CHEMOENZYMATIC FUNCTIONALIZATION OF CYCLIC 1,2-DIKETONES

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IŞIL BİÇER

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Prof. Dr. Canan ÖZGEN
Director

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

Prof. Dr. Hüseyin İŞÇİ
Head of Department

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

Prof. Dr. Ayhan S. DEMİR
Supervisor

Examining Commitee in Charge

Prof. Dr. Engin U. Akkaya (METU, CHEM) _____

Prof. Dr. Ayhan S. Demir (METU, CHEM) _____

Prof. Dr. Simeon Arseniyadis (CNRS, ICSN) _____

Assoc. Prof. Dr. Özdemir Doğan (METU, CHEM) _____

Assist. Prof. Dr. Servet Tural (Dicle Univ, CHEM) _____

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Name, Last name: Işıl BİÇER
Signature :

ABSTRACT

CHEMOENZYMATIC FUNCTIONALIZATION OF CYCLIC 1,2-DIKETONES

Biçer, Işıl

M. S., Department of Chemistry

Supervisor: Prof. Dr. Ayhan S. Demir

May 2006, 79 pages

Chiral hydroxylated cyclopentane derivatives are important structural units in many biologically active compounds and are also important synthons for the asymmetric synthesis of natural products. Synthesis of these types of compounds in optically pure form found increased interest in pharmaceutical chemistry. For this purpose 5-acetoxy-3-methyl-2-methoxy-2-cyclopentene-1-one and 5-acetoxy-3-ethyl-2-methoxy-2-cyclopentene-1-one were acetoxylated using manganese (III) acetate at α' positions. Enzyme catalyzed enantioselective hydrolysis of hydrolyzed acetoxy derivatives gives the corresponding hydroxylated diketones in optically pure form.

Key words: Manganese(III) acetate, lipase, enzymatic kinetic resolution.

ÖZ

SİKLİK 1,2-DİKETONLARIN KEMOENZİMATİK YÖNTEMLERLE FONKSİYONLANDIRILMASI

Biçer, Işıl

Yüksek Lisans, Kimya Bölümü

Tez Yöneticisi: Prof. Dr. Ayhan S. Demir

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Kiral hidroksi siklopentan türevleri biyolojik aktivite gösteren moleküllerin anahtar bileşikleridirler. Bu bileşiklerin kiral sentezleri farmasotik kimyada büyük öneme sahiptir. 3-Substitüe 1,2-Siklopentandion önce mangan asetat ile reaksiyona sokularak asetoksi türevlerine çevrilmiştir daha sonra enzim katalizörlüğünde seçici olarak hidroliz edilip hidroksi türevlerine dönüştürülmüştür.

Anahtar Sözcükler: Mangan (III) asetat, lipaz, enzimatik kinetik ayrıştırma.

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INTRODUCTION

1.1. Biotransformations in Organic Chemistry

Biotransformations are defined as the use of biological systems to bring about chemical changes on compounds that are not their natural substrates.[1] The use of natural catalysts for the transformation of non-natural man-made organic compounds is not all new as they have been used for more than one hundred years. Because of the enormous complexity of biochemical reactions compared to the classical organic reactions, it follows that most of these reactions have a strong empirical aspect. However, a lack of complete understanding of the mechanisms of these reactions did not deter the organic scientists from using them if their usefulness has been established.

Using enzymes might be a good choice for the transformation of organic compounds. Enzymes have many advantages compared to other catalysts. They are very good biocatalysts as the rates of enzyme-mediated processes are accelerated by a factor of 10^8-10^{10}, compared to those of the corresponding reactions. Another advantage is that enzymes are environmentally acceptable because of their complete degradability. In addition, enzymes act under mild conditions which minimize problems of undesired side-reactions like decomposition, isomerization, racemization and rearrangement. They also exhibit a high substrate tolerance by accepting a large variety of unnatural, man-made, substances. Some of them can work outside aqueous environment although loss of activity can be observed. Most importantly, since the purpose of an enzyme is to act on a single type of functional group, other sensitive functionalities, which would normally react to a certain extent under chemical catalysis, survive. This brings chemoselectivity. The complex three-dimensional structures of enzymes also bring regio- and diastereoselectivity as they may distinguish between functional groups which are

chemically situated in different regions of the same substrate molecule. Almost all them are made from L-amino acids and are chiral catalysts. That is why, upon the formation of enzyme-substrate complex, any type of chirality present in the substrate molecule is recognized. So a prochiral substrate might be transformed into an optically active compound upon an asymmetrization process.

Producing optically pure products is important as opposite enantiomers interact differently within an organism and can display various activities. Some differences are enormous such as distinguishable smells and flavours or teratogenic effects (Figure1).[2]

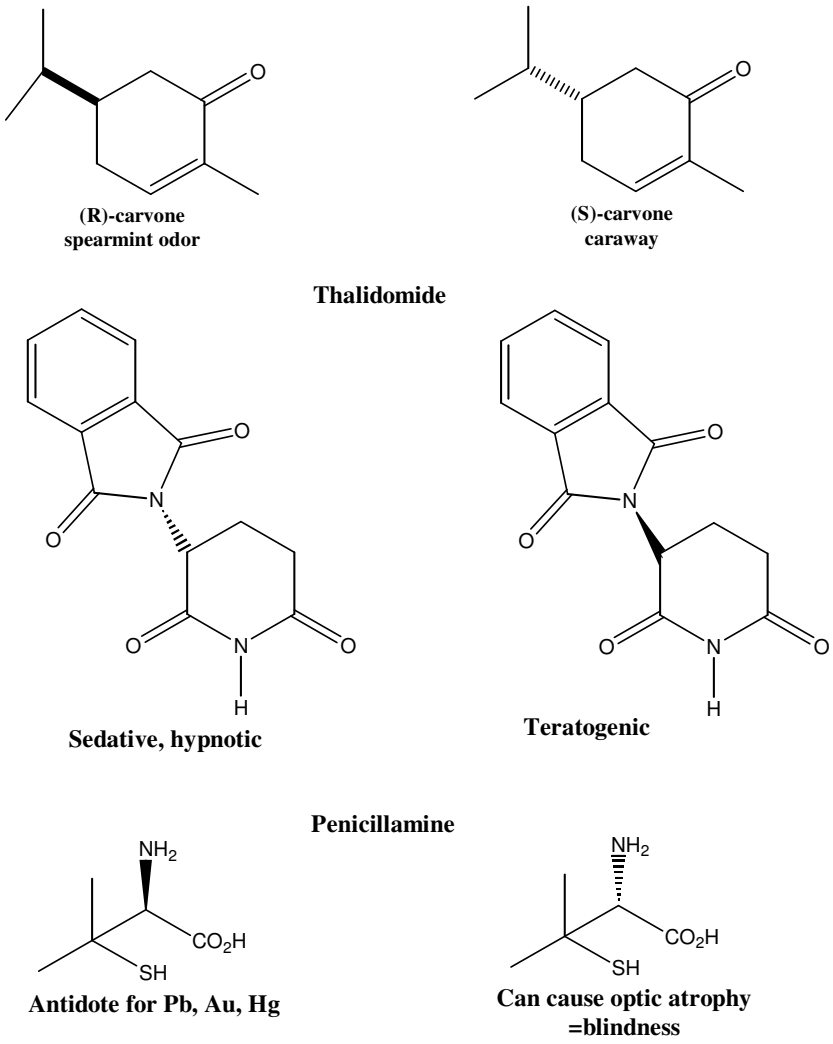


Figure 1: Biological effects of the enantiomers

Enzymes certainly have some disadvantages too. For instance, enzymes are provided by Nature in only one enantiomeric form. Moreover, they require narrow operation parameters like elevated temperatures and high pH which might result with the deactivation of the enzyme. They also show their highest catalytic activity in water. This is a disadvantage as the majority of organic compounds are poorly soluble in the aqueous media.

Infact whole organisms can also be used in such transformation reactions. It has its own advantages such as no cofactor recycling is needed. Generally, the work-up is easier and fewer by-products are formed. Also, higher activities can be obtained by growing cultures and cell re-use is possible with the immobilized cells.

There are also some disadvantages of using whole cells such as the low tolerance of organic solvents, side reactions due to uncontrolled metabolism, expensive equipment and lower concentration tolerance, eventually low productivity.[3]

1.2. Chirality and Asymmetric Synthesis

The word chiral comes from the Greek word 'cheir' meaning hand. A molecule is chiral if it can not be superimposed on its mirror image. The central carbon atom is known as the chiral or stereogenic center. Our hands are chiral as the right hand is a mirror image of the left and one cannot be superimposed on the other.

Amino acid alanine is a good example for chirality as it has four different groups attached to a carbon atom. (R)-alanine and (S)-alanine are mirror images of each other and in our cells we find only one form of this amino acid, (S)-alanine (Figure 2).

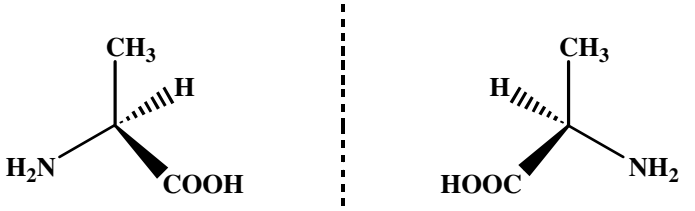
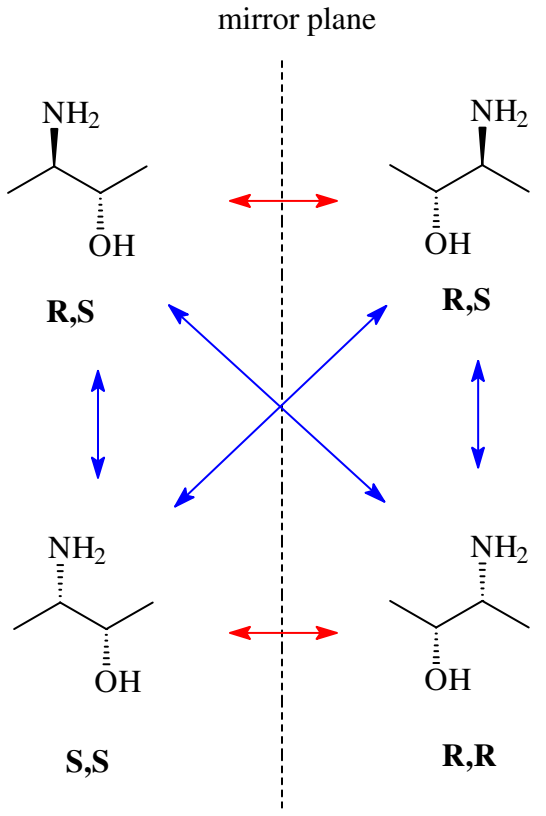


Figure 2: Two enantiomers of the alanine

When four different atoms or group of atoms are attached to a carbon atom, two different arrangements, called as enantiomers may exist. Enantiomers are simply non-superimposable mirror images of one another. This simply means that even if they are twisted or turned, they never overlap exactly. Assosiation of enantiomers with another chiral molecule which is enantiomerically pure gives diastereomers. Diastereomers are no longer mirror images of one another (Figure 3). Unlike enantiomers, diastereomers differ in physical properties. So they can be separated by physical methods such as distillation and crystallization.



Red arrows indicate **enantiomers**

Blue arrows indicate **diastereomers**

Figure 3: The relationship between enantiomers and diastereomers

Each enantiomer exhibits what is called optical activity, meaning that each isomer of the pair is capable of rotating plane polarized light. One isomer rotates the light to the clockwise direction, (+), and the other isomer of the pair rotates this light to the opposite direction, (-), for the same number of degrees. This is the only difference in the two isomers. All other physical and chemical properties are exactly the same such as solubility, melting point, chromatographic retention time, IR and NMR spectra. If one wants to determine the proportion of the two enantiomers in a mixture, the normal chromatographic and NMR methods must be modified to introduce an external chiral influence. By this way, enantiomers behave differently from each other.

L and D, or (R) and (S) the symbols used to define the difference between the two enantiomers. (R) comes from rectus, Latin, meaning "right" and (S) comes from sinister, Latin meaning "left". In the R, S system, groups are assigned priority using the Cahn-Ingold-Prelog sequence rules. To assign (R) or (S) configuration, firstly the stereogenic center(s) is found. Then the four atoms or groups attached to the stereogenic carbon are ranked according to the decreasing atomic number of the first bound atom. If no distinction is found at the first atom, atoms at increasing distances should be considered. Then the molecule is projected so that the group or the atom of the lowest priority is to the rear. Finally, the group or the atom of the highest priority is selected and a curved arrow is drawn toward the group (atom) of the next highest priority. Clockwise orientation is (R). Counterclockwise direction is (S).

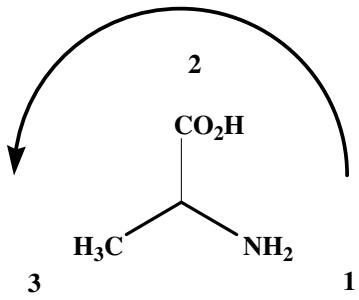


Figure 4: (S) Alanine

It should be remembered that the absolute configuration of an enantiomer has no bearing on its direction of rotation of plane-polarized light. This means that the (R) enantiomer of one compound could be dextrorotatory (+) while the (R) enantiomer of another chiral compound could be levorotatory (-).

Although the majority of optically active compounds contain at least one asymmetric carbon atom, the presence of asymmetric atoms is not a necessary condition for the existence of optical activity.[4] The necessity for a molecule to show optical activity is that such a molecule should not be superimposable on its mirror image. This means that the disymmetric molecules which lack one or more elements of symmetry can also be chiral. The requirement for chirality can be defined as follows: molecules which do not possess rotation-reflection axes (S axes) are chiral. Simply, the molecules that belong to the C_n or D_n point groups are chiral. The compound trans-2,5-dimethylpiperidine can be a good example as it contains a two-fold rotation axis, belongs to the point group C_2 and is chiral (Figure 5).

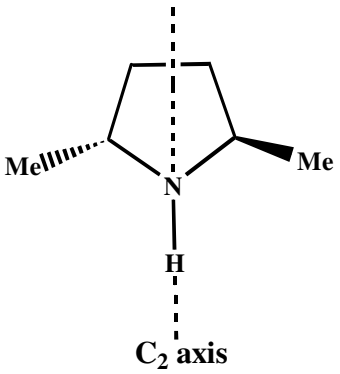


Figure 5: Two fold rotation axis of trans-2,5-dimethylpyrrolidine

There are several methods for obtaining enantiomerically pure materials. One is asymmetric synthesis which is the conversion of an achiral starting material to a chiral product in a chiral environment. This method involves the stereocontrolled formation of

the new stereogenic center. In this strategy, as the chiral element is formed 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. Another method is obtaining enantiomerically pure compounds through resolution of the corresponding racemic species performed at the end of the synthetic sequence. The 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. Other methods involve the conversion or derivatization of readily available natural chiral compounds (chiral pools) such as amino acids, tartaric and lactic acids, terpenes, carbohydrates, and alkaloids.

1.2.1 General Methods for Asymmetric Synthesis

Asymmetric synthesis is a term which has been used to describe stereocontrolled synthetic processes in many differing ways. Marckwald's classical definition of asymmetric synthesis embraced those which produce optically active substances from symmetrically constituted compounds with the intermediate use of optically active materials but with the exclusion of all analytical processes.[5] A more inclusive definition can be: An asymmetric synthesis is a process in which a prochiral unit is converted by a reactant into a chiral unit in such a manner that the stereoisomeric products are produced in unequal amounts.

To obtain enantioselective synthesis, at least one of the agents in the system must be chiral. That is why, the way of introducing a chiral center into a molecule is important. This can be done by an action of a chiral reagent, auxiliary or catalyst.

There are mainly two methods for asymmetric synthesis; the chemical and the biotechnological methods.

1.2.1.1. Chemical Methods

For obtaining enantiomerically pure materials, either reagent-controlled conditions or substrate-controlled conditions are used.

1.2.1.1.1. Reagent-Controlled Conditions

Chiral Reagents

A chiral reagent is used in order to obtain an enantiomerically enriched product. Considerable effort and expense might be required in the preparation of the reagent, and the stochiometric amounts are needed. The need for protection should be carefully considered as this could lead to the introduction of extra steps. The reagent must be selective both in terms of induction and functional group specificity.

Chiral Catalysts

Chiral catalysts cause one enantiomer to be selectively converted or only one enantiomer to be formed. Only catalytic amount of chiral catalyst is needed to produce large amount of chiral product. Transition metal catalysts are generally used and they have the advantage that the catalyst properties can be carefully tuned by changing the ligands around the metal atom.

1.2.1.1.2. Substrate-Controlled Conditions

Chiral Substrates

Having a chiral starting material to control the stereoselection of the reaction is another option. Nature produces chiral materials and a number of these are available in

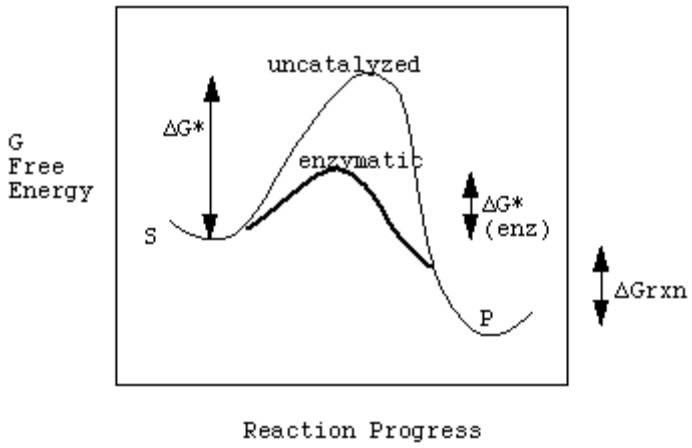
quantity which make up the 'chiral pool'. This can be a disadvantage as there are limited amounts of natural products available. The number of steps necessary to convert natural product into a useful starting material for synthesis and the price should also be considered. If all of the parameters are favourable, 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.[6]

Chiral Auxiliaries

A chiral auxiliary is a chiral molecule which can be covalently attached to an achiral molecule. They induce selectivity through a subsequent chemical reaction to afford diastereoselectivity. The reactions are often highly predictable and reliable. However, such processes need two extra synthetic operations: one to introduce the chiral auxiliary, and other one to remove the chiral originator. Another inherent drawback is that, the chiral auxiliary should be recoverable economically, in good yield and without racemization.

1.2.1.2. Biotechnological Methods

Using biocatalysts is another method to obtain enantiomerically pure products. Biocatalyst encompasses catalysis by bacteria, fungi, yeast, or their true catalytic components: enzymes.[7] Enzymes are proteins that are capable of accelerating reactions under mild reaction conditions by lowering the activation energy (Figure 6).



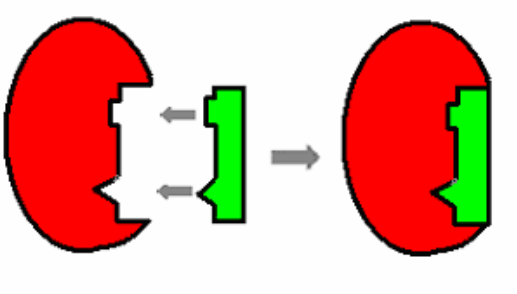
Delta G* is the activation energy

Delta G is negative overall for forward reaction

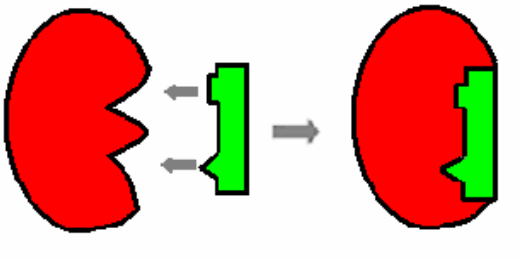
Figure 6: Free energy vs. reaction progress graphs for uncatalyzed and enzymatic reactions.

Enzymes function as catalysts by forming complexes with the reacting molecules, by increasing the local concentration of the molecule, by orienting the molecule correctly so that reaction can take place most efficiently and by distorting the shape of the molecule slightly, thereby changing their energy content and helping them reach the transition state.

Enzymes have a particular shape with an active site. Active site is the place where the enzyme binds to a substrate. Among the numerous theories which have been developed in order to understand enzyme catalysis, the most illustrative models are the "Lock and Key" mechanism and the Induced-Fit mechanism (Figure7).



a) Lock and key model



b) Induced-fit model

Figure 7: The "Lock and Key" mechanism and the Induced-Fit mechanism

The "Lock and Key" mechanism is the first proposal for a general mechanism of enzymatic action which was developed by E. Fischer in 1894. According to this mechanism, an enzyme acts as a lock, while the substrate acts as a key. This assumption was quite sophisticated at that time but it assumes a completely rigid enzyme structure so it cannot explain why many enzymes act on large substrates, while they are inactive on smaller, similar counterparts. Moreover, the hypothesis cannot explain why many enzymes can convert not only their natural substrates but also numerous non-natural compounds possessing different structural features.

Another important mechanism is the Induced Fit mechanism which was developed by Koshland Jr. in the late 1960s. It assumes that enzymes are not completely rigid but rather represent delicate and soft structures. The substrate comes into contact with the active site and the active site induces a fit around the substrate. The enzyme acts as the chemical operator and the substrate forces the enzyme to adapt a different active

conformation. Only then the active groups of the enzyme are positioned in the right way to effect catalysis. This rationale can explain the reason why in many cases several structural features on a substrate are required in addition to the reactive group. These structural features may be located at quite a distance from the actual site of the reaction.

Lipases are the most typical “Induced-Fit” enzymes. They are widely used in organic synthesis as they are stable, can easily be found and accept a broad range of substrates. They can convert a large variety of artificial substrates which possess structures that do not have much in common with the natural substrates.

Three major types of selectivities are displayed during the biocatalyst catalyzed processes. In chemoselective reactions purifications of product(s) from impurities is easier and side reactions can be omitted. Thus reactions are generally cleaner. Because of their three-dimensional structure, enzymes can distinguish functional groups, which are chemically situated in different regions of the same substrate, so they can show regioselectivity and diastereoselectivity. Biocatalysts are also enantioselective because they are chiral catalysts. Any type of chirality present in the substrate is recognized and prochiral compound is transformed into optically active product.

Another method is the kinetic resolution. When the rates are comparable, one of the enantiomers is transformed faster, and therefore an excess of the less reactive substrate gradually builds up. This excess goes through a maximum and disappears on completion of the reaction. If the reaction is interrupted before completion or if less than the necessary amount of reagent is applied, the result is a non-racemic mixture of the starting material and of a product in which an excess of the more reactive enantiomer of the substrate is incorporated. This way of partial separation of enantiomers is called kinetic resolution. The attractiveness of this method is diminished by the fact that a maximum of 50% yield can be obtained. A further disadvantage is that the other enantiomer often ends up in waste.

Enantiomeric excess (or ee) is defined as the excess of one enantiomer over the other, expressed as a percentage of the whole.

ee = (R-S): (R+S) (where R and S are the amounts of the two enantiomers)

It is possible to classify the enzymes according to the reactions they catalyze. Hydrolytic enzymes are the biocatalysts most commonly used in organic synthesis. They are able to speed up hydrolytic reactions. Amidases, proteases, esterases and lipases are examples of hydrolytic enzymes.[8] In general there are four types of proteases; serine proteases, thiol proteases, metalloproteases and aspartyl proteases. Examples of serine proteases include trypsin, chymotrypsin, pig liver esterase, and lipases. The catalytic cycle for serine proteases is illustrated in Figure 8. This catalytic cycle is also representative for most lipases.

