CHEMOENZYMATIC SYNTHESIS OF CHIRAL HYDROXYMETHYL CYCLOALKENOLS

A THESIS SUBMITTED TO
THE GRADUATE SCHOOL OF NATURAL AND APPLIED SCIENCES
OF
MIDDLE EAST TECHNICAL UNIVERSITY

BY

DENİZ ŞENOCAK

IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF
MASTER OF SCIENCE
IN
CHEMISTRY

JUNE 2004

Approval of the Graduate School of Natural and Applied Sciences

_____

Prof. Dr. Canan Özgen

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

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Chair of Chemistry Department

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

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ABSTRACT

CHEMOENZYMATIC SYNTHESIS OF CHIRAL HYDROXYMETHYL CYCLOALKENOLS

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Chiral cyclic alkenols with hydroxymethyl functionality are important structural units in many biologically active natural compouds such as prostaglandins, sesquiterpene antiviral agents, pentenomycins, xanthocidin, sarkomycin, etc.

1,3-cycloalkanediones are converted into bicyclic polyoxo derivatives with formaldehyde and trioxane in the presence of Lewis acid. Selective oxidation of the bicyclic compounds by using manganese(III)acetate followed by enzyme-catalyzed kinetic resolution afforded chiral bicyclic hydroxy ketones. Reduction of carbonyl group and cleavage of the ether functionality furnished the desired chiral cycloalkanols with hydroxymethyl group. This study is a model for the synthesis of these type of compounds.

Key words: α′-acetoxy enones, manganese(III)acetate mediated oxidation, enzymatic kinetic resolution

ÖZ

KİRAL HIDROKSIMETIL SİKLO ALKENOLLERİİN KEMOENZİMATİK SENTEZİ

Şenocak, Deniz
Yüksek Lisans, Kimya Bölümü
Tez Yöneticisi: Prof. Dr. Ayhan S. Demir
Haziran 2004, 71 pages

Hidroksimetil fonksiyonuna sahip kiral siklik alkenoller prostaglandin, seskueterpen antiviral ajanlar, pentenomisinler, xanthokidin, sarkomisin gibi biyolojik aktiviteye sahip bir çok doğal üründe bulunan önemli yapılardır.

1,3-sikloalkandionlar formaldehit ve trioksanla Lewis asitler varlığında reaksiyona sokularak bisiklik poliokso türevlerine dönüştürülmüştür. Mangan asetat oksidasyonu ile elde edilen asetoksi ketonlar enzimle seçici hidroliz yapılarak kiral hidroksi ve asetoksi türevlerine dönüştürülmüştür. Bu ürünlerin keton gruplarının indirgenmeleri ve hidrolizleri sonucu kiral, hidroksimetil grubu içeren sikloalkanoller sentezlenmiştir. Bu çalışma, bu türde maddelerin sentezi için model bir yöntemdir.

Anahtar kelimeler: α′-asetoksi enonlar, mangan(III)asetat oksidasyonu, enzimatik kinetik resolüsyon.

To My Parents

ACKNOWLEDGMENTS

I would like to express my deepest gratitude to my supervisor Prof. Dr. Ayhan S. Demir for his guidances, advices, criticisms and encouragements and insight throughout this research.

I also would like to thank Prof. Dr. Dinçer Ülkü and Dr. Cengiz Arıcı from Hacettepe University, Department of Physics for the X-Ray crystallographic analysis.

I am also grateful to my lab mates Çiğdem İğdir and Mustafa Emrullahoğlu for their warm friendship, help and collaboration.

I also want to thank my friends in the organic research group for their wonderful friendship and help during this study.

Finally I would like to thank my lovely sister Derya Şenocak and my parents for their love, moral support and encouragements.

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INTRODUCTION

1.1. Biotransformations in Organic Synthesis

Incorporation of biotransformation steps, using microorganisms and/or isolated enzymes, is increasingly being exploited both in industry and academic synthesis laboratories. The primary consideration for incorporation of a biotransformation in a synthetic sequence is the regio- and stereo-control that can be achieved using an enzyme-catalyzed reaction. Biotransformations are becoming accepted as a method for generating optically pure compounds and for developing efficient routes to target compounds. Biotransformations provide an alternative to the chemical synthetic methodology that is sometimes competitive, and thus represent a section of the tools available to the synthetic chemist.

Isolated enzyme systems or intact whole organisms may be used for such transformations. The majority of useful biotransformations carried out in organic synthesis are by the hydrolase class of enzyme. The oxidoreductases are a mediocre second, and the remaining classes are of low, but increasing utility. Each approach has its advantages and disadvantages. The advantages of enzymes in synthesis include that:

- They are effcient catalysts; the rates of enzyme-mediated processes are accelerated compared with chemical catalysts.

- Enzymes act in a pH range of about 5-8, typically around 7 and they can be carried out at ambient temperature and atmospheric pressure.

- They catalyse a broad range of reactions; enzyme-catalysed processes exist for a wide range of reactions and can often promote reactions at ostensibly non-activated sites in a substrate.

- They display selectivity; such as chemoselectivity, regioselectivity, diastereoselectivity and enantioselectivity.

- They are not restricted to their natural substrates; the majority of enzymes display high specifcity for a specific type of reaction while generally accepting a wide (although sometimes narrow) variety of substrates.

