CHEMOENZYMATIC SYNTHESIS OF 2-ETHYL-5-HYDROXY-3-METHOXY-CYCLOPENT-2-ENONE

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ABSTRACT

CHEMOENZYMATIC SYNTHESIS OF 2-ETHYL-5-HYDROXY-3-METHOXY-CYCLOPENT-2-ENONE

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Chiral hydroxylated cyclopentane derivatives are important precursors for biologically active compounds. Synthesis of these types of compounds in optically pure form found increased interest in pharmaceutical chemistry. 2-ethyl-cyclopentane-1.3-dione was acetoxylated using manganese III acetate at preferred positions. Enzyme catalyzed enantioselective hydrolysis or enantioselective acetoxylation of hydrolyzed acetoxy derivatives gives the corresponding hydroxylated diketones in optically pure form.

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

ÖZ

2-ETİL-5-HİDROKSİ-3-METOKSİ-SİKLOPENTENON TÜREVLERİNİN KEMOENZİMATİK SENTEZİ

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Kiral hidroksi çiklopentan türevleri biyolojik aktivite gösteren moleküllerin anahtar bileşikleridirler ve bu bileşiklerin kiral sentezleri farmasotik kimyada büyük öneme sahiptir. 1.3-Ciklopentandion 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, enzymatic kinetic ayrıştırma

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INTRODUCTION

1.1. Bioconversion in Organic Chemistry

Bioconversion is the chemical conversion of substances by living organisms or enzyme preparations. Compounds relevant to biology challenges to non-biological synthetic methods, but are natural targets for biological methods. For some types of compound, it may only be possible to synthesize these molecules by biological methods; for others, both biological and non-biological methods may offer synthetic routes, but it may simply be much more convenient to use enzymes. The ability to carry out synthetic transformations that are otherwise impossible or impractical, especially in key areas of biochemistry, is clearly one of the best opportunities now available to chemistry.[1]

Biotransformation has a number of advantages when viewed alongside the corresponding chemical methods. Many biotransformations are not only regio- and stereospecific but are also enantiospecific allowing the production of chiral products from racemic mixtures.[2] The high interests in enantioselective synthesis provides reason for considering enzymes as catalyst. The active sites of enzymes are chiral, and enzymes are now well accepted as catalyst for reactions generating the enantiomerically pure intermediates and products demanded by the pharmaceutical industry.[1]

Now synthetic methods incorporating new catalysts are also necessary to deal with the increasing constraints imposed by environmental concerns. Many of the new reagents and catalysts that have been benefited organic synthesis in the last years have contained transition metals or heavy elements. When these materials are used

with great efficacy, their handling and disposal poses problems, and their replacement with environmentally acceptable catalyst would always be an advantage. Enzymes are intrinsically environmentally materials that operate best in water. [1]

Enzymes catalyze only the reactions of very narrow ranges of reactants (substrates), which may consist of a small number of closely related classes of compounds. This means that the chosen reaction can be catalyzed to the exclusion of side-reactions, eliminating undesirable by products. Thus, higher productivities may be achieved, reducing material costs. Most enzymes operate at room temperature in aqueous solution at pH 7; they are, as a group, intrinsically compatible with one another. Numbers of enzymes can therefore be used together, to accomplish multisitep reaction sequences in a single reaction vessel. In contrast, many useful non-biological catalysts are intrinsically incompatible with one another, or operate under incompatible conditions, and opportunities for using multiple non-biological catalysts at the same time are relatively limited.[1] Enzymes can work outside an aqueous environment; although some loss of activity is usually observed some of them can operate in organic solvents.[3]

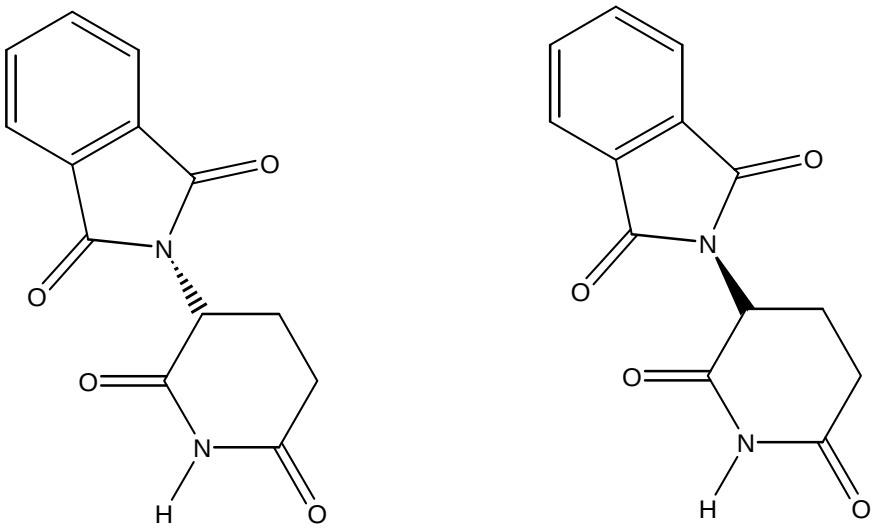
The main disadvantages of enzymes in synthesis are that enzymes are usually made from L-amino acids and thus it is impossible to invert their chiral induction on a reaction. Enzymes require a narrow operation range; elevated temperatures and extremes in pH or high salt concentrations all lead to deactivation of the enzyme. [3]

To synthesize chiral drug as the single appropriate enantiomer is very important issue because the world around us is chiral and most of the important building blocks that make up the biological macromolecules of living systems are found in one enantiomeric form. Therefore, a biologically active chiral compound such as a drug will interact with living systems in different ways and may lead to different effects.

A good example is the drug thalidomide, it was prescribed to pregnant women for morning sickness and was used as a sleeping pill but it turned out to be a

teratogen (creating malinformation in embryos) having caused serious birth defects in more than 10.000 babies because one of the enantiomer is therapeutic drug while the other is toxic. This event has shown for many pharmaceuticals that only one enantiomer contains all the desired activity, and the other is either totally inactive or toxic. (Figure 1)

Thalidomide

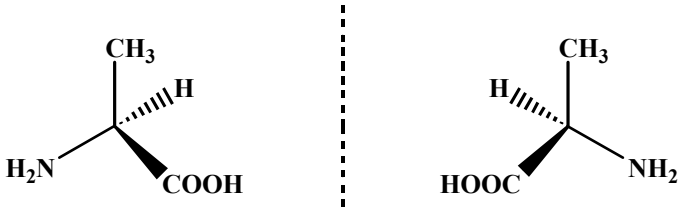


Sedative, hypnotic **Teratogenic**

Figure 1: Biological effects of the enantiomers

1.2. Chirality and Asymmetric Synthesis

Chirality is a symmetry property of three-dimensional objects. An object is said to be chiral if it cannot be superimposed upon its mirror image. Chiral is a deviation of chiros (Greek for hand) and derives from the fact that left and right hands are examples of chiral objects and cannot be superimposed on each other. A carbon atom attached to four different groups will be chiral e.g. the amino acid alanine.



S-(+)-alanine mirror image R-(-)-alanine

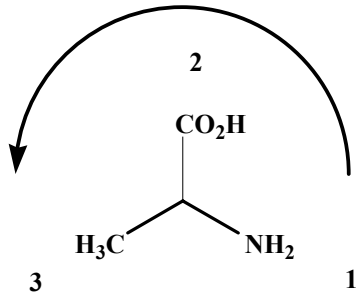
Figure 2: Two enantiomers of the alanine

If the four attached atoms are all different, then they can be arranged in two distinct ways, which are, mirror images known as enantiomers. However, the mirror images are twisted or turned, they never overlap exactly. This is because the three-dimensional arrangements.

An enantiomer is one of a pair of stereoisomer that is related as non-superimposable mirror images. Enantiomers have identical chemical and physical properties in the absence of an external chiral influence. This means that both will have the same melting points, solubility, chromatographic retention time, IR and NMR spectra. If we want to determine the proportion of the two enantiomers in a mixture, the normal chromatographic and NMR methods must be modified to introduce an external chiral influence. Only then, will the enantiomers behave differently from each other and would analysis be possible.

There is one property in which enantiomers do differ and that is the direction in which they rotate the plane of plane-polarized light. This phenomenon of optical activity provides the basis for the nomenclature of enantiomers. The enantiomer which rotates the plane-polarized light in a clockwise direction is denoted (+), while the other which has an equal and opposite rotation is denoted (-). This (+) and (-) nomenclature must not be confused with the (R) and (S) nomenclature. In this nomenclature in order to categorize stereoisomers, it is necessary to prioritise different atomic substituents using the Cahn-Ingold and Prelog sequence rules that are applied in order until a distinction is found. The substituents on the stereogenic

center are ranked in order of decreasing atomic number of the first bound atom. If no distinction is possible at the first atom, consider atoms at increasing distances until a difference is found. After viewing the stereogenic center with the lowest priority substituent pointing away; if the order of priority of three remaining substituents decreases in a clockwise manner the centre is defined as (R) (rectus, Latin for "right"). If the order of priority decreases in an anti-clockwise direction the centre is defined as (S) (sinister, Latin for "left").



Anti-clockwise direction (S) alanine

Figure 3: (S) Alanine

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 necessary and sufficient condition for a molecule to show optical activity is that such a molecule should not be superimposable on its mirror image. So dissymmetric molecules with lack of 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 axis (S axes) are chiral. Based on point groups; molecules that belong to the C_n or D_n point groups are chiral. 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 optically active.[5] However a molecule that has a plane of symmetry, a centre of symmetry or

an alternating axis of symmetry is superimposable on its mirror image and cannot be chiral.

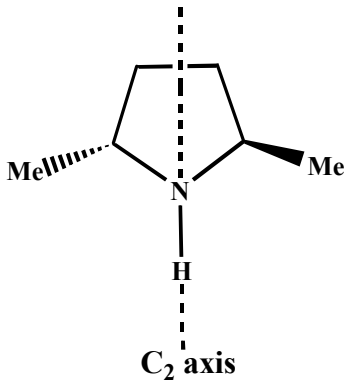
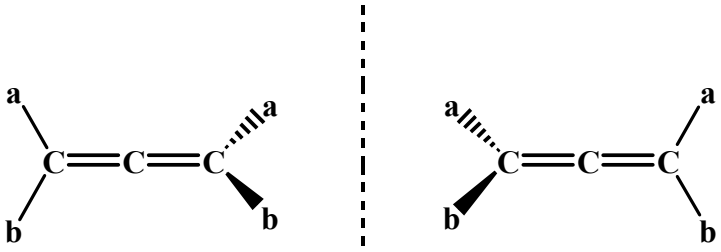


Figure 4: Two fold rotation axis of trans-2,5-dimethylpiperidine

The allenes shown in (Figure 5) will be dissymmetric provided that a is not equal to b.

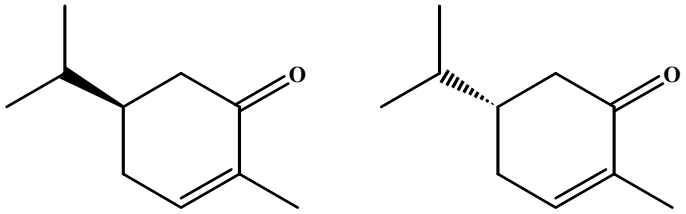


enantiomeric allenes

Figure 5: Dissymetric chiral molecules

It can be thought that both mirror images of chiral molecules (enantiomers) would be equally common in living systems. Actually, this is not the case. In humans, amino acids, proteins and enzymes consist of only one of the two mirror images. The same holds for DNA, RNA and carbohydrates, biopolymers constructed from single enantiomers of small building blocks. Chirality is a property often determines the actions and behavior of molecules in rather unexpected ways. Lemons

and oranges both contain lemonene, the different enantiomers giving rise to subtle changes in the aroma properties of these fruits. Similarly, R- and S- carvone have different tastes: the former tasting of spearmint and the latter of caraway (Figure 6). [5]



(R)-carvone (S)-carvone

Figure 6: R- and S- carvone

As natural products and their derivatives and analogues find wide use in our everyday life, from medicines to food additives, it is understandable that for the production of these compounds by synthetic means we need to secure them in enantiopure form. If in a reaction one stereoisomer is produced in excess the reaction is stereoselective and a stereospecific process is 100% stereoselective.

Since the attainment of chiral compounds in enantiopure form is desired, we need to consider the various ways available for achieving this goal. One way is to isolate the compound from the natural sources and than this natural chiral compound can be converted into the desired new building block. Examples of chiral synthons from nature are amino acids, carbohydrates and terpenes. The stereochemistry of the starting material determines the stereochemistry of the product that is a limitation of this approach as one can only synthesize one enantiomer. Another method relies on resolution. One synthesizes the target compound in racemic form, and then breaks the racemate to obtain the desired enantiomer. Resolution can be performed by enzymatic or chemical methods. 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. Racemic compound can also

be separated by converting them to a mixture of diastereomers. The last approach to obtain enantiopure product is asymmetric synthesis starting from a prochiral compound. In this approach stereocontrolled formation of the new stereogenic center is performed.

1.2.1 General methods for asymmetric synthesis

Asymmetric synthesis is a reaction or reaction sequence that selectively creates one configuration of one or more new stereogenic elements by the action of a chiral reagent or chiral auxiliary, acting on heterotopic faces, atoms or groups of a substrate.[6] Before embarking on a synthesis, careful thought must be given to how a chiral centre will be introduced into a molecule.[7] In order to obtain enantioselective synthesis, at least one of the agents in the system must be chiral. 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 perform this, especially at the beginning of the synthetic sequence, few options are available. Nature produces chiral materials which make up the "chiral pool" and a number of these are available in quantity. This approach is often limited to the amount of the natural product available and its price. Another consideration is the number of steps necessary to convert natural product into a useful starting material for synthesis. 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.

Chiral Auxiliaries: Since the number of useful natural products available is not large, to convert a cheap, readily available one to a useful intermediate in a synthesis may require many steps; some of these steps may involve expensive reagents; another approach can be used. A number of chiral groups have 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 centre from a starting material can be transferred to another part of the molecule. This new chiral centre then provides control for a stereoselective reaction, where a new centre of asymmetry can be established, or the chirality at the centre of the original starting material can be re-established. Invariably, a cyclic system is involved.

Chiral Reagents: In this approach, the prochiral substrate is treated with a chiral reagent in order to obtain enantiomerically enriched product. 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 centre. 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.

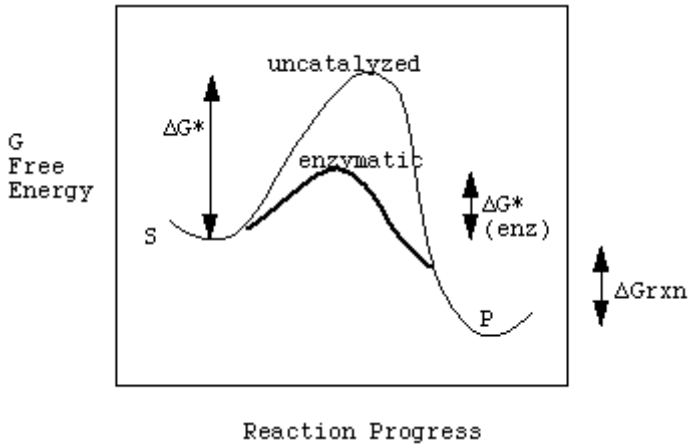
Chiral Catalysts: It is also possible to use chiral catalysts. On using a catalyst, two diastereomeric transition states are involved and an excess of one product will be delivered via the lowest energy transition state. Often transition metal catalysts are used and these have the advantage that the catalyst properties can be carefully tuned by changing the ligands around the metal atom.

1.2.2. Asymmetric Synthesis Using Biotechnological Methods

Thus far, only chemical methods have been considered; biocatalysts can also be used to obtain enantiomerically pure products from prochiral substrates. The use of biological methods to bring about chemical reactions forms a bridge between chemistry and biochemistry. Biocatalyst encompasses catalysis by bacteria, fungi, yeast, or their true catalytic components: enzymes.[8] Enzyme-catalyzed chemical transformations have been recognized as practical alternatives to conventional organic synthesis. In general this catalysis is inexpensive and in many cases able to accept a wide range of structurally different substrates. Moreover, biocatalysts are ecologically beneficial natural catalysts.

Enzymes are proteins that are capable of accelerating reactions under mild reaction conditions (pH range about 5-8 and in a temperature range of 20-40°C). Other advantages are the high degrees of substrate-, chemo-, regio- and stereoselectivity and high efficiency.

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.



Delta G* is the activation energy

Delta G is negative overall for forward reaction

Figure 7: Free energy vs. reaction progress graphs for uncatalyzed and enzymatic reactions.