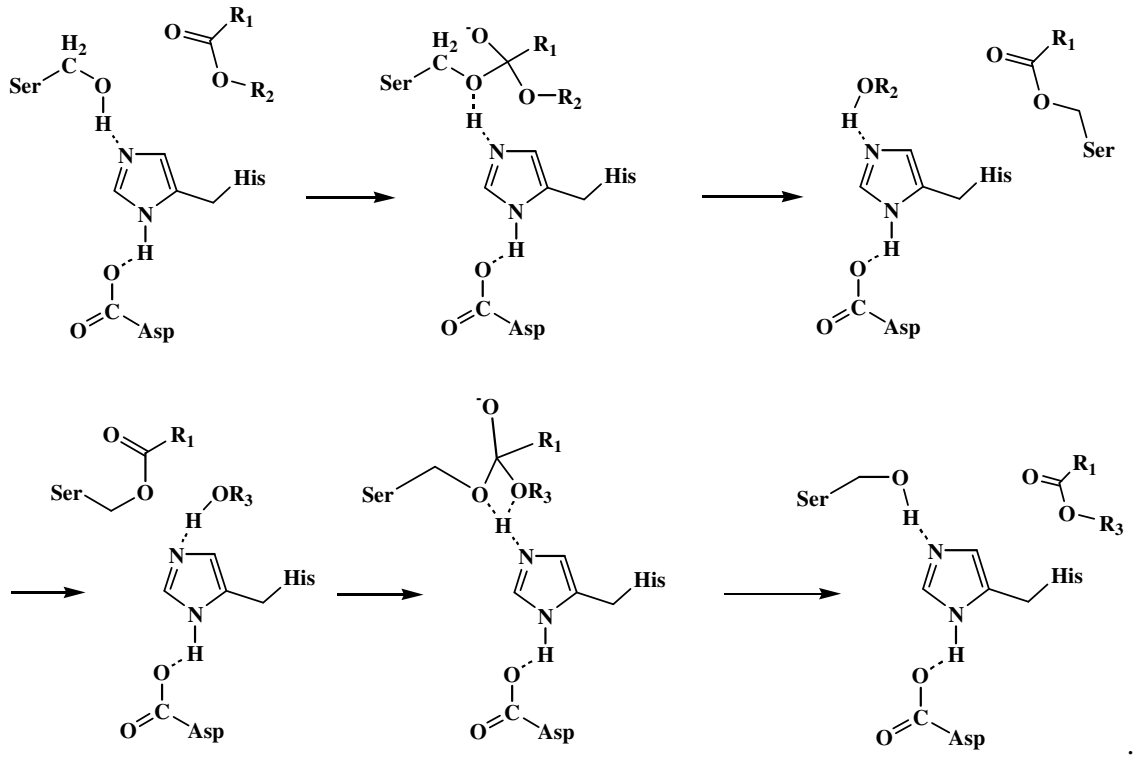


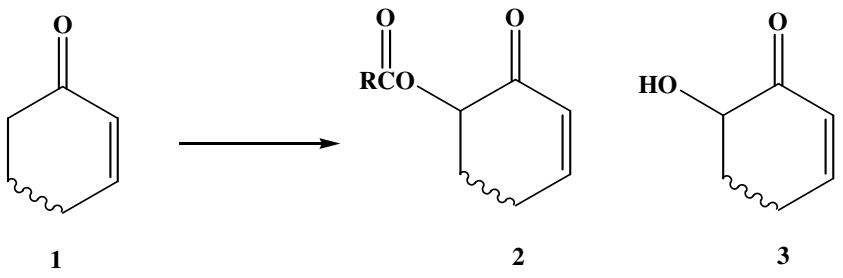
Figure 8: Catalytic cycles for serine proteases.

Serine proteases contain a catalytic triad in the active site composed of the amino acids, Asp, His and Ser. In figure 8, Ser reacts as a nucleophile with a substrate molecule. Here being an ester. During substrate binding 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_2 = H, hydrolysis) or a second alcohol molecule (transesterification) to yield the product of the reaction, being either an acid or an ester.[9]

1.3. α' Oxidation of Enones

Selective oxidations giving α'-hydroxy and acetoxy α,β-unsaturated ketones has been of continuous interest as they are an important class of compounds in natural product and drug synthesis.

In the literature, there are methods available for the α' oxidation of **1** to α'-acetoxy-α,β-unsaturated ketones **2** using lead (IV), mercuric (II), and manganese (III) carboxylates or to α'-hydroxy-α,β-unsaturated ketones **3** using direct enolate oxidations (Scheme 1).



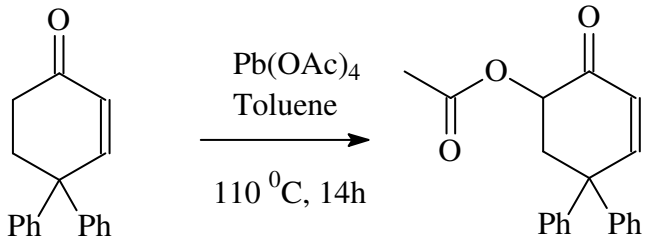
Scheme 1

Using Lead (IV) Acetate

The regioselective oxidation of enones to α'-acetoxy enones using lead(IV) acetate, in acetic acid, benzene or toluene gave yields varying from poor to acceptable.

The mechanism for the α'-acetoxylation of enones by lead (IV) acetate is proposed by Henbest and co-workers[10], and Marshal and Bundy[11]. It involves the formation of an enol-lead triacetate derivative directly from the enone followed by intramolecular acetate transfer.

An example of the acetoxylation of an α,β-unsaturated ketone using lead(IV) acetate is given in Scheme 2. The reaction of the α,β-unsaturated ketone **4** afforded the expected α'-acetoxy derivative **5** in 56% yield.[12]



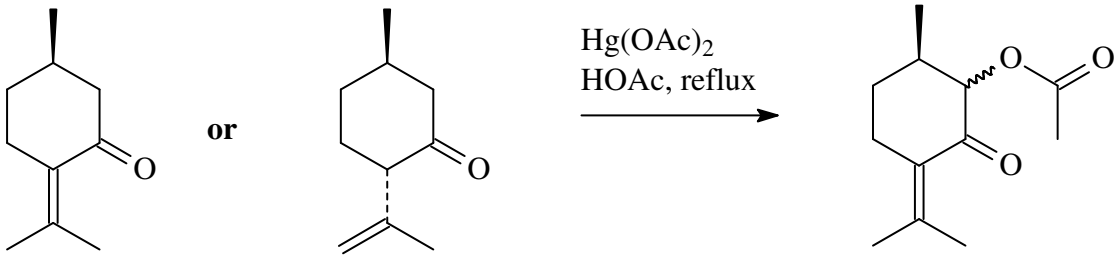
4 **5**

Scheme 2

Lead (IV) acetate α'-acetoxylation of enones is a promising method but it has a drawback that lead (IV) is highly toxic.

Using Mercury(II) Acetate

The oxidation of (+)-pulegone **6** or the deconjugated isomer **7** with mercury(II) acetate in refluxing acetic acid provided α'-acetoxyenone **8** in modest yield (Scheme 3). However, since lead(IV) acetate produced comparable yields of **8** and since the scope of this oxidation was unexplored, there is little to recommend mercury(II) acetate for this type of oxidation.[13]



6 **7** **8**

Scheme 3

Other Methods for the Synthesis of α-Hydroxy Ketones

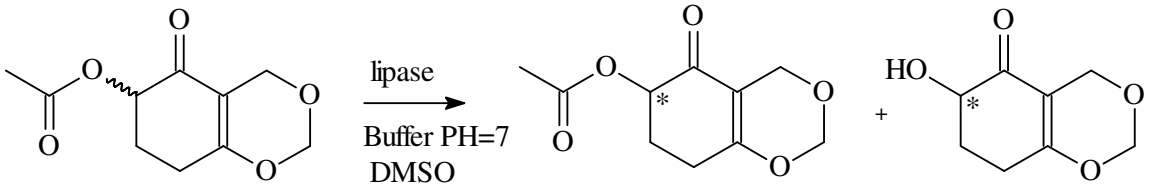
Heavy metal-containing oxidants such as MoO_5.Py.HMPA[14] and CrO_2Cl_2[15] are studied for the synthesis of α-hydroxy ketones. However, as these agents are potentially contaminating, alternative methods should be considered.

Biotechnological Methods

As can be done by chemical methods, optically active α-hydroxy ketones can also be prepared enzymatically by the reduction of the α-diketones with yeast as the biocatalyst.[16] The disadvantages of this enzymatic method are; the further reduction of diketone to vic-diol, formation of both regioisomeric α-hydroxy ketones and moderate chemical yields.

Esterases and lipases such as PLE (Pig liver esterase), PPL (Porcine pancreatic lipase), CCL (Candida cylindracea lipase) and PCL (Pseudomonas cepasia lipase) are the enzyme systems for hydrolysis which play an important role in the preparation of chiral molecules for synthesis. Lipases have been widely used for the synthesis of optically active alcohols, carboxylic acids and esters via enantioselective esterification and transesterification in organic solvents.

An example is the report which has been presented by Adam et al. about the kinetic resolution of racemic α-hydroxy ketones by lipase-catalyzed irreversible transesterification with isopropenyl acetate in organic media.[17] In addition, Demir et. al. reported in several examples the enzymatic hydrolysis of racemic ά-acetoxy enones, for example racemic ά-acetoxy-4,6,7,8-Tetrahydro-5H-1,3-benzodioxin-5-one using lipase type enzymes (Scheme 4).[18]

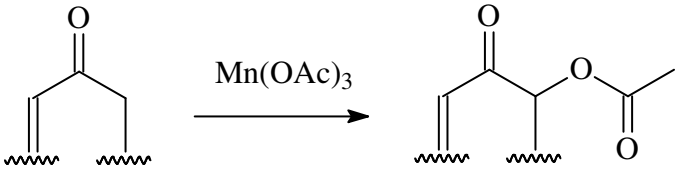


Scheme 4

1.4. $Mn(OAc)_3$ Mediated Acetoxylation of Enones

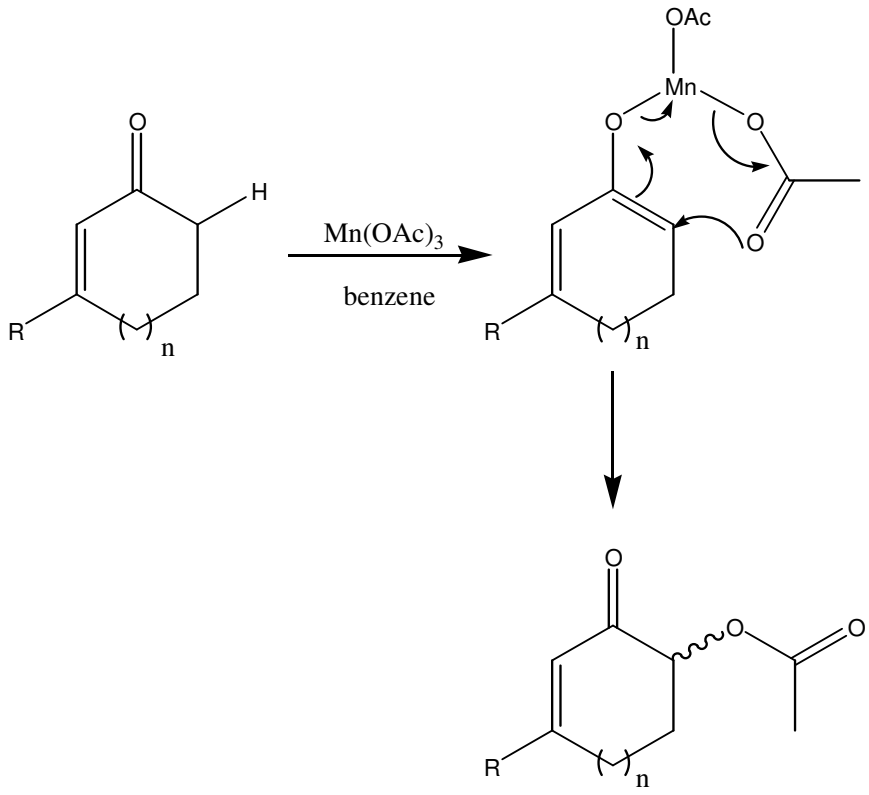
Procedures for the selective oxidation of common functional groups occupy a central position in the synthesis of complex natural products. Literature methods gave unsatisfactory results for the preparation of α'-acetoxyenones.[19]

In 1976, Williams and Hunter reported that the oxidation of enones to ά-acetoxyenones using manganese (III) acetate in acetic acid in a 6-36 % yield.[20] Watt and co-workers greatly improved the yields by using excess dried manganese (III) acetate in refluxing benzene.[21] Also, Demir and his co-workers studied on the oxidation of α,β-unsaturated enones using manganese(III) acetate.[22-24] They got satisfactory results for the preparation of α'-acetoxy enones (Scheme 5).



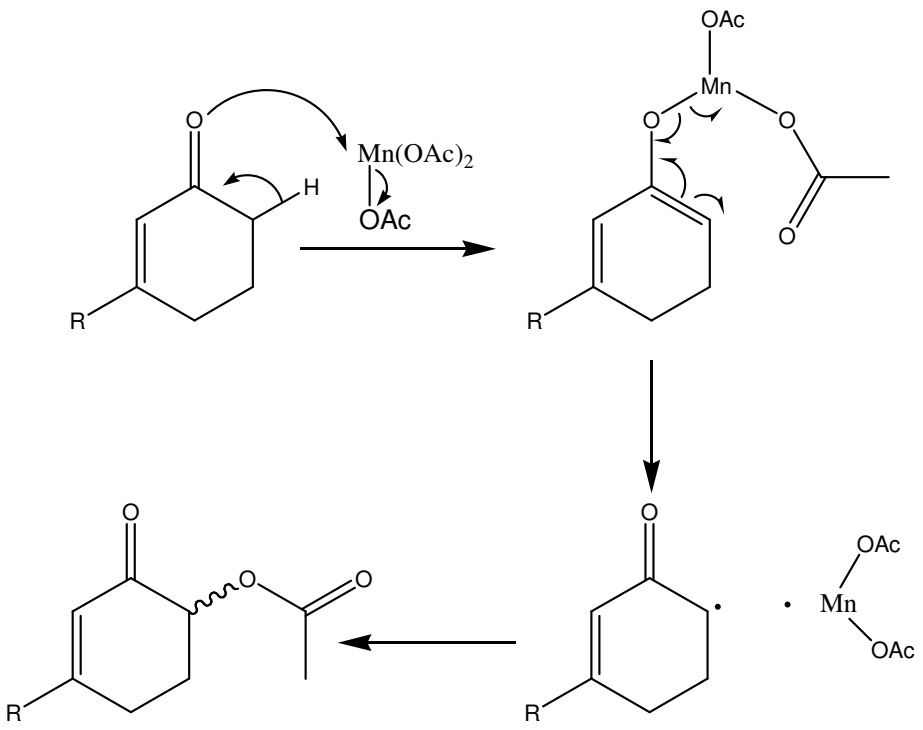
Scheme 5

Recently Demir, Reis and Igdir reinvestigated the synthetic and mechanistic aspects of Mn (III) acetate mediated oxidation of enones in benzene.[25] There are mainly two proposed mechanisms. One is the mechanism of the ligand transfer via metal-enolate intermediate (Scheme 6).



Scheme 6

The other one is the radical mechanism which is the formation of an α-oxo radical followed by ligand transfer (Scheme 7).



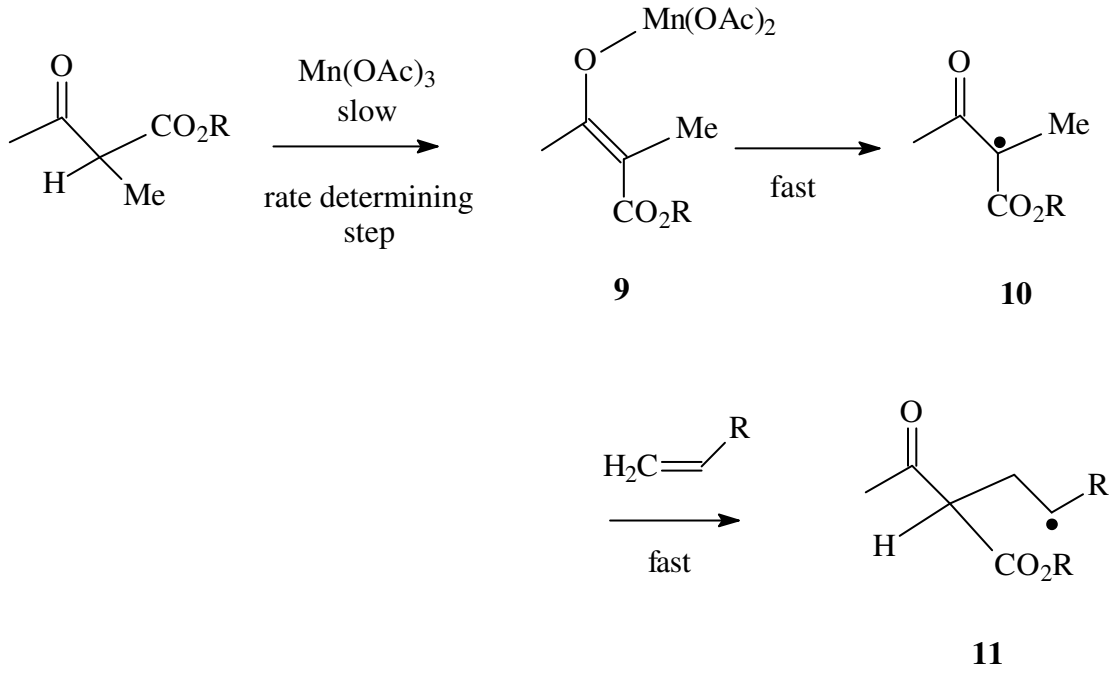
Scheme 7

Since $Mn(OAc)_3$ is a single electron oxidant and a vast majority of the reactions mediated by it has been shown to be taking place via a radical mechanism, formation of radical is widely accepted.

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.

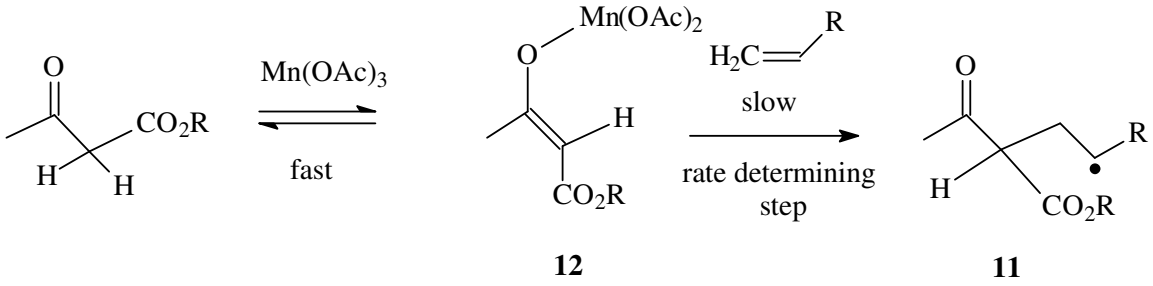
Today, manganese (III) acetate oxidation is one of the most useful methods as it has the advantages of higher chemical yields, higher α'-regioselectivity and milder reaction conditions, tolerating many sensitive functional groups.

The mechanism of oxidation of monocarbonyl substrates with $Mn(OAc)_3.2H_2O$ has been extensively studied and a mechanism has been suggested by Snider[26], which is operative in the oxidation of α-alkyl-β-keto esters (Scheme 8).



Scheme 8

The formation of **9** is slow; whereas the electron transfer with loss of Mn (II) to give **10** is fast. Therefore the rate of reaction is independent of alkene concentration. This indicates that free radical **10** is involved in the Mn (III)-mediated oxidations. Another observation is that the enolization of α-unsubstituted β-keto esters is fast and reversible, and electron transfer to give the radical is very slow (Scheme 9).



12 **11**

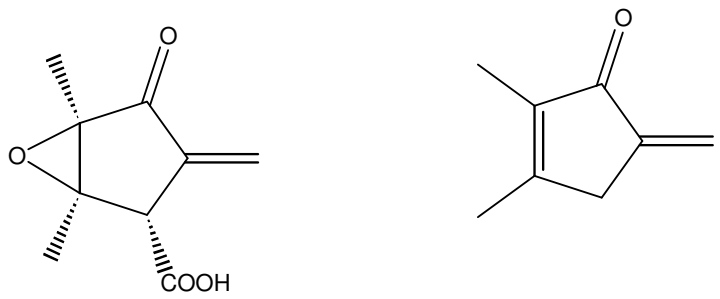
Scheme 9

Here the rate-determining step depends on alkene concentration and is presumably the reaction of the Mn (III) enolate **12** with the alkene to give radical **11** with the loss of Mn (II). β-Keto ester radicals analogous to **10** do not appear to be intermediates in these reactions. They concluded that the nature of the reaction depends 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.

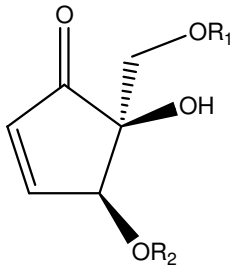
1.5 The Importance of Polyoxo Cyclopentenones

Polyoxo-cyclopentenones can be used in many synthetic applications as they have many functional groups. These groups can be changed by any other functional groups which make them important structural units in many biologically active compounds.

One of the examples of the important biologically active compounds that can be synthesized from cyclopentenones is Pentenomycin and methlenomycin A and B (Figure 9). They are the antibiotics which show antibacterial activity.