- They can work outside an aqueous environment; although some loss of activity is usually observed some enzymes can operate in organic solvents.

- They are environmentally acceptable; unlike heavy metals biocatalysts are environmentally benign reagents since they are completely degradable.

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.

The use of whole cells has the advantages that co-factor recycling is not required or that higher activities can be obtained with growing cultures or that immobilized whole cells have possible re-use. The disadvantages of whole cells include the technical expense of equipment, the technical problems when dealing with large volumes, lower concentration tolerance, lower tolerance to organic solvents, large biomass production with growing cultures and thus more by-products, and the low activities of immobilized cells [1].

1.2. Asymmetric Synthesis

1.2.1. Chirality

In 1874, the Dutch chemist Van‘t Hoff and the French chemist Le Bel independently of each other, considered a molecule formed of a carbon atom linked by chemical bonds to four other atoms at the four corners of a tetrahedron. Thus the molecule is said to be chiral, and the central carbon atom is known as the chiral or stereogenic center. This proposal of van't Hoff and Le Bel was, as it turns out, completely correct; indeed, the molecule CHFClBr is today the simplest known optically active molecule (Figure 1). This breakthrough formed the basis of modern stereochemistry.

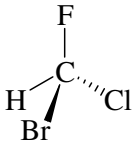


Figure 1 Optically active molecule

The word chiral comes from the Greek word “cheir”, which means hand. Chirality is often referred to as handedness. Hands are chiral and right and left hands cannot be superimposed on each other. Figure 2 is an example of a chiral object.

Right-handed helix left-handed helix

Figure 2 Chiral object

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 in space are different.

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 and the proteins and enzymes constructed from these building blocks, consist of only one of the two mirror images. L and D, or (R) and (S) are symbols used to designate the difference between enantiomers. Laboratory syntheses provide, in the absence of special chiral influences, equal quantities of (R) and (S) enantiomers. This 1:1 mixture of enantiomers is called a racemate.

The same holds for DNA, RNA and carbohydrates, biopolymers constructed from single enantiomers of small building blocks. Most physiological phenomena arise from highly precise molecular interactions, in which chiral host molecules recognize two enantiomeric guest molecules in different ways. Opposite enantiomers interact differently within the organism and can display various activities. Drugs work by interacting with the body's enzymes and receptors so some form of chiral selectivity is to be expected. Chiral receptor sites in the human body interact only with drug molecules having the proper absolute configuration, which results in marked differences in the pharmacological activities of enantiomers (Figure 3).

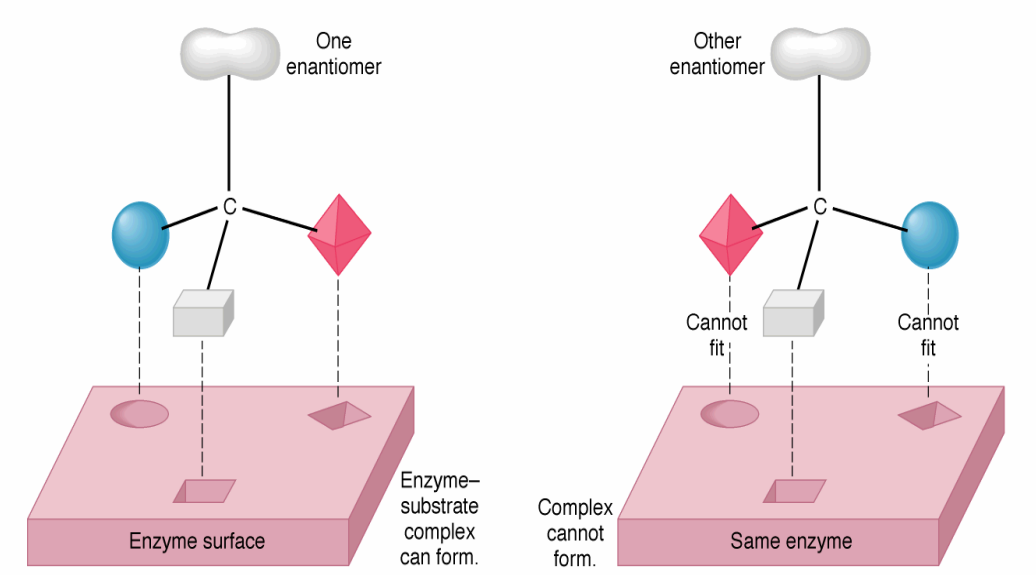


Figure 3 Model of enzyme substrate complex formation

An example of the relationship between pharmacological activity and molecular chirality was provided by the tragic administration of thalidomide to pregnant women in the 1960s. (R)-Thalidomide contained the desired therapeutic activity, while its S enantiomer is teratogenic and induces fetal malformations [2]. Another example is the odorant; limonene. (R)-(+)-limonene has a distinct orange odor whereas (S)-(-)-limonene has a characteristically lemon odor. Thus differences of the enantiomers range from teratogenic effects to distinguishable smells and flavors (Figure 4).

