1}; R_f: for (-)-**12**: 12.1 min; $[\alpha]_D^{20}$ = -63 (c 0.5, $CHCl_3$); (+)-**12**: 13.2 min; $[\alpha]_D^{20}$ =61 (c 0.4, $CHCl_3$); R_f: for (+)-**7a**: 21.4 min; $[\alpha]_D^{20}$ = 54 (c 2, $CHCl_3$); (-)-**7a**: 23.4 min; m $[\alpha]_D^{20}$ =-57 (c 2,$CHCl_3$).

3.2.3.2 Synthesis of (-)-2-hydroxy-6-methoxy tetralone, (-)-23

The unreacted acetate (+)-**16** and the product (-)-**23** were separated by flash column chromatography (1:4 EtOAc:Hex). The ee's of the ester and the alcohol were determined by chiral HPLC.

HPLC: Chiralcel OD column for the acetoxy, UV detection at 254 nm, eluent: hexane/2- propanol=9:1, flow 0.8 ml min^{-1}; R_f: for (-)-**16**: 21.5 min; (+)-**16**: 24.5 min. Chiralcel OJ column for the hydroxy, UV detection at 254 nm, eluent: hexane/2- propanol=9:1, flow 0.8 ml min^{-1}; R_f: for (+)-**23**: 27.2 min; (-)-**23**: 30.0 min.

3.2.3.3 Synthesis of (+)-2-Hydroxy-6-fluoro-chroman-4-one, (+)-24

The unreacted acetate (-)-**18** and the product (+)-**24** were separated by flash column chromatography (1:4 EtOAc:Hex). The ee's of the ester and the alcohol were determined by chiral HPLC.

HPLC: Chiralcel OD column for the acetoxy, UV detection at 254 nm, eluent: hexane/2- propanol=9:1, flow 0.8 ml min^{-1}; R_f: for (-)-**18**: 11.6 min; (+)-**18**: 13.4 min. Chiralcel OJ column for the hydroxy, UV detection at 254 nm, eluent: hexane/2- propanol=99:1, flow 0.8 ml min^{-1}; R_f: for (+)-**24** : 52.4 min; (-)-**24**: 57.4 min.

3.2.4 General Procedure for the Lipase-Catalyzed Asymmetric Transesterification of Racemic α-Hydroxy Ketones

Racemic alcohol (328 mg) and vinyl acetate were dissolved in organic solvent (8ml) and lipase (200-300 mg) was added. The reaction mixture was stirred at RT. The reaction was monitored by TLC and when 50% conversion was reached, the reaction was terminated by filtration. Substrate and product were separated by flash column chromatography.

3.2.4.1 Transesterification of (±)-3-hydroxy-2,3-dihydro-4H-chromen-one, (+)7a

Substrate (-)-**7a** and product (+)-**12** were separated by flash column chromatography (1:4 EtOAc: Hex). The ee's of the ester and the alcohol were determined by chiral HPLC.

HPLC: Chiralcel OD column, UV detection at 254 nm, eluent: hexane/2-propanol=9:1, flow 0.8 ml min^{-1}; R_f: for (-)-**12**: 12.1 min; (+)-**12**: 13.2 min; R_f: for (+)-**7a**: 21.4 min; (-)-**7a**: 23.4 min.

3.2.4.2 Transesterification of (±)-2-hydroxy-6-methoxy tetralone, (±)-23

Substrate (+)-**23** and product (-)-**16** were separated by flash column chromatography (1:4 EtOAc: Hex). The ee's of the ester and the alcohol were determined by chiral HPLC.

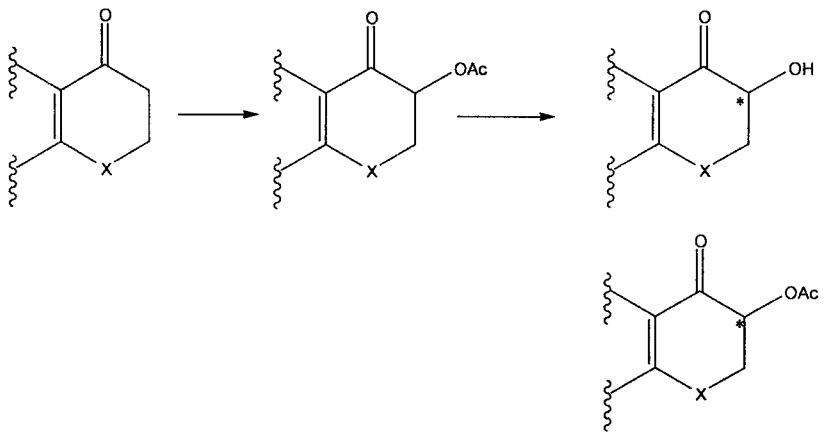
HPLC: Chiralcel OD column for the acetoxy, UV detection at 254 nm, eluent: hexane/2- propanol=9:1, flow 0.8 ml min^{-1}; R_f: for (-)-**16**: 21.5 min; (+)-**16**: 24.5 min. Chiralcel OJ column for the hydroxy, UV detection at 254 nm, eluent: hexane/2- propanol=9:1, flow 0.8 ml min^{-1}; R_f: for (+)-**23**: 27.2 min; (-)-**23**: 30.0 min.