Metylenomycin A B



a) R_1=R_2=H

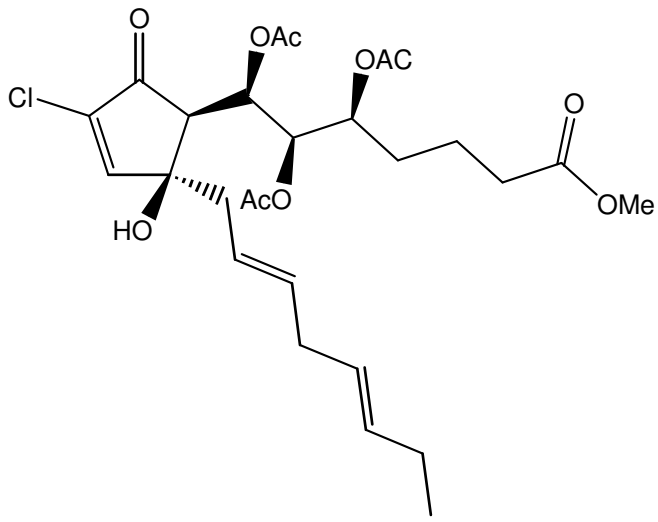
b) R_1=H, R_2=Me

c) R_1=Ac, R_2=H

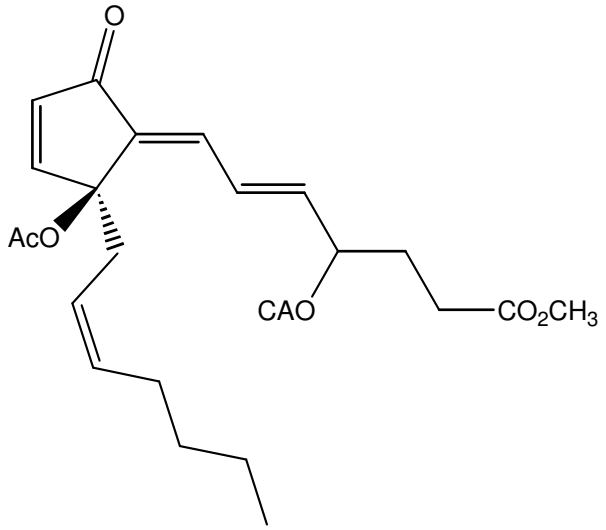
Pentenomycin

Figure 9: Metylenomycin A-B and Pentenomycin

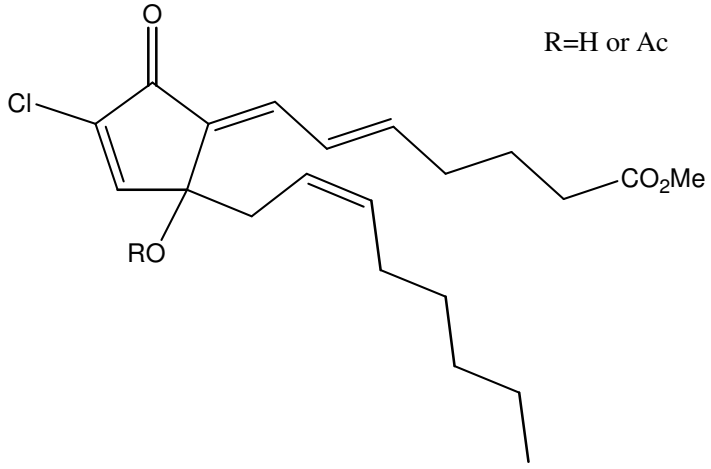
Another example is the halogenated prostanoids such as chlorovulones. They are especially of interest due to their strong antiproliferative activity against tumor cells. Other groups that show antitumor activity are punaglandin, clavulone and sarkomycin. (Figure 10)



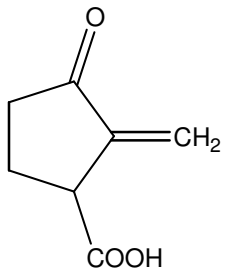
Punaglandin



Clavulone



Chlorovulone

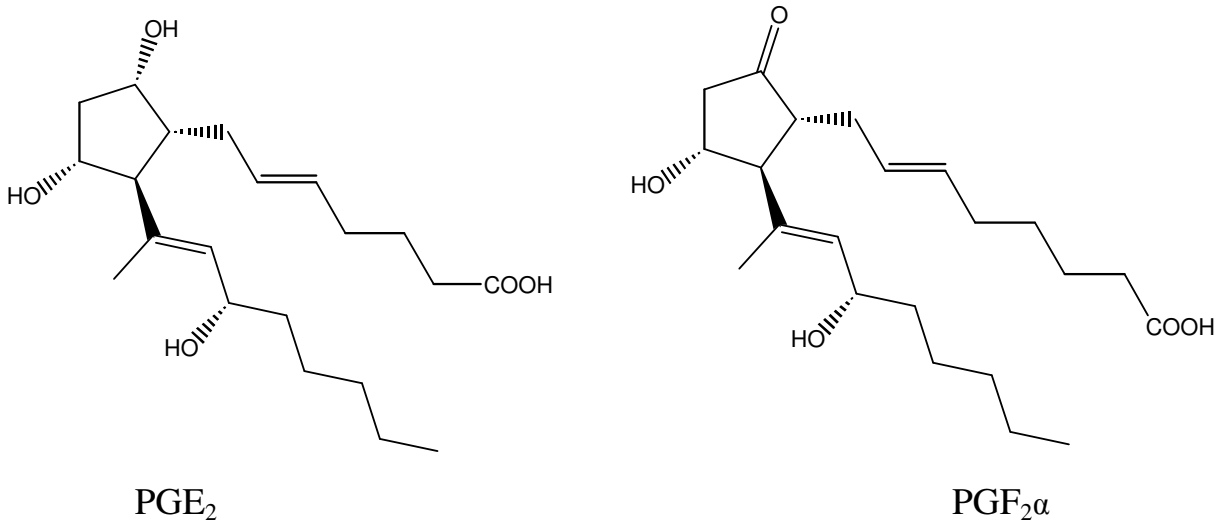


Sarkomycin

Figure 10: Cyclopentanoid structures that show antitumor activity

Another biologically important compound is prostaglandins. Many prostaglandins and their analogues are used as drugs. They have many applications such as therapeutic

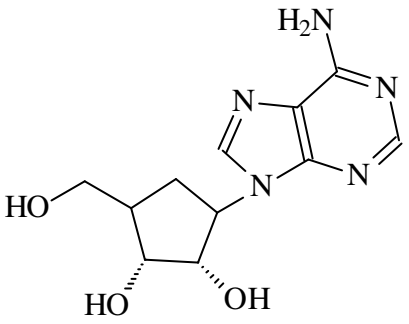
applications in the field of gynaecology for the termination of unwanted pregnancy. (Figure 11)[27,28]



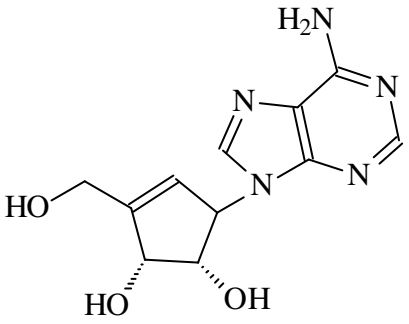
PGE_2 $PGF_2\alpha$

Figure 11: Prostaglandins

Neplanocin A which can be synthesized from a cyclopentenone derivative, is a potent antitumor agent. It can easily be converted to aristeromycin. Aristeromycin is a nucleoside antibiotic. It exhibits a variety of interesting biological activities such as inhibition of cell division and elongation in rice plants, inhibition of AMP synthesis in mammalian cells and inhibition of the enzyme S-adenosylhomocysteine.[29,30]



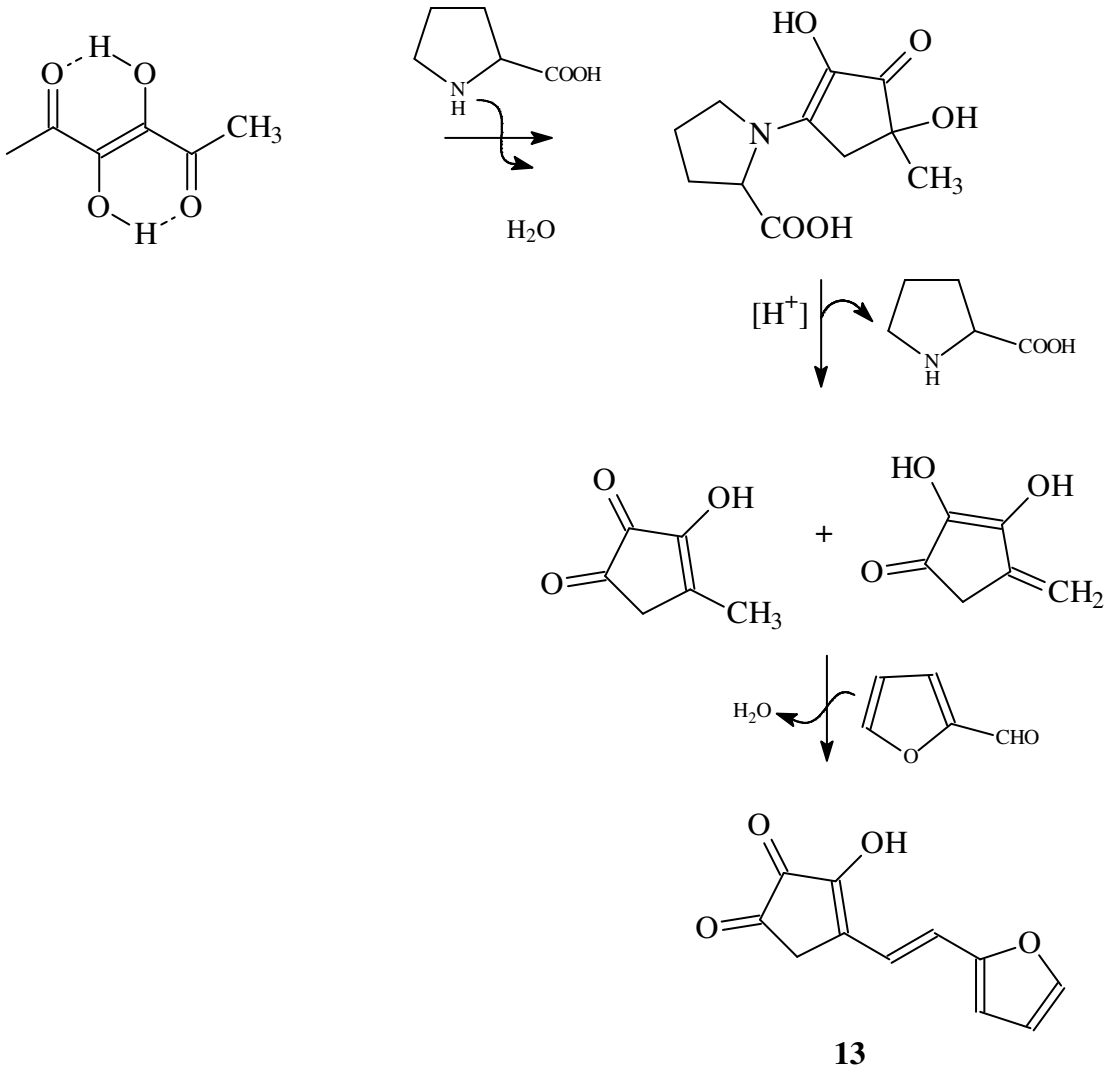
aristeromycin



neplanocin A

Figure 12: Aristeromycin and neplanocin A

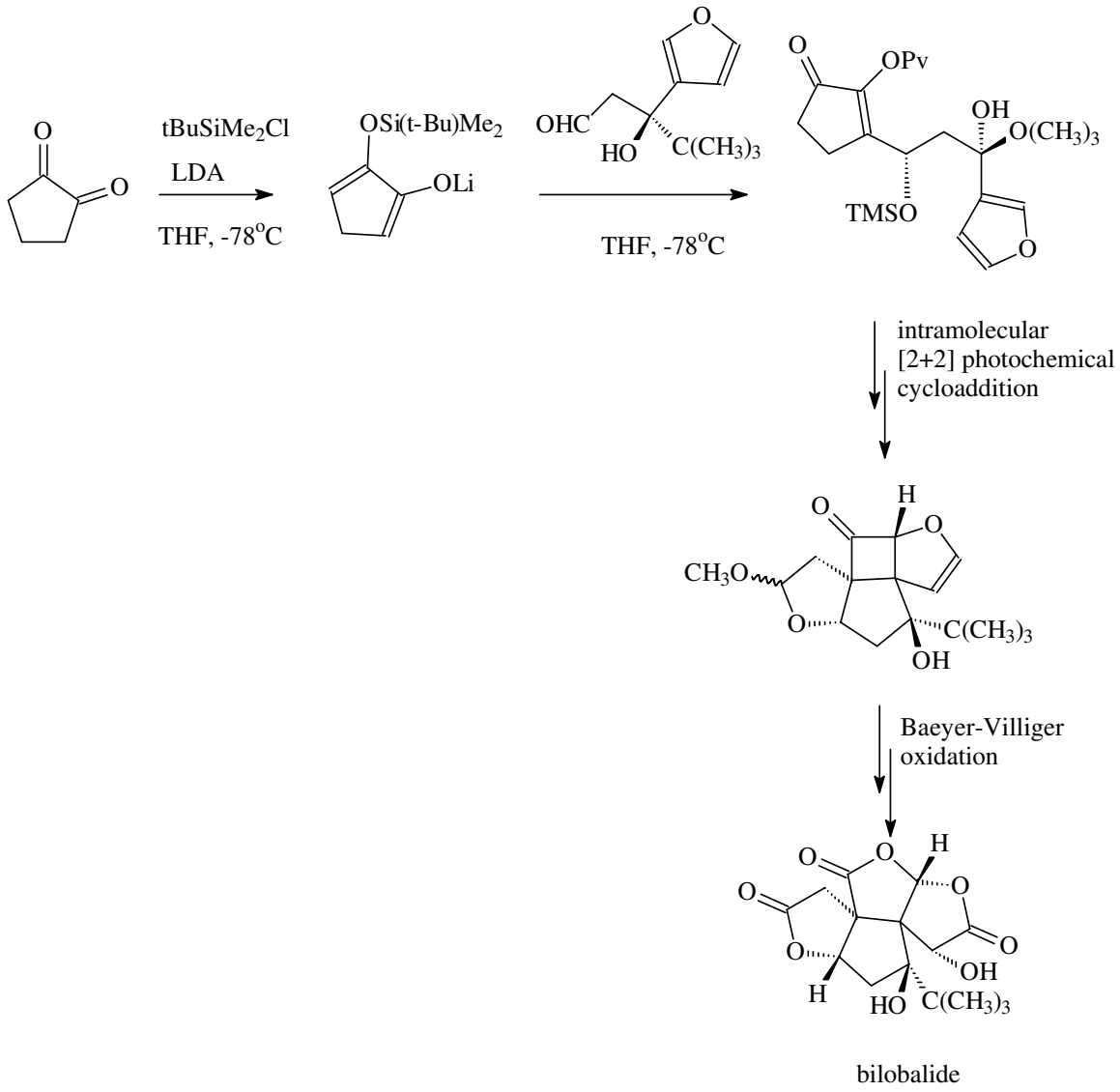
Besides the unique aroma, the typical brown color in, for example, coffee, maple syrup, bread crust or roasted meat is highly desirable. The orange-coloured 3-hydroxy-4-[(E)-(2-furyl)methylidene]methyl-3-cyclopentene-1,2-dione **13** is used in non-enzymatic browning.[31] The synthesis of this compound is shown in scheme 10.



13

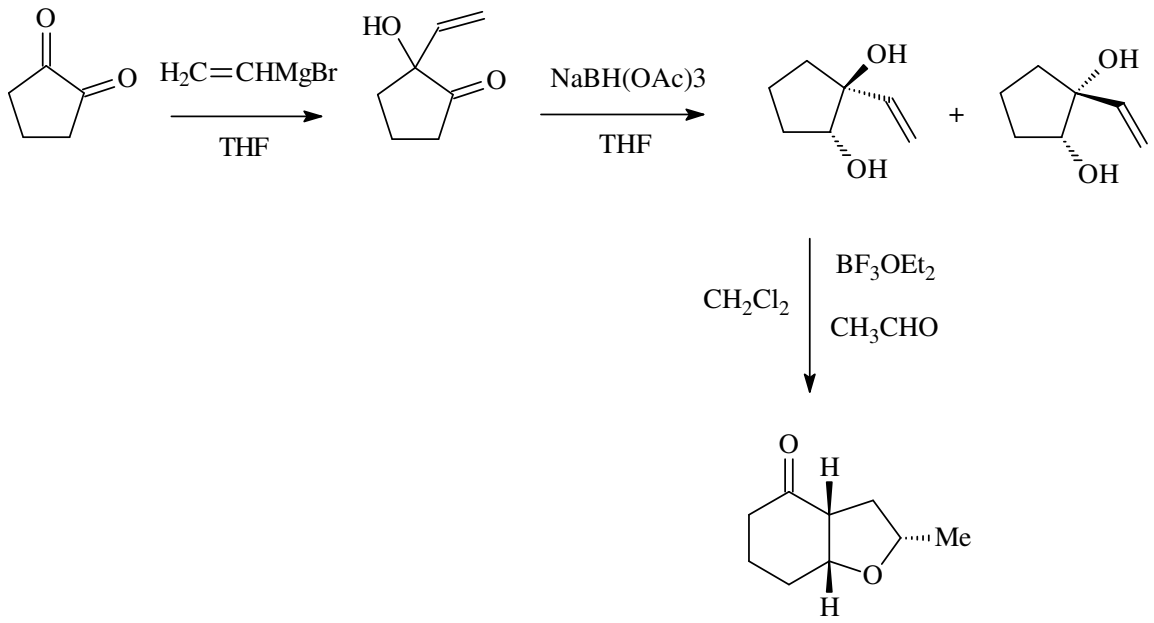
Scheme 10

Ginkgo biloba is an ancient plant species whose extracts have been used as medicinal agents for approximately 5000 years. It was found that one of the compounds responsible from the healing powers of the ginkgo extracts is bilobalide. The total synthesis of bilobalide can be done starting from 1,2-cyclopentanedione with 77% yield.[32] The synthesis of this compound is shown in scheme 11.



Scheme 11

Cyclic diketones can also be used in the ring-enlarging furan annulations.[33] For instance, the synthesis of cis hydrobenzofurans which are known as the precursors to oxacyclic marine natural products can be done by using 1,2-cyclopentanediols that carry an unsubstituted vinyl substituent at C(1).[34] An example to this synthesis is shown in scheme 12.



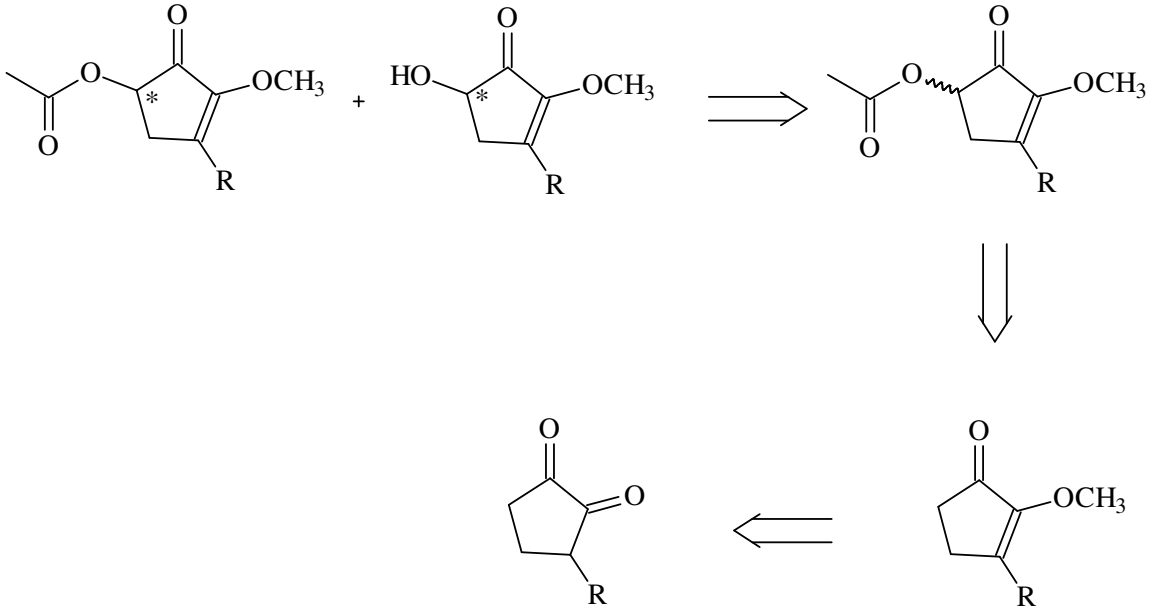
Scheme 12

1.6 Aim of the Work

The major aim of this research is to develop simple and selective method for the synthesis of chiral 3-substituted cyclic polyoxo-ketones. The oxidation products of cyclic 1,2-diketones are important compounds as they are found in the structure of many natural products. Particularly for the poly-oxygenated cyclopentane rings, it is a great advantage that these functional groups can be changed with any other functional groups, leading to many pharmacologically important products. For this purpose the synthesis of 5-acetoxy -3-methyl-2-methoxy-2-cyclopentene-1-one **14,** 5-hydroxy -3-methyl-2-methoxy-2-cyclopentene-1-one **15,** 5-acetoxy -3-ethyl-2-methoxy-2-cyclopentene-1-one **16** and 5-hydroxy -3-ethyl-2-methoxy-2-cyclopentene-1-one **17** were choosen as a model study as they have not been synthesized before in enantiomerically pure forms.

There is no chiral preparation of **14, 15, 16** and **17** published in the literature so far. There is no racemic preparation of **15, 16** and **17** either. But there is one report of Demir and Saatcioglu for the racemic preparation of **14.**[35]

The aim of this work is shown retrosynthetically in scheme 13.



Scheme 13

Our first approach to optically active **14, 15, 16** and **17** is to synthesize the 3-alkyl-2-methoxy-2-cyclopentene-1-one starting from 3-alkyl-1,2-cyclopentenedion, which will be followed by α-oxidation of enone by using manganese(III) acetate. Then, enzymatic bioconversion of racemic 5-acetoxy -3-alkyl-2-methoxy-2-cyclopentene-1-one can be done by lipases.

CHAPTER 2

RESULTS AND DISCUSSION

2.1 Perspective of the Work

Poly oxygenated cyclopentane derivatives are important chiral synthons for the construction of chiral organic compounds due to reactive functional groups like OMe, acetoxy, double bond, carbonyl and hydroxyl groups. These functional groups can be transformed to other functional groups which opens many applications in organic synthesis.

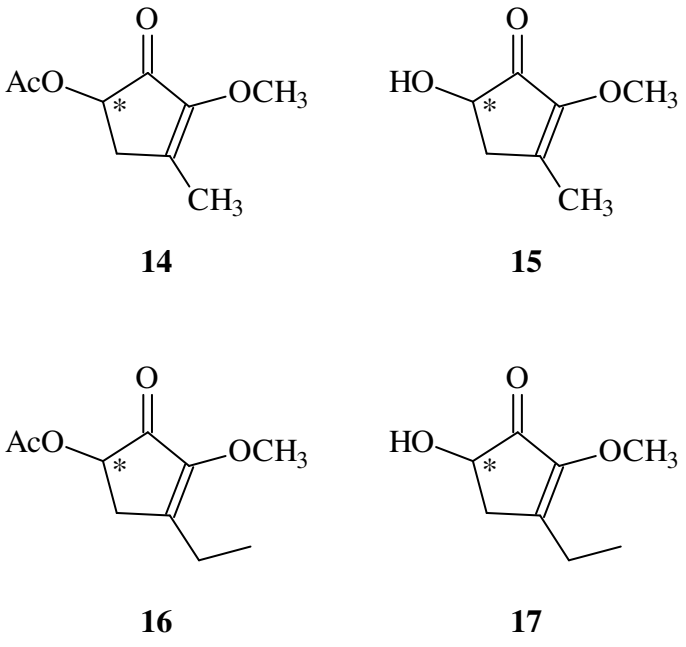


Figure 13: Polyoxo ketones

A chemoenzymatic method for the synthesis of **14, 15, 16** and **17** was developed starting from substituted 1,2-cyclopentanedione. Protection of hydroxyl group, manganese (III) acetate mediated oxidation followed by enzymatic kinetic resolution showed to provide the desired compounds **14-17** in enantiomerically pure form.

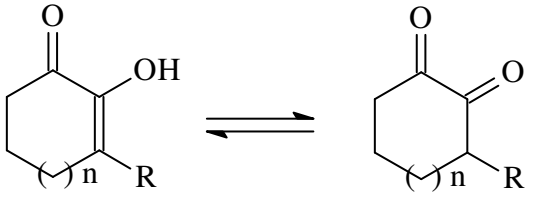
Lipase type enzymes are widely used for the synthesis of enantiomerically pure compounds via the resolution of racemic mixture. The high stereoselectivity in organic media and their low cost make them very useful catalysts for enantioselective resolution.