Enzymes have a particular shape with an active site to bind a substrate. To understand relation between active site and substrate, the most illustrative models are Lock and Key mechanism and Induced-Fit mechanism.[9]

In 1884 E. Fisher developed the first proposal mechanism of enzymatic action. According to this mechanism, an enzyme and its substrate mechanistically interact like a lock and key, respectively. In spite of this assumption being quite sophisticated at that time, it assumes a completely rigid enzyme structure. Therefore, it cannot explain why many enzymes do act on large substrates, while they are inactive on smaller, similar counterparts. Furthermore, the hypothesis cannot explain why many enzymes can convert not only their natural substrates but also numerous non-natural compounds possessing different structural features.

Induced-Fit mechanism was developed by Koshland Jr. in the late 1960s. According to this, enzymes are not entirely rigid but rather represent delicate and soft structures. It assumes that upon approach of a substrate during the formation of the enzyme-substrate complex, the enzyme can change its conformation under the

influence of the substrate structure so as to wrap itself around its guest. This advanced model can indeed explain 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.[10]

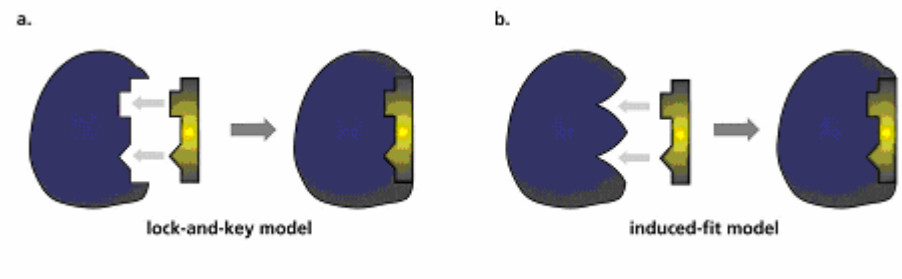


Figure 8: Lock and key model and Induced fit model

During the biocatalyst catalyzed processes three major types of selectivities are displayed. In chemoselective reactions purifications of product(s) from impurities is easier and side reactions can be omitted. Thus reactions are generally cleaner. As mentioned before 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. So any type of chirality present in the substrate is recognized and prochiral compound is transformed into optically active product.

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.

Kinetic resolution is an approach in which 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. 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. As this is a resolution, only 50% of the substrate can be converted to the desired product.

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)

Because of the chirality of the enzymes active site, enzyme fits one enantiomer better than the other enantiomer. Therefore, it is converted at a higher rate, which forms enantiomeric differentiation. (Figure 9)

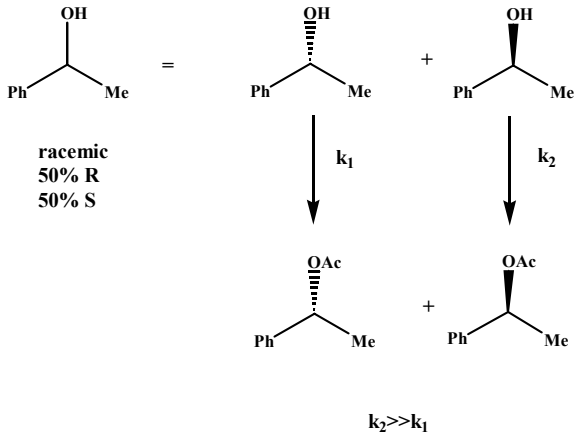


Figure 9 Enantiomeric differentiation

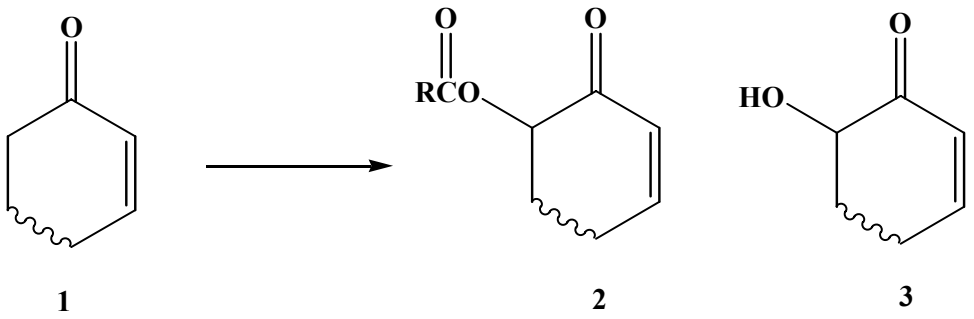
There are many enzyme systems available and quite a number can be obtained from commercial suppliers. Enzymes can be classified according to the

reactions they catalyze. Among the biocatalyst in organic synthesis, lipases are the most frequently used. In particular this class of enzyme is able to perform enantioselective hydrolytic reactions.[11] Moreover; lipases accept a wide variety of substrates while maintaining their regioselectivity and stereoselectivity. In nature, lipases catalyze hydrolysis of triglycerides to the corresponding fatty acids and glycerol. In addition to hydrolytic reaction, lipases catalyze reactions involving esterification, interesterification, amidation and so forth. [12]

1.3 α' Oxidation of Enones

Procedures for the selective oxidation of common functional groups occupy a central position in the synthesis of complex natural products.[13] The synthesis of α-hydroxy and acetoxy carbonyl derivatives has been of continuous interest to organic chemists since the beginning of the century. Selective oxidations giving α'-hydroxy and acetoxy α,β-unsaturated ketones possess a central role in synthetic methodology.

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).



1 **2** **3**

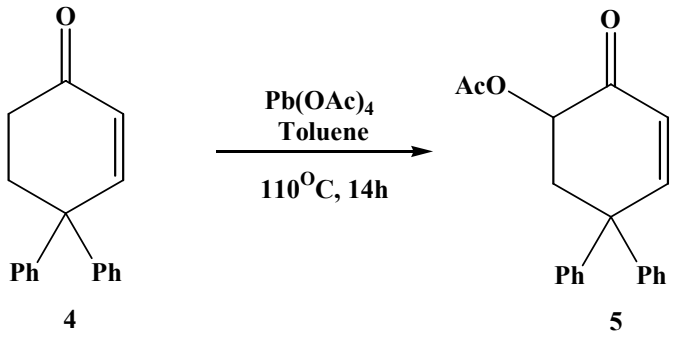
Scheme 1

Using Lead (IV) Acetate

The regioselective oxidations of enones using lead(IV) acetate in acetic acid, benzene or toluene provided the α'-acetoxy-enones in yields varying from poor to acceptable. Henbest and co-workers reported that various Lewis acids gave improved yields.

The mechanism for the α'-acetoxylation of enones by lead(IV) acetate as proposed by Henbest and co-workers[14], and Marshal and Bundy[15], involves formation of an enol-lead triacetate derivative directly from the enone followed by intramolecular acetate transfer.

It is reported that the lead(IV) acetate method is applied to the α,β-unsaturated ketone **4** and the reaction afforded the expected α'-acetoxy derivative **5** in 56% yield[16] (Scheme 2).



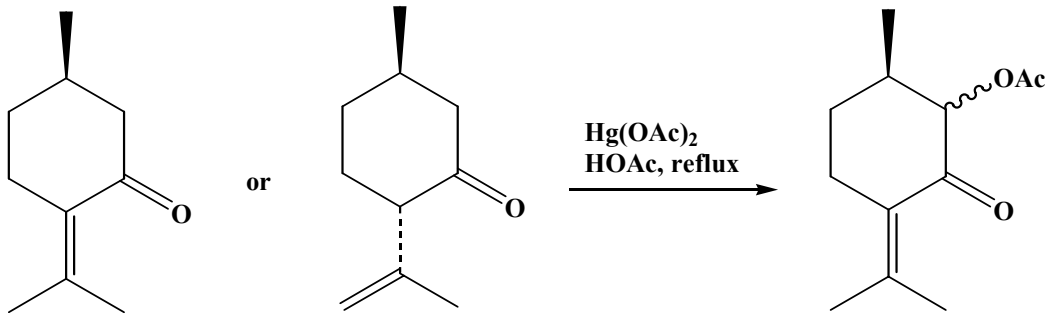
4 5

Scheme 2

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

Classical methods have been supplemented in more recent years by several heavy metal-containing oxidants such as MoO_5.Py.HMPA[17] and CrO_2Cl_2[18], but these type agents are potentially contaminating so chemists are trying to minimize the use of them.

A number of studies on this subject apart from metal-containing oxidation have been reported. According to one of them, the complex, HOF.MeCN made directly by bubbling fluorine through aqueous acetonitrile, reacts quickly and efficiently with enolic forms of ketones to produce α-hydroxy ketones.[19] This complex is rapidly evolving as the best possible oxygen transfer agent, since it contains a truly electrophilic oxygen.

Other study is asymmetric oxidation of ester and amide lithium enolates to α-hydroxy carbonyl compounds using (camphorsulfonyl) oxaziridines, the

configuration of whose three-membered ring determines the product stereochemistry.[20]

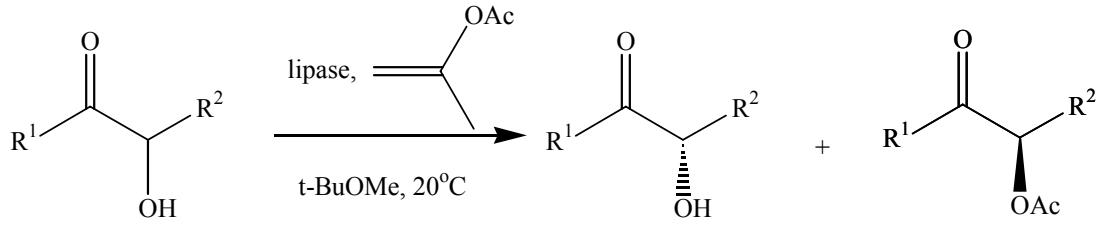
Biotechnological Methods

Alternative to the chemical methods, optically active α-hydroxy ketones can be prepared enzymatically by reduction of the α-diketones with yeast as the biocatalyst.[21] However; this enzymatic method possesses the following disadvantages: further reduction of diketone to vic-diol, formation of both regioisomeric α-hydroxy ketones and moderate chemical yields.

Esterases and lipases have a major application in the preparation of chiral molecules for synthesis. For the most part these hydrolyses are kinetic resolutions rather than complete resolutions and some experimentation is necessary to establish the optimum conditions to obtain the highest enantiomeric excess.[2] PLE (Pig liver esterase), PPL (Porcine pancreatic lipase), CCL (Candida cylindracea lipase), α-chymotrypsin, and PCL (Pseudomonas cepasia lipase) are some commonly used enzyme systems for hydrolysis.

Lipases 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 the resolution of α-hydroxy aryl ketones (precursors of chiral azole antifungal reagents) by lipase catalyzed hydrolysis of the corresponding acetates in phosphate buffer; nevertheless, the irreversible transesterification route of this enzymatic reaction appears not to be known.[22] Also, Demir et. al. has described that the lipase Amano PS, PPL, PLE and CCL-catalyzed asymmetric ester hydrolysis and transesterification afforded enantiomers of 3-hydroxy-2,3-dihydro-4*H*-chromen-4-one and 4-oxo-3,4-dihydro-2-chromen-3-yl acetate with high enantiomeric excess

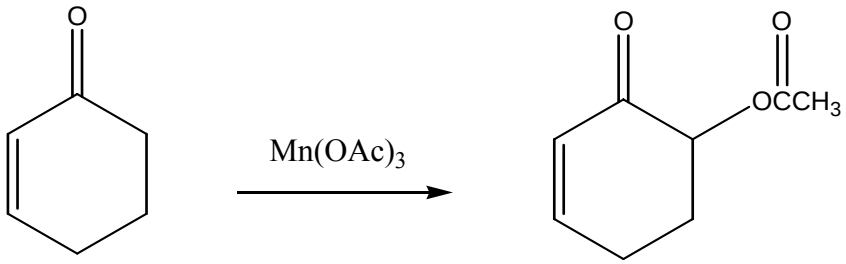
(up to 97% ee) and in good yields.[23] In another report by Demir et. al. it was described that, Mn $(OAc)_3$ oxidation of aromatic ketones afforded the α-acetoxy ketones in good yield and selective hydrolysis of the acetoxy ketones by the fungus *Rhizopus oryzae* yields (*R*)-hydroxy ketones in high enantiomeric excess.[24] 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). [25]



Scheme 4

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

Literature methods gave unsatisfactory results for the oxidation of an enone to an α'-acetoxyenone.[26] To overcome this problem, Demir and his co-workers studied on the oxidation of α,β-unsaturated enones using manganese(III) acetate.[27-29] They got satisfactory result for the preparation of α'-acetoxy enones (Scheme 5).

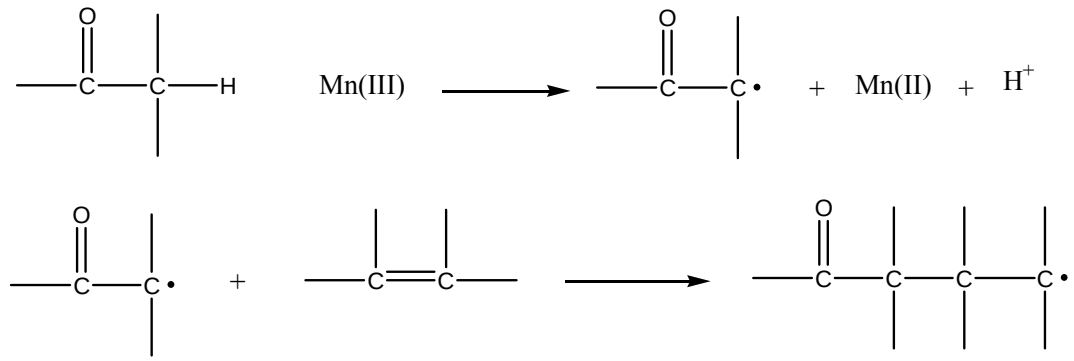


Scheme 5

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

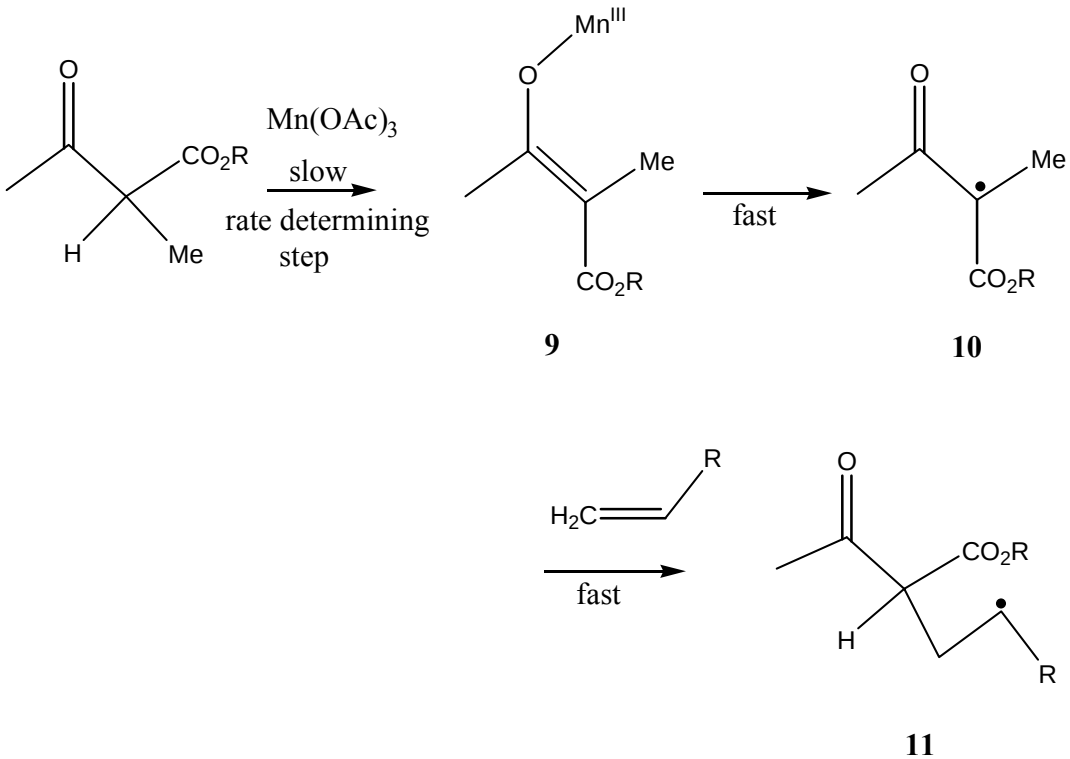
The fate of the primary radical adduct strongly depends on reaction conditions and the nature of the substrate. Manganese (III) acetate can be used as a free radical generator if substrates are less reactive to common oxidants. The one

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.[30]

In general, manganese (III) acetate oxidations are characterized by higher chemical yields, higher α'-regioselectivity and milder reaction conditions, tolerating many sensitive functional groups.