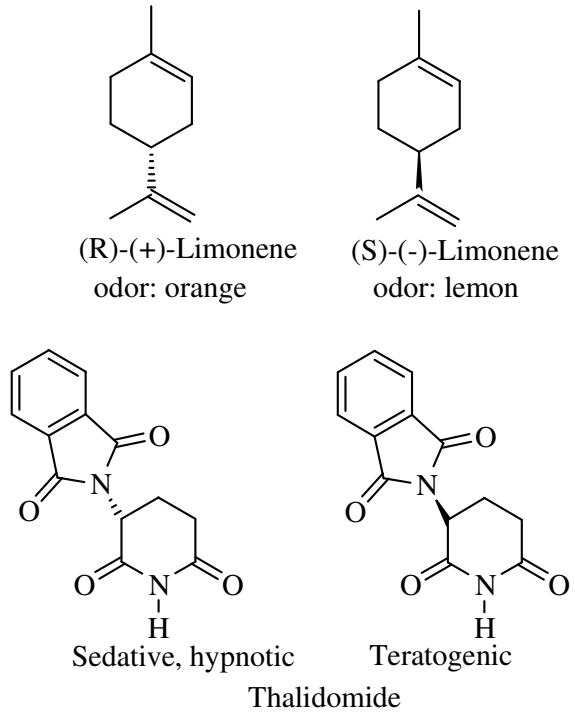


Figure 4 Biological effects of the enantiomers

Enantiomers have identical physical properties, such as melting point, boiling point etc. The difference between enantiomers can only be observed in a chiral environment, for example in a chiral solvent, in the presence of another chiral molecule, in the presence of circularly polarized light or in other chiral surroundings such as the human body. Enantiomers are capable of rotating the plane of polarized light and are therefore optically active. Association of enantiomers with another chiral molecule that is enantiomerically pure leads to diastereomers. Diastereomers are no longer mirror images as shown in figure 5, hence they differ in physical properties and can thus be separated by physical methods such as crystallization, distillation, etc.

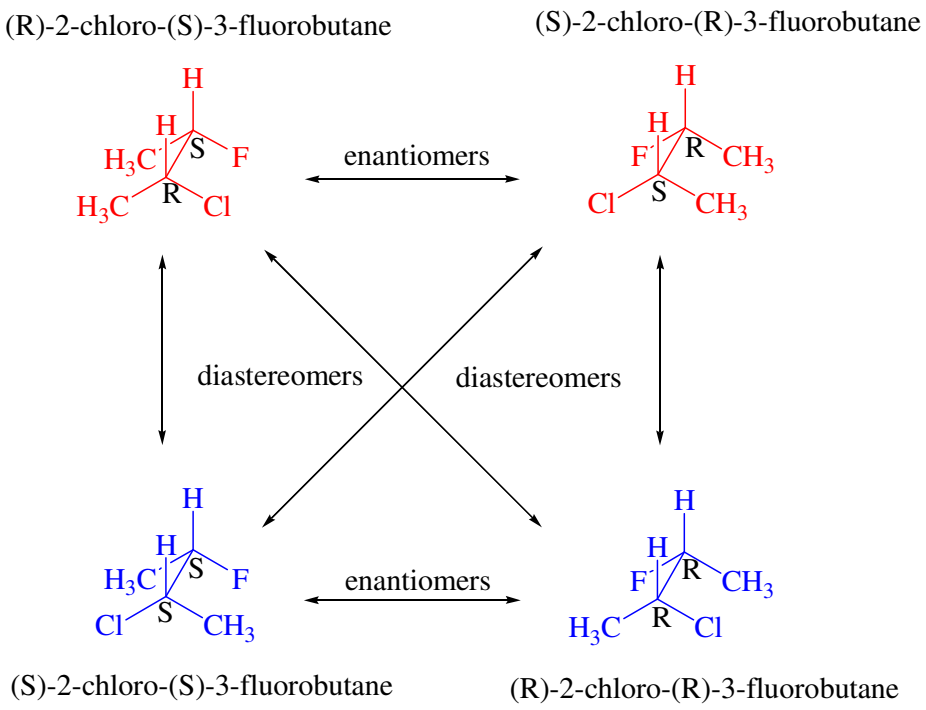


Figure 5 Enantiomer and diastereomer differentiation

In general, there are three routes to enantiopure compounds. They can be obtained from the chiral pool, from prochiral substrates or from a racemic mixture (Figure 6) [3,4]