To find a suitable experimental setup for the lipase catalyzed resolutions of **14, 15, 16** and **17,** we have tried to find the right reaction conditions that give the best results in the enzyme catalyzed hydrolysis. It was also necessary to find a proper way to determine both conversion of the reaction and the enantiomeric excess values of the acetate (remaining substrate) and the alcohol (product). For the determination of the ee of the acetate and the alcohol in the kinetic resolutions several chiral HPLC columns were used. Determination of the ee by this technique was always first performed on the racemate to optimize base-line separation. To determine the conversion, the reaction was controlled by 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.

Even very small amount of samples are enough for HPLC analysis, so it was possible to perform the hydrolysis on an analytical scale. By this way, the screening of a large number of enzymes becomes fast and easy.

2.2 Synthesis of α-Acetoxy Ketones from Cyclic 1,2-Diketones

Cyclic 1,2-diketones are structural units found in a number of biologically active compounds. This class of molecules is characterized by an equilibrium between the mono-enol and di-keto forms (Scheme 14).



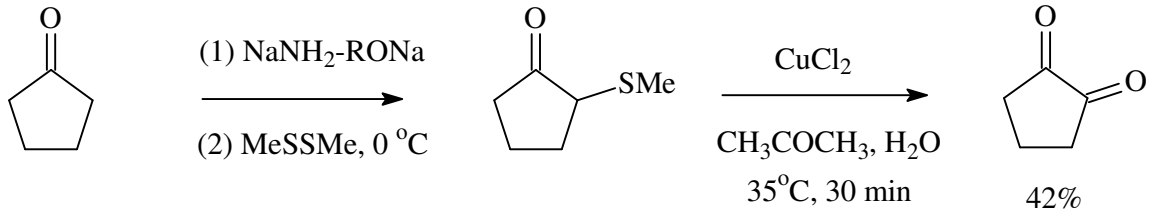
Scheme 14

In smaller rings (5,6) these compounds exist in the mono-enol form almost exclusively, however as ring size increases and becomes more flexible, the amount of the di-keto form increases. As evidence of this, the intensity of the infrared O-H absorption is sharply reduced when the ring size is increased from six to seven and completely disappears when it is ten.[36]

Enolization is most extensive in the smaller rings because the two carbonyl groups are confined to a planar cis-configuration which results in unfavorable dipole interactions between the two carbonyl groups. Conjugation between the remaining carbonyl and the double bond in the ring also favors the mono-enol from. When R=alkyl, the diketone enolizes to give the more substituted enol exclusively. This allows for the facile discrimination between the two carbonyl groups.

Cyclic 1,2-diketones can be prepared in numerous ways including the oxidation of cycloalkanones, direct alkylation of 1,2-cycloalkanedione and reaction of

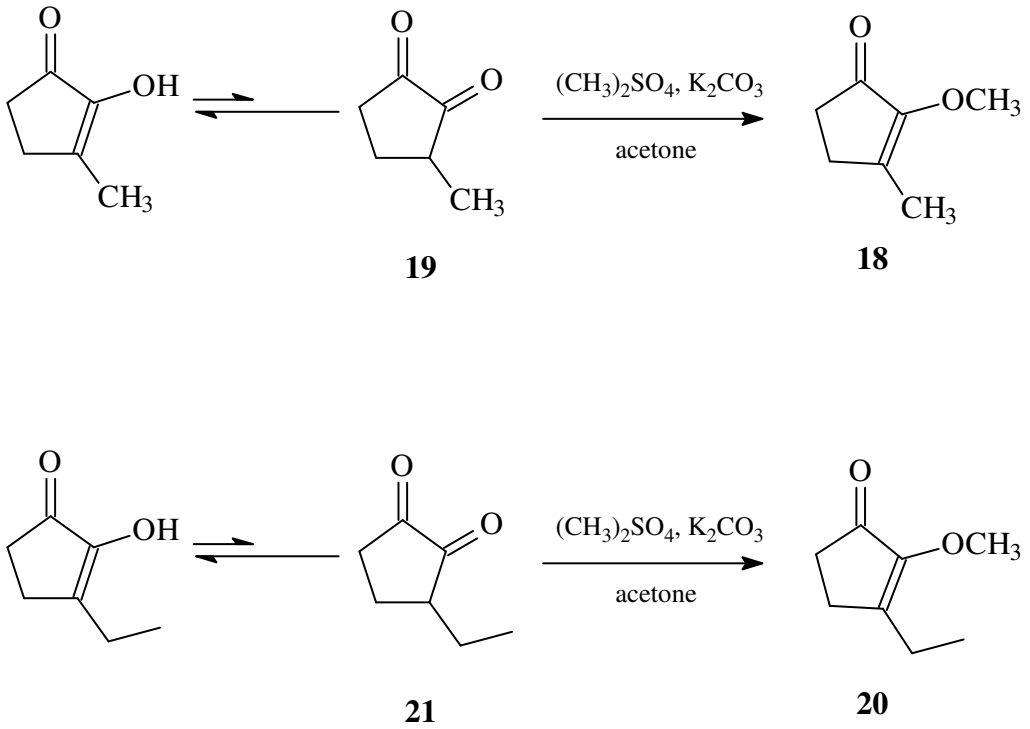
bromocycloalkanones with aqueous sodium hydroxide. An example is shown in scheme 15.[37]



Scheme 15

2.2.1 Protection of Cyclic 1,2-Diketones

For the synthesis of chiral α-acetoxy and α-hydroxy ketones, protection was performed on the diketone. As the diketones 3-methyl-1,2-cyclopentanedione, **19** and 3-ethyl-1,2-cyclopentanedione, **21** stay, 100% in the enol form, the acidity of the OH group is high (pka = 9.60). That is why using a weak base and a methyllation reagent was suitable for the reaction. $(CH_3)_2SO_4$ was used in the reaction as the methyllation reagent. In this method, diketone was dissolved in dry acetone and $(CH_3)_2SO_4$ and K_2CO_3 were added to the solution. The mixture was heated under reflux. The reaction was monitored by TLC (Silica gel, EtOAc/Hex 1:1). After the work up, product was purified with column chromatography (Silica gel, EtOAc/Hex 1:1). Desired products 2-methoxy-3-methyl-2-cyclopenten-1-one, **18** and 2-methoxy-3-ethyl-2-cyclopenten-1-one, **20** were obtained in 89% and 80% yield respectively (Scheme 16). As an evidence to this formation, typical (OCH_3) signals were observed for both **18** and **20** (3.85 for **18** and 3.80 for **20**).

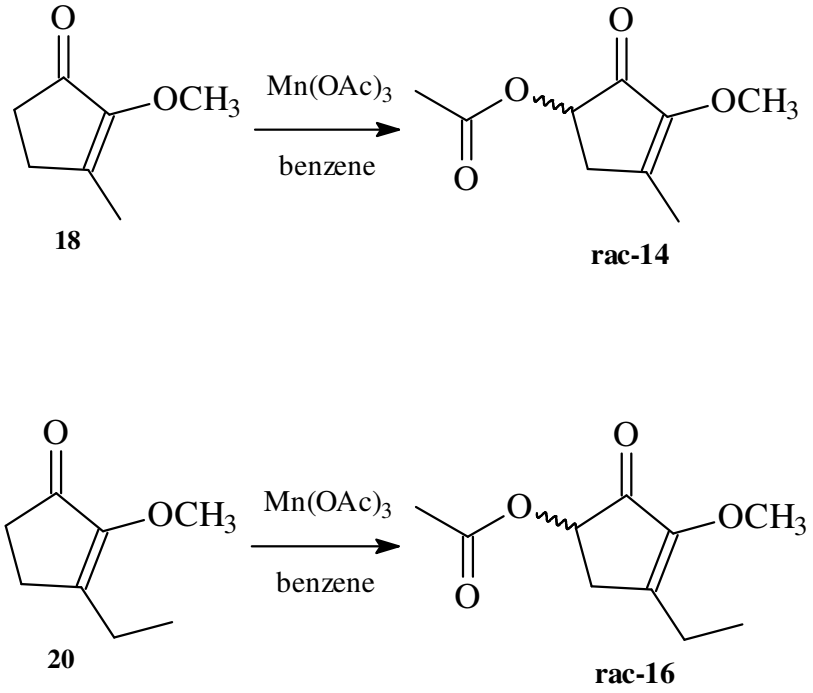


Scheme 16

2.2.2 $Mn(OAc)_3$ Mediated Acetoxylation of α-Alkoxy Enones

$Mn(OAc)_3.2H_2O$ was synthesized by using $Mn(OAc)_2.4H_2O$ and $KMnO_4$ in glacial acetic acid according to the procedure in the literature.[38] $Mn(OAc)_3$ was then dried over P_2O_5 under high vacuum to remove water. $Mn(OAc)_3$ was further dried in a heating gun (refluxing xylene) under high vacuum to obtain a dark brown colored $Mn(OAc)_3$. $Mn(OAc)_3$ from a commercial source was also used and provided similar results as long as it was extensively dried.

For the acetoxylation reaction, enone was allowed to react with 3 equivalents of $Mn(OAc)_3$ in benzene and refluxed under a Dean-Stark trap to give the desired acetoxy derivative in racemic form (Scheme 17).



Scheme 17

The reaction was monitored by TLC (Silica gel, EtOAc/Hex 1:2). After the work-up and purification of the crude products by column chromatography (EtOAc/Hex 1:2), the desired products, racemic 5-acetoxy-3-methyl-2-methoxy-2-cyclopenten-1-one **14** and 5-acetoxy -3-ethyl-2-methoxy-2-cyclopentene-1-one **16** were obtained as yellow oil in 88% and 89% yield respectively. The products were identified by using NMR spectroscopy.

From the ^{1}H-NMR spectrum of **14**, we observed a singlet at 1.89 ppm from the –CH_3 group and at 5.00 ppm dd, (J=2.6 and 6.8 Hz) for the α-proton. From the ^{13}C-NMR spectrum, we observed a peak at 13.8 ppm for the CH_3 carbon and a peak at 169.1 ppm for the O<u>C</u>OCH_3 carbon.

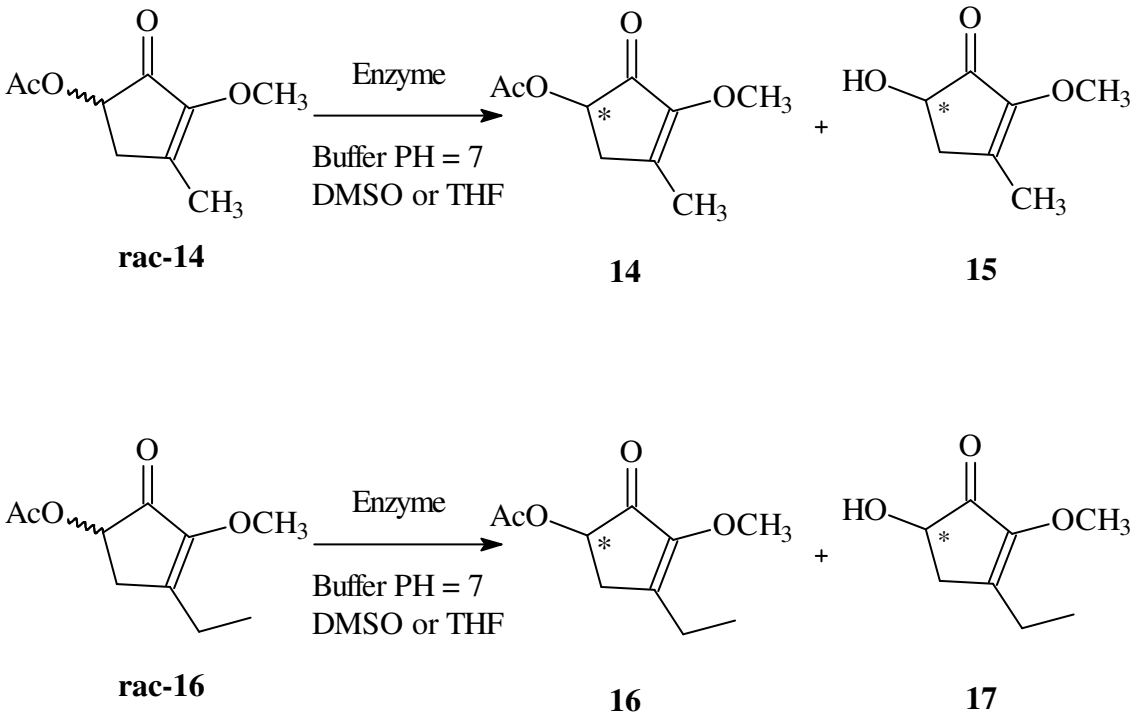
From the ^{1}H-NMR spectrum of **16**, we observed a triplet at 1.00 ppm (J=7.5 Hz) for the $CH_2\underline{C}H_3$ carbon and at 5.05 ppm dd, (J = 2.7, 6.7 Hz) for the α'-proton. From the ^{13}C-NMR spectrum, we observed a peak at 11.2 ppm for the $CH_2\underline{C}H_3$ carbon and a peak at 170.3 ppm for the $O\underline{C}OCH_3$ carbon.

The acetoxylation reactions of **18** and **19** gave **rac-14** and **rac-16** with high yields. However these yields could only be obtained when $Mn(OAc)_3$ was well dried. Also, the way of synthesizing $Mn(OAc)_3$ is important as its reactivity is effected.

2.3 Enzyme Mediated Hydrolysis of Acetoxy Ketones

Hydrolytic enzymes are one of the most important biocatalysts in organic synthesis. The most commonly used hydrolytic enzymes are; amidases, proteases, esterases and lipases. These enzymes catalyze the hydrolysis and formation of ester and amide bonds. Lipase-mediated resolutions of chiral alcohols, either by acyl transfer methods or by hydrolysis of their corresponding esters, are probably the most commonly described biotransformation in modern literature. Both the regio and enantioselectivity of biocatalyst makes the process even more attractive.[9]

In the enzymatic hydrolysis, **rac-14** and **16** were used to obtain chiral α'-acetoxy and α'-hydroxy enones (Scheme 18).



Scheme 18

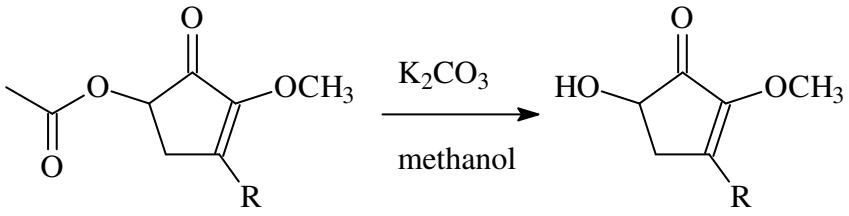
As to be understood from scheme 18, one enantiomer of **rac-14** was accepted as substrate by the enzyme whereas the other enantiomer was not accepted. That is because, the rate of the reaction with one enantiomer is much faster then the other one. Therefore the result is the non-racemic mixture of the starting material (the less reactive enantiomer) and the hydrolyzed product (more reactive enantiomer). Same things are also true for **rac-16.**

Firstly, to obtain the optimum conditions for the enzymatic hydrolysis of **rac-14** and **16,** we carried out the analytical screening. To do that, the reactions were performed in analytical scale. The lipases used for enantioselective hydrolysis of α-acetoxy enones include; Amano PS, Porcine Pacreatic Lipase (PPL), Candida Cylindracea Lipase (CCL), and Pig Liver Esterase (PLE). Because of the poor solubility of the substrate in aqueous

medium, a few milliliters of organic solvent was also necessary. Either DMSO or THF were used for this purpose. About 5 mg of acetoxy enone was dissolved in minimum amount of organic solvent. All reactions were carried out in phosphate buffer (pH=7) at room temperature. For 5 mg of acetoxy enone, 300-µL phosphate buffer was added. After that, the enzyme was added to the mixture and the mixture was stirred at room temperature. The reaction was monitored by TLC. At the point where approximately 50% conversion was observed, chloroform was added for terminating the reaction. Organic phase was separated from the water phase and the ee values were determined by HPLC. Many chiral columns were tried in HPLC for the best separation of the enantiomers. Chiralcel AD column was chosen as it was able to separate both enantiomers of α'-acetoxy and α'-hydroxy enones.

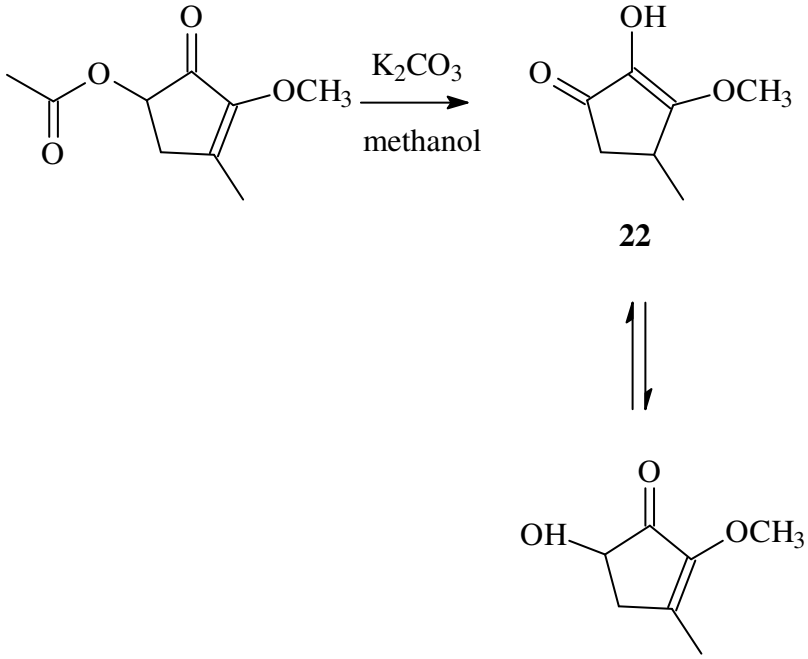
For both enantiomers of products, racemic forms of them were analyzed first in order to decide the R_f values. For **rac-14,** Rf for (-)-**14** was 38.31 min and Rf for (+)-**14** was 46.83 min (Figure 14). For **rac-16,** Rf for (-)-**16** was 24.81 min and Rf for (+)-**16** was 28.15 min (Figure 15).

Compounds **rac-14** and **rac-16** were also hydrolyzed chemically to completely understand which peak corresponds to the hydroxyl enone in the HPLC spectra. The chemical hydrolysis was done by using K_2CO_3 in methanol (Scheme 19).



Scheme 19

However, it was recognized that while **rac-16** gave the expected hydroxy enone, **rac-14** gave isomerized product **22** as the major product (Scheme 20).



Scheme 20

The HPLC spectrum of the racemic isomerized product **22** is shown in figure 16.

The isomerized product **22** was also characterized by using NMR spectroscopy. From the ^{1}H-NMR spectrum, we observed the characteristic peaks of a doublet from the –CH_3 group at 1.15 ppm (J=7.2Hz), a multiplet at 2.55 ppm for the CH- group and a singlet at 4.15 ppm for the CH_3O- group. From the ^{13}C-NMR spectrum, we observed a peak at 18.6 ppm for the CH_3 carbon, a peak at 30.8 ppm for CH carbon, a peak at 38.9 ppm for CH_2 carbon, and a peak at 59.1 for CH_3O- carbon. The double bond carbons gave peaks at 131.9 and 167.2 ppm and carbonyl carbon gave a peak at 199.2 ppm.

The best results for **rac-14** were obtained with Amano PS (in THF and DMSO) and CCL (in THF) for the high enantioselectivity of acetoxy enone and with CCL (in DMSO,THF), PLE and Amano PS (in DMSO) for the high enantioselectivity of hydroxyl enone (Table 1). CCL in THF was used for the preperative scale synthesis. Monitoring of the reactions with TLC furnished the (+)-**14** (87% ee) and (-)-**15** (85% ee).

Table 1. Enzymatic hydrolysis of (±)-**14**-5-acetoxy-3-methyl-2-methoxy-2-cyclopenten-1-one

Enzyme	Reaction time (h)	Solvent	Conversion (%)	Acetate		Alcohol		E
				ee %	Yield %	ee %	Yield %	
CCL	6	DMSO	14	15		96		56
PLE	41	DMSO	43	63		83		20
Amano PS	1	DMSO	51	87	46	85	43	34
CCL	21	THF	51	87	47	82	42	28
Amano PS	1	THF	56	87		67		13
PLE	82	THF	56	32		25		2.2

The E value in table 1 stands for the enantiomeric ratio. The enantiomeric ratio is the ratio of the percentage of one enantiomer in a mixture to that of the other one. If we suppose that A and B are the fast and slow reacting enantiomers that compete for the same site on the enzyme and VA, KA and VB, KB denote maximal velocities and Michaelis constants of the fast- and slow-reacting enantiomers, respectively, the enantiomeric ratio, E is dependent on the ratio of the specificity constants (V/K) and is independent of substrate concentrations. The E value can be calculated using the following formula.[39]

$$\frac{\ln [1-c(1+ee(P))]}{\ln [1-c(1-ee(P))]} = \frac{VA/KA}{VB/KB} = E$$

Where c is the extent conversion, ee(P) is the enantiomeric excess of the product fraction and and V/K is the ratio of the specificity constants.

The best results for **rac-16** were obtained with CCL in DMSO and PLE in THF for high enantioselectivity of hydroxyl enone and with PLE in DMSO for high enantioselectivity of acetoxy enone (Table 2). PLE in DMSO and THF was used for the preperative scale synthesis of acetoxy enone. Monitoring of the reactions with TLC furnished the (+)-**16** (93% ee) and (+)-**17** (95% ee). Chiralcel AD column was also suitable for this separation.