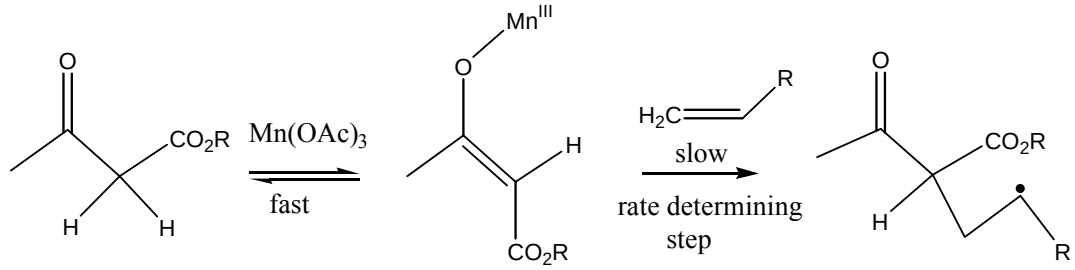
Manganese (III) acetate can be used for initiating the addition of aldehydes to olefinic unsaturated systems, the addition of ketones to olefinic unsaturated systems, the addition of haloalkanes to unsaturated systems, aromatic substitution reactions.

The mechanism of oxidation of monocarbonyl substrates with $Mn(OAc)_3.2H_2O$ has been extensively studied. Snider[31] has suggested a mechanism, which is operative in the oxidation of α-alkyl-β-keto esters (Scheme 7).



Scheme 7

Enolization to give **9** is slow; electron transfer with loss of Mn (II) to give **10** is rapid. The rate of reaction is therefore independent of alkene concentration. This indicates that free radical **10** 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 8).



12 **11**

Scheme 8

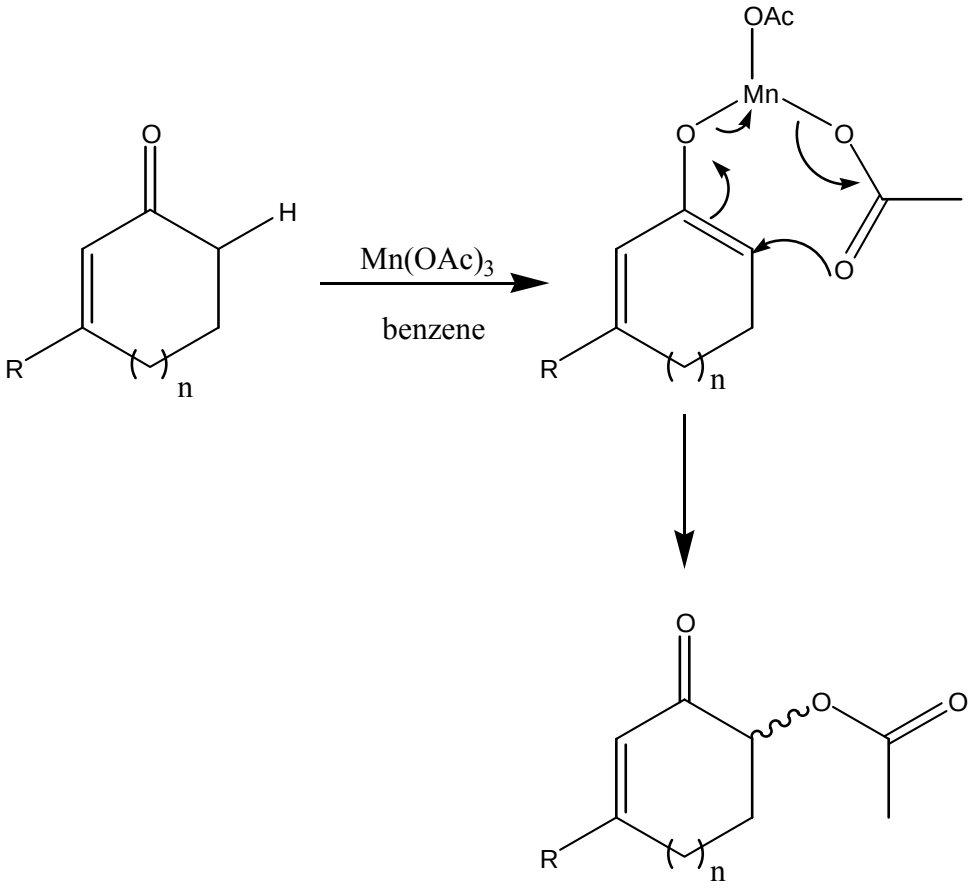
The rate-determining step depends on alkene concentration and is presumably the reaction of the Mn (III) enolate **12** with the alkene to give radical **11** with loss of Mn (II). β-Keto ester radicals analogous to **10** do not appear to be intermediates in these reactions. They concluded that the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

Although successful α'-acetoxylation of a great variety of substrates have been reported, there are some problems associated with the use of $Mn(OAc)_3$. A brief list of them is as follows: (1) excess $Mn(OAc)_3$ (4-6 equiv.) is generally used for acceptable yields and reaction times; (2) many contradictory results can be seen when literature reports are closely inspected. [32]

These inconsistencies and the use of an undesirable amount of $Mn(OAc)_3$ reduced the value of the method. Considering that there are not many simple methods for the direct acetoxylation of enones, optimization of $Mn(OAc)_3$ mediated α'-acetoxylation of enones and reaching its maximum potential has a great importance from a synthetic and economical point of view. Demir and his co-workers reported their investigation towards understanding the nature of this reaction together with increasing its efficiency and reproducibility. They have presented[32] an improved procedure based on the use of acetic acid as a co-solvent. According to this procedure, AcOH shortens the reaction time and increases yields. The role of acetic acid could be related to an increased solubility of $Mn(OAc)_3$ in the reaction mixture. From a synthetic point of view, excellent results were obtained for a variety of structurally diverse and synthetically important enones under optimized conditions. Although benzene is the most frequently, used solvent cyclohexane and MeCN can also be used instead of benzene and acetic anhydride can be used instead of acetic acid.

The mechanism of manganese (III) acetate oxidations in benzene remains uncertain, but it seems reasonable based on related oxidations of lead (IV) acetate.[14] The mechanism for the α'-acetoxylation of enones by lead (IV) acetate as proposed by Henbest and co-workers and Marshall and Bundy involves formation of an enol lead triacetate derivative directly from the enone followed by intramolecular acetate transfer.

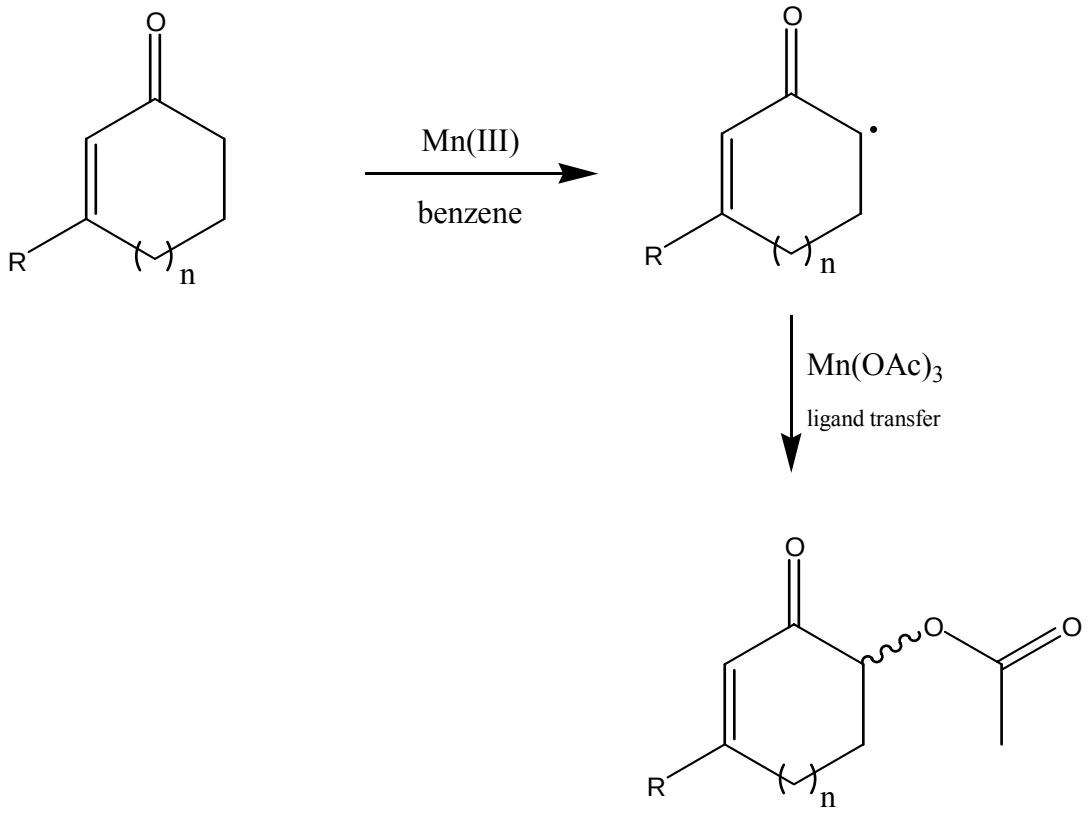
The interaction of the enol or enolate of aromatic ketone with manganese (III) acetate would result in acetate transfer (Scheme 9).



Sceheme 9

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 10).

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. Different behavior of enones and saturated ketones might be the indication of different mechanisms depending on the types of substrates and solvents.



Scheme 10

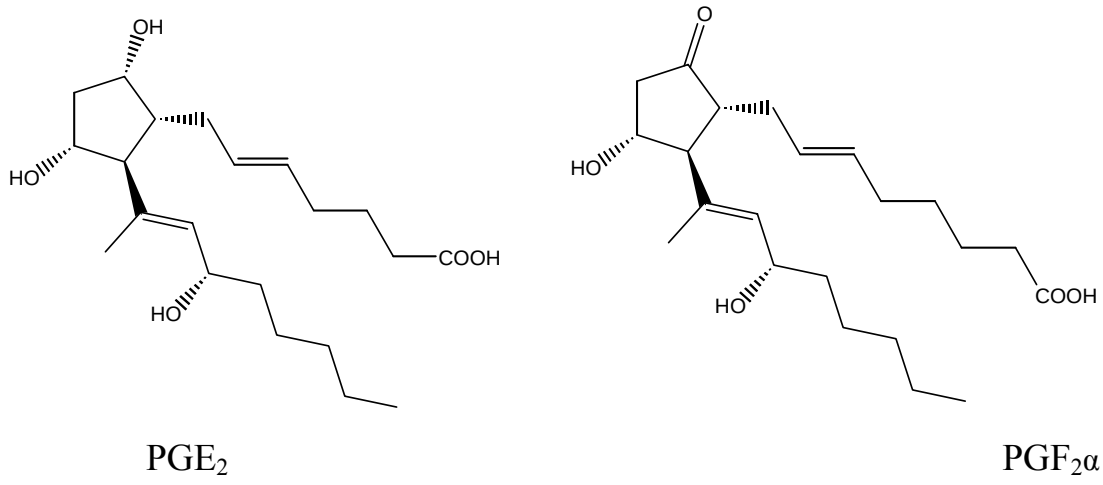
Presence of water causes disproportionation of trivalent manganese into Mn (IV) and Mn (II) and alternative two-electron oxidants may take place by Mn (IV). Therefore, complications may arise in the presence of water.

1.5 The Importance of Polyoxo Cyclopentenones

The synthesis of cyclopentenones has been a subject of intensive investigations. The biological importance and great structural diversity of cyclopentanoid natural products have made these compounds valuable synthetic targets.[33]

Cyclic polyoxo-cyclopentenones have many functional groups in spite of their small structures. These functional groups can be changed with any other functional groups and they can be used in many synthetic applications.

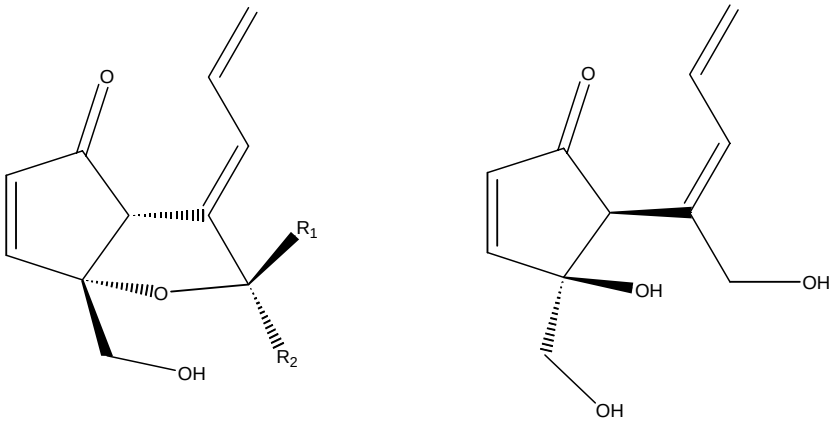
One of the examples of the important biologically active compounds that can be synthesized from cyclopentenones is prostaglandins. Several natural prostaglandins and analogues are used as drugs. In recent years new indications, especially for the E and F types, have been clinically introduced. Several studies on asthmatic subjects have shown highly protective effects of prostaglandins of the E type. They have a therapeutic application in the field of gynaecology such as termination of unwanted pregnancy. (Figure 10)[34]



PGE_2 $PGF_{2\alpha}$

Figure 10: Prostaglandins

Another group containing this structure is didemnenones. Every carbon atom in this group is functionalized so they display a rich abundance and variety of functionality. They show a broad range of biological activities including toxicity against leukemia as well as antimicrobial and antifungal activities.[35] (Figure 11)

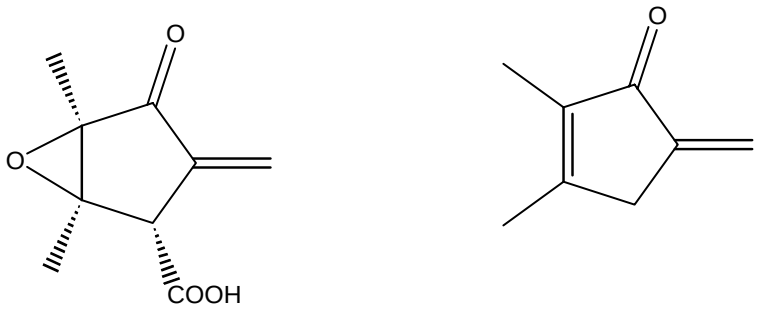


R_1=H, R_2=OH A

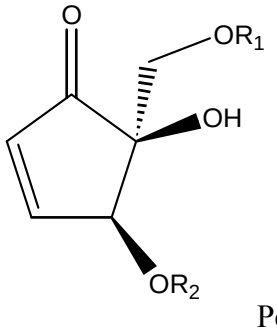
R_1=OH, R_2=H B

Figure 11: Didemnenones

Pentenomycin and methlenomycin A and B are antibiotics and shows antibacterial activity. (Figure 12)



Metylenomycin A B



a) R_1=R_2=H

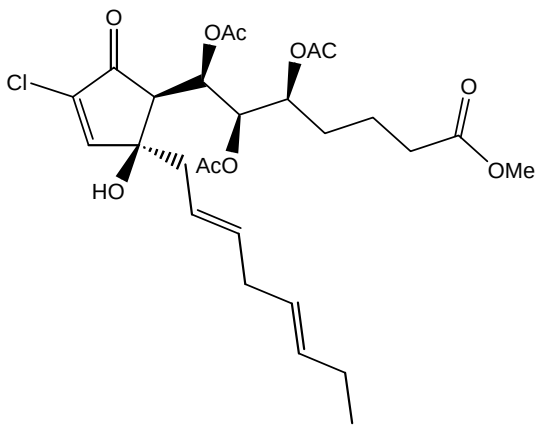
b) R_1=H, R_2=Me

c) R_1=Ac, R_2=H

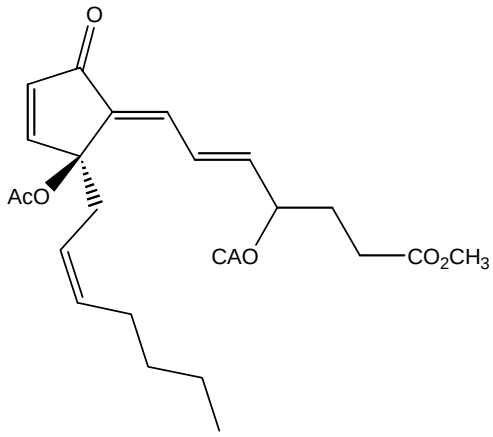
Pentenomycin

Figure 12: Metynomycin A-B and Pentenomycin

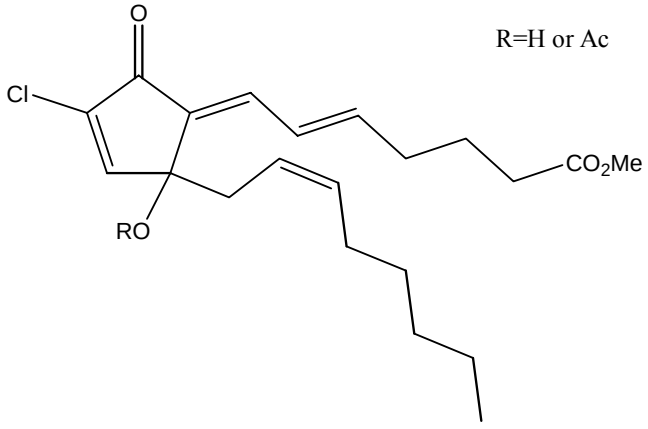
Halogenated prostanoids such as chlorovulones are especially of interest due to their strong antiproliferative activity against tumor cells. Other groups that show antitumor activity are punaglandin, clavulone and sarkomycin. (Figure 13)