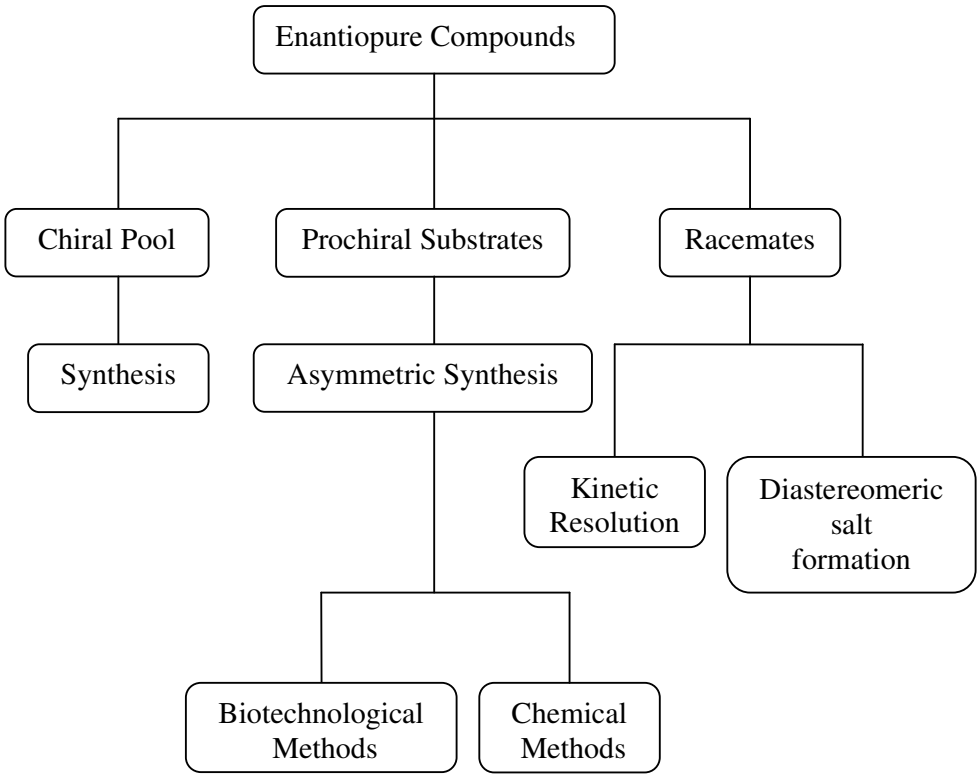


Figure 6 Flowchart of enantiopure compounds

In the chiral pool approach, naturally occurring, enantiomerically pure non-racemic compounds that are commercially available are used as the source of the desired asymmetry (chirality). These 'synthons' are transformed into the desired product via synthetic methods. Examples of chiral synthons from nature are amino acids, carbohydrates and terpenes. Clearly, all transformations should be carried out with high stereoselectivity in order to maintain the original enantiomeric or diastereomeric purity. The natural compound is used stoichiometrically and the stereochemistry of the starting material determines the stereochemistry of the product which is a limitation of this approach as one can only synthesize one of the enantiomers.

1.2.2. General Methods for Asymmetric Synthesis

In order to obtain enantioselective synthesis, at least one of the agents in the system must be chiral. Asymmetric synthesis involves the introduction of chirality by action of a chiral reagent, auxiliary or catalyst which is not incorporated in the final product. This process is probably the choice which provides the widest of possibilities.

A molecule is said to be prochiral if it can be converted to a chiral molecule in a single reaction step. If a carbon atom is sp^2-hybridized and bears three different substituents, the two sides of the carbon available for addition of a fourth substituent are prochiral. A tetrahedral carbon with two identical substituents is prochiral and replacement or change of one of these substituents leads to a chiral compound. Prochirality can only be recognized in a chiral operation.

Mainly there are two methods for asymmetric synthesis; chemical and biotechnological.

1.2.2.1. Chemical Methods

In order to prepare optically pure compounds through synthesis, chemists make use of either reagent-controlled (chiral reagents, chiral catalysts) or substrate-controlled (chiral substrates, chiral auxiliaries) conditions.

1.2.2.1.1. Reagent-Controlled Conditions

Chiral Reagents:

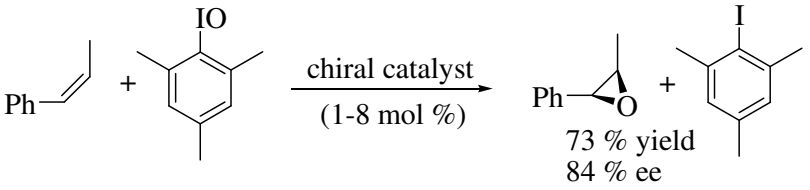
In this approach, the prochiral substrate is treated with a chiral reagent in order to obtain enantiomerically enriched product. The chiral reagent is used stoichiometrically which is usually rather costly. In many ways, this is the approach of choice as nature utilizes this methodology through enzymes. 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 [5].

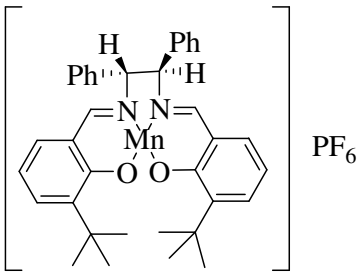
Chiral Catalysts:

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.

An example to a catalytic asymmetric epoxidation reaction was described by Jacobsen who uses manganese complexes of chiral Schiff bases. The epoxidation of olefin with iodosylmesitylene as the oxygen atom source and 1-8 mol % catalyst (Figure 7) to give the epoxide in moderate yield and high enantiomeric excess and iodomesitylene (Scheme 1)[6].



Scheme 1



(*R*),(*R*)

Figure 7 Manganese complex of chiral schiff base

1.2.2.1.2. Substrate-Controlled Conditions

Chiral Substrates:

The best way to control the stereoselection of the reaction is to start with a chiral starting material. Nature produces chiral compounds that make up the "chiral pool". This approach is often limited to the amount of the natural product available and its price. With a chiral starting material in hand, a well-designed synthesis should then reduce to the control of relative stereochemistry, the natural product's chirality inducing the appropriate stereochemistry at the new center(s).