Table 2. Enzymatic hydrolysis of (±)-**16**-5-acetoxy-3-ethyl-2-methoxy-2-cyclopenten-1-one

Enzyme	Reaction time (min)	Solvent	Conversion c[a] (%)	Acetate		Alcohol		E
				ee %	Yield %	ee %	Yield %	
CCL	85	DMSO	44	79		99		>200
PLE	85	DMSO	64	93	50	52	47	10
Amano PS	25	DMSO	60	61		41		4.3
PPL	85	DMSO	39	50		79		13
PLE	110	THF	35	51	42	95	47	64
Amano PS	25	THF	58	75		55		7.5
PPL	52 h	THF	30	12		28		2

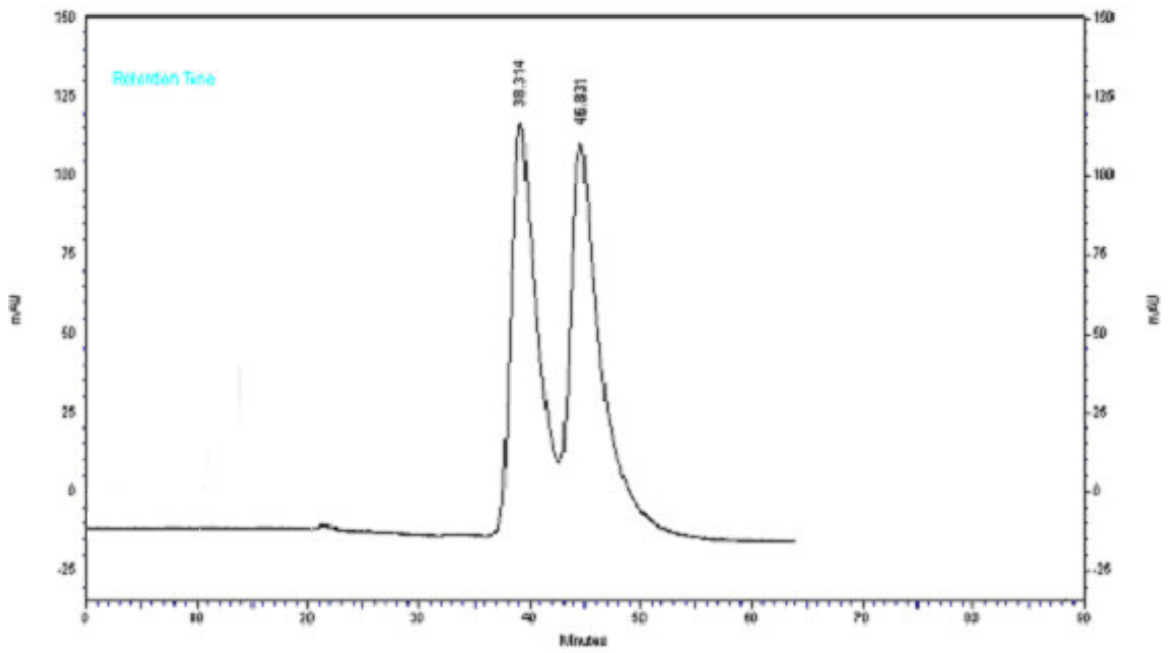


Figure 14: Chiral HPLC chromatogram of racemic α'-acetoxy enone (±)-**14**

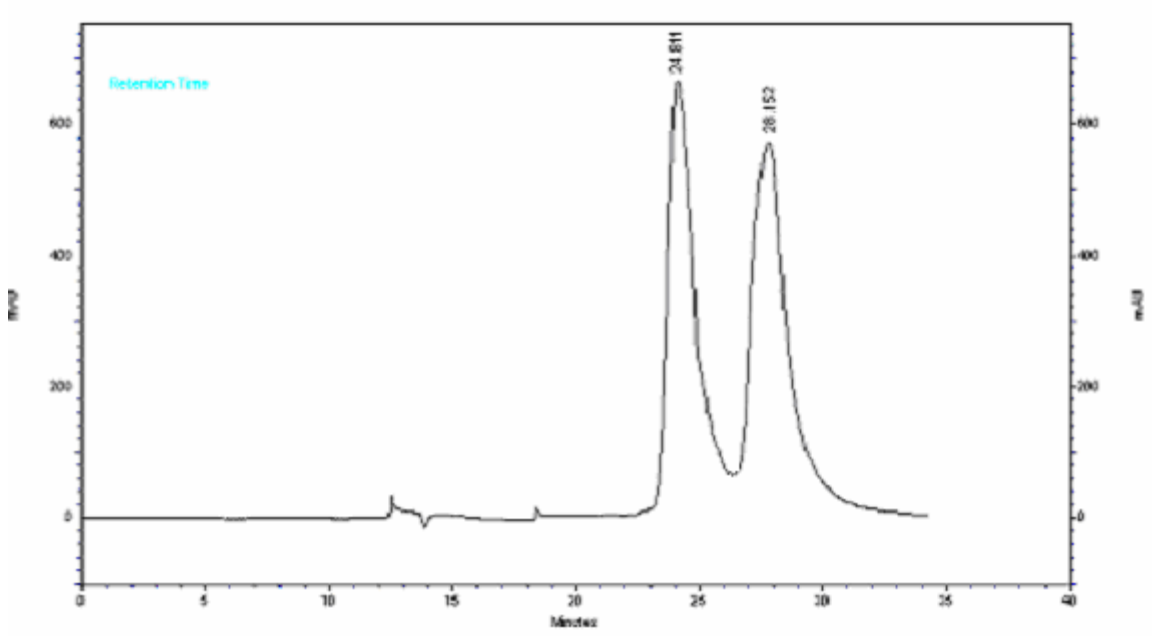


Figure 15: Chiral HPLC chromatogram of racemic α'-acetoxy enone (±)-**16**

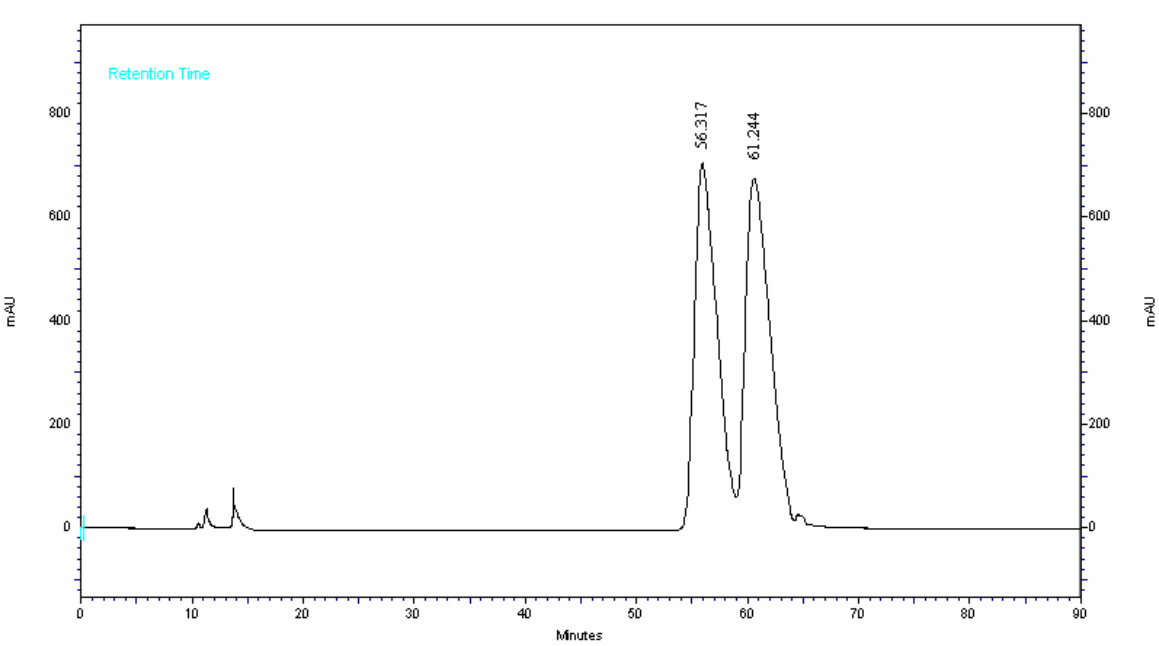


Figure 16: Chiral HPLC chromatogram of the isomerized product, **rac-22**.

After that, all of the enzymes were analyzed and the enantiomeric excess values were determined with HPLC (For **rac-14;** Chiralcel AD column, eluent: hexane/2-propanol=98:2, flow 0.3 mLmin-1, 20°C and for **rac-16;** Chiralcel AD column, eluent: hexane/2-propanol=96:4, flow 0.3 mLmin-1, 20°C) by using peak area %'s of the enantiomers.

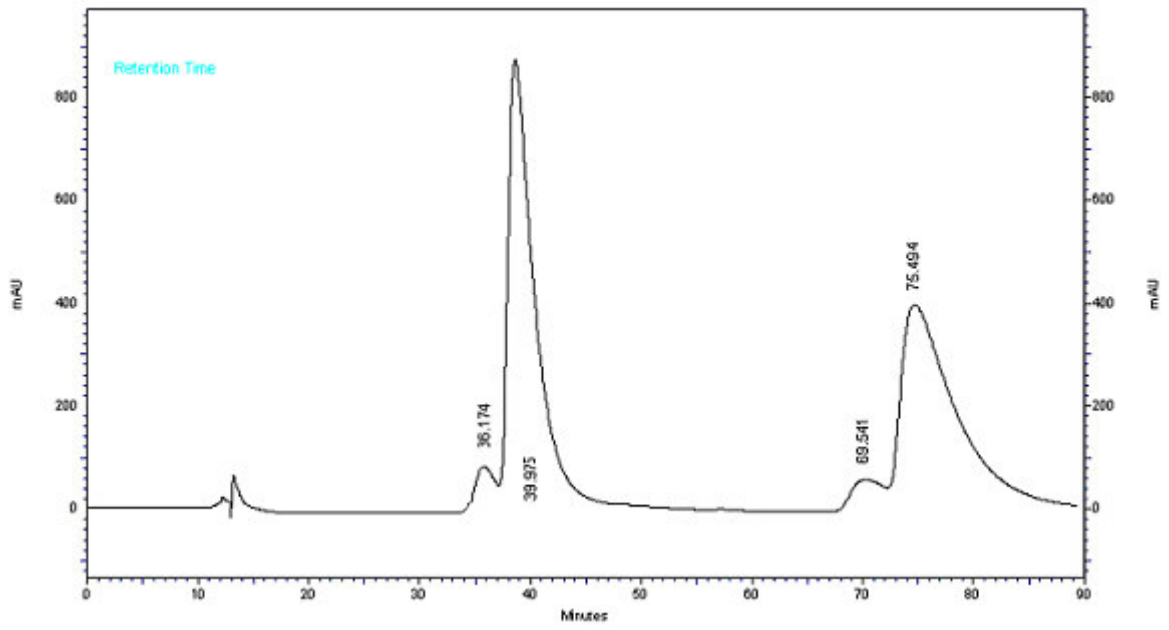


Figure 17: HPLC results for the CCL catalyzed hydrolysis of **rac-14** in THF

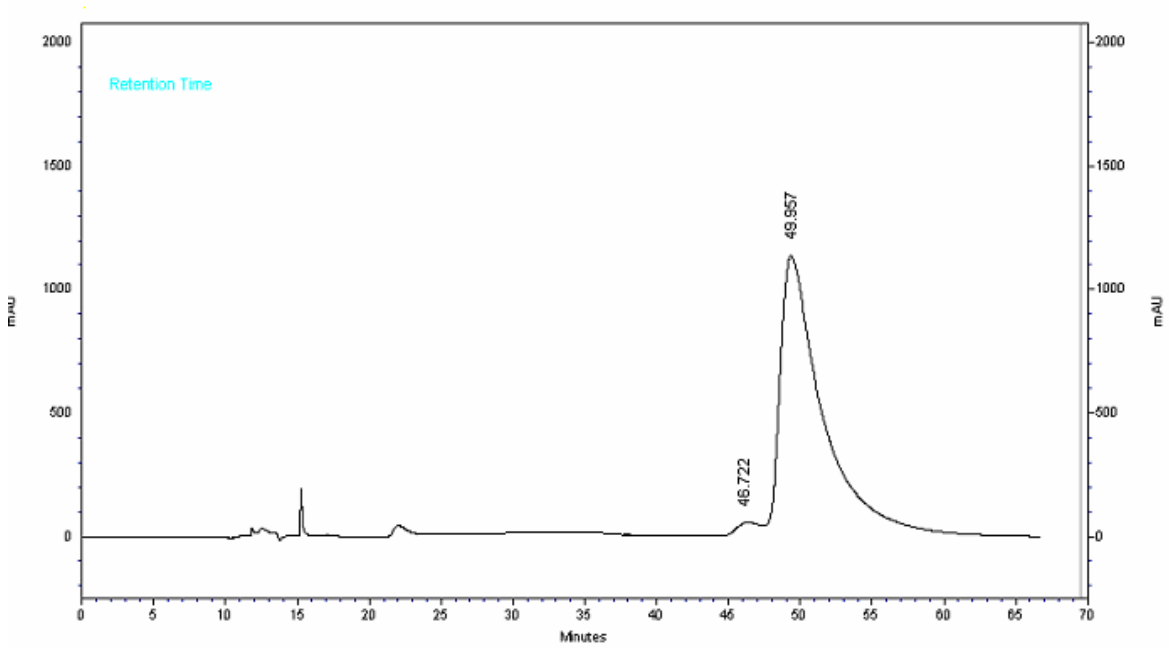


Figure 18: HPLC results for the PLE catalyzed hydrolysis of **rac-16** in THF

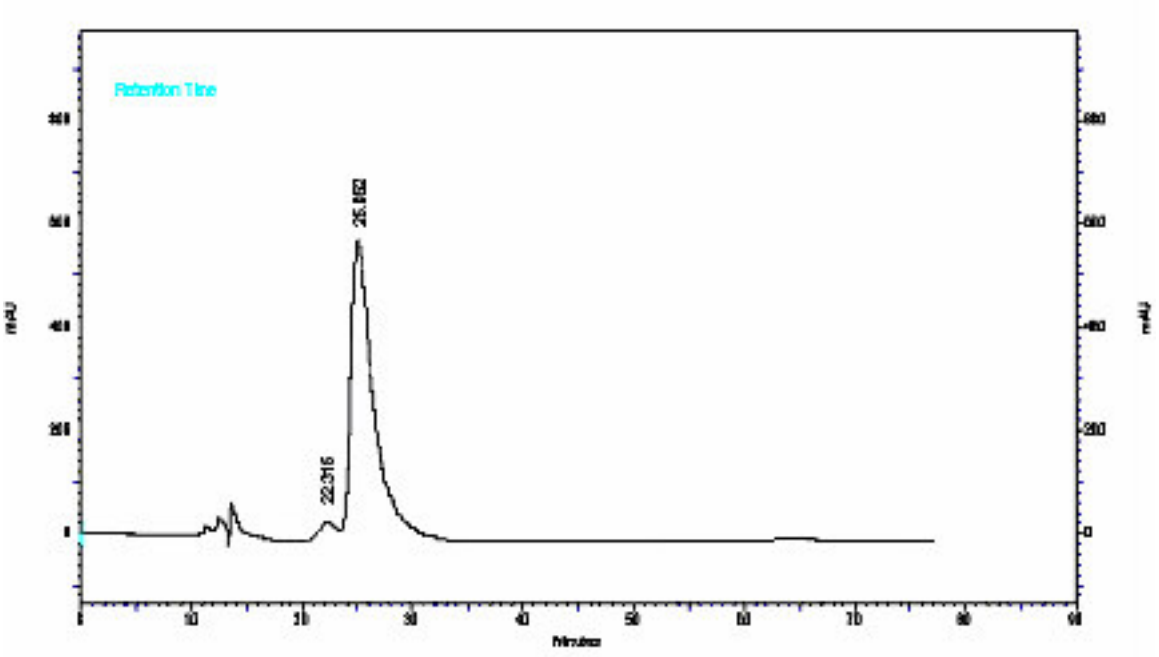


Figure 19: HPLC results for the PLE catalyzed hydrolysis of **rac-16** in DMSO**.**

The products were identified by using NMR spectroscopy. From the ^{1}H-NMR spectrum of (+)-**14**, we observed a singlet at 1.89 ppm from the –CH_3 group and at 5.00 ppm dd, (J=2.6 and 6.8 Hz) for the α-proton. From the ^{13}C-NMR spectrum, we observed a peak at 13.8 ppm for the CH_3 carbon and a peak at 169.1 ppm for the O<u>C</u>OCH_3 carbon. From the ^{1}H-NMR spectrum of (-)-**15**, we observed a singlet at 1.90 ppm from the –CH_3 group and at 4.05 ppm dd, (J=7.1 and 19.4 Hz) for the α-proton. From the ^{13}C-NMR spectrum, we observed a peak at 70.0 ppm for the for –CH-OH carbon.

From the ^{1}H-NMR spectrum of (+)-**16**, we observed a triplet at 1.00 ppm (J=7.5 Hz) for the CH_2<u>C</u>H_3 carbon and at 5.05 ppm dd, (J = 2.7, 6.7 Hz) for the α-proton. From the ^{13}C-NMR spectrum, we observed a peak at 11.2 ppm for the CH_2<u>C</u>H_3 carbon and a peak at 170.3 ppm for the O<u>C</u>OCH_3 carbon. From the ^{1}H-NMR spectrum of (+)-**17,** we observed a triplet at 1.05 ppm (J=7.5 Hz) for the CH_2<u>C</u>H_3 carbon and at 4.02 ppm dd, (J=2.5, 6.5 Hz) for the α-proton. From the ^{13}C-NMR spectrum, we observed a peak at 69.9 ppm for the for –CH-OH carbon.

2.4 Summary of Chemoenzymatic Synthesis of α'-Acetoxy and α'-Hydroxy Enones

In summary, this work describes here as the model study for the first efficient synthesis of **14, 15, 16** and **17** from the enantioselective hydrolysis of **rac-14** and **rac-16.**

Firstly, the commercially available 3-methyl-1,2-cyclopentanedione and 3-ethyl-1,2-cyclopentanedione which stay in the enol form exclusively were converted to 5-hydroxy-3-methyl-2-methoxy-2 cyclopentene-1-one and 5-hydroxy-3-ethyl-2-methoxy-2-cyclopentene-1-one respectively. After that, with three equivalents of manganese (III) acetate (in benzene), acetoxylation reactions were performed to obtain the desired α'-acetoxy ketones.

Before the enzymatic hydrolysis, **rac-14** and **rac-16** were analyzed first to decide the correct R_f values. In addition the racemic compounds were also hydrolyzed chemically to understand the peak of the hydroxyl enone in the HPLC spectra. An interesting observation was the chemically hydrolysis of **rac-14** as the main product was the isomerized product **22** instead of the expected hydroxyl enone.

The enzymatic hydrolysis of the acetates **rac-14** and **rac-16**, in aqueous organic medium furnished **14, 15, 16** and **17** in high ee. The best results for **rac-14** were obtained with Amano PS (in THF and DMSO) (87% ee) and CCL (in THF) (87% ee) for the high enantioselectivity of acetoxy enone and with CCL (in DMSO, THF) (96% ee and 82% ee respectively), PLE and Amano PS (in DMSO) (83% ee and 85% ee respectively) for the high enantioselectivity of hydroxyl enone. The best results for **rac-16** were obtained with CCL in DMSO (99% ee) and PLE in THF (95% ee) for high enantioselectivity of the hydroxyl enone and with PLE in DMSO (93% ee) for high enantioselectivity of acetoxy enone. The yields were around 50% which means that no side products were observed.

These results show that, this method provides a simple new entry for the synthesis of cyclic hydroxy enones which are important precursors for pharmacologically interesting compounds.

As the absolute configurations of the chiral compounds studied are unknown the studies in this field will continue. There are many ways to find the absolute configuration of the stereogenic centers such as the X-ray determination, vibrational circular dichroism and enzymatic resolutions.[40] One way is the determination in the presence of a chiral solvating agent by low temperature and low concentration ^{1}H-NMR analysis.[41]

In addition, the formation of the isomerized product as the major product in the chemical hydrolysis of **rac-14** is highly attractive as such thing does not happen for **rac-16**. So the studies on these formations will also take place as a future work.

CHAPTER 3

EXPERIMENTAL

3.1 Materials and Methods

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

TLC was carried out on aluminum sheets precoated with silica gel $60F_{254}$ (Merck), and the spots were visualized with UV light (λ= 254 nm).

Flash column chromatographies were done for purifying the products by using silica gel 60 (partical size 40-63 μm).

Optical rotations were measured with a Kruss P3002RS automatic polarimeter. Enantiomeric excesses were determined by HPLC analysis using a Thermo Quest (TSP) equipped with an appropriate column packed with an optically active material.

3.2 General Procedures

3.2.1 Synthesis of Protected Cyclic 1,2-Diketones

3.2.1.1 Synthesis of 2-Methoxy-3-Methyl-2-Cyclopenten-1-one (18)

To a well stirred solution of 3-methyl-1,2-cyclopentanedione, **19** (1.0 g, 8.91 mmol) in dry acetone (75 ml) containing anhydrous potassium carbonate (1.1g, 7.97 mmol), 0.75 ml $(CH_3)_2SO_4$ was added dropwise during 30 min, maintained under gentle reflux. The mixture was then heated under gentle reflux for 2 h and the reaction was monitored by TLC. For the work up the mixture was cooled and filtered. Evaporation of the acetone left a residue which was purified by column chromatography (Silica gel, EtOAc/Hexane 1:1).

The product was isolated as a yellow oil after column chromatography with 89% yield.

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

δ (ppm): 1.85 (s, 3H)
2.20 (m, 2H)
2.30 (m, 2H)
3.78 (s, 3H)

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

δ (ppm): 202.3, 153.4, 152.6, 57.9, 32.9, 27.0, 14.6

3.2.1.2 Synthesis of 2-Methoxy-3-Ethyl-2-Cyclopenten-1-one (20)

To a well stirred solution of 3-ethyl-1,2-cyclopentanedione, **21** (1.0 g, 7.93 mmol) in dry acetone (75 ml) containing anhydrous potassium carbonate (1.1g, 7.97 mmol), 0.75 ml $(CH_3)_2SO_4$ was added dropwise during 30 min, maintained under gentle reflux. The mixture was then heated under gentle reflux for 2 h and the reaction was monitored by TLC. For the work up the mixture was cooled and filtered. Evaporation of the acetone left a residue which was purified by column chromatography (Silica gel, EtOAc/Hexane 1:1).

The product was isolated as a colorless oil after column chromatography with 80% yield.

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

δ (ppm): 1.08 (t, 7.8 Hz, 3H)
2.28 (m, 2H)
2.36 (m, 2H)
2.40 (m, 2H)
3.82 (s, 3H)

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

δ (ppm): 202.6, 158.0, 152.2, 58.1, 32.9, 24.4, 21.9, 11.6

3.2.2 Synthesis of Acetoxy Ketones

3.2.2.1 Synthesis of Manganese (III) Acetate

$Mn(OAc)_2.4H_2O$ (19.6 g, 113mmol) was added to glacial acetic acid (200ml) and stirred for 1 h at R.T. To the well stirred mixture, powdered $KMnO_4$ (3.1g, 19.5mmol) was added in small portions. The mixture was stirred for another 1 h and distilled water (3ml) was added. After 48 h the cinnamon brown crystalline precipitate was collected on a glass filter and washed with glacial acetic acid (100 ml). $Mn(OAc)_3$ was then dried over P_2O_5 under high vacuum to remove water. $Mn(OAc)_3$ was further dried in a heating gun (refluxing xylene) under high vacuum to obtain a dark brown colored $Mn(OAc)_3$.

3.2.2.2 Synthesis of 5-Acetoxy -3-Methyl-2-Methoxy-2-Cyclopentene-1-one (14)

A solution of **18** (1.0g, 7.93 mmol) and $Mn(OAc)_3$ (5.7g, 24.0 mmol) in benzene/AcOH (10:1) (200 mL) were heated under reflux (using Dean-Stark apparatus) for 4 days. The reaction was monitored by TLC. After all starting material was consumed, the reaction mixture was cooled and diluted with ether, filtered; then washed with saturated $NaHCO_3$ solution. The solution was dried over $MgSO_4$, concentrated under vacuum and purified by column chromatography (1:2 EtOAc: Hexane), to yield 88% of racemic **14** as a yellow oil.

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

δ (ppm): 1.89 (s, 3H, CH_3)
2.08 (s, 3H, $COCH_3$)
2.24 (d, J=17.4 Hz, 1H, H-4)
2.76 (dd, J=6.7, 17.5 Hz, 1H, H-4)
3.85 (s, 3H, OCH_3)
5.00 (dd, J=2.59, 6.76 Hz, H-5)

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

δ (ppm) 195.7, 169.1, 150.0, 149.5, 69.3, 57.2, 34.2, 19.7, 13.8.

3.2.2.3 Synthesis of 5-Acetoxy -3-Ethyl-2-Methoxy-2-Cyclopentene-1-one (16)

A solution of **20** (1.0g, 7.14 mmol) and $Mn(OAc)_3$ (5.2g, 22.0 mmol) in benzene/AcOH (10:1) (200 mL) were heated under reflux (using Dean-Stark apparatus) for 4 days. The reaction was monitored by TLC. After all starting material was consumed, the reaction mixture was cooled and diluted with ether, filtered; then washed with saturated $NaHCO_3$ solution. The solution was dried over $MgSO_4$, concentrated under vacuum and purified by column chromatography (1:2 EtOAc: Hexane), to yield 89% of racemic **16** as a yellow oil.