CHAPTER 4

CONCLUSION

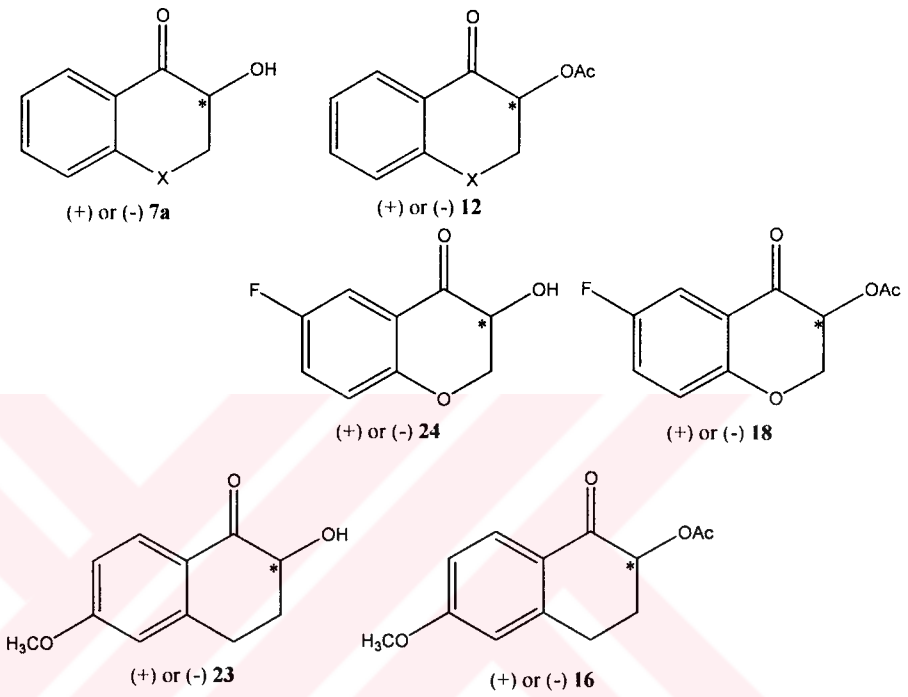
Chiral α-hydroxy ketones are very important intermediate molecules for the synthesis of biologically active compounds such as auerolic acid antibiotics, blowing agents, anti-bacterial cephalosporins, anti-depressive, anti-viral and cardiag drugs, steroids, potassium channel openers, such as cromakalim and its analogues and HIV protease inhibitors.

A new route is developed for the synthesis of optically active α-hydroxy ketones. The ketones are converted into their acetoxy derivatives using $Mn(OAc)_3$ in good yield. The acetoxy ketones are then converted to chiral α-hydroxy ketones by using different lipases as biocatalysts (Scheme 34).



Scheme 34

Simple, mild, efficient and highly enantioselective synthesis of α-hydroxy ketones which are important starting materials for different biologically active compounds, is realized and following compounds are obtained in good yield and high ee.