For small scale reactions, with few cost constraints, thousands of chiral materials are readily available. However, not many chiral materials are available on a multi-ton scale. Amino acids provide a wide variety of functionality in the side chain. The amino and carboxylic acid groups can be transformed into a wide range of other functional groups as well as aiding the formation of carbon-carbon bonds. Carbohydrates are also available in large amounts. However, unless the carbon skeleton is close to the desired target structure, the number of other reactions required to utilize these chirons often make them less desired as the starting material. 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 [5].

Chiral Auxiliaries:

Chiral auxiliaries play an important role in asymmetric syntheses of optically active compounds. A chiral auxiliary is a chiral molecule that can be covalently attached to a prochiral substrate as a ‘chiral handle’ so that the subsequent reaction proceeds with high diastereoselectivity. After the diastereoselective reaction, the auxiliary should be removable and recoverable in good yield and without racemization so that they can be reused. This extra two steps in the synthesis, is the major drawback of this approach. The pioneering work of Professor David A. Evans in the field of chiral auxiliaries has revolutionized the art of organic synthesis. Evans’ chiral oxazolidinones are frequently found in total syntheses and due to their widespread use and ease of preparation, have become commercially available.

1.2.2.2. Biotechnological Methods

Thus far, only chemical methods have been considered; biocatalysis can also be used to obtain enantiomerically pure products from prochiral substrates and encompasses catalysis by enzymes from bacteria, fungi or yeast [7,8]. An advantage of these biocatalysts is the high selectivity and high efficiency.

Enzymes have an active site to bind a substrate. Among the numerous theories and rationales which have been developed in order to understand relation between active site and substrate, the most illustrative models for the organic chemist are 'Lock and Key' mechanism and Induced-Fit mechanism [9]. (Figure 8)

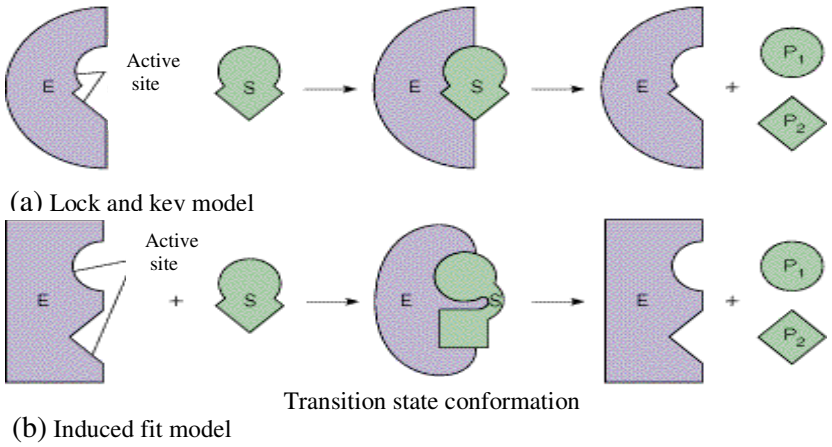
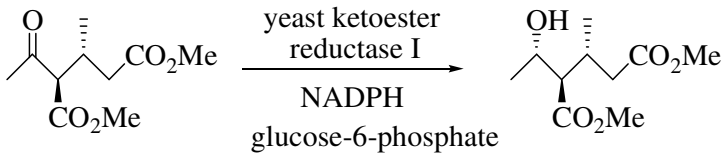


Figure 8 Lock and key model and Induced fit model

Weak binding forces stabilise the three-dimensional structure of an enzyme. These forces are Van Der Waals interactions of aliphatic chains, π-π stacking of aromatic units, salt bridges between charged parts of the molecules, covalent –S-S- disulfide bridges, and the layer of water that covers the surface of an enzyme, called the 'structural water'. These features are essential for maintaining the three dimensional structure of the enzyme and thus its catalytic activity. In order for the synthetic aim of the organic chemist to be achieved with a biotransformation step the variety of factors that infuence the enzyme's structure and thus catalytic activity and specificity must be considered.

An example to a reduction reaction was given in Scheme 2. In 1998 Kawai *et al.* performed the reduction of a β-keto-ester **1** substituted by a secondary alkyl group at the alpha position by using a NADPH-dependent reductase from bakers' yeast. The corresponding β-keto-ester **2**, methyl-2-alkyl-3-hydroxybutanone having three consecutive chiral centers is obtained in excellent stereoselectivity [1].



1 **2**

Scheme 2

Another important and the most exploited method is the kinetic resolution. It is based on differences in rates of the reaction with the particular enantiomer under the chiral recognition ability of the biocatalyst. In general the enantiomeric excess of the starting material will increase as the reaction progresses, while the ee of a chiral product will decrease. As this is a resolution, only 50 % of the substrate can be converted to the desired product [5]. The enzymes commonly used for such resolutions are hydrolases, mostly lipases. These enzymes are usually applied to separate chiral alcohols, organic acids as well as their esters in processes of ester hydrolysis, esterification and transesterification.

Enzymes can be classified according to the reactions they catalyze [10]. Hydrolytic enzymes, such as lipases are able to speed up hydrolytic reactions. This class of 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. Figure 9 illustrates a catalytic cycle for serine proteases, which is representative for most lipases.