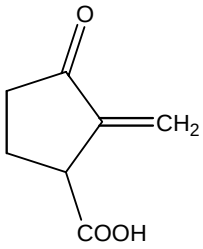
Punaglandin



Clavulone



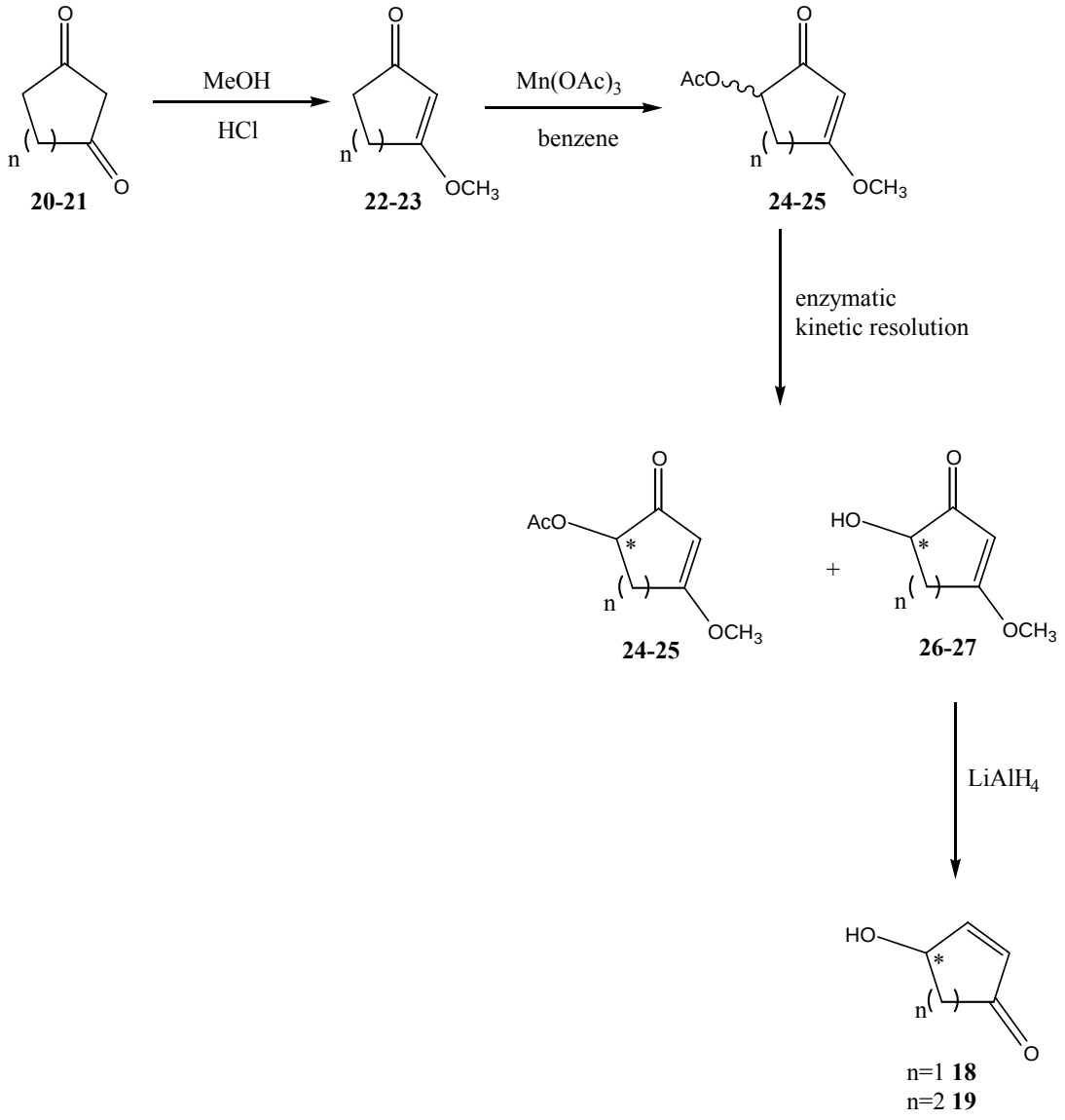
Chlorovulone



Sarkomycin

Figure 13: Cyclopentanoid structures that show antitumor activity

Demir and Sesenoglu studied on the synthesis of 4-hydroxy-2-cylopenten-1-one **18** and 4-hydroxy-2-cylohexene-1-one **19.** [36-37] The synthesis was accomplished in four steps starting from 1,3-cyclopentanedione **20** and 1,3-cyclohexandione **21**. During this study 1,3-diketones were converted to the 3-methoxy-2-cyclopenten-1-one **22** and 3-methoxy-2-cyclohexen-1-one **23**. This products are then converted to the α'-acetoxy ketones **24** and **25** using manganese (III) acetate oxidation. It was followed by enzymatic kinetic resolution and α'-acetoxy ketones are hydrolyzed to α'-hydroxy ketones **26** and **27** enantioselectively using lipases. The reduction of this compound with $LiAlH_4$ yielded the γ-hydroxy enones **18** and **19.** Scheme 11

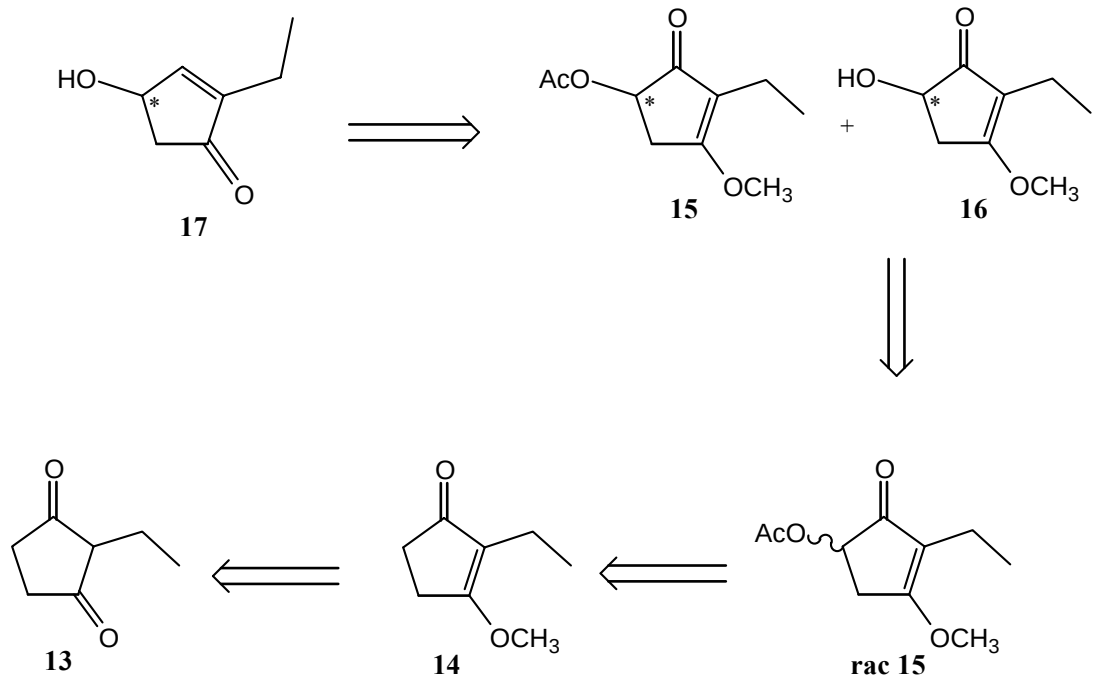


Scheme 11

1.6 Aim of the work

The major aim of this research is to develop simple and selective method for the synthesis of chiral 2-substituted cyclic polyoxo-ketones and γ-hydroxy enones which are important intermediates for the asymmetric synthesis of many natural products.

For this purpose the synthesis of 5-acetoxy and 2-ethyl-5-hydroxy-3-methoxy-2-cyclopentene-1-ones **15, 16** and 2-ethyl-4-hydroxy-2-cyclopentene-1-one **17** was chosen as a model study. The aim of this work is shown retrosynthetically in scheme 12.



Scheme 12

There is no method for the enantioselective synthesis of 5-acetoxy and 2-ethyl-5-hydroxy-3-methoxy-2-cyclopentene-1-ones **15, 16** and 2-ethyl-4-hydroxy-2-cyclopentene-1-one **17** in the literature.

Our first approach to optically active **15, 16** and **17** is to protect one of the carbonyl groups and synthesize 2-ethyl-3-methoxy-2-cyclopentene-1-one starting from 2-ethyl-1,3-cyclopentenedione that will be followed by α-oxidation of enone by using manganese(III) acetate. Then enzymatic bioconversion of racemic 5-acetoxy-2-ethyl-3-methoxy-2-cyclopentene-1-one **15** can be performed by lipases. Lastly, chiral hydroxy ketone can be reduced using to afford 4-hydroxy-enone **17.**

It was also aimed to find the optimum conditions for enzymatic bioconversions for good optical yield. To do this, several lipases will be tried throughout the enzymatic kinetic resolution step. Alternative to the chemical methods, the regioselective one-step α-oxidation of manganese(III) acetate and enantioselective hydrolysis by using lipases should provide a low cost production of the α-hydroxy ketones.

CHAPTER 2

RESULTS AND DISCUSSION

2.1 Perspective of the Work

Polyoxo ketones and γ-hydroxy enone are versatile chiral synthons for the construction of chiral organic compounds due to reactive functional groups: OMe, acetoxy, double bond, carbonyl, hydroxyl groups. These functional groups can be changed with any other functional groups and they can be used in many synthetic applications.

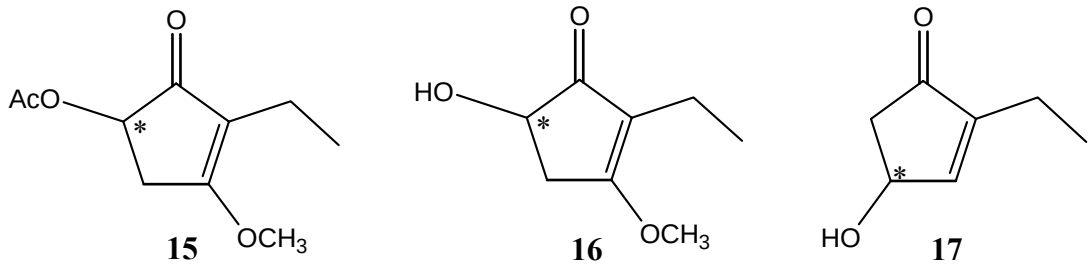


Figure 14: Polyoxo ketones and γ-hydroxy enone

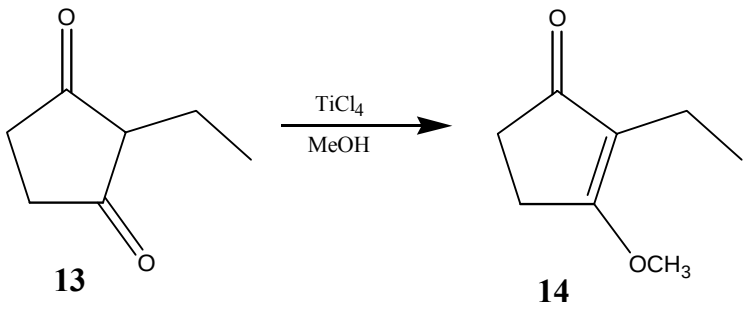
Many biologically active compounds contain these structures; such as didemnenones, prostaglandins, sarkomycin, punoglandin etc.

Based on preliminary information available to us from previous work with biocatalyst-mediated reactions, a chemoenzymatic method for the synthesis of **15** and **16** by protection, manganese(III) acetate mediated acetoxylation followed by hydrolysis using lipase were developed.

2.2 Synthesis of α-Acetoxy Ketones

2.2.1 Protection of Cyclic 1,3-diketones

For the synthesis of chiral α-acetoxy and α-hydroxy ketones, protection was first performed on the diketone. We performed the protection reaction with $TiCl_4$ according to the procedure in the literature.[38] In this method; diketone was dissolved in methanol and allowed to react with $TiCl_4$ under argon. The reaction is monitored by TLC (Silica gel, EtOAc/Hex 3:1). After the work up, product is purified with column chromatography (Silica gel, EtOAc/Hex 3:1). Desired product 2-ethyl-3-methoxy-2-cyclopenten-1-one **13** was obtained as yellow oily compound in 88% yield. (Scheme 13)



13 **14**

Scheme 13

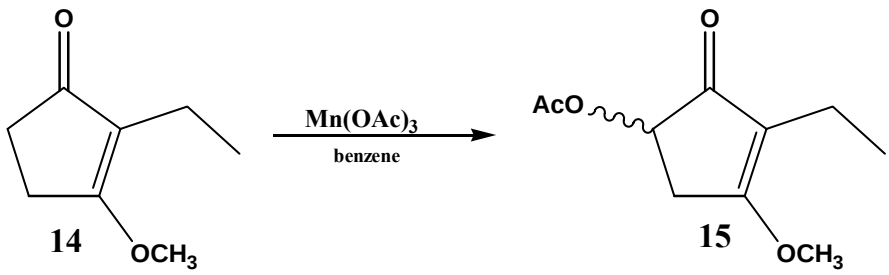
The product was identified by using NMR spectroscopy. From the ^{1}H-NMR spectrum we observed a singlet at 3.95 ppm from the $–OCH_3$ group. From the ^{13}C-NMR spectrum, we observed a peak 56.1 ppm for the carbon of $–OCH_3$ group.

There are some procedures, reported in the literature for the protection of 1,3-dicarbonyl compounds in enol ether form. As a first example, 1,3-dicarbonyl compound was dissolved in methanol and HCl gas was passed through the solution for the formation of enol ether. For another published procedure diketone was allowed to react with 1 equivalent of diazomethane.[39] Also methyl iodide and alkoxide were used for making enol ethers.[40]

2.2.2 $Mn(OAc)_3$ Mediated Acetoxylation of β-alkoxy enone

2.2.2.1 $Mn(OAc)_3$ Mediated Acetoxylation of β-alkoxy enone in Benzene

2-Ethyl-3-methoxy-2-cyclopenten-1-one **14** was allowed to react with 4 equivalents of $Mn(OAc)_3$ in benzene for the acetoxylation reaction. The reaction was performed in benzene's reflux temperature under a Dean-Stark trap to obtain the desired acetoxy derivative **15** in racemic form. (Scheme 14)



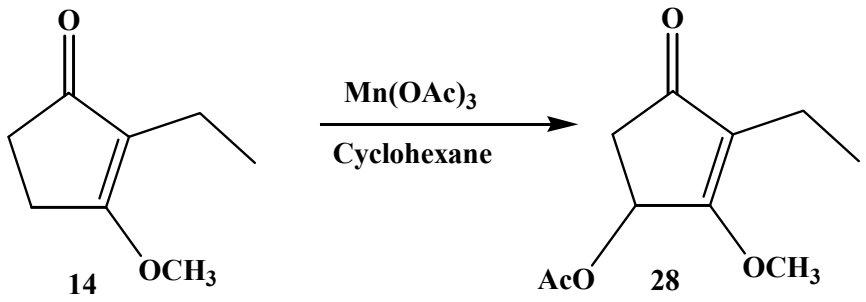
Scheme 14

The reaction is monitored by TLC (Silica gel, EtOAc/Hex 1:1). After the work-up and purification of the crude product by column chromatography, the desired product, racemic 5-acetoxy-2-ethyl-3-methoxy-2-cyclopenten-1-one **15** was obtained as yellow oil in 70% yield.

The product was identified by using NMR spectroscopy. From the ^{1}H-NMR spectrum we observed a singlet at 2.13 ppm from the $-CH_3$ group and at 5.08 ppm dd, (J=2.6 and 6.9 Hz) for the α-proton. From the ^{13}C-NMR spectrum, we observed a peak at 19.6 ppm for the CH_3 carbon and a peak at 169.2 ppm for the $O\underline{C}OCH_3$ carbon.