Scheme 35

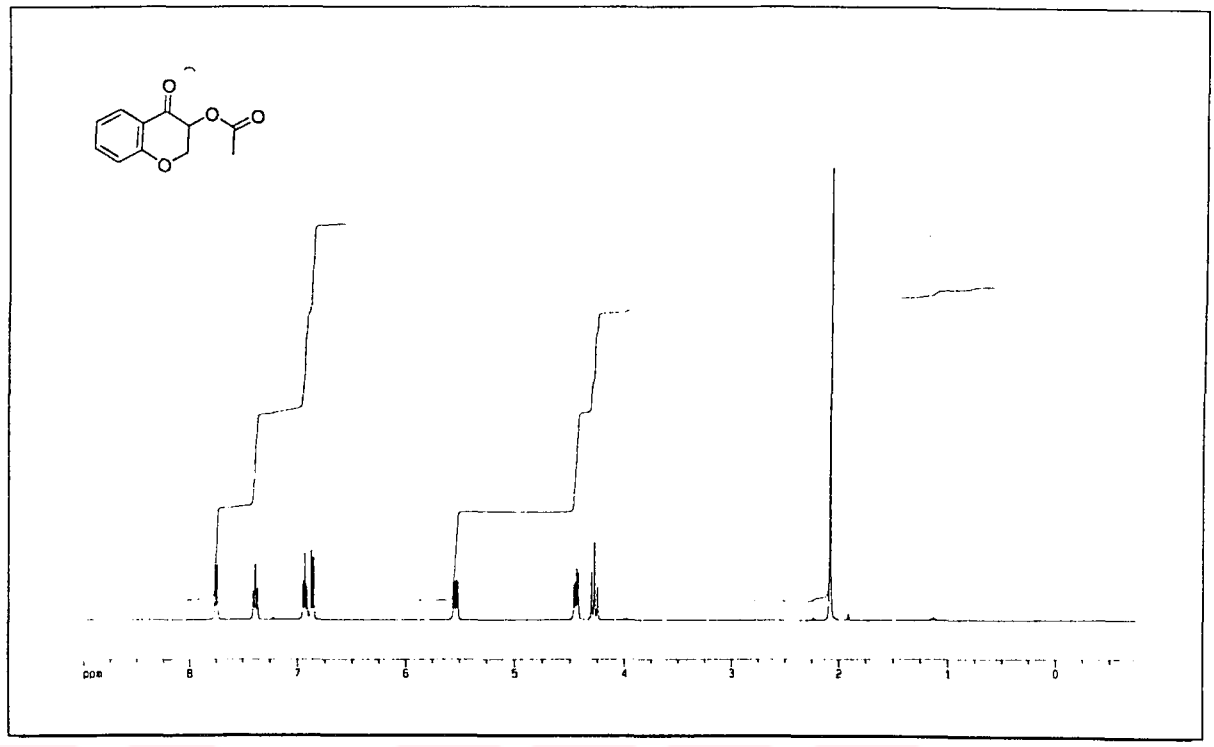


Figure 8: ^{1}H-NMR spectrum of 4-oxo-3,4-dihydro-2-chromen-3-yl acetate

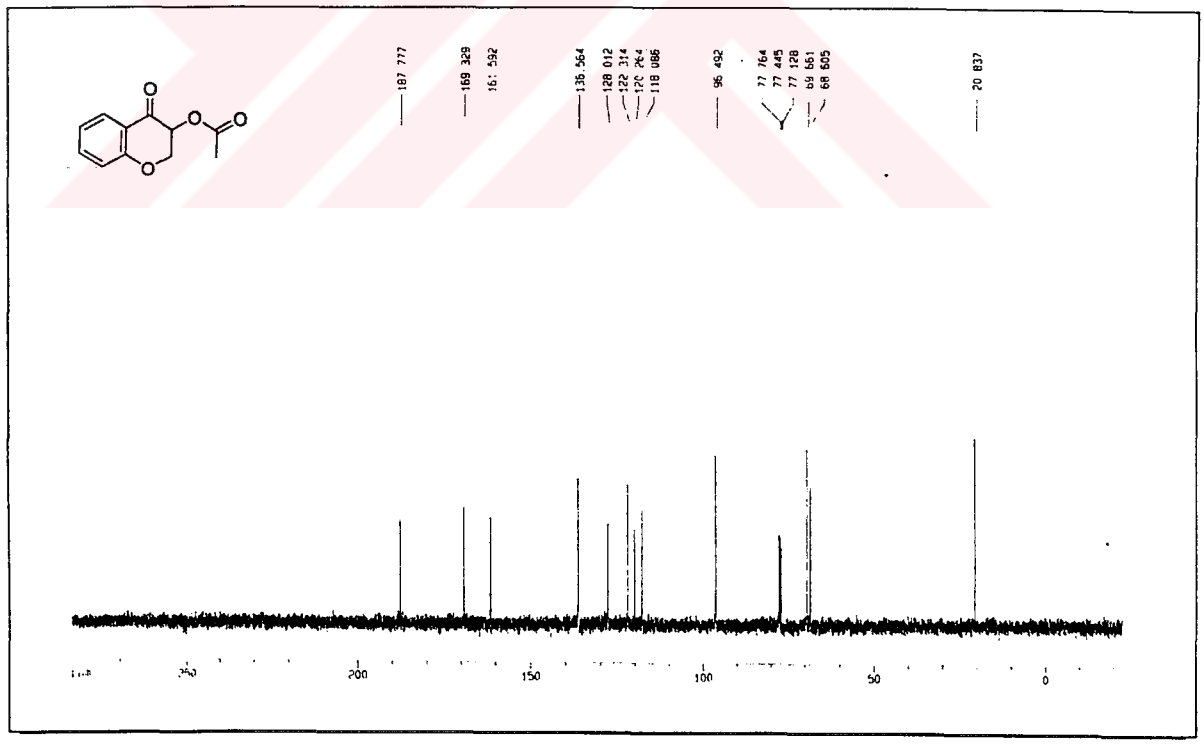


Figure 9: ^{13}C-NMR spectrum of 4-oxo-3,4-dihydro-2-chromen-3-yl acetate

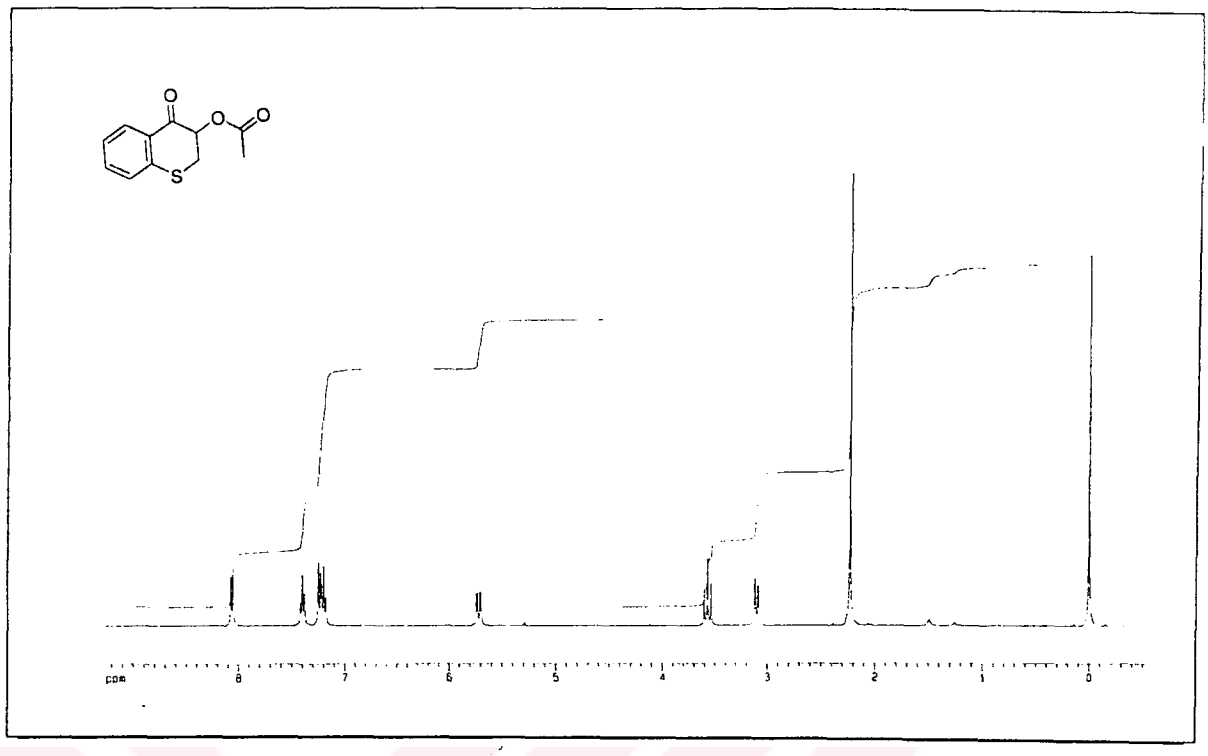