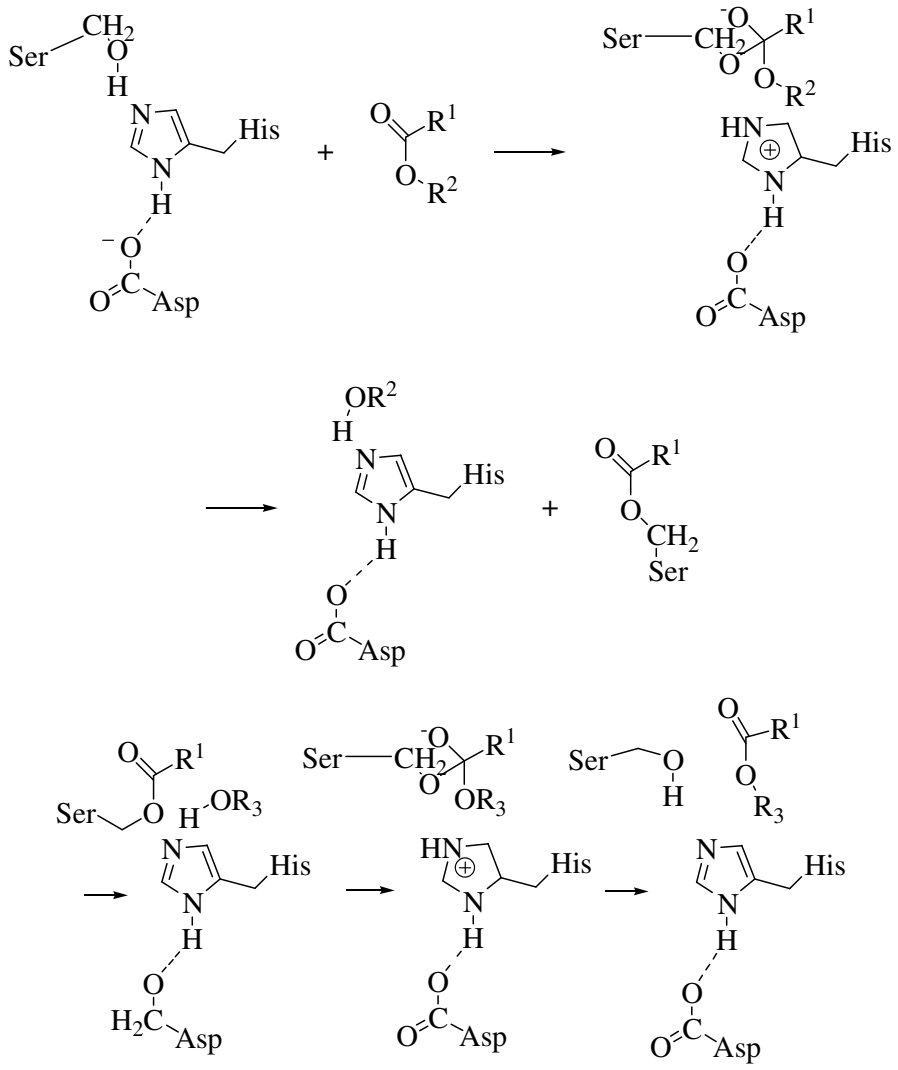


Figure 9 Catalytic cycles for serine

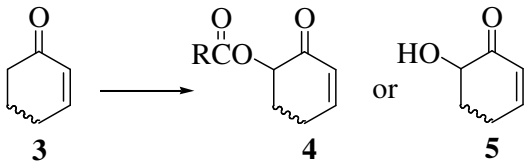
In serine proteases a catalytic triad consisting of the amino acids serine, histidine and aspartic acid are responsible for the catalysis. In figure 9 Ser reacts as a nucleophile with a substrate molecule. Here being an ester. During substrate binding a proton is transferred from Ser to His, making Ser more nucleophilic. The positive charge of the protonated imidazole ring is stabilized by interaction with the carboxylate group of Asp. A tetrahedral intermediate is formed in which the enzyme and substrate are covalently linked (enzyme-substrate transition state). The proton on His binds to the alkoxy group that is then eliminated as an alcohol molecule. An acyl

enzyme is formed as a covalent intermediate. The highly reactive intermediate formed may react with water (R_3 = H, hydrolysis) or a second alcohol molecule (transesterification) to yield the product of the reaction, being either an acid or an ester [11].

1.3. α′ Oxidation of Enones

1.3.1. General Methods

Selective oxidations giving α′-hydroxy α,β-unsaturated ketones possess a central role in synthetic methodology. The regioselective α′-oxidation of enones to α′-acetoxy enones constitutes a valuable procedure for manipulating a common functional group.



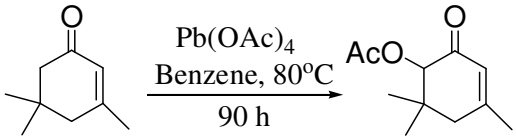
Scheme 3

In the literature, there are methods available for the α′ oxidation of **3** to α′-acyloxy-α,β-unsaturated ketones **4** using lead (IV), mercuric (II), and manganese (III) carboxylates or to α′-hydroxy-α,β-unsaturated ketones **5** using direct enolate oxidations.

Using Lead (IV) Acetate:

The regioselective oxidation of enones in both steroidal and non-steroidal groups using lead (IV) acetate in acetic acid, benzene or toluene provided the α′-acetoxy-enones in yields varying from poor to acceptable.