2.2.2.2 $Mn(OAc)_3$ Mediated Acetoxylation of β-alkoxy enone in Cyclohexane

2-Ethyl-3-methoxy-2-cyclopenten-1-one **14** was allowed to react with 4 equivalents of $Mn(OAc)_3$ in cyclohexane. The reaction was performed in cyclohexane's reflux temperature under a Dean-Stark trap monitoring of the reaction by TLC showed that the product have different R_f value as in former reaction, carried out in benzene. (Scheme 15)



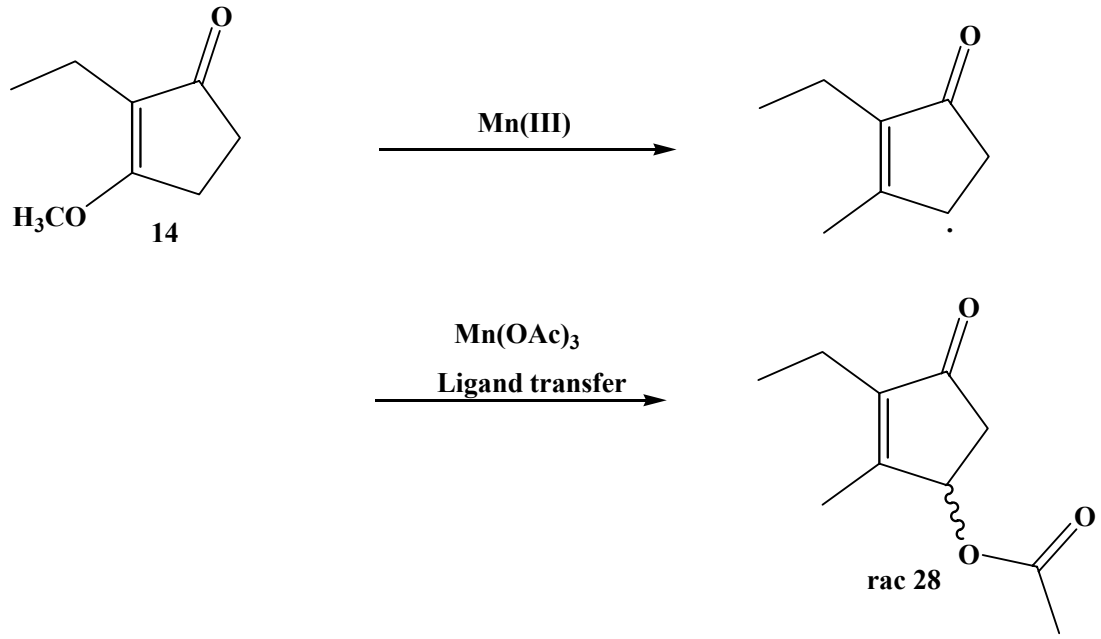
Scheme 15

After the work-up and purification of the crude product by column chromatography, the product is identified as racemic 4-acetoxy-2-ethyl-3-methoxy-2-cyclopenten-1-one **28** (yellow oil in 80% yield). It was observed that when cyclohexane is used as a solvent instead of benzene γ-acetoxylation product is obtained according to the spectroscopic data.

From the ^{1}H-NMR spectrum we observed a singlet at 2.06 ppm from the $-CH_3$ group and at 5.78 ppm d, (J=6.3 Hz) for the α-proton. From the ^{13}C-NMR spectrum, we observed a peak at 20.8 ppm for the CH_3 carbon and a peak at 169.5 ppm for the $O\underline{C}OCH_3$ carbon.

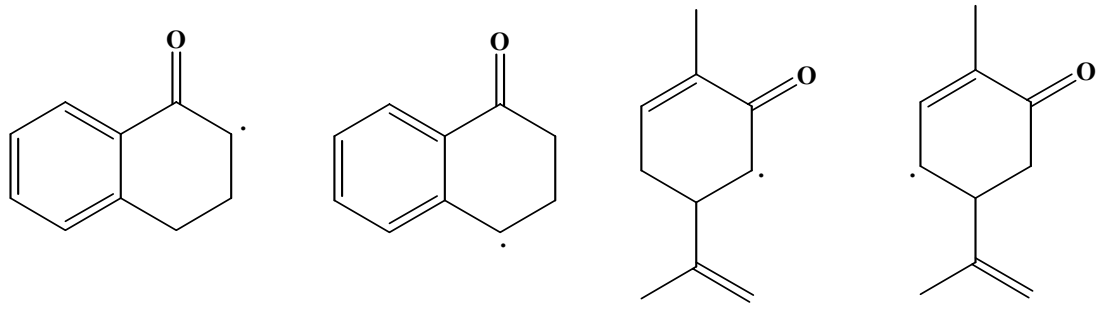
Since the oxidation of carbonyl compounds with manganese(III) acetate has been reported to involve an α-oxo radical resulting from the oxidation of an enol or

enolate anion by Mn(III), it is possible that this reaction proceeds via the formation of an β-keto radical which can be preferred in cyclohexane, followed by ligand transfer (Scheme 16) to yield the product.



Scheme 16

A theoretical analysis involving α-tetralone and carvone have been reported.[41] According to this study all the calculated data suggest that (at least in the case of α-tetralone and carvone); if the manganic oxidations of enones followed by ligand transfer-oxidations to the product would occur, then not the α' but the γ-position with respect to the keto group would be the susceptible site for the reaction. Molecular orbital calculations for the radicals **29a-b** and **30a-b** (α-tetralone and carvone) indicate that radicals **29b** and **30b** are more stable than their counterparts **29a** and **30a**. (Scheme 17) The stable radicals of the present concern are characterized with more negative total and binding energies. Moreover, their heats of formation (ΔH_f) are exothermic while the corresponding values for the less stable radical (**29a** and **30a**) indicate endothermic (ΔH_f).



29a **29b** **30a** **30b**

Scheme 17

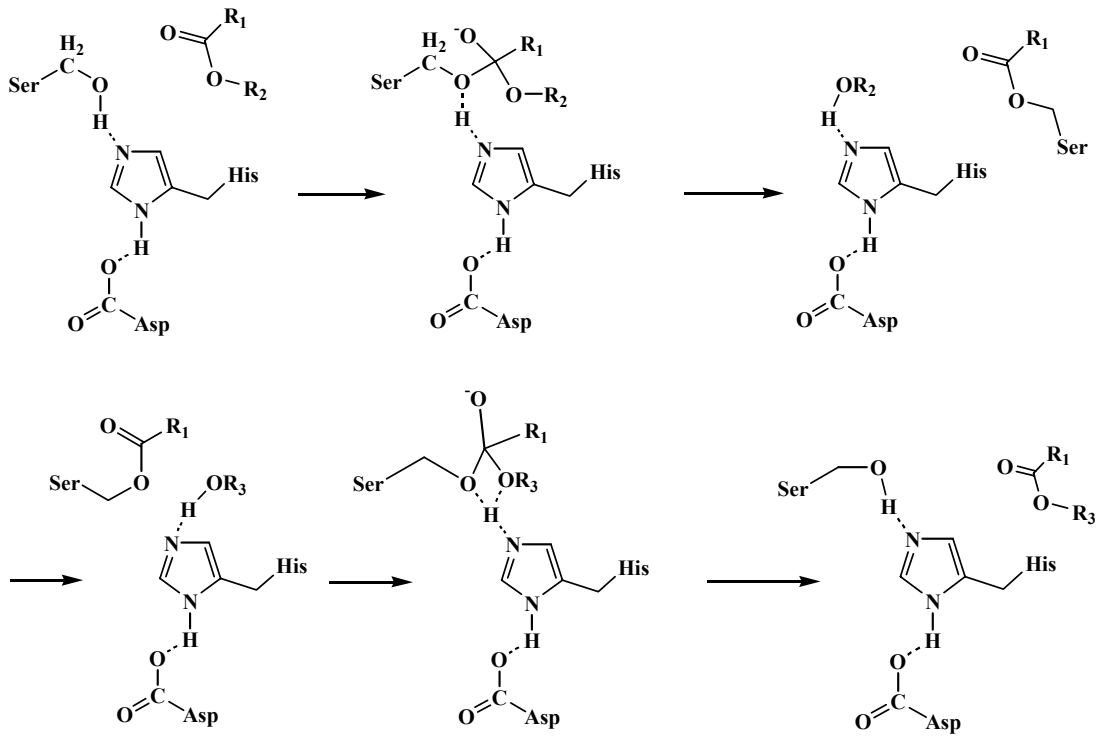
The two reactions, carried out in benzene and cyclohexane, are showing the solvent dependent regioselectivities by the oxidation of enone 2-ethyl-3-methoxy-2-cyclopenten-1-one **14** with $Mn(OAc)_3$.

2.3 Enzyme Mediated Hydrolysis of Acetoxy Ketones

The most commonly used biocatalysts are the hydrolytic enzymes 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. Lipase-mediated resolutions of chiral alcohols, either by acyl transfer methods or by hydrolysis of their corresponding esters, are probably the biotransformation most commonly described in modern literature. Both the regio and enantioselectivity of biocatalyst makes the process even more attractive. 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.[10]

Hydrolytic enzymes can be divided into 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. Scheme 18 illustrates a catalytic cycle for serine proteases, which is representative for most lipases.

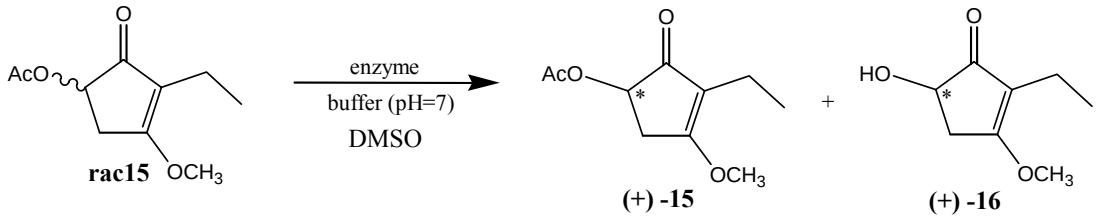


Scheme 18

In serine proteases, a catalytic triad consisting of the amino acids serine, histidine and aspartic acid are responsible for the catalysis. In Scheme 18 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 His binds to the alkoxy group that is then eliminated as an alcohol molecule. An acyl

enzyme is 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.

Racemic 5-acetoxy-2-ethyl-3-methoxy-2-cyclopenten-1-one **15** is used in the enzymatic hydrolysis to obtain chiral α'-acetoxy and α'-hydroxy enones. (Scheme 19)



Scheme 19

To obtain optimum conditions for the enzymatic hydrolysis of acetoxy enone **15** analytical screening was carried out. Hence the reaction was performed in analytical scale. Because of the poor solubility of the substrate in aqueous medium, a few milliliters of DMSO was used as an organic solvent. About 5 mg. of acetoxy enone **15** was dissolved in minimum amount of DMSO and then 300-μL buffer was added. The mixture was stirred at room temperature in the presence of enzyme. The reaction was monitored by TLC, and when approximately 50% conversion was observed; it is terminated by adding chloroform. After the separation of organic phase from water, the ee values were determined by HPLC. (Table 1)

Table 1. Enzymatic hydrolysis of (±)-**15**-5-acetoxy-2-ethyl-3-methoxy-2-cyclopenten-1-one

			acetoxy		*hydroxy*	
Enzyme	Reaction time	Conversion %	ee %	yield %	ee %	yield %
CCL (Candida Cylindracea Lipase)	174 h	50	>97	46	>93	48
Amano (Amano PS)	30 h	56	99	44	>65	48
PPL (Porcine Pancreatic Lipase)	160 h	48	75	47	69	45
PLE (Pig Liver Esterase)	22 h	52	85	46	83	47

From this table, it can be seen that best results were obtained using CCL (Candida Cylindracea Lipase). Careful monitoring of the reactions with TLC furnished the (+)-**15,** (>97% ee) and (+)-**16,** (>93% ee) by using CCL (Candida Cylindracea Lipase). Several types of chiral HPLC columns were tried for the best separation of enantiomers. Chiralcel OD column was chosen because it was able to separate both enantiomers of α'-acetoxy and α'-hydroxy enones.

In order to decide R_f values for both enantiomers of products, racemic forms of them were analyzed first. After analysis of all of the enzymes, enantiomeric excess values were determined with HPLC (Chiralcel OD column, eluent: hexane/2-propanal=98:2) by using peak area %'s of the enantiomers. For racemic acetoxy enone R_f for (-)-**15** was 55 min and R_f for (+)-**15** was 98 min (Figure 15) and for

racemic hydroxy enone (±)-**16** R_f values were determined; R_f for (-)-**16** was 58 min and R_f for (+)-**16** was 65 min. (Figure 16).

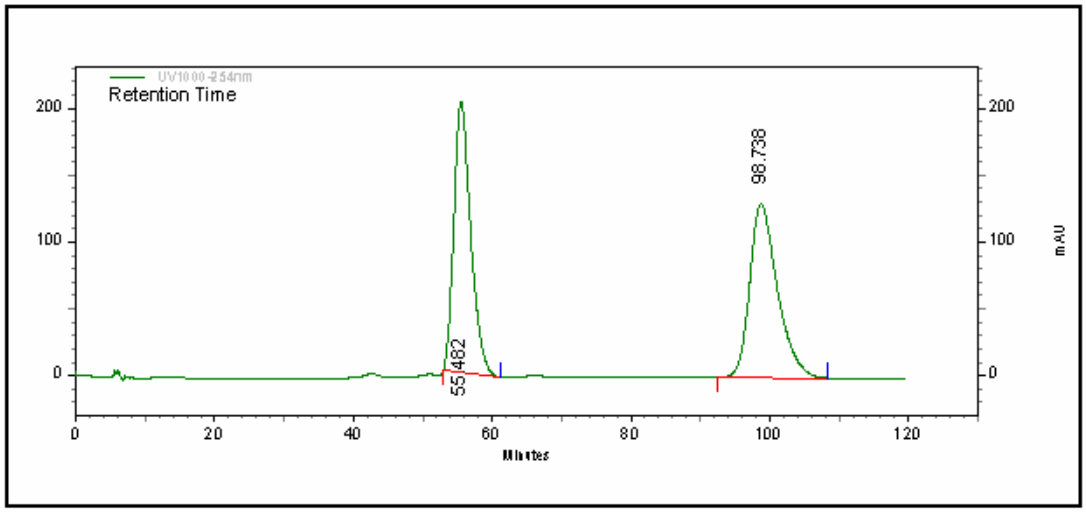


Figure 15: Chiral HPLC Chromatogram of racemic α'-acetoxy enone (±)-**15** (Chiralcel OD column, eluent: hexane/2-propanal=98:2, flow 0.7 mL min^{-1})

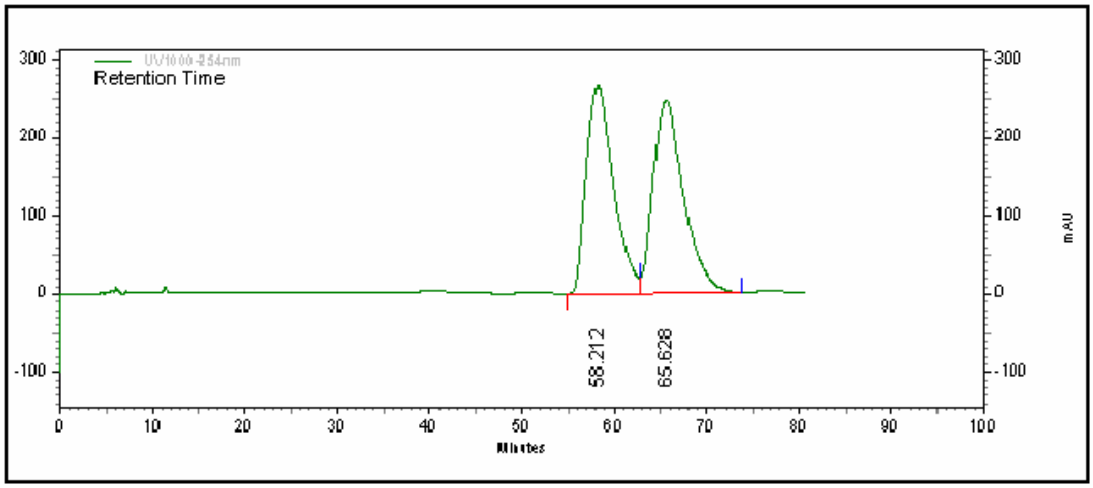


Figure 16: Chiral HPLC chromatogram of racemic α'-hydroxy enone (±)-**16** (Chiralcel OD column, eluent: hexane/2-propanal=98:2, flow 0.7 mL min^{-1})

According to HPLC data, the enzyme, which gave the highest ee value, was chosen for the preparative synthesis. (Figure 17)