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

δ (ppm): 1.00 (t, J=7.5 Hz, 3H, CH_3)
2.08 (s, 3H, $COCH_3$)
2.30 (d, J = 7.3 Hz, 1H, H-4)
2.40 (m, 2H, CH_2)
2.88 (dd, J = 6.7, 17.7 Hz, 1H, H-4)
3.80 (s, 3H, OCH_3)
5.05 (dd, J = 2.7, 6.7 Hz, H-5)

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

δ (ppm) 197.3, 170.3, 156.3, 150.0, 70.2, 58.2, 32.5, 21.7, 20.7, 11.2.

3.2.3 Chemical Hydrolysis

3.2.3.1 Chemical Hydrolysis of 5-Acetoxy-3-Methyl-2-Methoxy-2-Cyclopentene-1-one (14)

To a stirred solution of **rac-14** (100mg, 0.54 mmol) in methanol (20 ml), K_2CO_3 (140mg, 1.0 mmol) was added and the mixture was stirred for 2h at R.T. The reaction was monitored by TLC. For the work up, methanol was evaporated and the mixture was extracted with ether. Purification was done by column chromatography (1:1 EtOAc: Hexane), to yield 89% of the isomerized product **rac-22** as the main product.

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

δ (ppm): 1.15 (d, J=7.2 Hz, 3H)
1.90 (d, J=18 Hz, 1H)
2.55 (m, 1H)
2.65 (m, 1H)
4.15 (s, 3H)
5.80 (s, OH)

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

δ (ppm) 199.2, 167.2, 132.0, 59.1, 38.9, 30.8, 18.6.

3.2.3.2 Chemical Hydrolysis of 5-Acetoxy-3-Ethyl-2-Methoxy-2-Cyclopentene-1-one (16)

To a stirred solution of **rac-16** (100mg, 0.50 mmol) in methanol (20 ml), K_2CO_3 (140mg, 1.0 mmol) was added and the mixture was stirred for 2h at R.T. The reaction was monitored by TLC. For the work up, methanol was evaporated and the mixture was extracted with ether. Purification was done by column chromatography (1:1 EtOAc: Hexane), to yield 87% of **rac-17**.

3.2.4 Enzyme Catalyzed Kinetic Resolution

3.2.4.1 Enzyme Catalyzed Hydrolysis of 5-Acetoxy-3-Methyl-2-Methoxy-2-Cyclopentene-1-one (14)

To a stirred solution of 5-acetoxy-3-methyl-2-methoxy-2-cyclopentene-1-one**, rac-14,** (100mg, 0.54 mmol) in either DMSO or THF (1 ml) and phosphate buffer (pH 7.0, 30 mL) enzyme (Candida Cylindracea Lipase 100-200 mg) was added in one portion and the reaction mixture was stirred at R.T. Conversion was monitored by TLC and when 50% conversion was established, the reaction was terminated by adding $CHCl_3$ (20 ml). The organic layer was separated, dried over $MgSO_4$, and concentrated.

The unreacted acetate (+)-**14** and the product (-)-**15** were seperated by column chromatography (1:1 EtOAc: Hexane). The product was isolated as a white solid with 42% yield.

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

δ (ppm): 1.90 (s, 3H, CH_3)
2.30 (d, J = 17.2, 1H, H-4)
2.70 (dd, J = 6.55, 17.3 Hz, 1H, H-4)
3.50 (s, OH)
3.85 (s, 3H, OCH_3)
4.05 (dd, J = 7.07, 19.4 Hz, 1H, H-5)

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

δ (ppm): 202.7, 151.0, 150.2, 70.0, 58.0, 36.8, 14.9.

The ee's of the acetate and the alcohol were determined by HPLC using chiralcel AD column.

Chiralcell AD column, UV detection at 254 nm, eluent: hexane/2-propanol= 98:2, flow 0.3 mL min^{-1}, 20°C, R_f : for (-)-**14:** 38.31 min, (+)-**14:** 46.83 min, $[\alpha]_D^{20}$ = + 1.06 (c=0.6 $CHCl_3$); R_f for (-)-**15:** 75.48 min, (+)-**15** 69.53 min, $[\alpha]_D^{20}$ = - 0.96 (c=0.6, $CHCl_3$).

3.2.4.2 Enzyme Catalyzed Hydrolysis of 5-Acetoxy-3-Ethyl-2-Methoxy-2-Cyclopentene-1-one (16)

To a stirred solution of 5-acetoxy-3-ethyl-2-methoxy-2-cyclopentene-1-one**, rac-16,** (100mg, 0.50 mmol) in either DMSO or THF (1 ml) and phosphate buffer (pH 7.0, 30 mL) enzyme (Pig liver esterase 100-200 mg) was added in one portion and the reaction mixture was stirred at R.T. Conversion was monitored by TLC and when 50% conversion was established, the reaction was terminated by adding $CHCl_3$ (20 ml). The organic layer was separated, dried over $MgSO_4$, and concentrated.

The unreacted acetate (+)-**16** and the product (+)-**17** were seperated by column chromatography (1:1 EtOAc: Hexane). The product was isolated as a colorless oil with 47% yield.

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

δ (ppm): 1.05 (t, J = 7.5 Hz, 3H, CH_3)
2.28 (dd, J = 6.6, 17.3 Hz, 1H, H-4)
2.40 (m, 2H, CH_2)
2.70 (dd, J=6.5, 17.3 Hz, 1 H, H-4)
3.65 (s, OH)
3.80 (s, 3H, OCH_3)
4.02 (dd, J = 2.5, 6.5 Hz, 1H, H-5)

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

δ (ppm) 202.9, 156.1, 149.2, 69.9, 57.8, 33.9, 21.8, 11.3.

The ee's of the acetate and the alcohol were determined by HPLC using chiralcel AD column.

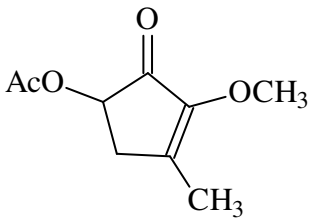
Chiralcell AD column, UV detection at 254 nm, eluent: hexane/2-propanol= 96:4, flow 0.3 mL min^{-1}, 20°C, R_f : for (-)-**16:** 24.81 min, (+)-**16:** 28.15 min, $[\alpha]_D^{20}$ = +3.2 (c=0.5 $CHCl_3$); R_f for (-)-**17:** 46.72 min, (+)-**17** 49.96 min, $[\alpha]_D^{20}$ = +1.5 (c=0.4 $CHCl_3$).

CHAPTER 4

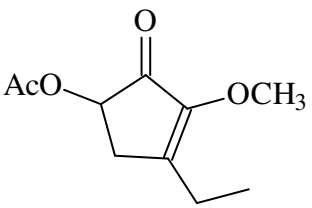
CONCLUSION

Poly oxygenated cyclopentane derivatives are important class of molecules for the construction of chiral organic compounds due to reactive functional groups. These functional groups can be transformed to other functional groups which make them potential building blocks in the synthesis of complex molecules. They are the structural units found in a number of biologically active compounds. Some examples are pentenomycin and metylenomycin which are known as antibiotics, sarkomycin, clavulone and punaglandin which are known to exhibit antitumor activity, prostaglandins which are used in the therapeutic applications in the field of gynaecology of unwanted pregnancy and neplanocin A which is used as a potent antitumor agent.

A new and efficient chemoenzymatic route is developed for polyoxo cyclopentanones. Compounds **14** and **16** are chosen as a model study as they have not been synthesized before in the enantiomerically pure form.



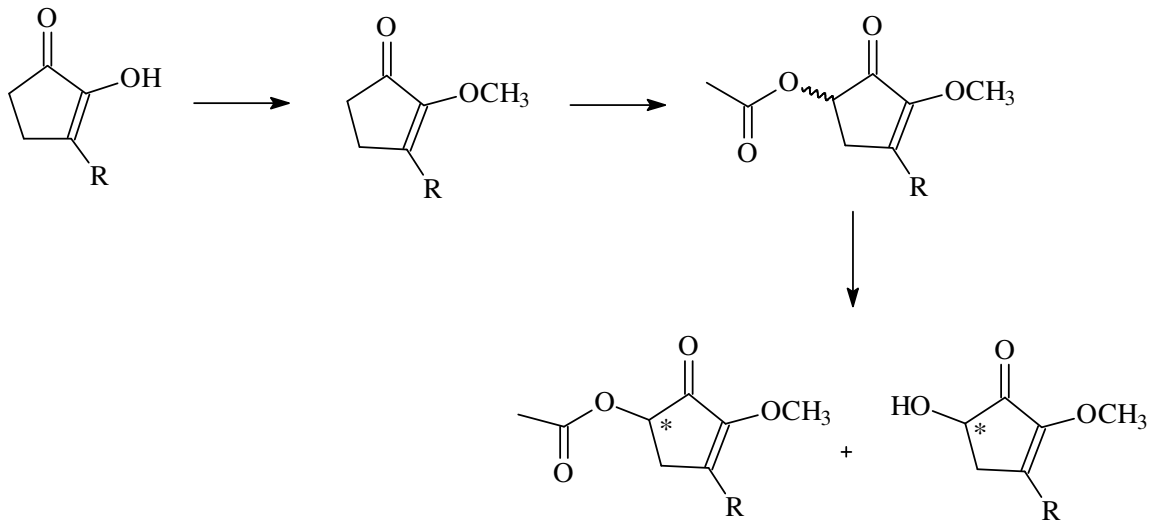
14



16

Figure 20: Polyoxo cyclopentanones

The 3-substituted 1,2-diketone is first protected by a methylation reagent. Protected enone is converted to its acetoxy derivative using $Mn(OAc)_3$ in good yield. The acetoxy enone is then converted to chiral α'-hydroxy enone by using different lipases as biocatalysts with high enantiomeric excesses (upto 99%) in good yields. For **rac-14,** the best results were obtained with Amano PS (in THF and DMSO) and CCL (in THF) for the high enantioselectivity of acetoxy enone and with CCL (in DMSO, THF), PLE and Amano PS (in DMSO) for the high enantioselectivity of hydroxyl enone. The best results for **rac-16** were obtained with CCL in DMSO and PLE in THF for high enantioselectivity of hydroxyl enone and with PLE in DMSO for high enantioselectivity of acetoxy enone.



Scheme 21

These results show that manganese (III) acetate mediated acetoxylation of protected 1,2-diketones followed by enzyme mediated hydrolysis of the acetoxy group provides hydroxyl enones and acetoxy enones with high enantiomeric excesses and in good chemical yields. So this method provides a new entry to the synthesis of cyclic enones which are used in the synthesis of many pharmacologically important compounds.

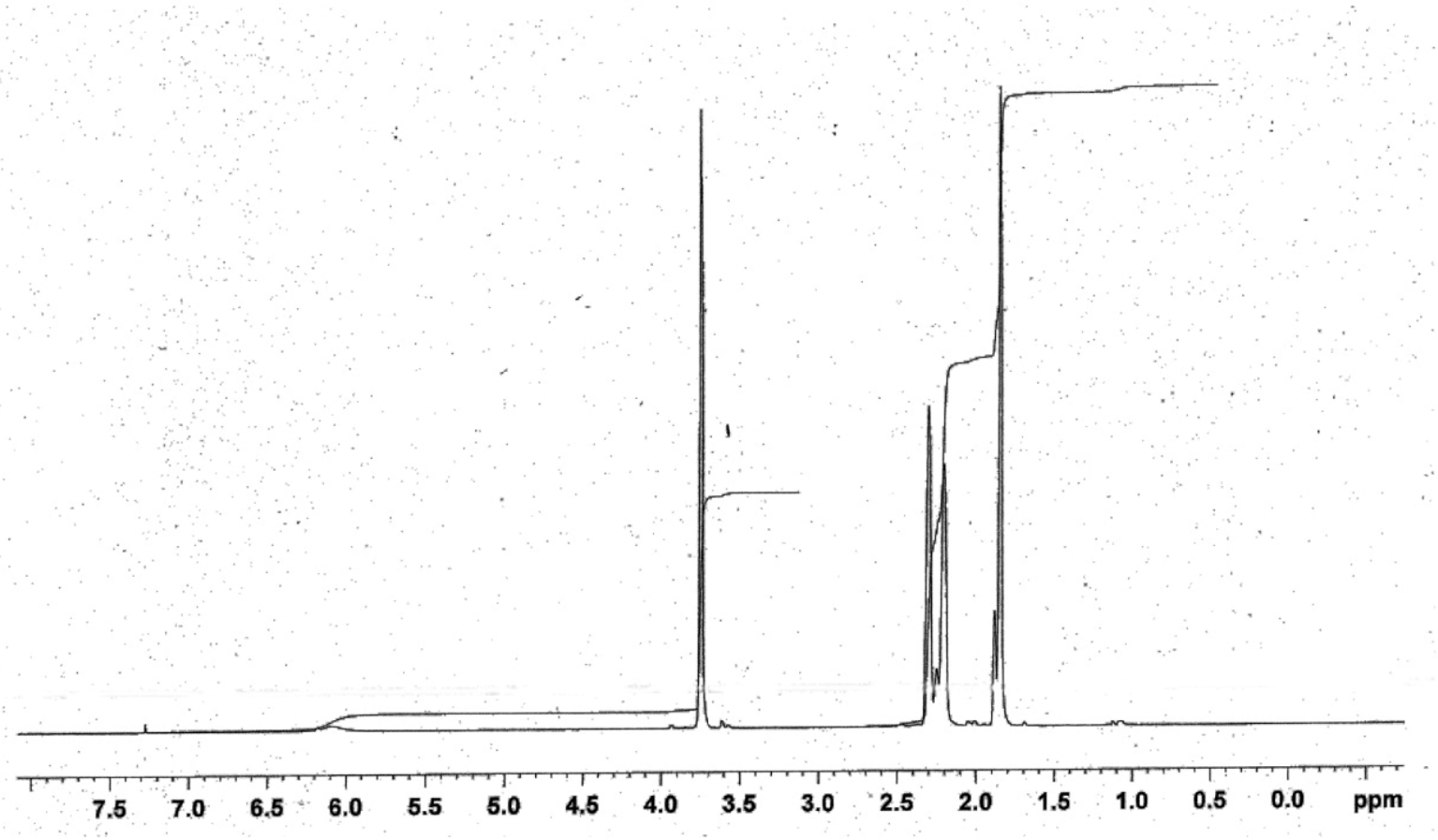


Figure 21: ^{1}H-NMR spectrum of 2-methoxy-3-methyl-2-cyclopenten-1-one (**18**)

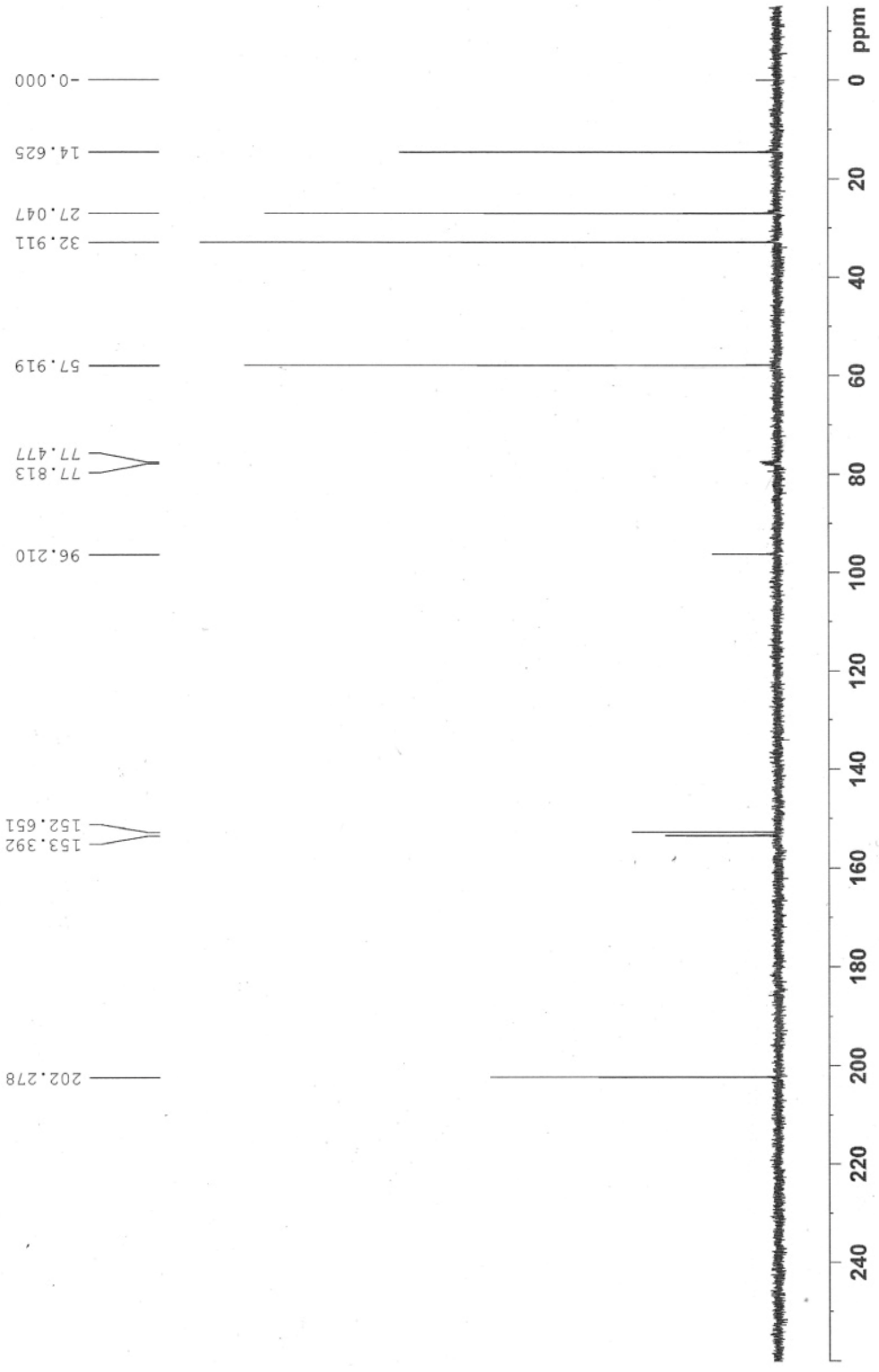


Figure 22: ^{13}C-NMR spectrum of 2-methoxy-3-methyl-2-cyclopenten-1-one (**18**)

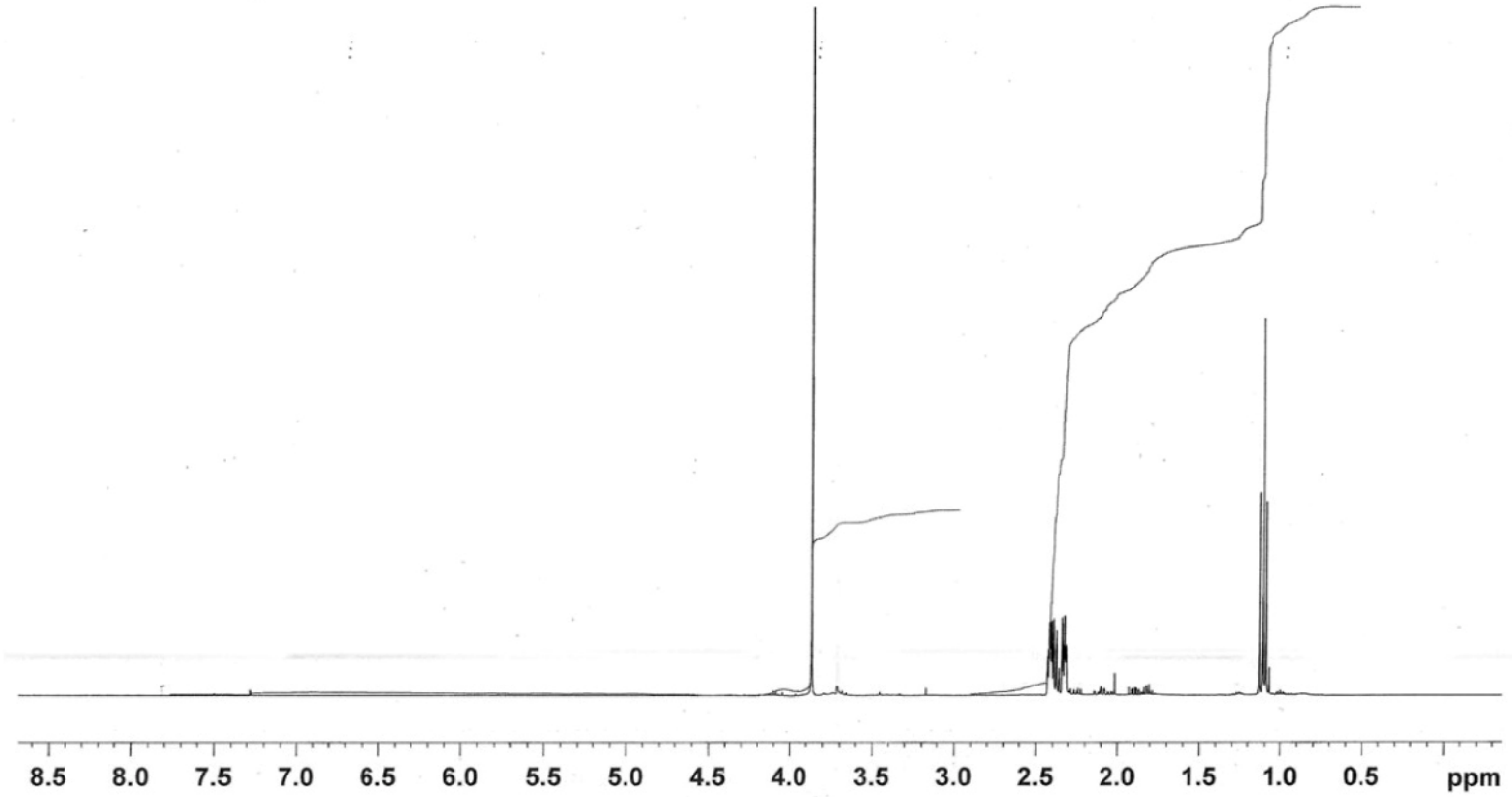


Figure 23: ^{1}H-NMR spectrum of 2-methoxy-3-ethyl-2-cyclopenten-1-one (**20**)