Figure 10: ^{1}H-NMR spectrum of 4-oxo-3,4-dihydro-2-thiochromen-3-yl acetate

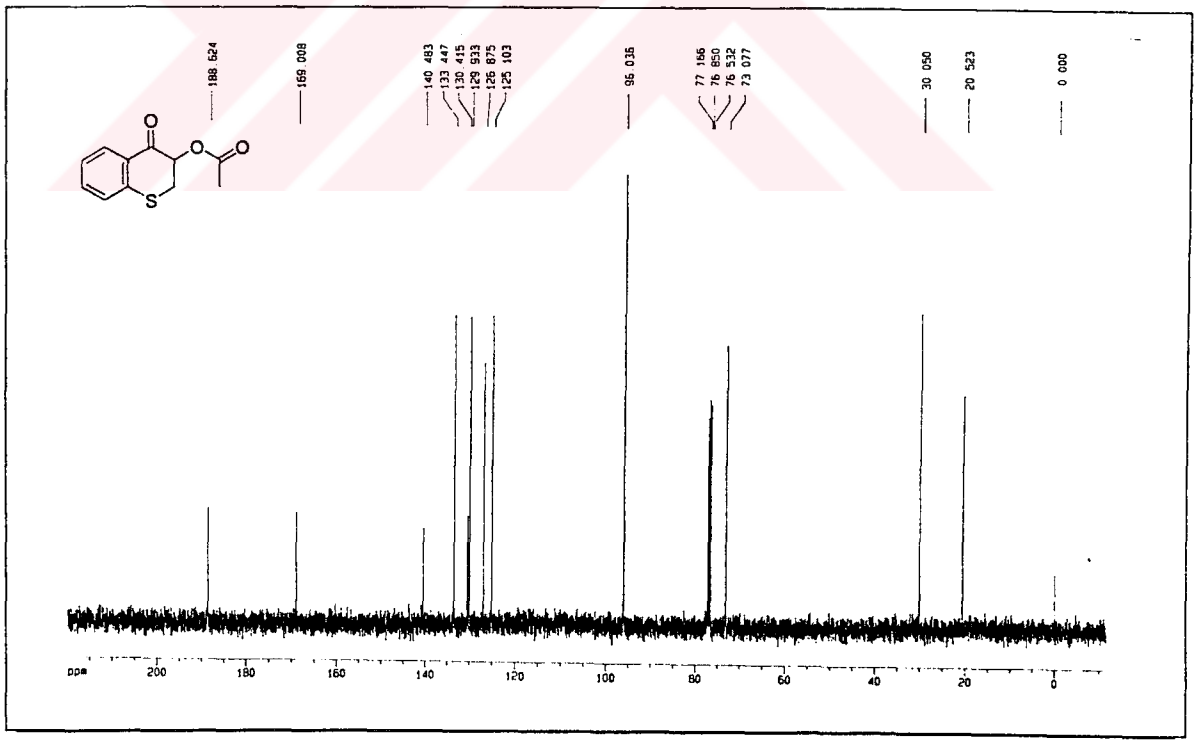


Figure 11: ^{3}C-NMR spectrum of 4-oxo-3,4-dihydro-2-thiochromen-3-yl acetate

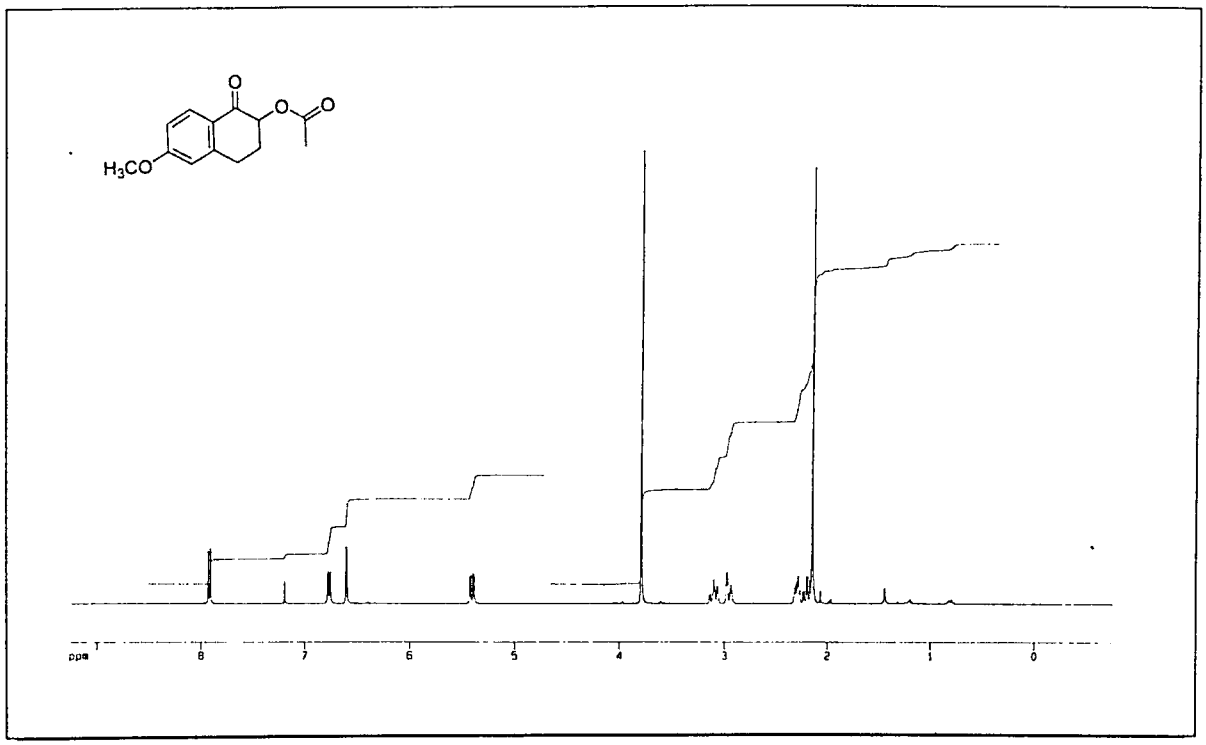