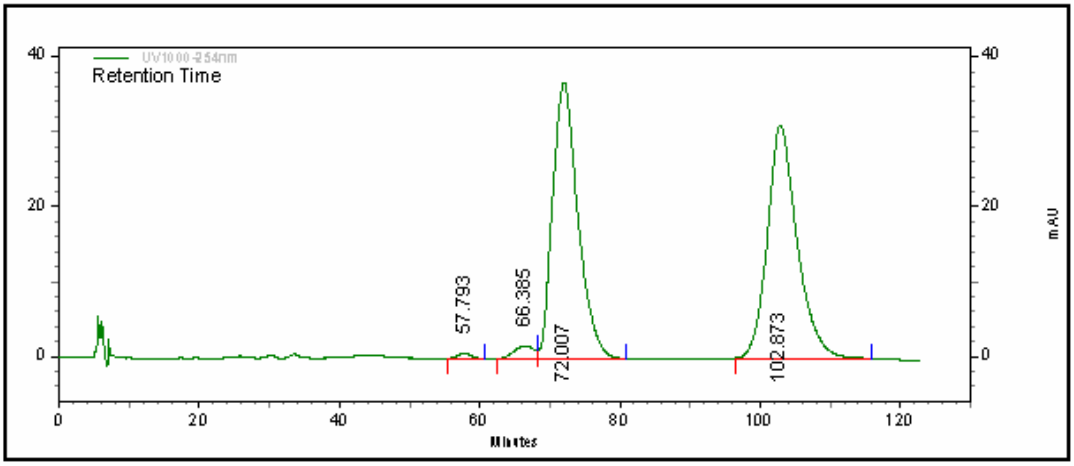


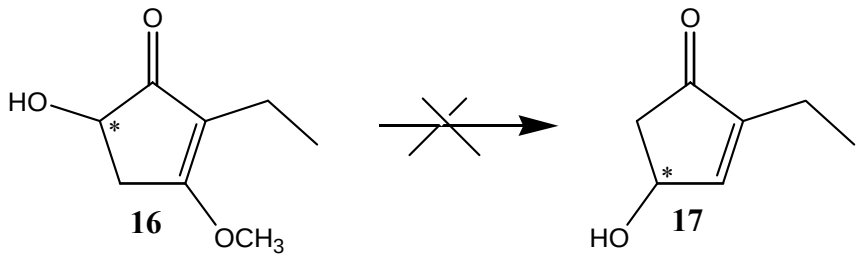
Figure 17: Chiral HPLC chromatogram of both enantiomers of α'-acetoxy enone and α'-hydroxy enone
(Chiralcel OD column, eluent: hexane/2-propanal=98:2, flow 0.7 mL min^{-1})

The products were identified by using NMR spectroscopy. From the ^{1}H-NMR spectrum of (+)-**15,** we observed a singlet at 2.13 ppm from the $–CH_3$ group and at 5.08 ppm dd, (J=2.6 and 6.9 Hz) for the α-proton. From the ^{13}C-NMR spectrum, we observed a peak at 19.6 ppm for the CH_3 carbon and a peak at 169.2 ppm for the $O\underline{C}OCH_3$ carbon. From the NMR spectrum of (+)-**16,** we observed a d at 4.29 ppm (J = 6.6 Hz) for the α-proton and a broad singlet at 1.25 ppm for the –OH proton. From the ^{13}C-NMR spectrum, we observed a singlet at 70.7 ppm for –CH-OH carbon.

According to published procedures racemization-free conversion of acetoxy to hydroxyl enone and vice versa gives possibility to obtain all of the enantiomers of acetoxy and hydroxy enones.[36-37]

2.4 Conversion of α-Hydroxy Enone to γ-Hydroxy Enone

α'-Acetoxy enones and α'-hydroxy enones are potential precursors for poly-hydroxy cyclopentenones. In general, reduction of carbonyl group into alcohol then hydrolysis of formed enol ether followed by elimination of water should give γ-hydroxy enone. In this step, although we have tried several methods according to the published procedures we could not perform conversion of α-hydroxy enone to γ-hydroxy enone. (Scheme 20)



Scheme 20

As a first example to a suspension of $LiAlH_4$ in anhydrous Et_2O α-hydroxy enone **16** was added at 25 ^{0}C over 30 min. The mixture is refluxed for two hours, cooled to 0 ^{0}C and quenched with water and aqueous H_2SO_4.[27] This method was also tried with α-acetoxy enone **15.**

According to another method two equivalence of $LiBH_4$ is reacted with the α-hydroxy enone **16** in methanol at 0^0 C.[42] The same procedure was tried with the $NaBH_4$. This method was also tried with α-acetoxy enone **15.**

In another method, enone **16** in THF was added to stirred suspension of $LiAlH_4$ in Et_2O. The reaction mixture was refluxed and allowed to cool. H_2O and NaOH is added.[43]

Lastly, α-hydroxy enone **16** in dry ether under argon was cooled in an ice-water bath and lithium aluminum hydride was added to the stirred solution. After 1 hour, it is treated with water and NaOH. [44]

None of these methods gave the desired γ-hydroxy enone. As a result of all these methods; from NMR spectrum a lot of unwanted products were observed.

It may be possible that the hydroxy enone isomerize to endiole, during the reaction and reduction elimination step can not take place.

2.5 Summary of Chemoenzymatic Synthesis of α-Acetoxy, γ-Acetoxy, α-Hydroxy, and γ-Hydroxy Enones

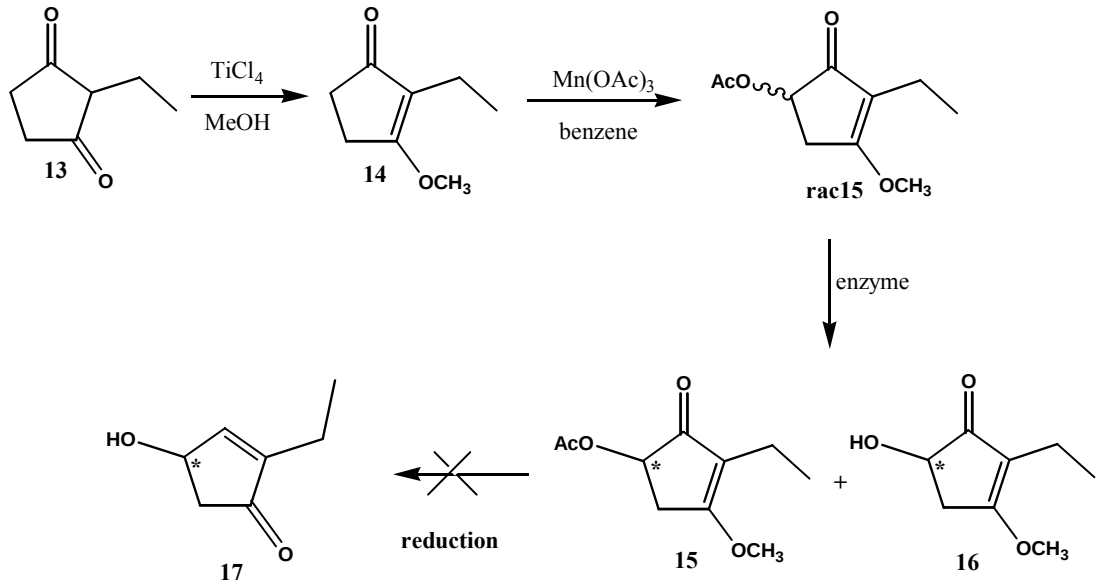
In summary, it is described here, the model study for the synthesis of chiral poly-functionalized cyclopentenone **15, 16** via enzymatic kinetic resolution.

According to the method firstly, the commercially available 2-ethyl-1,3-cyclopentanedione was converted to 2-ethyl-3-methoxy-2-cyclopenten-1-one. Then to obtain desired α'-acetoxy ketone the oxidation was performed with four equivalents of manganese (III) acetate in benzene. When the same reaction is performed in cyclohexane, the product was γ-acetoxy enone **28**.

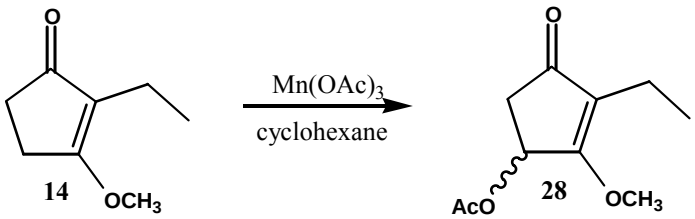
The enzymatic hydrolysis of the acetate (±)**-15**, in aqueous organic medium furnished **(+)-15** and **(+)-16** in high ee. High enantioselectivities can be achieved by an appropriate choice of an enzyme. The best results in the enzymatic hydrolysis of the racemic **15** were obtained with CCL (>97% ee for **(+)-15** and >93% ee for **(+)-16**) and with Amano PS (99% ee for **(+)-15** and >65% ee for **(+)-16)** in DMSO. The yields were around 50%, which means that no side products were observed.

For the synthesis of γ-hydroxyl enone, **(+)-16**, which is obtained from the reaction of racemic acetoxy enone with CCL is used. However, synthesis of the γ-

hydroxyl enone could not be performed although several methods were tried. (Scheme 21-22)



Scheme 21



Scheme 22

These results show us that, the same experiments can be applied to the synthesis of any other 2-substituted poly-oxo ketones, which are important intermediates for the asymmetric synthesis of many natural products.

CHAPTER 3

EXPERIMENTAL

3.1 Materials and Methods

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

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 Sucromat polarimeter. Enantiomeric excesses were determined by HPLC analysis using a Thermo Quest (TSP) equipped with an appropriate optically active column.

3.2 General Procedures

3.2.1 Synthesis of 2-ethyl-3-methoxy-cyclopent-2-enone (14)

To a well stirred solution of 2-ethyl-1,3-cyclopentanedione (1g 7.94 mmol) in dry methanol (90mL), 1.5 mL 1.0 M $TiCl_4$ solution is added dropwise under argon at RT. The reaction mixture was then stirred for 28 h. and reaction monitored by TLC. After work up it is purified by column chromatography (3:1 EtOAc:Hexane).

The product was obtained as yellow oil after column chromatography. (980 mg, 88% yield)

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

δ (ppm): 1.07 (t, J=7.6, 3H)
2.22 (q, J=7.6, 2H)
2.50 (t, J=4.8, 2H)
2.73 (t, J=4.8, 2H)
3.95 (s, 3H)

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

δ (ppm): 12.7, 14.6, 24.4, 33.4, 56.1, 122.4, 183.4, 204.0

3.2.2 Synthesis of acetoxy ketones

3.2.2.1 Synthesis of 3-Ethyl-4-methoxy-2-oxo-cyclopent-3-enyl acetate (15)

A solution of **14** (1.0g 7.14 mmol) and $Mn(OAc)_3$ (7.3g 28.6 mmol) in benzene (200 mL) were heated under reflux for 4 days. The reaction was monitored by TLC. After cooling, the reaction mixture was filtered; then washed with saturated $NaHCO_3$ solution. The solution was dried over $MgSO_4$, concentrated and purified by column chromatography (1:1 EtOAc: Hexane), to yield 990 mg, 70% of the product as a yellow oil.

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

δ (ppm): 1.01 (t, J=7.6, 3H, CH_3)
2.13 (s, 3H, $COCH_3$)
2.16 (q, J=7.6, 2H, CH_2)
2.48 (m, 1H, CH_2)
3.19 (dd, J= 17.5, 6.9, 1H, CH_2)
3.95 (s, 3H, OCH_3)
5.08 (dd, J= 2.6, 6.9, 1H, CH)

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

δ (ppm): 11.4, 13.5, 19.6, 31.7, 55.5, 69.9, 120.4, 169.2, 179.5, 197.0

3.2.2.2. Synthesis of 3-Ethyl-2-methoxy-4-oxo-cyclopent-2-enyl acetate (28)

A solution of **14** (1.0g 7.14 mmol) and $Mn(OAc)_3$ (7.3g 28.6 mmol) in cyclohexane (200 mL) were heated under reflux for 4 days. The reaction was monitored by TLC. After cooling, the reaction mixture was filtered; then washed with saturated $NaHCO_3$ solution. The solution was dried over $MgSO_4$, concentrated and purified by column chromatography (1:1 EtOAc: Hexane), to yield 1130mg, 80% of the product as a yellow oil.

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

δ (ppm): 0.97 (t, J=7.7, 3H, CH_3)
2.06 (s, 3H, $COCH_3$)
2.15 (d, J=19.0, CH_2, 1H)
2.16 (q, J=7.7, 2H, CH_2)
2.79 (dd, J=6.3, 19.0, 1H, CH_2)
3.90 (s, 3H, OCH_3)
5.78 (d, J=6.3, 1H, CH)

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

δ (ppm): 12.9, 14.9, 20.8, 41.8, 57.1, 67.7, 125.5, 169.5, 176.2, 199.6

3.2.3 Enzyme Catalyzed Kinetic Resolution

To a stirred solution of (±)-3-ethyl-4-methoxy-2-oxo-cyclopent-3-enyl acetate (100 mg 0.5 mmol) in DMSO (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 room temperature. Conversion was monitored by TLC and when it was 50%, the reaction was terminated by adding 20ml. $CHCl_3$. The organic layer was separated, dried over $MgSO_4$, and concentrated.

The unreacted acetate and the product is separated by flash column chromatography (1:1 EtOAc:Hex) to obtain 46 mg, 46% of (+)-3-ethyl-4-methoxy-2-oxo-cyclopent-3-enyl acetate and 38 mg, 48% of 2-ethyl-5-hydroxy-3-methoxy-cyclopent-2-enone as a white solid. (M.P. = 94 ^{0}C)

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

δ (ppm): 0.99 (t, J=7.5, 3H, CH_3)
1.25 (s, OH)
2.15 (q, J=7.5, 2H, CH_2)
2.57 (d, J=17.1, 1H, CH_2)
3.07 (dd, J=6.6, 17.1, 1H, CH_2)
3.96 (s, 3H, OCH_3)
4.29 (d, J=6.6, 1H, CH)

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

δ (ppm): 12.4, 14.4, 33.8, 56.9, 70.7, 119.8, 182.5, 205.1

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

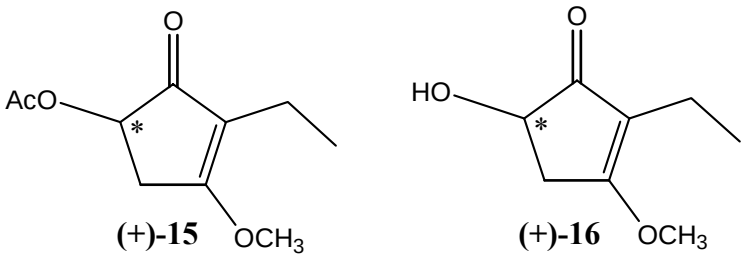
Chiralcel OD column, UV detection at 254 nm, eluent: hexane/2-propanol=98:2, flow 0.7 mL min^{-1}, using racemic compounds as references; R_f for **(-)15**=57 min, **(+)15**=102 min, $[\alpha]_D^{20}$ = +52.83 (c=0.6, $CHCl_3$); R_f for **(-)16**= 66 min, **(+)16**=72 min, $[\alpha]_D^{20}$ = +45.76 (c=0.33, $CHCl_3$).

CHAPTER 4

CONCLUSION

Polyoxo ketones are important intermediates for the construction of chiral organic compounds due to reactive functional groups: OMe, acetoxy, double bond, carbonyl, hydroxyl groups. These functional groups can be converted to other functional groups and they can be used in many synthetic applications. Many biologically active compounds contain these structures; such as didemnenones, prostaglandins, sarkomycin, punoglandin etc.