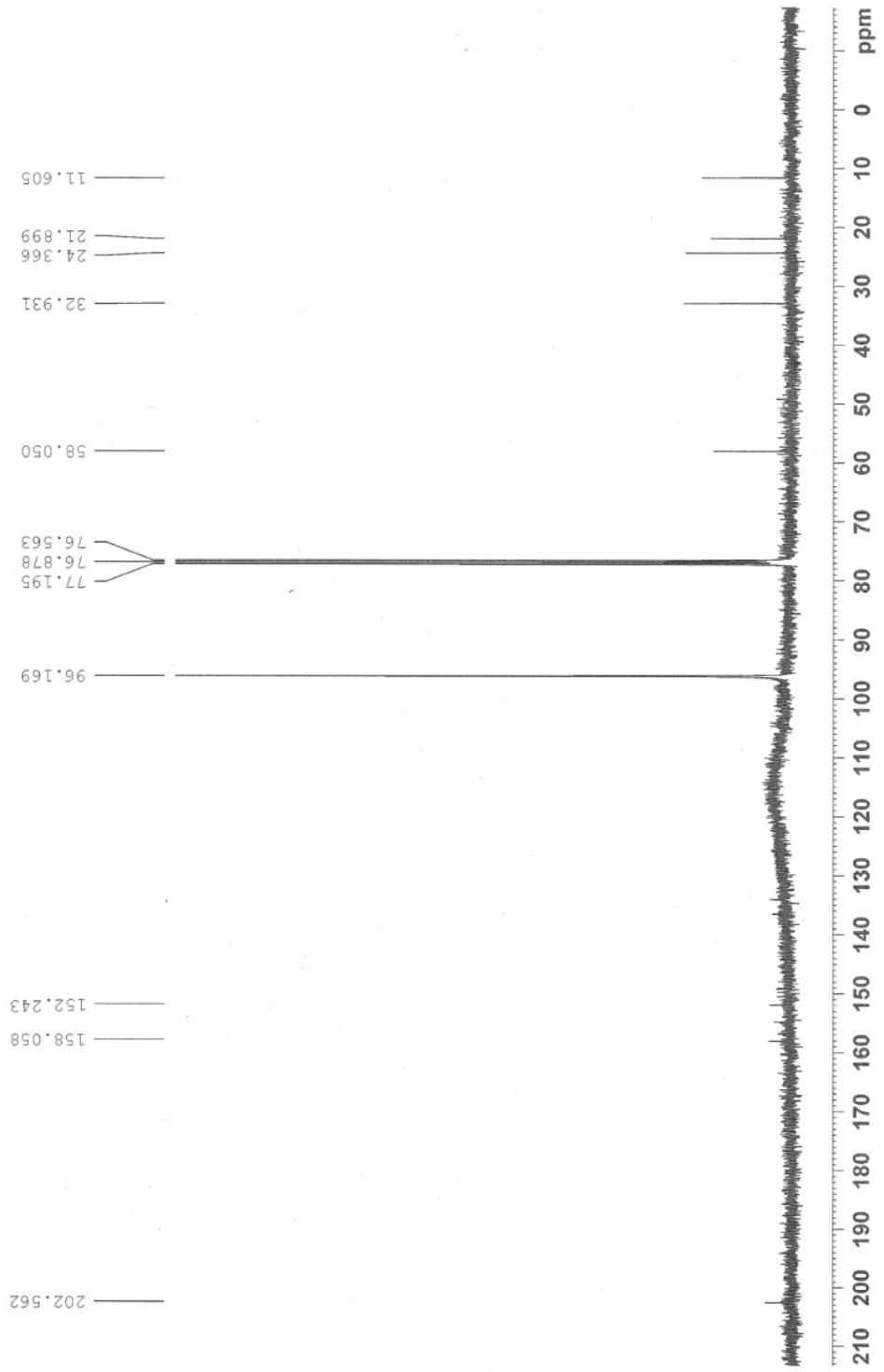


Figure 24: ^{13}C-NMR spectrum of 2-methoxy-3-ethyl-2-cyclopenten-1-one (**20**)

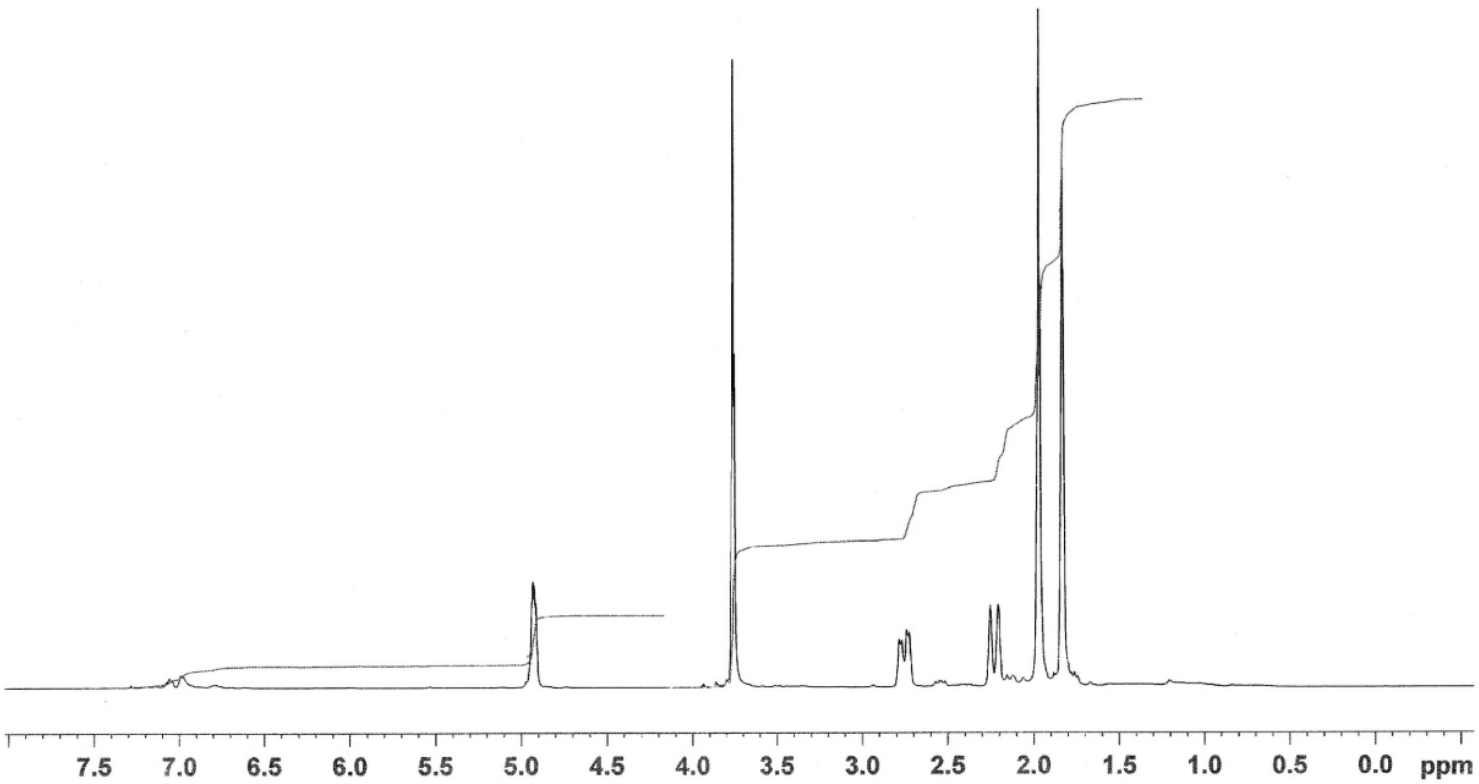


Figure 25: ^{1}H-NMR spectrum of 5-acetoxy -3-methyl-2-methoxy-2-cyclopentene-1-one (**14**)

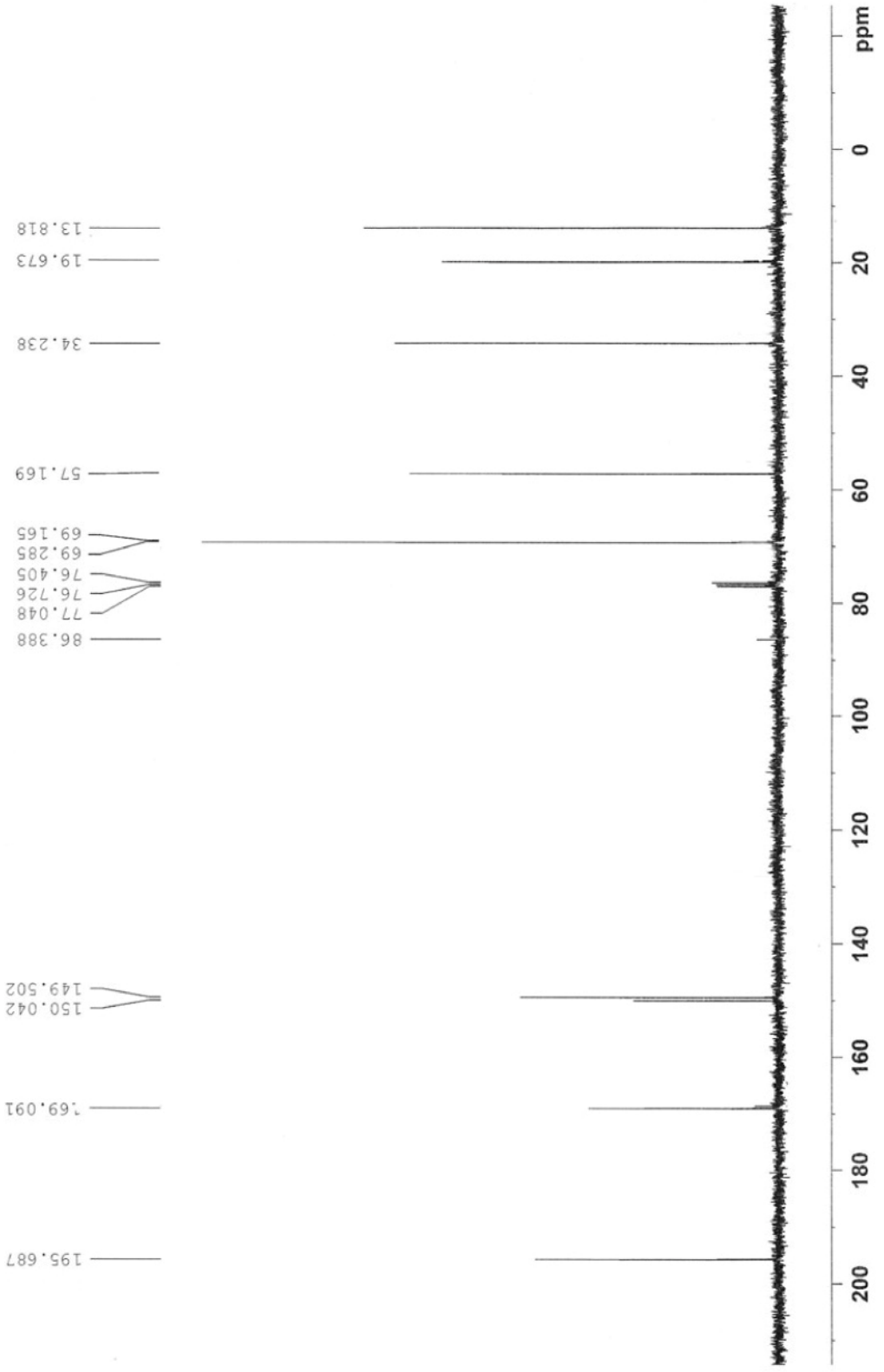


Figure 26: ^{13}C-NMR spectrum of 5-acetoxy -3-methyl-2-methoxy-2-cyclopentene-1-one (**14**)

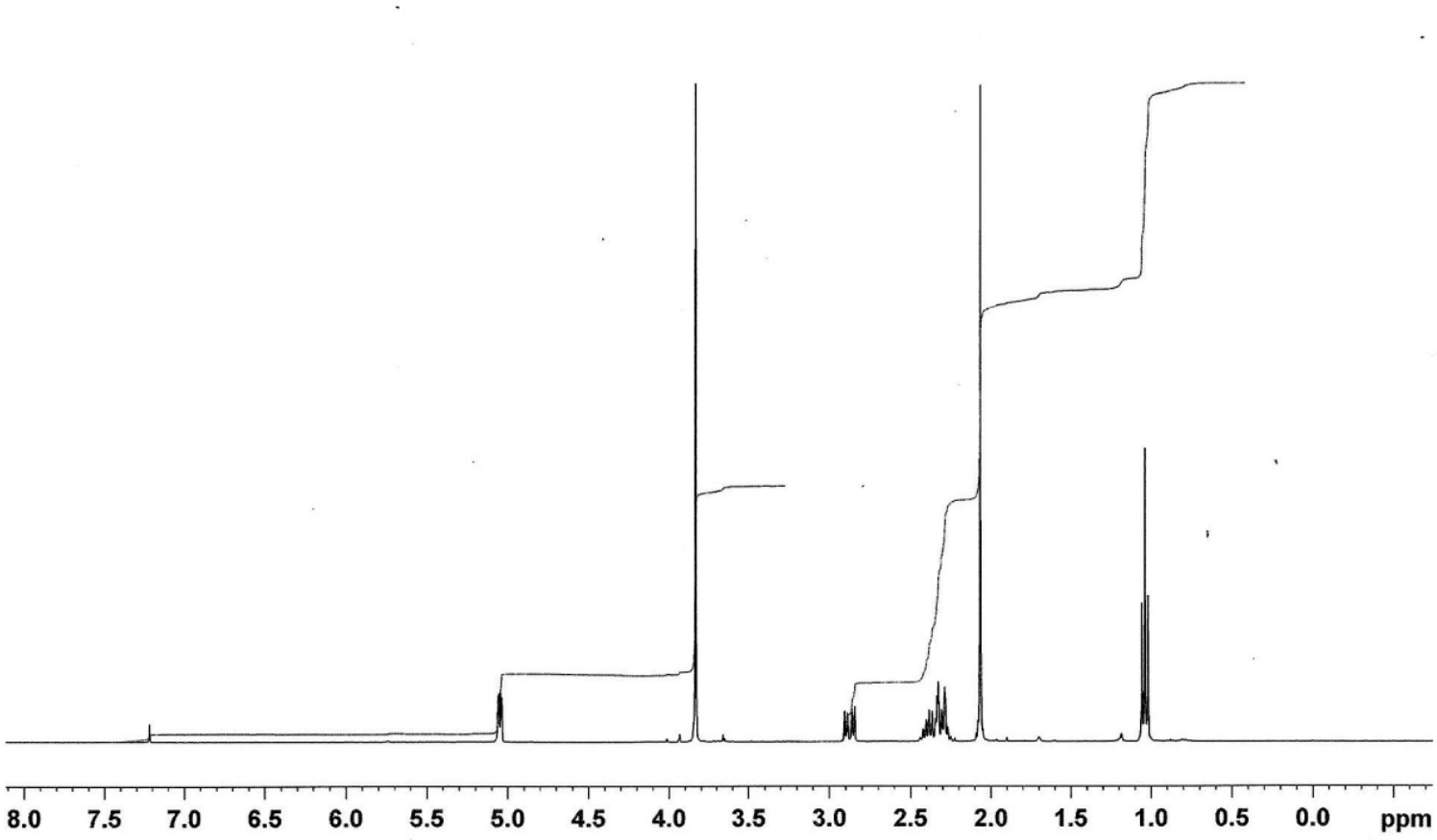


Figure 27: ^{1}H-NMR spectrum of 5-acetoxy -3-ethyl-2-methoxy-2-cyclopentene-1-one (**16**)

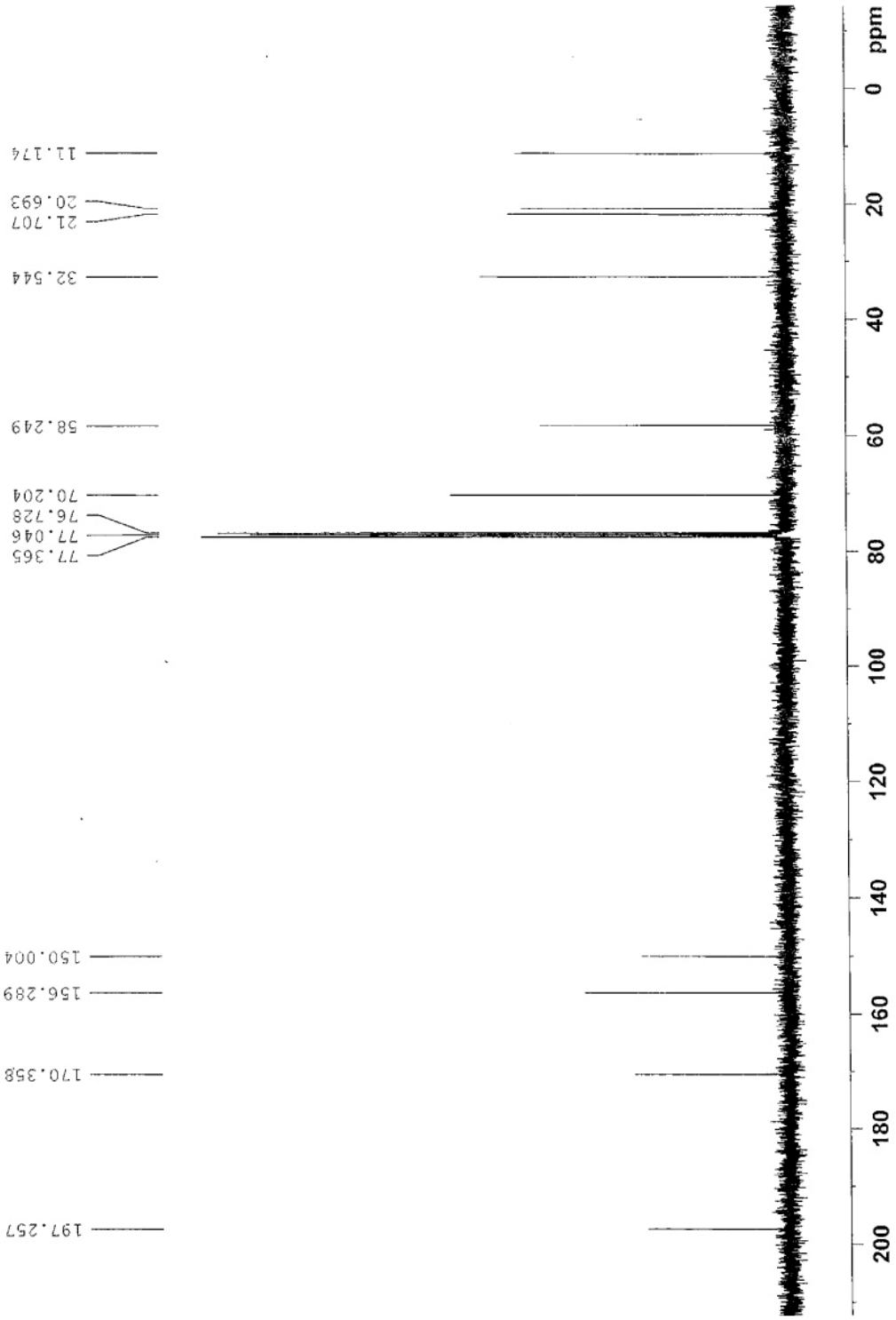


Figure 28: ^{13}C-NMR spectrum of 5-acetoxy -3-ethyl-2-methoxy-2-cyclopentene-1-one (**16**)