Figure 12: ^{1}H-NMR spectrum of 2-acetoxy-6-methoxy tetralone

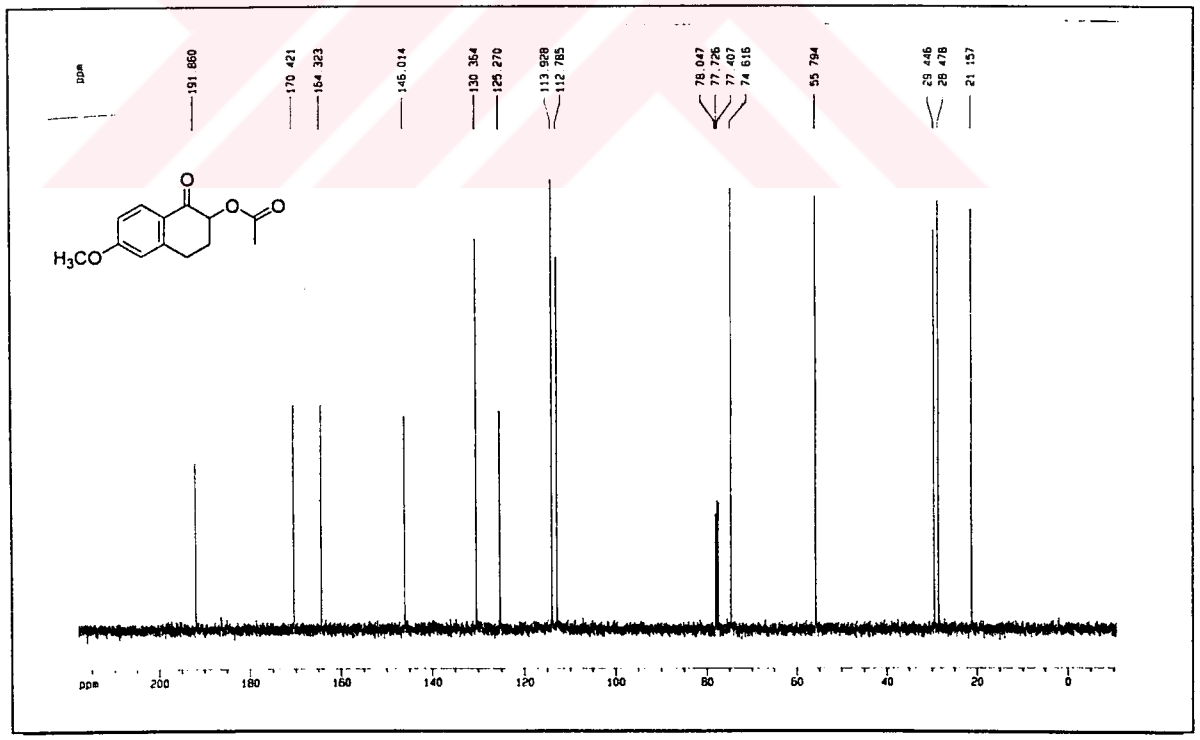


Figure 13: ^{13}C-NMR spectrum of 2-acetoxy-6-methoxy tetralone

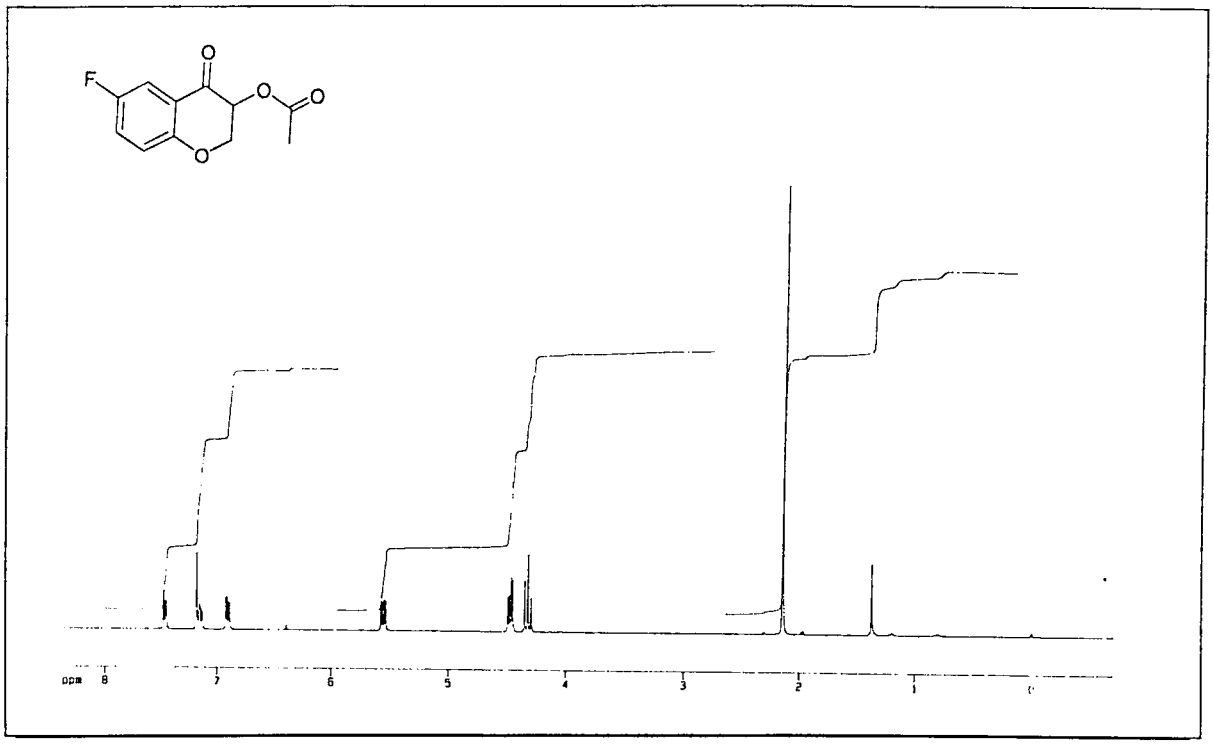


Figure 14: ^{1}H-NMR spectrum of 2-acetoxy-6-fluoro-chroman-4-one