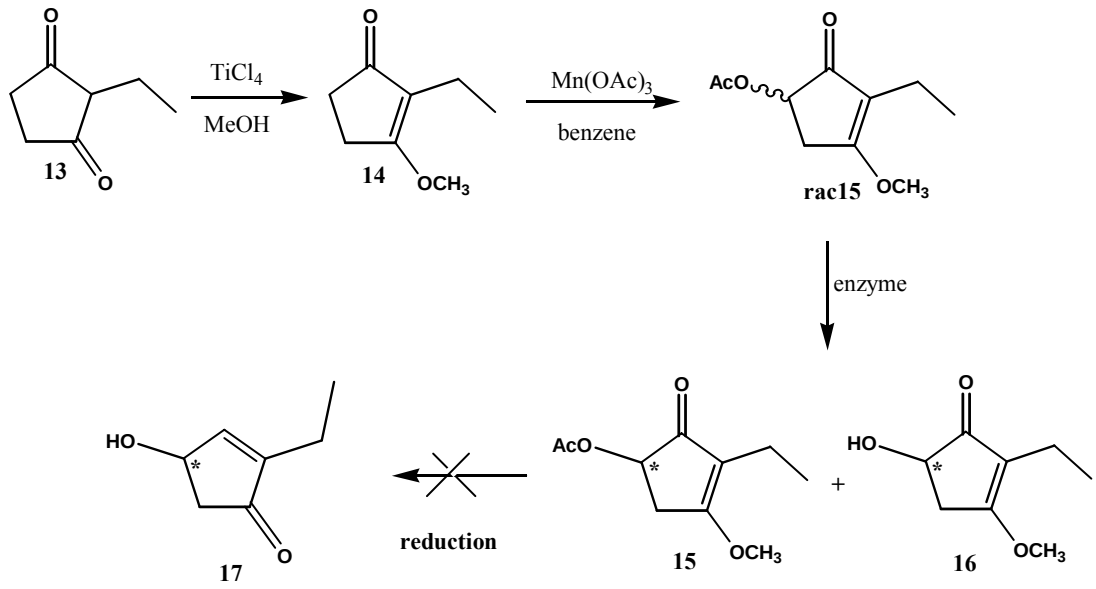
A new and efficient route is developed for the synthesis of optically active cyclopentenones. 2-Ethyl-1,3-cyclopentanedione is protected and converted into its acetoxy derivative using $Mn(OAc)_3$ in good yield. Acetoxylation reaction in benzene gives 3-Ethyl-4-methoxy-2-oxo-cyclopent-3-enyl acetate while the product of this reaction in cyclohexane is 3-Ethyl-2-methoxy-4-oxo-cyclopent-2-enyl acetate. Acetoxylation is followed by enzyme-mediated hydrolysis of the acetoxy group provides chiral α-hydroxy enone with 48% yield and 97% enantiomeric excess and chiral α-acetoxy enone 46% yield and 93% enantiomeric excess. (Scheme 23)



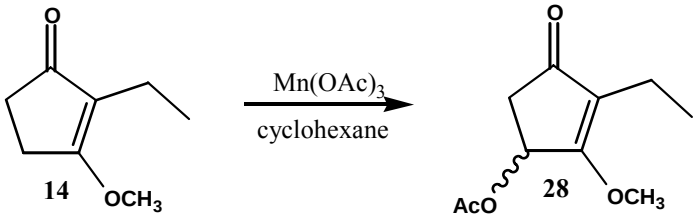
Scheme 23

For the synthesis of γ-hydroxyl enone, **(+)-16**, which is obtained from the reaction of racemic acetoxy enone with CCL is used. However, synthesis of the γ-hydroxyl enone could not be performed although several methods were tried.

These results show us that, the same experiments can be applied to the synthesis of any other 2-substituted poly-oxo ketones, which are important intermediates for the asymmetric synthesis of many natural products. (Scheme 24-25)



Scheme 24



Scheme 25

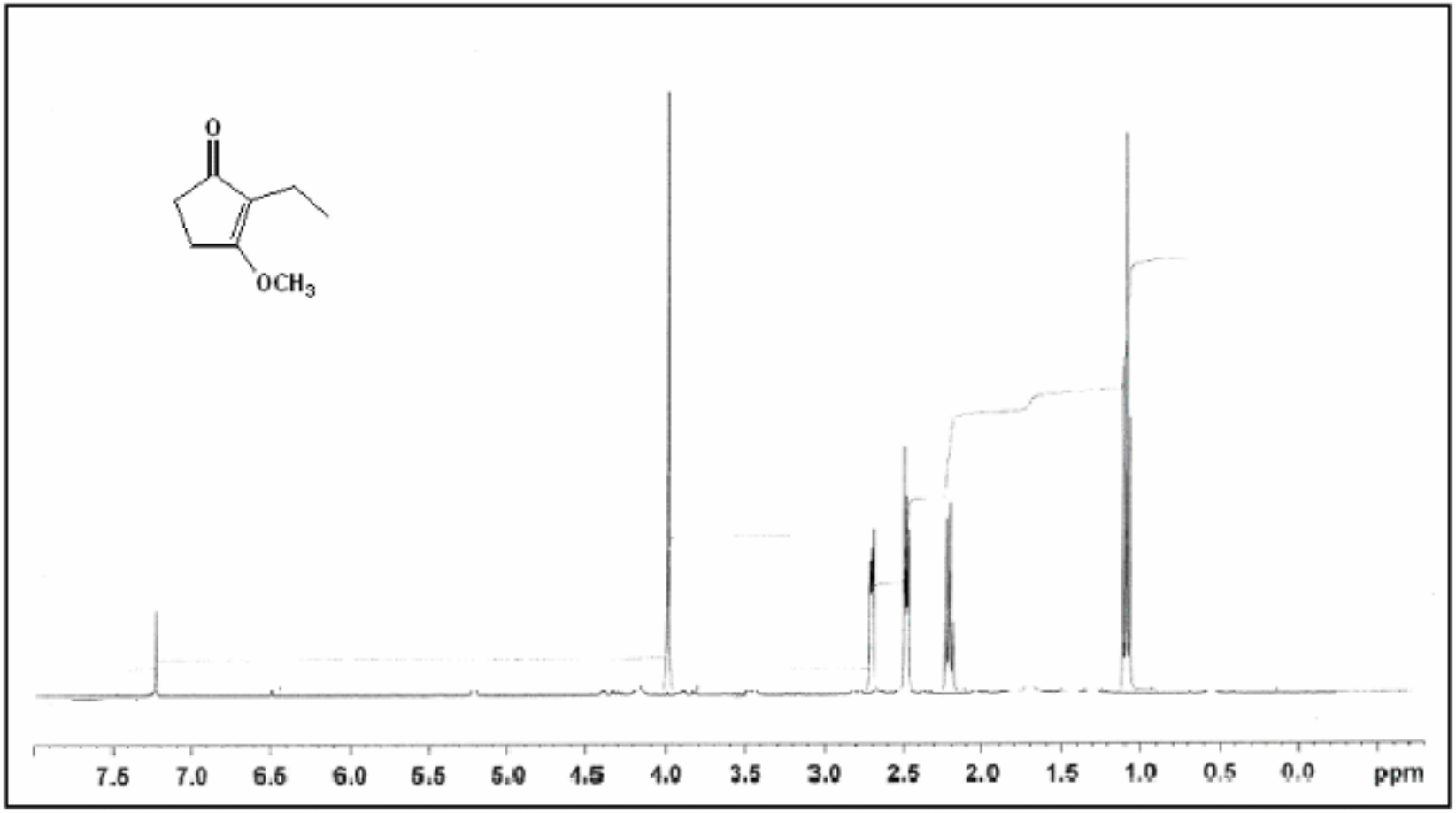


Figure 18: ^{1}H-NMR spectrum of 2-ethyl-3-methoxy-cyclopent-2-enone **(14)**

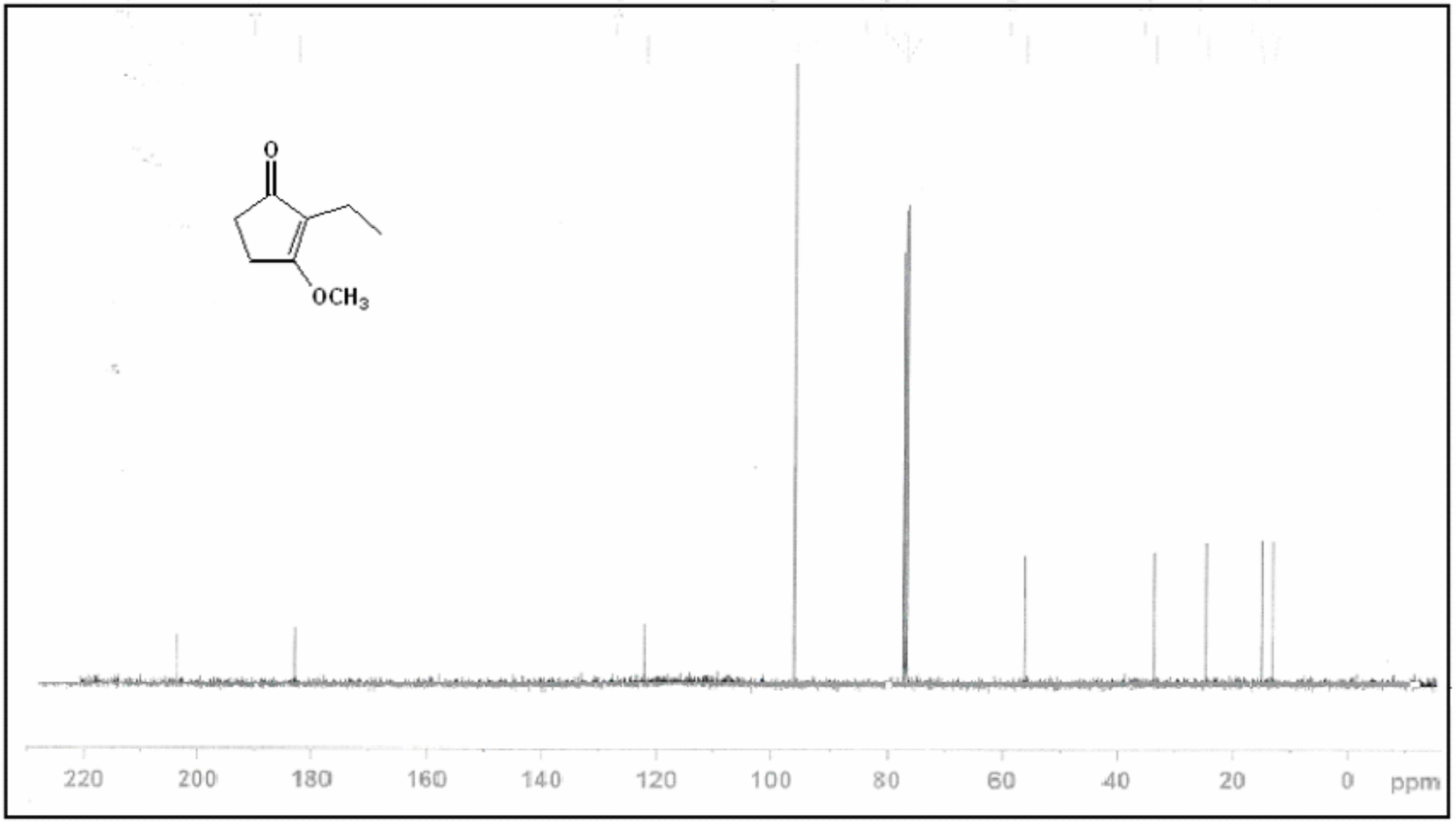


Figure 19: ^{13}C-NMR spectrum of 2-ethyl-3-methoxy-cyclopent-2-enone **(14)**

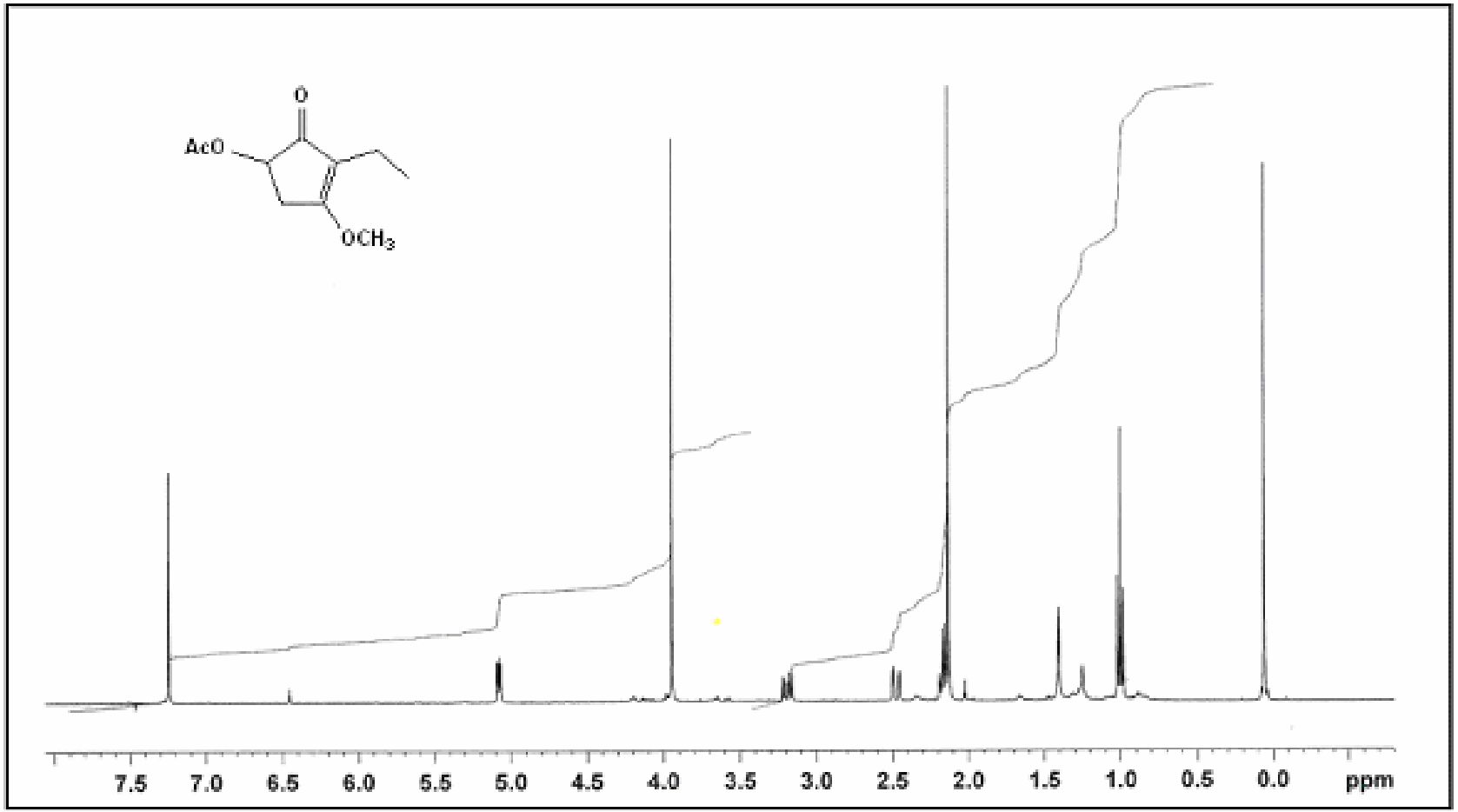


Figure 20: ^{1}H-NMR spectrum of 3-Ethyl-4-methoxy-2-oxo-cyclopent-3-enyl acetate **(15)**

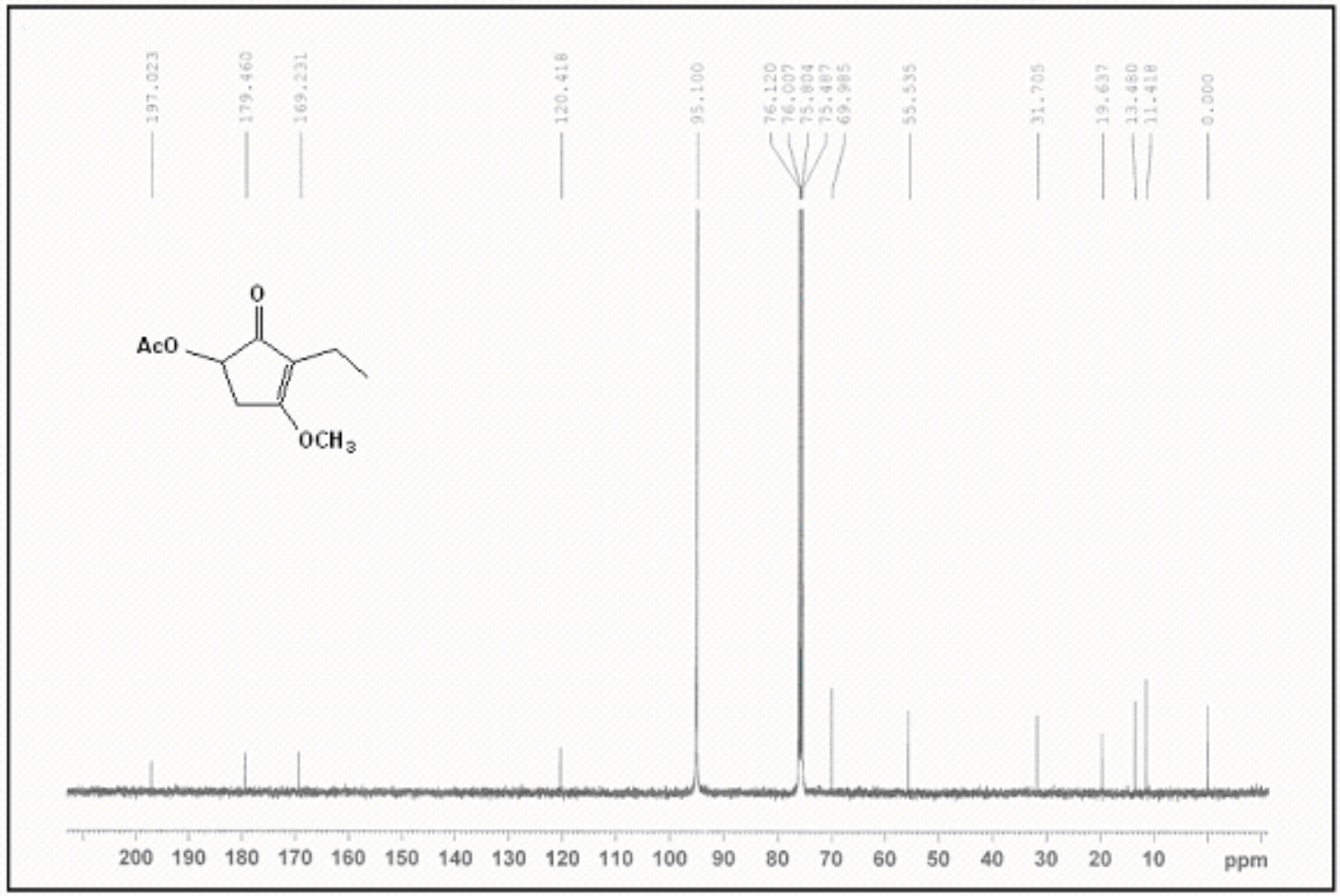


Figure 21: ^{13}C-NMR spectrum of 3-Ethyl-4-methoxy-2-oxo-cyclopent-3-enyl acetate **(15)**