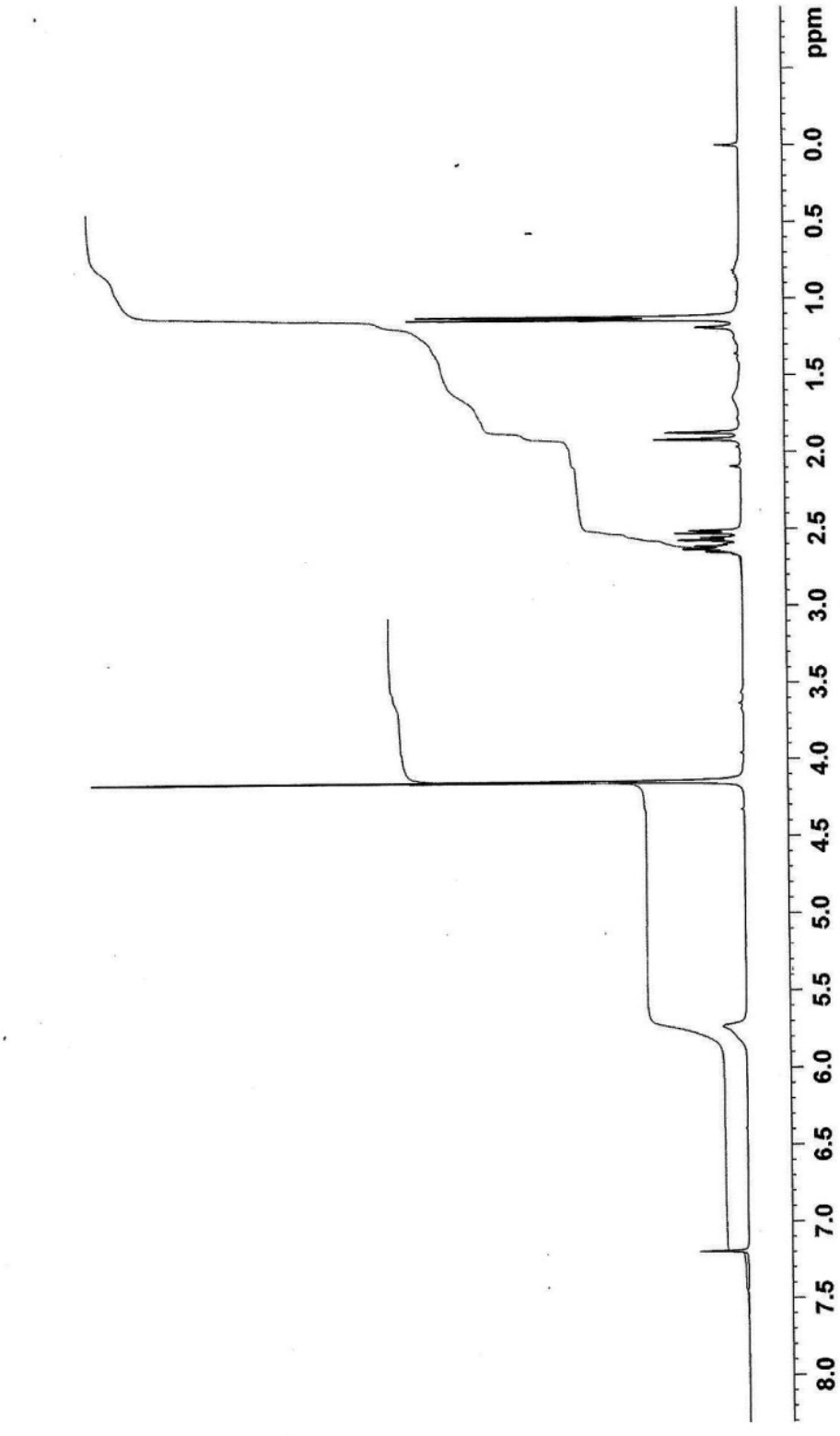


Figure 29: ^{1}H-NMR spectrum of the isomerized product **22**

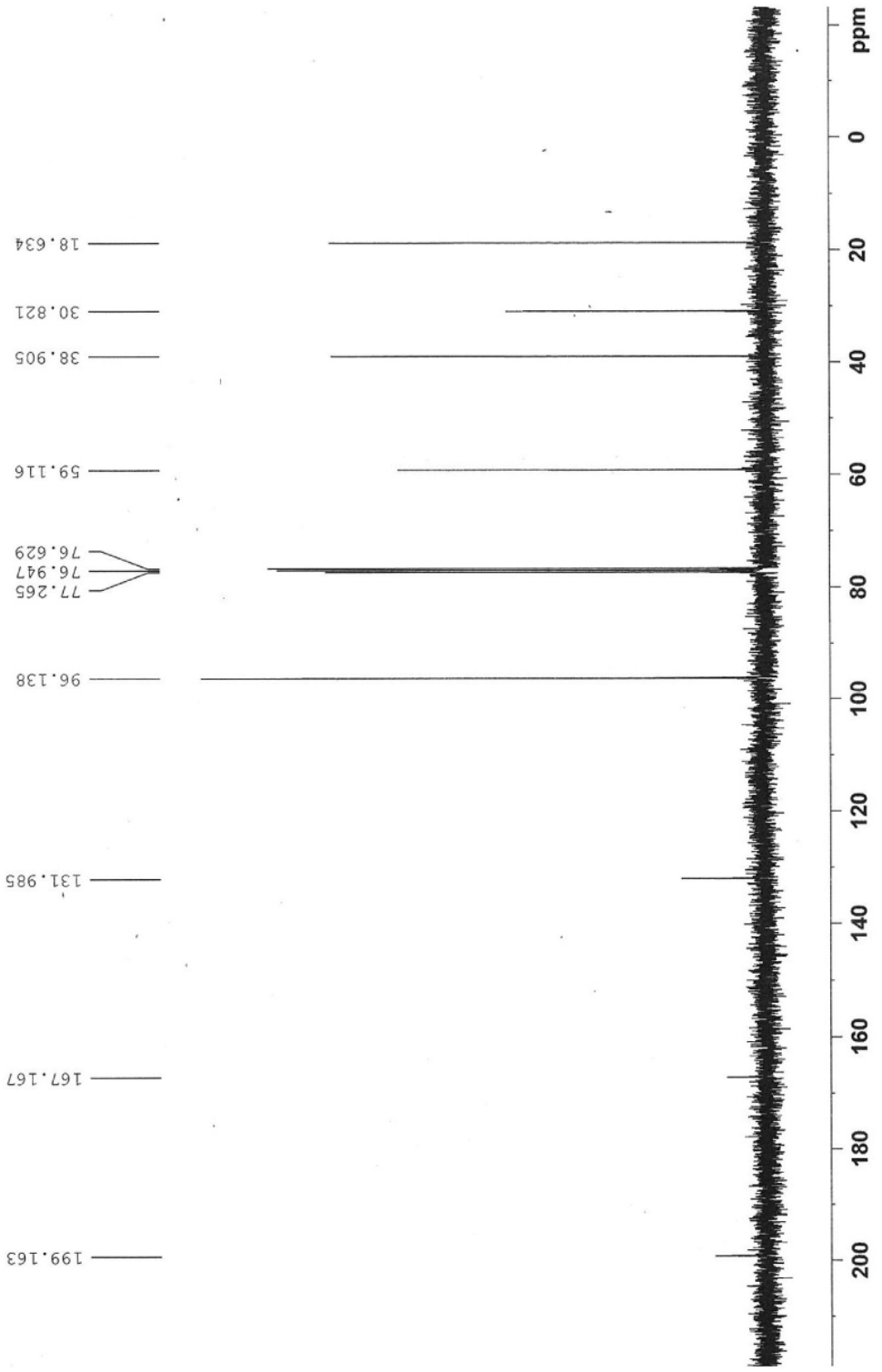


Figure 30: ^{13}C-NMR spectrum of the isomerized product **22**

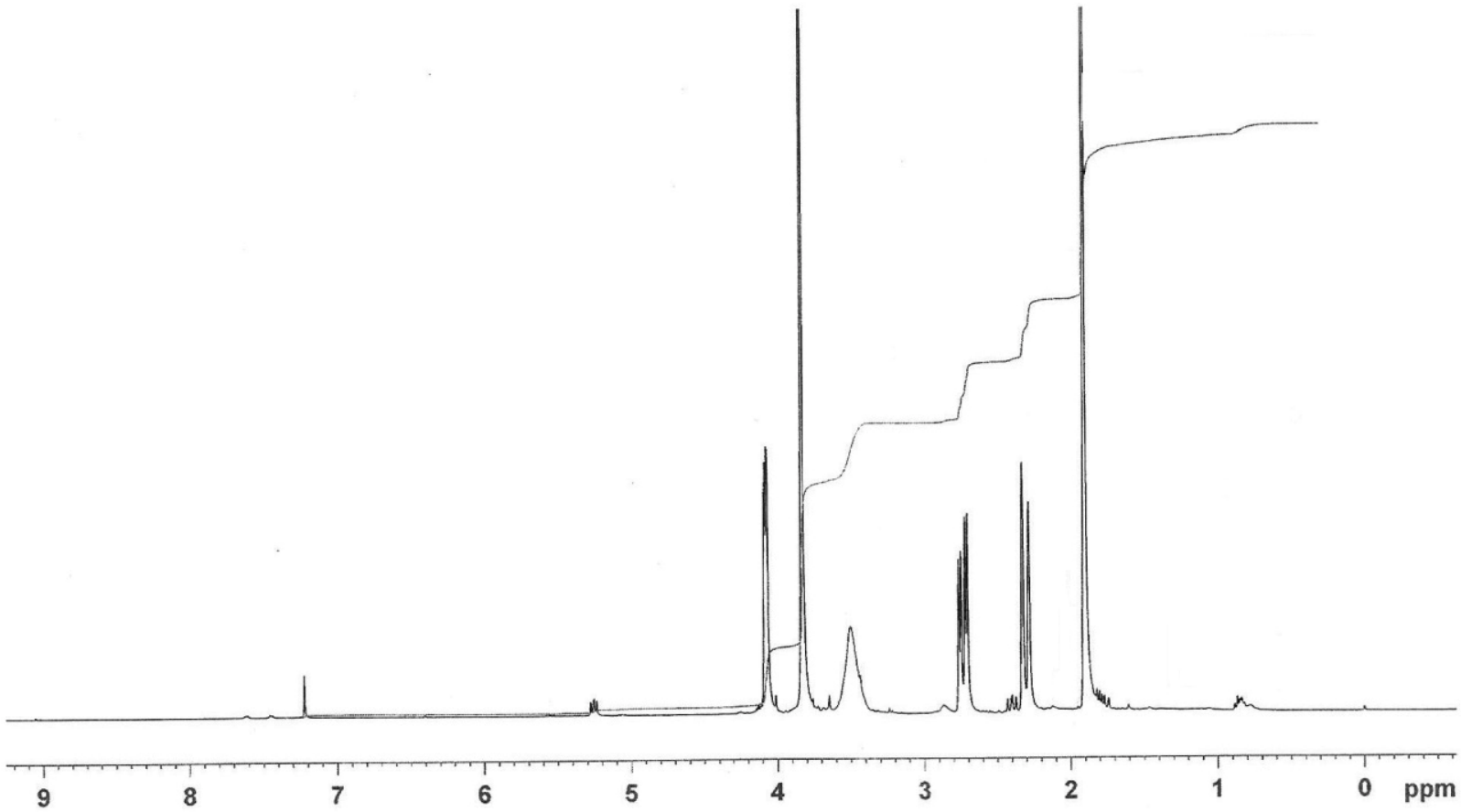


Figure 31: ^{1}H-NMR spectrum of (-)-5-hydroxy -3-methyl-2-methoxy-2-cyclopentene-1-one (**15**)

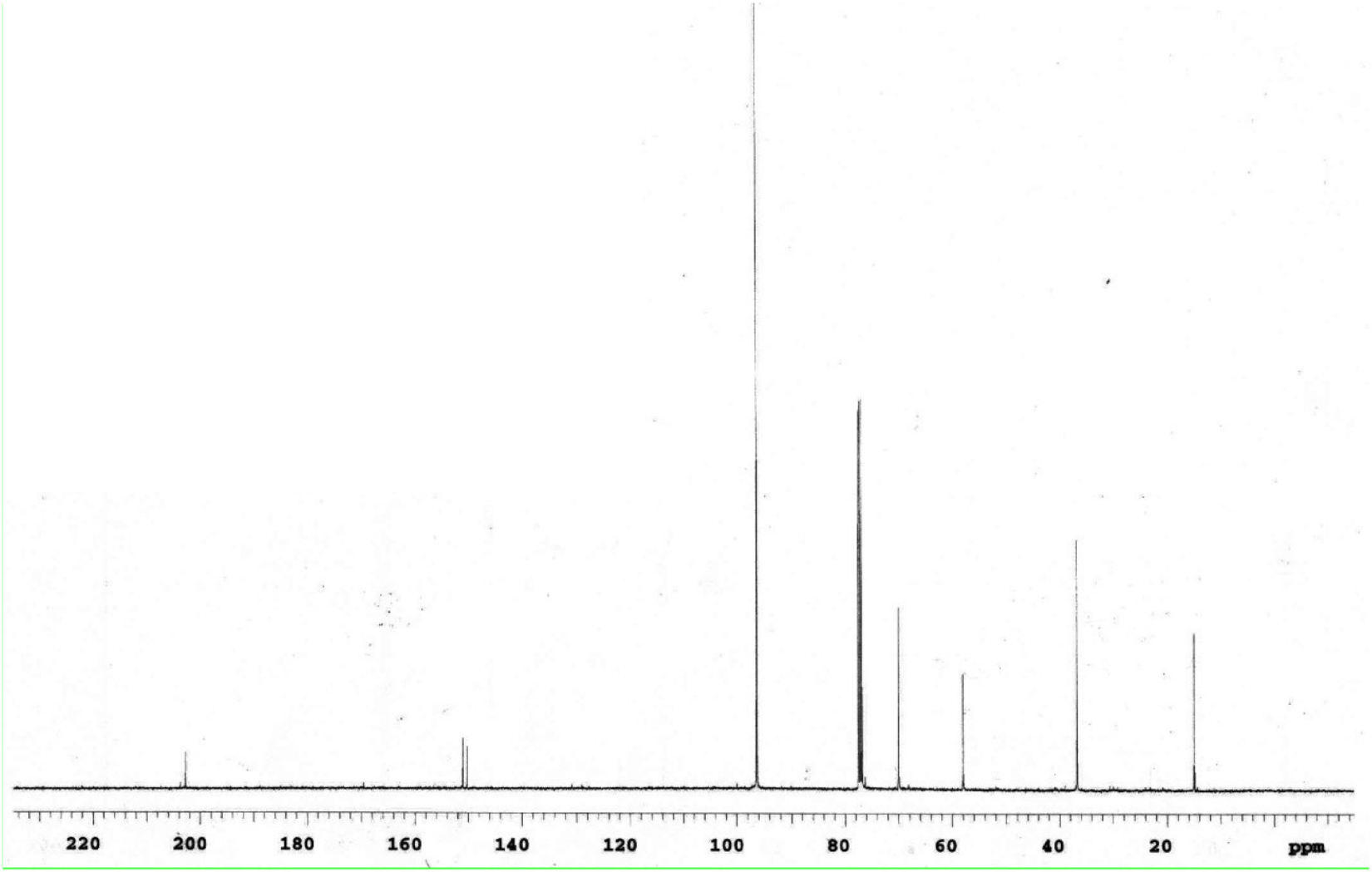


Figure 32: ^{13}C-NMR spectrum of (-)-5-hydroxy -3-methyl-2-methoxy-2-cyclopentene-1-one (**15**)

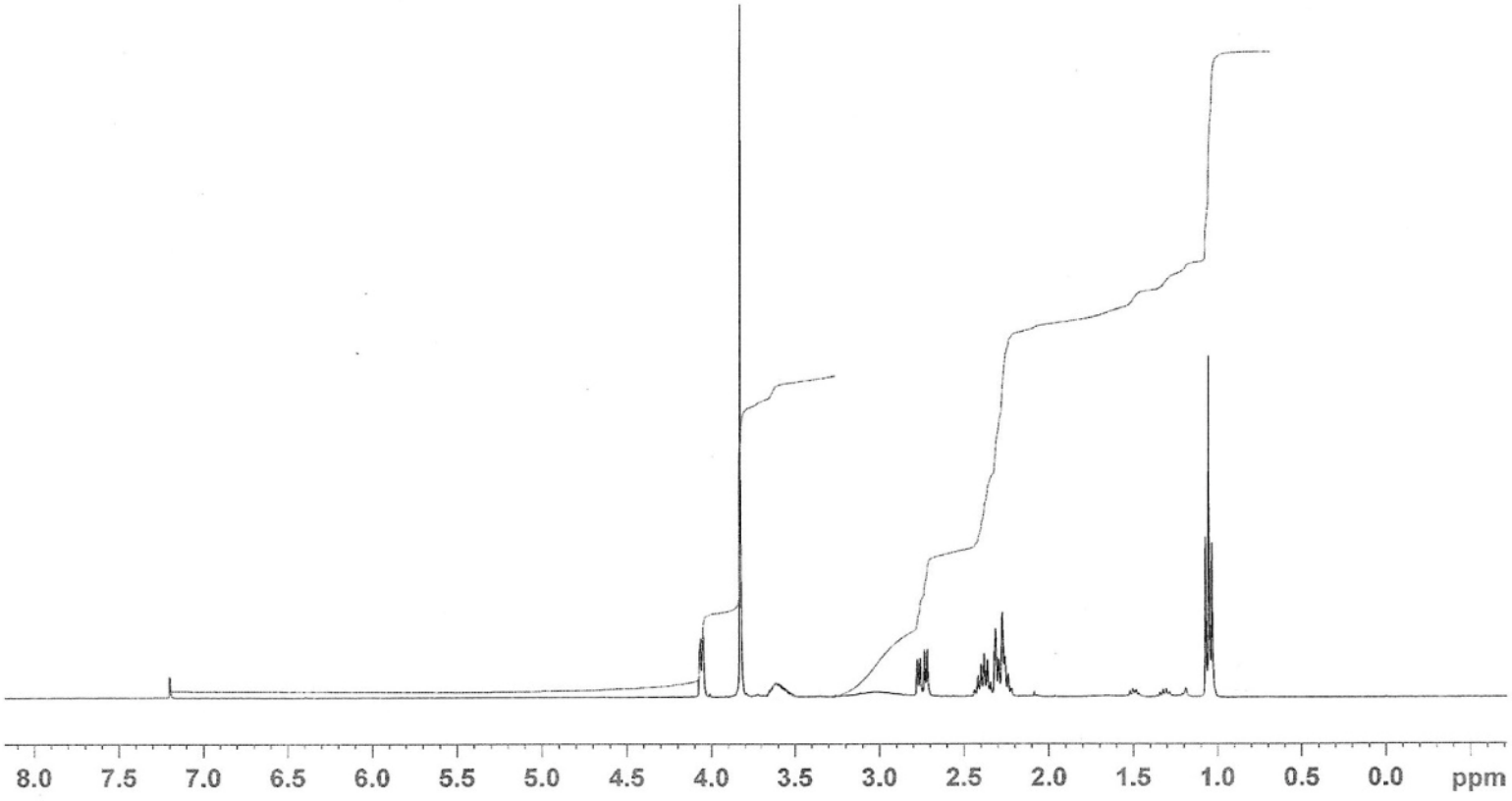


Figure 33: ^{1}H-NMR spectrum of (+)-5-hydroxy-3-ethyl-2-methoxy-2-cyclopentene-1-one (**17**)

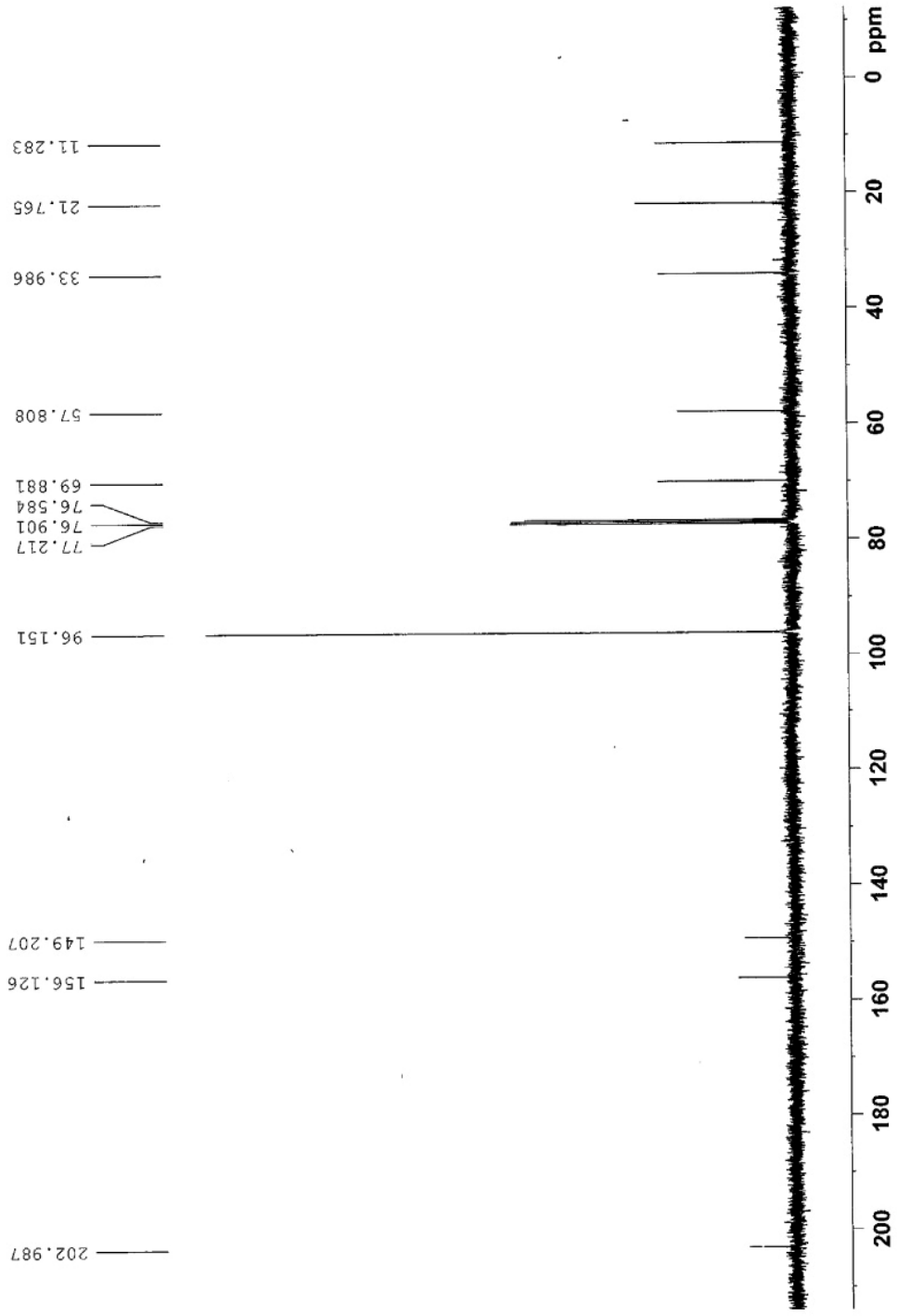


Figure 34: ^{13}C-NMR spectrum of (+)-5-hydroxy-3-ethyl-2-methoxy-2-cyclopentene-1-one (**17**)

REFERENCES

1. Hanson, J. R. *An introduction to Biotransformation in Organic Chemistry;* W. H. Freeman Spectrum, **1995.**

2. Sheldon, R. A. *Industrial Synthesis of Optically Active Compounds;* Marcel Dekker Inc., New York. **1993.**

3. Faber, K. *Biotransformations in Organic Chemistry*, Springer, **2000**.

4. Hallas, G. *Organic Stereochemistry;* McGraw-Hill: London, **1965.**

5. Koskinen, A. *Asymmetric Synthesis of Natural Products;* John Wiley & Sons Ltd. **1993.**

6. Ager, D. J.; East, M. B. *Asymmetric Synthetic Methodology;* CRC Press, **1995.**

7. Itoh, T.; Takagi, Y.; Tsukube, H. *J. Mol. Catal. B: Enzym.* **1997,** 3, 259.

8. Wong, C.; Whitesides, G. M. *Enzymes in Synthetic Organic Chemistry,* Tetrahedron Organic Chemistry series, vol 12.

9. Köse, E. *Chemoenzymatic synthesis of 4-Hydroxy Enones,* Ms. Thesis submitted to the graduated of natural and applied sciences of METU, **2004.**

10. Henbest, H. B.; Jones, D. N.; Slater, G. P. *J. Chem. Soc.* **1961,** 4472.

11. Marshall, J.A.; Bundy, G.L. *J. Chem. Soc., Chem. Commun*. **1966,** 4472.

12. Oppolzer, W.; Sarkar, T.; Mahalanabis, K. K. *Helv. Chim. Acta* **1976,** 59, 2012.

13. Demir, A. S.; Jeganathan, A. *Synthesis* **1992,** 235.

14. Vedejs, E.; Engler D.A.; Telschow J. E. *J. Org Chem.***1978,** 43,188.

15. Lee, T.V.; Toczek, J. *Tetrahedron Lett.* **1982**, 23, 2917.

16. Nakamura, K.; Kondo, S. L.; Kawai, Y.; Hida, K.; Kitano, K.; Ohno, A. *Tetrahedron: Asymmetry* **1996,** 7, 409.

17. Waldemar, A.; Diaz, M. T.; Rainer, T.; Chantu, R. *Tetrahedron: Asymmetry* **1996,** *7,* 2207.

18. Demir, A.S.; Şenocak, D. *Tetrahedron: Asymmetry* **2002** 15, 2641.

19. Danishevsky, S.; Bernarshi, M. *Tetrahedron Lett*. **1985**, *26*, 3411.

20. Williams, G.J.; Hunter, N.R. *Can. J. Chem*. **1976**, 3830.

21. Dunlap, N.K.; Sabol, M. R.; Watt, D. S., *Tetrahedron Lett.* **1984** 25, 5839.

22. Demir, A. S.; Sayrac, T.; Watt, D. S. *Synthesis* **1990,** 1119.

23. Demir, A. S.; Akgun, H.; Tanyeli, C.; Sayrac, T.; Watt D. S. *Synthesis* **1991,** 719.

24. Demir, A. S.; Alwarsamy, J.; Watt, D. S. *J. Org. Chem.* **1989,** *54,* 4020.

25. Demir, A. S.; Reis, Ö; Iğdir, A. C. *Tetrahedron* **2004,** 60, 3427.

26. Snider, B. B. *Chem Rev.* **1996,** *96,* 339.

27. Rodriguez, A.; Nomen, M.; Spur, B. W.; Godfroid J. *Eur. J. Org. Chem.* **1999,** 2655.

28. Roberts, S. M.; Roger, F. N. *Prostaglandins and Thromboxanes*, Butterworth and Co Ltd, **1982**.

29. Rajappon, V. P.; Yin, X.; Schreller, S. W., *Tetrahedron* **2002** 58, 9889.

30. Parry, R. J.; Jiang, Y.; *Tetrahedron Lett.* **1994** 52, 9665.

31. Marko, M.; Kemeny, E.; Bernady, M.; Habermayer, V.; Weyand, U.; Meiers, S.; Frank, O. ;Hofman, T. *Food and Chemical Toxicology* **2002** 40, 9.

32. Crimmins, M. T.; Jung, D. K.; Gray, J. L. *J. Am. Chem. Soc.* **1992** 114, 5445.

33. Brown, M. J.; Harrison, T.; Herrinton, P. M., Hopkins, M. H. *J. Am. Chem. Soc.* **1991** 113, 5365.

34. Brown, M. J.; Harrison, T.; Overman, L. E., *J. Am. Chem. Soc.* **1991** 111, 5378.

35. Demir, A. S. ; Saatcioglu, A. *Synth. Commun.* **1991** 23, 571.

36. Trost, B.M.; Schroeder, G.M. *J. Am. Chem. Soc.* **2000** 122, 3785.

37. Gregoire, B.; Carre, M.C.; Caubere, P. *J. Org. Chem.* **1986** 51, 1419.

38. de Klein, W. J. In Organic synthesis by oxidation with metal compounds; Mijs, W. J., de Jonge, C. R. H., Eds.; Plenum: New York, **1986**, 261.

39. Chen, C. S.; Fujimoto, Y.; Girdaukas, G.; Sih, C. S. *J. Am. Chem. Soc.* **1982** 104, 7294.

40. Roussel, C.; Rio, A. D.; Sanders, J. P.; Piras, P.; Vanthuyne, N. *J. Chromatogr. A* **2004** 1097, 311.

41. Jullian, J. C.; Franck, X.; Latypov, S.; Hocquemiller, R.; Figadere, B., *Tetrahedron: Asymmetry* **2003** 14, 963.