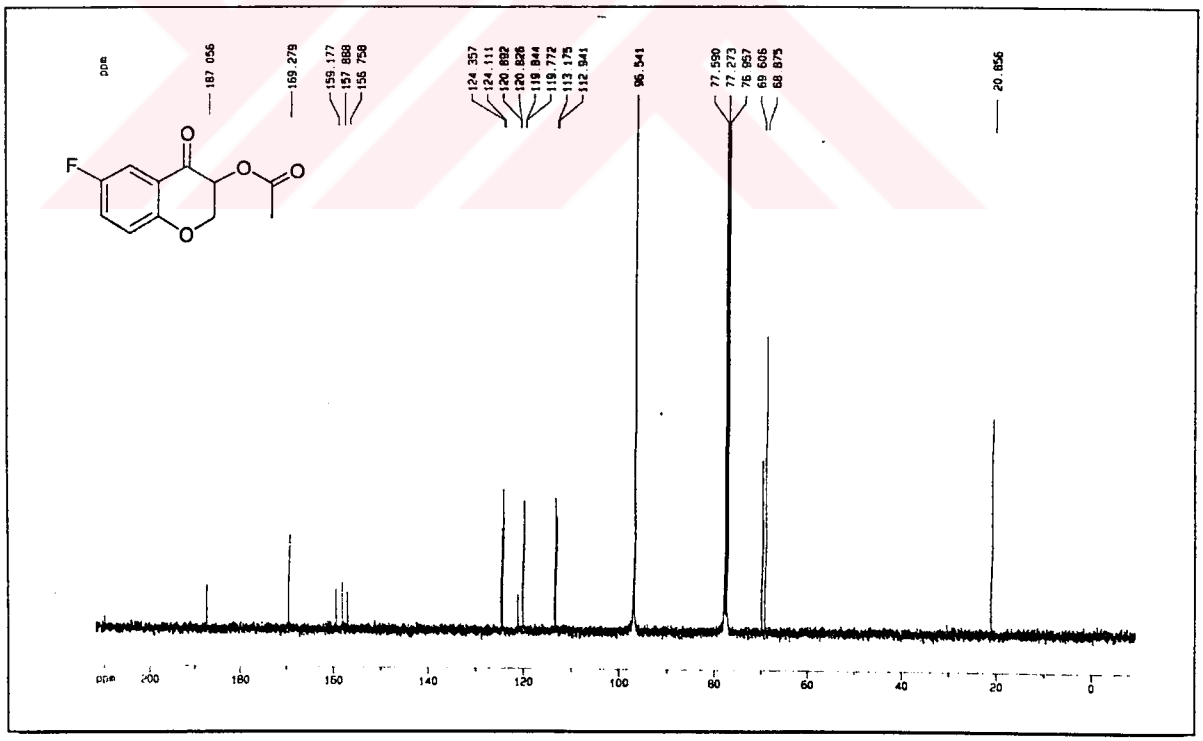


Figure 15: ^{13}C-NMR spectrum of 2-acetoxy-6-fluoro-chroman-4-one

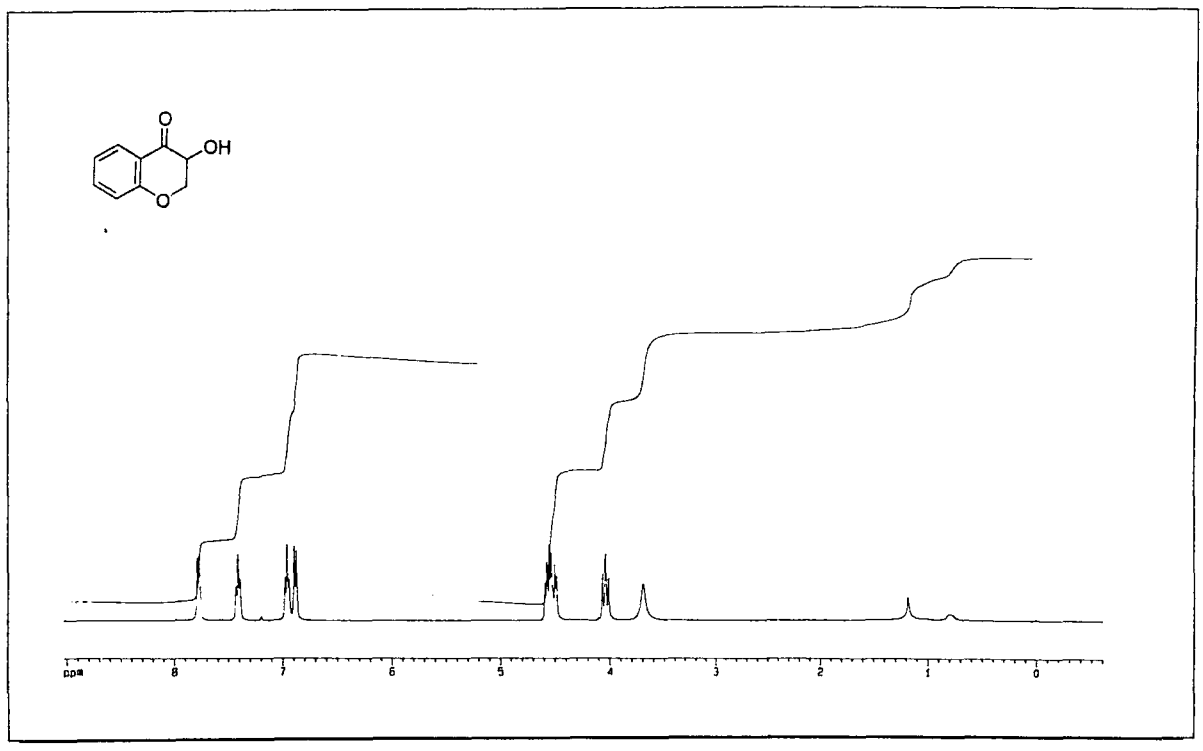


Figure 16: ^{1}H-NMR spectrum of 3-hydroxy-2,3-dihydro-4H-chromen-one

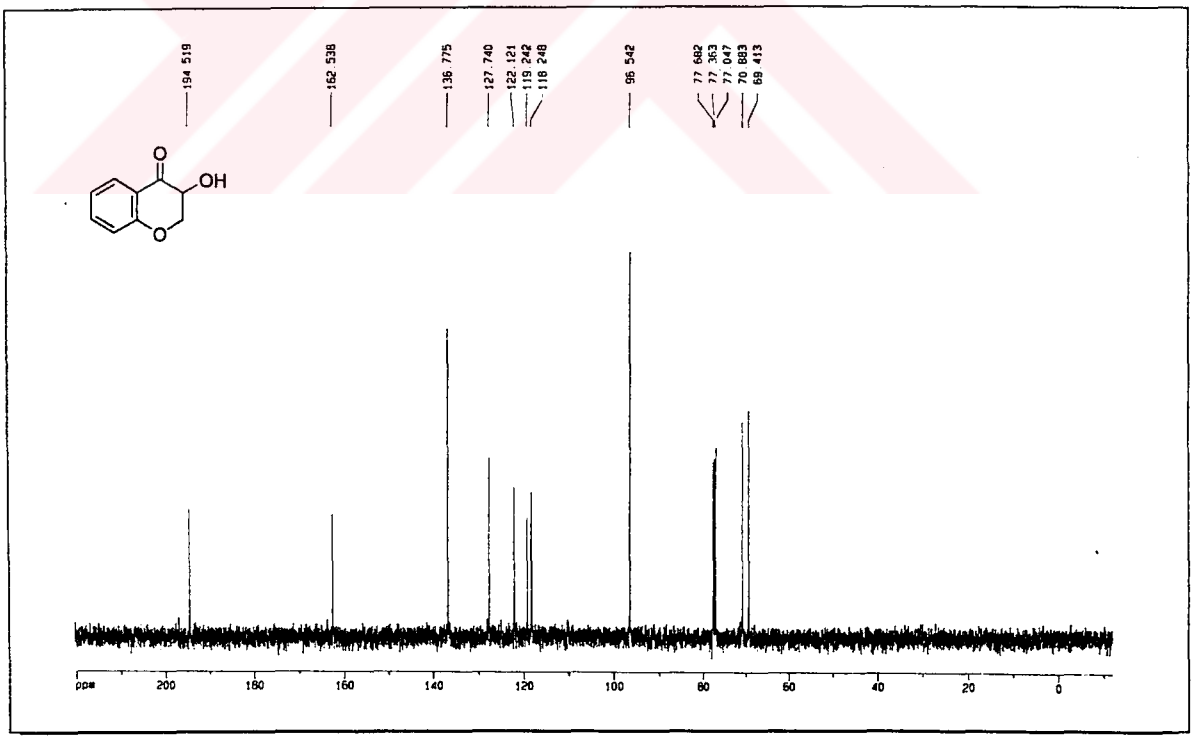