For instance in 1969, Ellis applied the lead (IV) acetate method to isophorone. This reaction afforded the expected 6-acetoxyisophorone in 78 % yield (Scheme 4) [12].

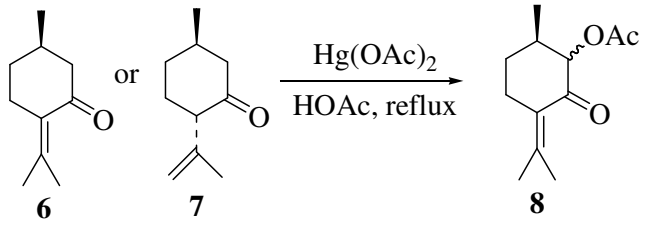


Scheme 4

The mechanism for the α′-acetoxylation of enones by lead (IV) acetate as proposed by Henbest and co-workers [13] and Marshall and Bundy [14] involves formation of an enol-lead triacetate derivative directly from the enone followed by intramolecular acetate transfer.

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 5). 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 [15].



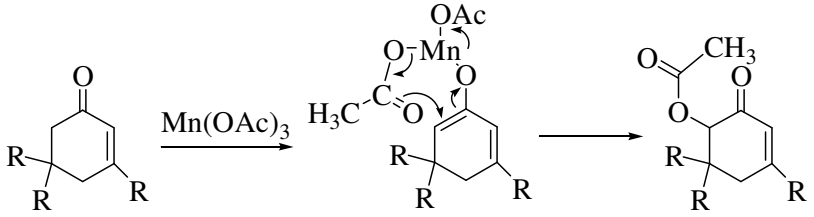
Scheme 5

1.3.2. α′- Oxidation of Enones via Manganese (III) Acetate

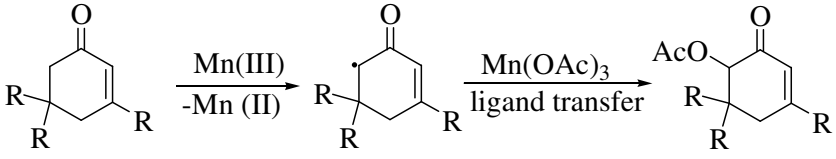
In 1976, Williams and Hunter reported that the manganese (III) acetate oxidation of enones in acetic acid at reflux temperature affords 6-36 % of α′-acetoxyenones [16].

Demir and his coworkers required a procedure for the oxidation of an enone **3** to an α′-acetoxyenone **4**, and in the face of unsatisfactory results using literature methods, they have comprehensively developed the α′- Oxidation of enones to α′-acyloxy enones by using manganese (III) acetate method discovered by Hunter [15].

The exact mechanism for oxidation of enones to α′- acetoxyenones is not fully mentioned. Recently Demir, Reis and Igdir reinvestigated the synthetic and mechanistic aspects of Mn(III) acetate mediated oxidation of enones [17]. There are mainly two proposed mechanisms. One explains the mechanism by the formation of a metal enolate followed by acetate transfer (Scheme 6). The other suggested mechanism is through the formation of an α-oxo radical followed by ligand transfer (Scheme 7).



Scheme 6



Scheme 7

Besides this, manganese(III) acetate can be used to generate α-oxo- and α,α-dioxoalkyl radicals by regioselective oxidation of carbonyl compounds such as aldehydes, ketones, acids, diketones, keto esters and diesters. The functionalization step which consists of the introduction of α-oxo- and α,α-dioxoalkyl moieties into multiple bond containing substrates, occurs in most cases with high regio-, chemo- and stereoselectivities. The extensive exploration of this field has uncovered its huge synthetic potential and resulted in various novel approaches to different classes of organic compounds [18].

Although manganese (III) acetate is inexpensive its use on an industrial scale is problematic because 2 equivalents of the reagent is usually required to bring about the desired transformation and this generates considerable amounts of metal wastes. Chemical and electrochemical methods have been investigated to regenerate the manganese (III) in situ [19].

1.4. The Importance of α-Hydroxymethyl-Cyclic Hydroxy Enones

The discovery and development of new methods for the synthesis of chiral synthons for complex molecules of natural and unnatural origin remains an enduring challenge [20]. The synthesis of enantiomerically pure cyclic alkenols (Figure 10) are very important in synthetic methodology. Five and six membered analogues hold considerable potential for the construction of both natural and unnatural carbocyclic products since they are important intermediates in the synthesis of many biologically active natural products.