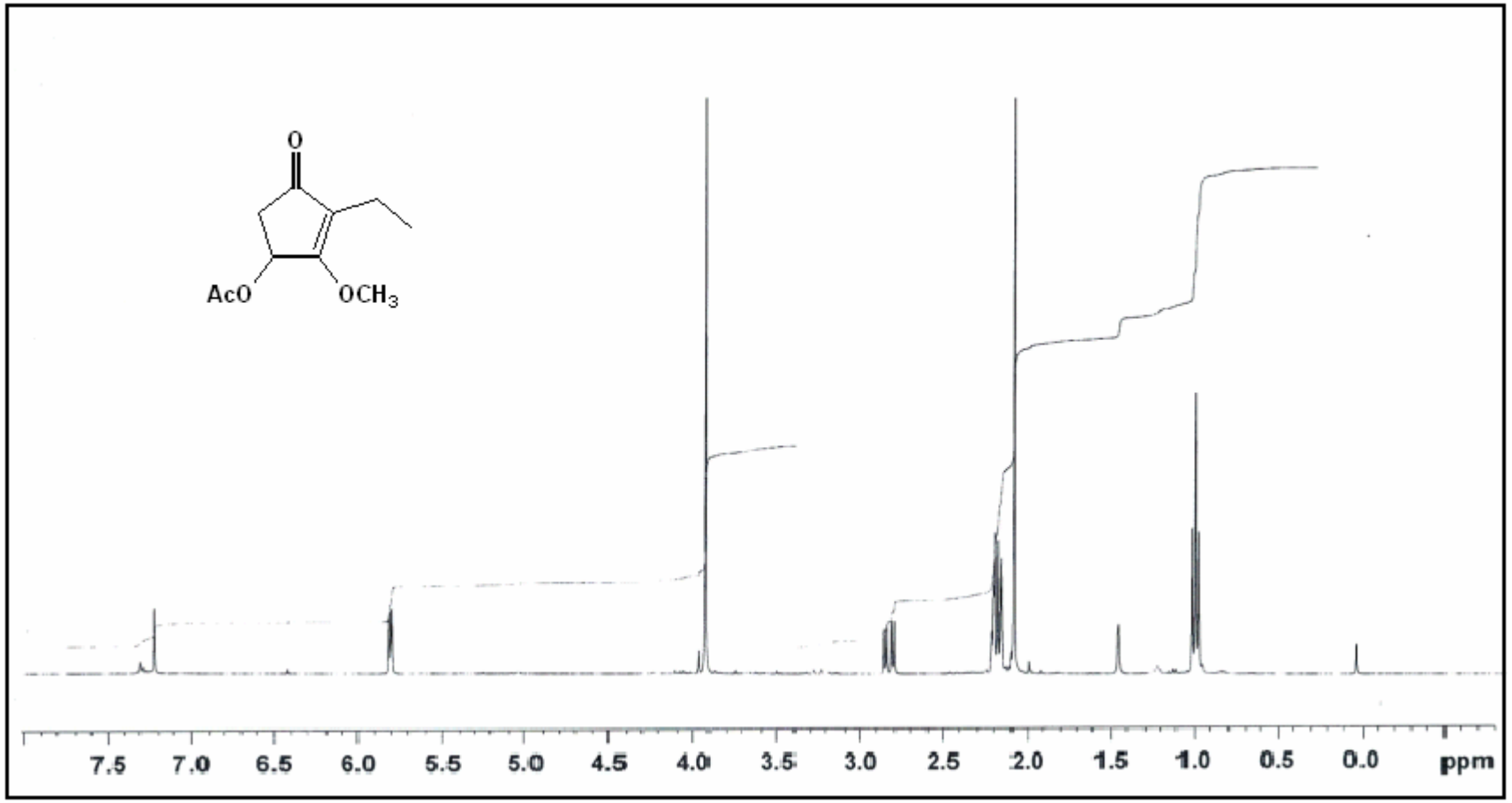


Figure 22: ^{1}H-NMR spectrum of 3-Ethyl-2-methoxy-4-oxo-cyclopent-2-enyl acetate **(28)**

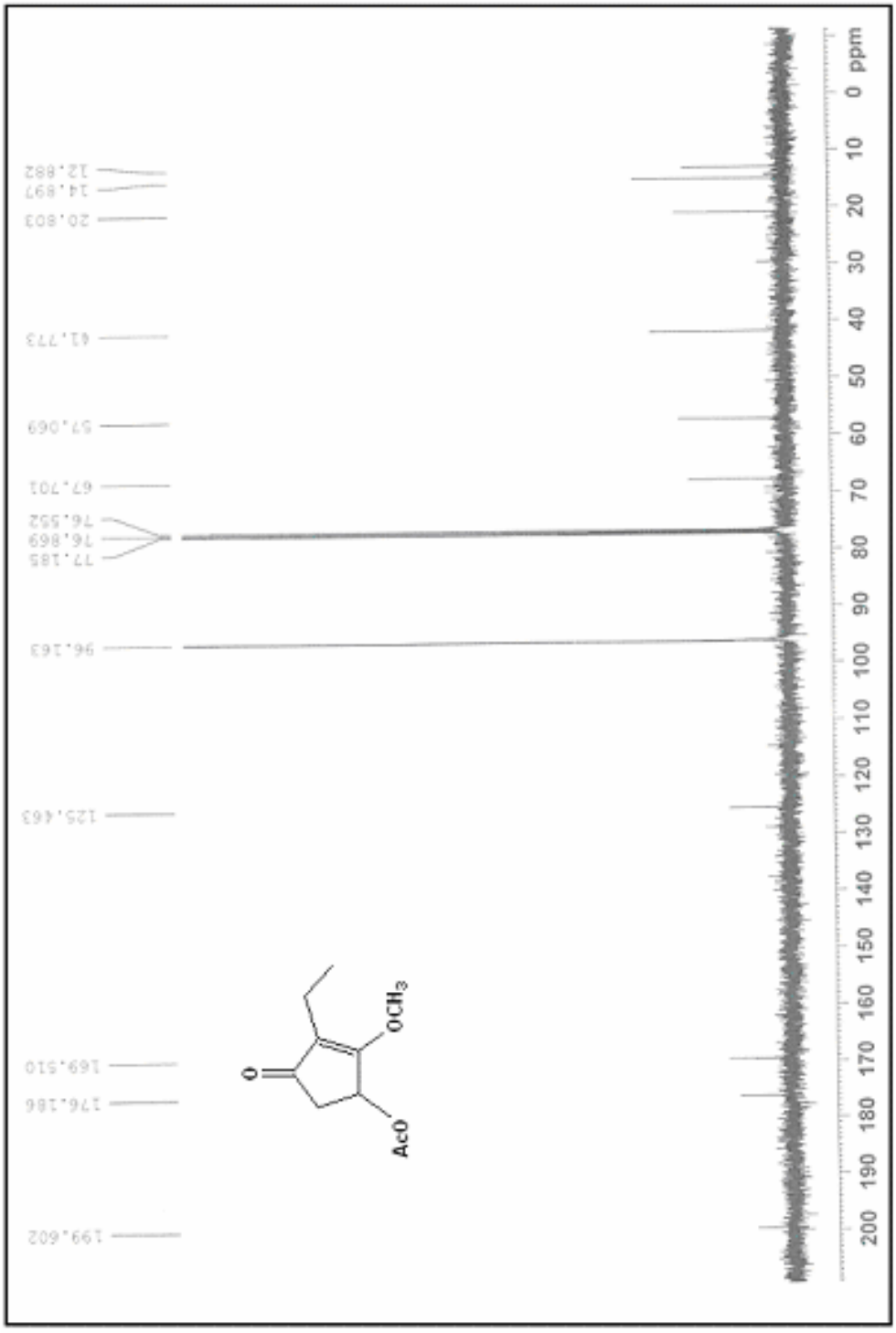


Figure 23: ^{13}C-NMR spectrum of 3-Ethyl-2-methoxy-4-oxo-cyclopent-2-enyl acetate **(28)**

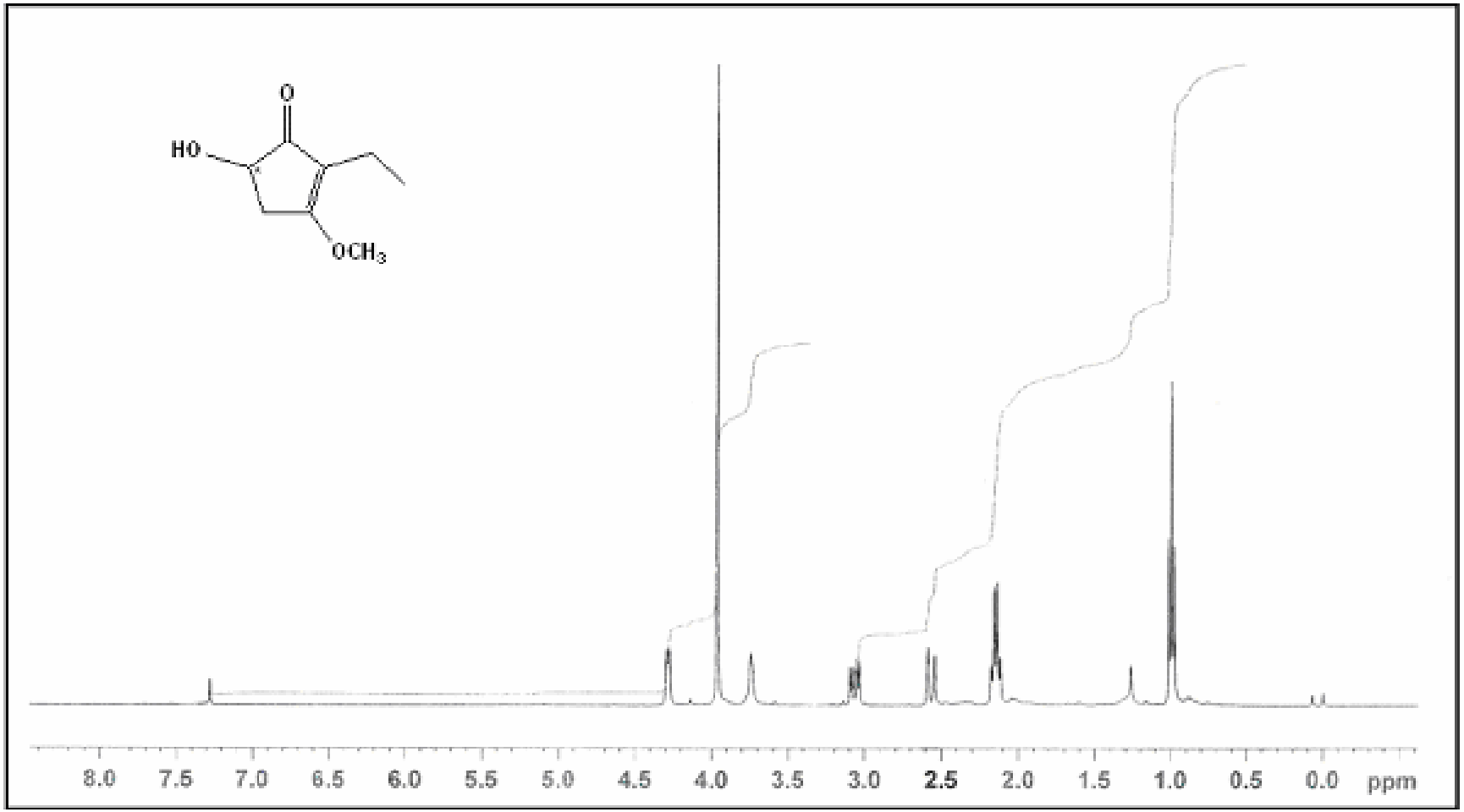


Figure 24: ^{1}H-NMR spectrum of 2-ethyl-5-hydroxy-3-methoxy-cyclopent-2-enone **(16)**

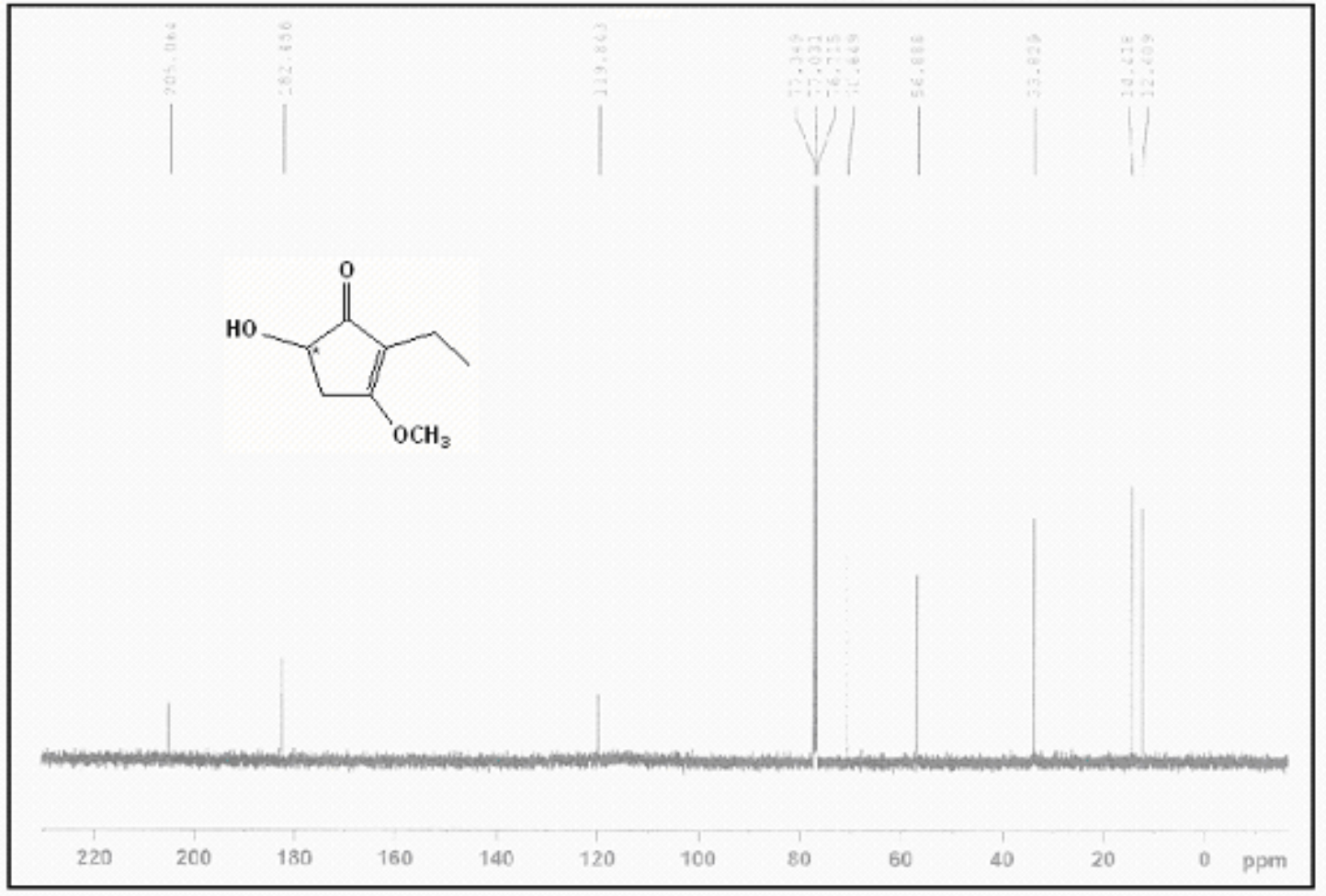


Figure 25: ^{13}C-NMR spectrum of 2-ethyl-5-hydroxy-3-methoxy-cyclopent-2-enone **(16)**

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