Figure 17: ^{13}C-NMR spectrum of 3-hydroxy-2,3-dihydro-4H-chromen-one

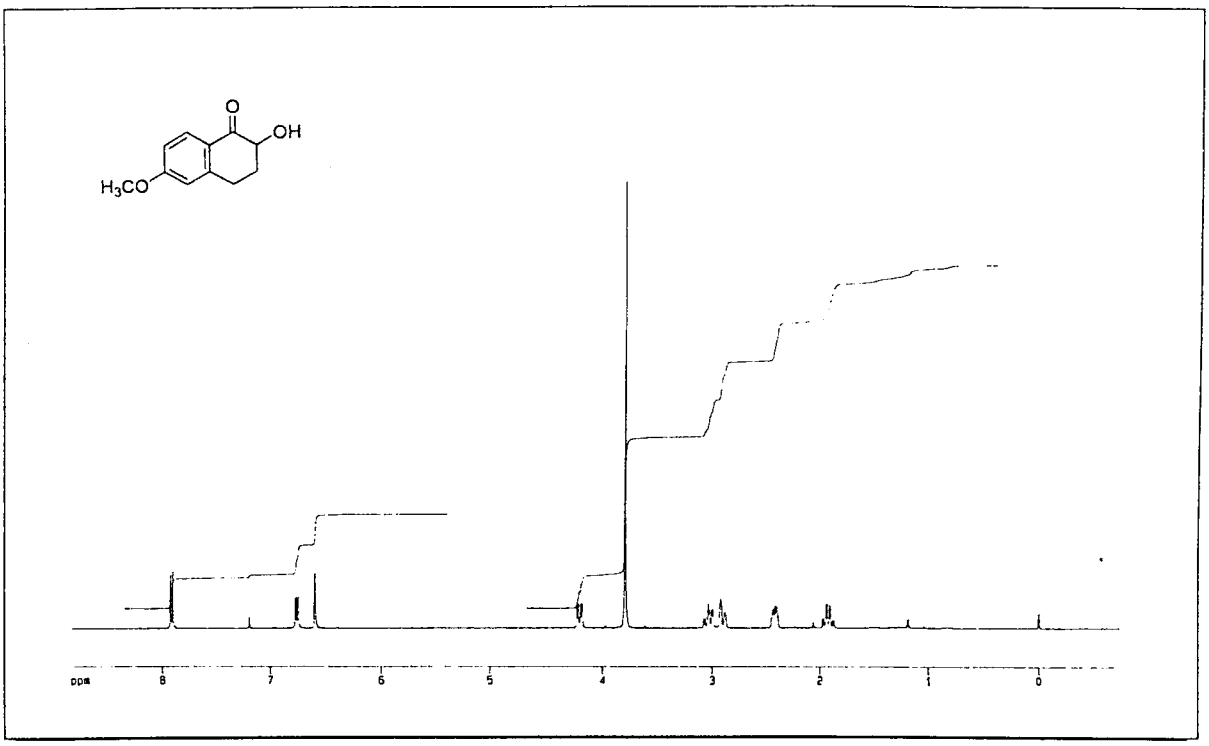


Figure 18: ^{1}H-NMR spectrum of 2-hydroxy-6-methoxy tetralone

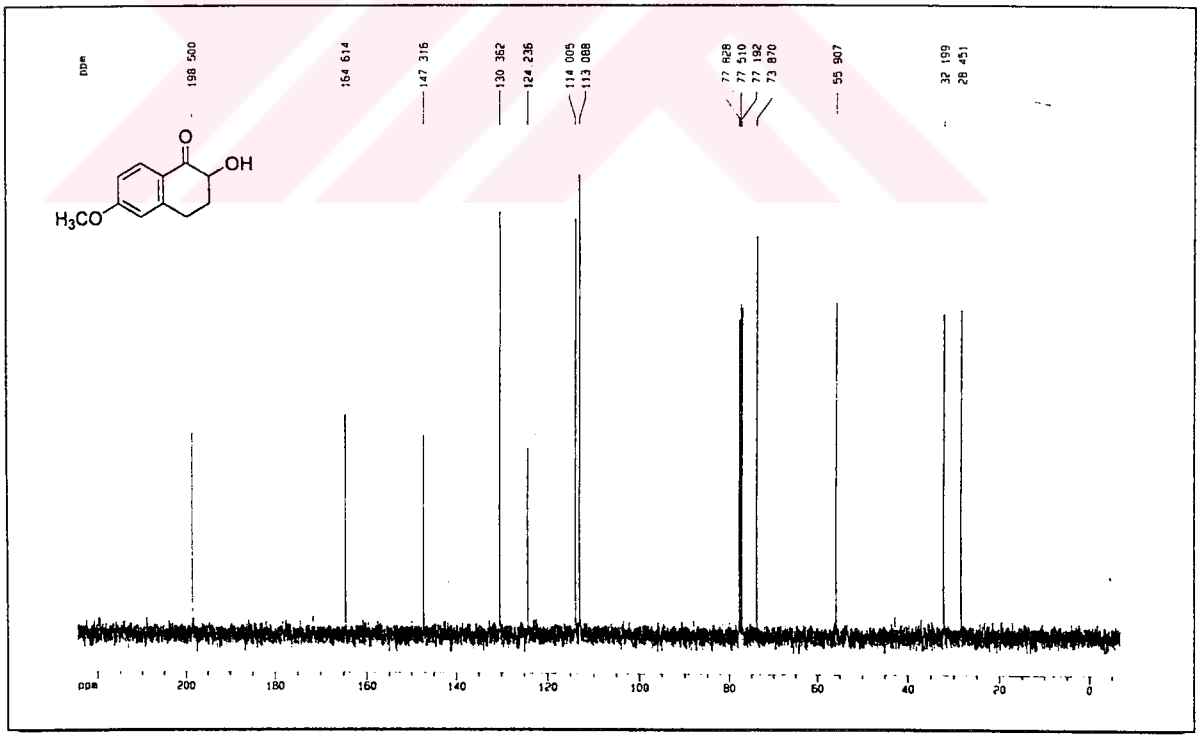


Figure 19: ^{13}C-NMR spectrum of 2-hydroxy-6-methoxy tetralone

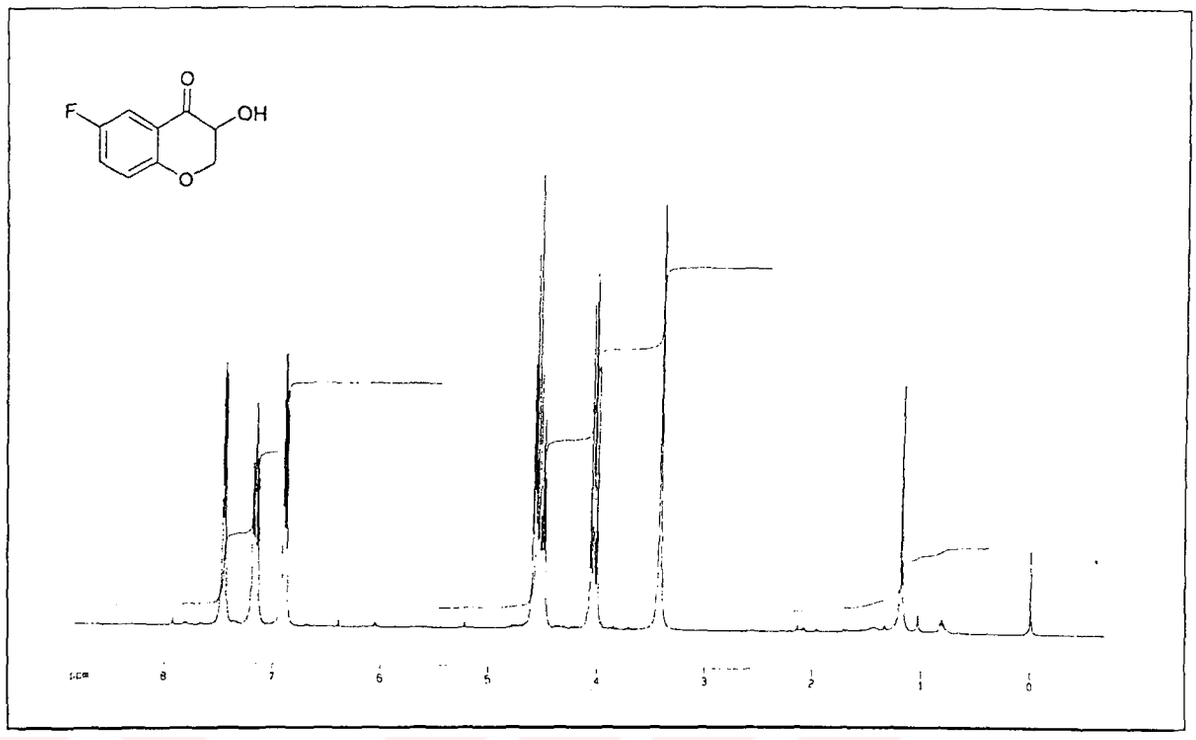


Figure 20: ^{1}H-NMR spectrum of 2-hydroxy-6-fluoro-chroman-4-one

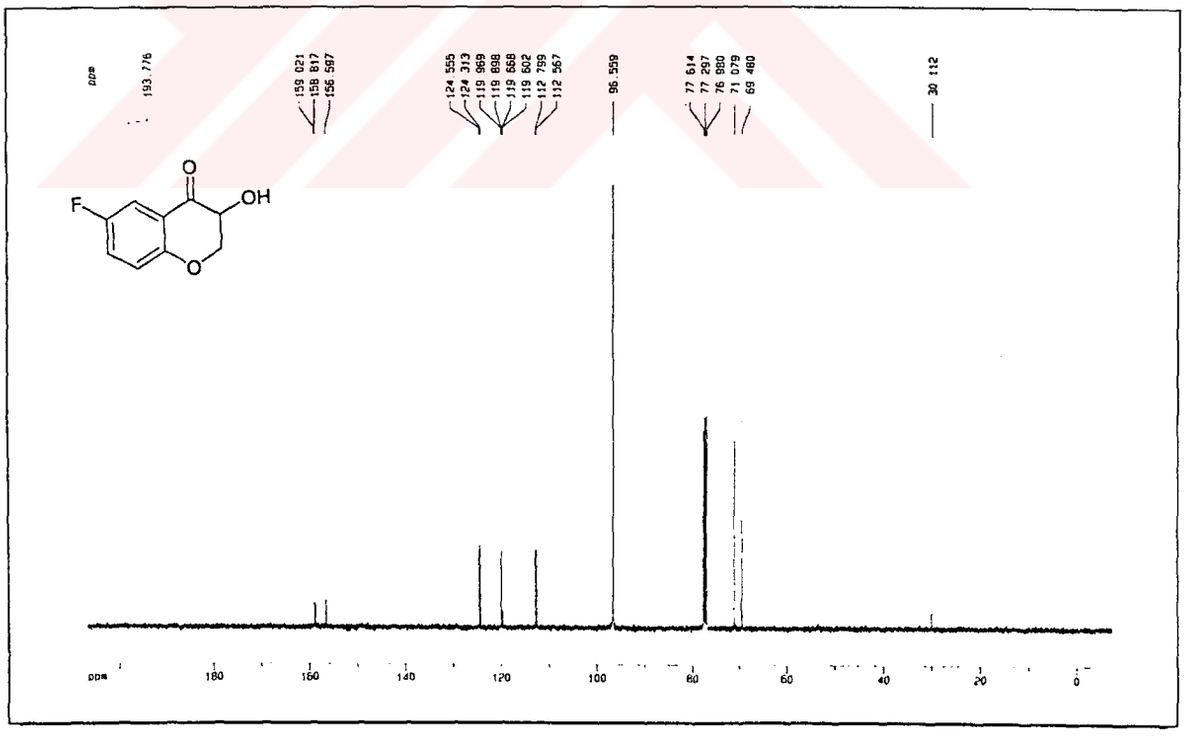


Figure 21: ^{13}C-NMR spectrum of 2-hydroxy-6-fluoro-chroman-4-one

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