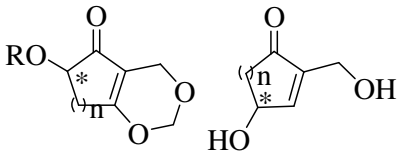
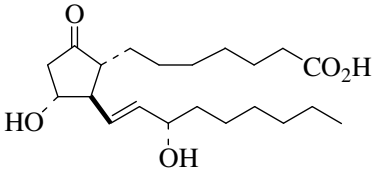


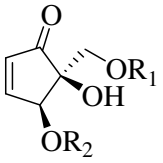
Figure 10 Enantiomerically pure cyclic alkenols

A wide range of biological properties are associated with several relatively simple compounds incorporating the cyclopentanone unit. Cyclopentenones are expected to be versatile intermediates in the synthesis of bioactive cyclopentanoid natural products [21]. For example, the prostaglandins, of which prostaglandin E_2 **9** is a familiar example, exert diverse physiological effects upon the mammalian respiratory, digestive, renal, reproductive, cardiovascular, endocrine and nervous systems. The pentenomycins **10**, dehydropentenomycin **12**, xanthocidin **11** and vertimycin **13** display anti-bacterial activity. Pentenomycin antibiotics constitute a family of natural hydroxylated cyclopentanoids which are produced by streptomyces species and show moderate activity against gram-positive and gram-negative bacteria. Cryptosporiopsin **14** is active against fungi and methylenomycin A **15** and sarkomycin **16** possess antitumour properties [22] (Scheme 8).



Prostaglandin E_2

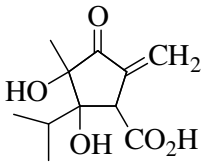
9



a). $R_1 = R_2 = H$
b). R_1=H, R_2 = Me
c). R_1 =Ac, R_2 = H

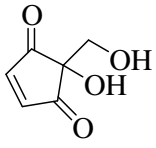
Pentenomycins

10



Xanthocidin

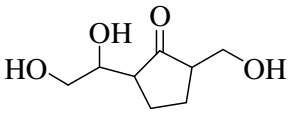
11



Dehydropentenomycins

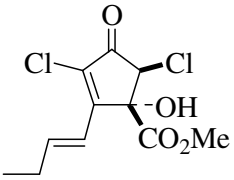
12

Scheme 8



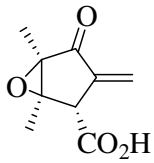
Vertimycins

13



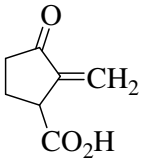
Cryptosporiopsin

14



Methlenomycin A

15



sarkomycin

16

Scheme 8 (continued)

Through the total synthesis of hydroxyjatrophone [23] used for the treatment of cancer, 4-hydroxy-2-hydroxymethylcyclopent-2-en-1-one was used as an intermediate (figure 11).

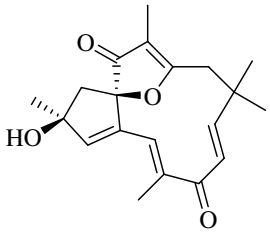
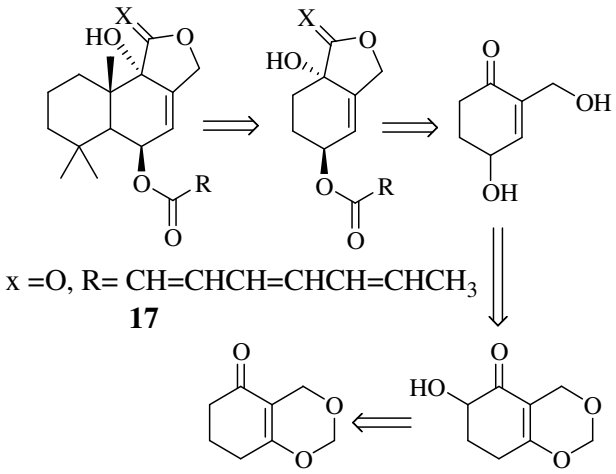


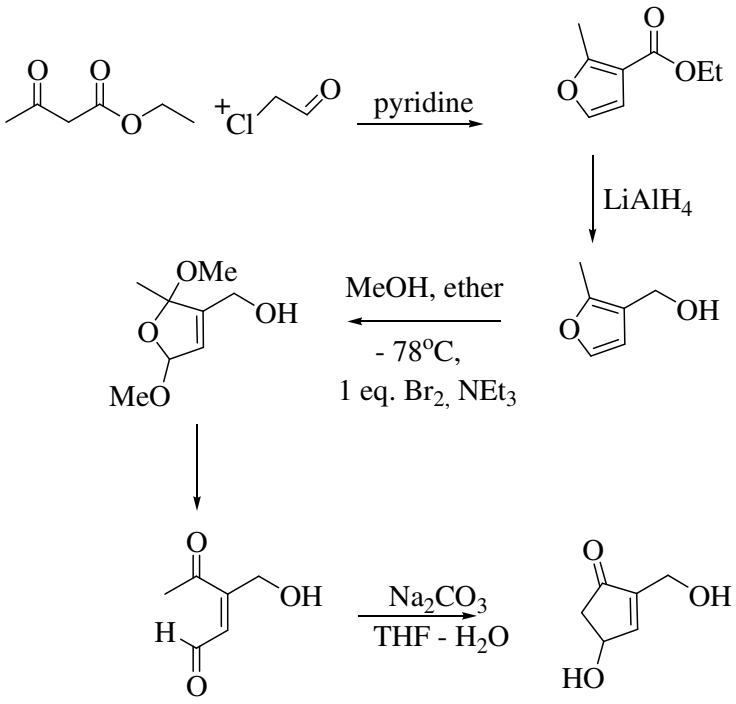
Figure 11 Hydroxyjatrophone

RES-1149-2 **17** exhibit endothelin receptor binding activity at the μg/mL level in the human ETA assay. It is a sesquiterpene antiviral agent that exert useful biological activity [24].

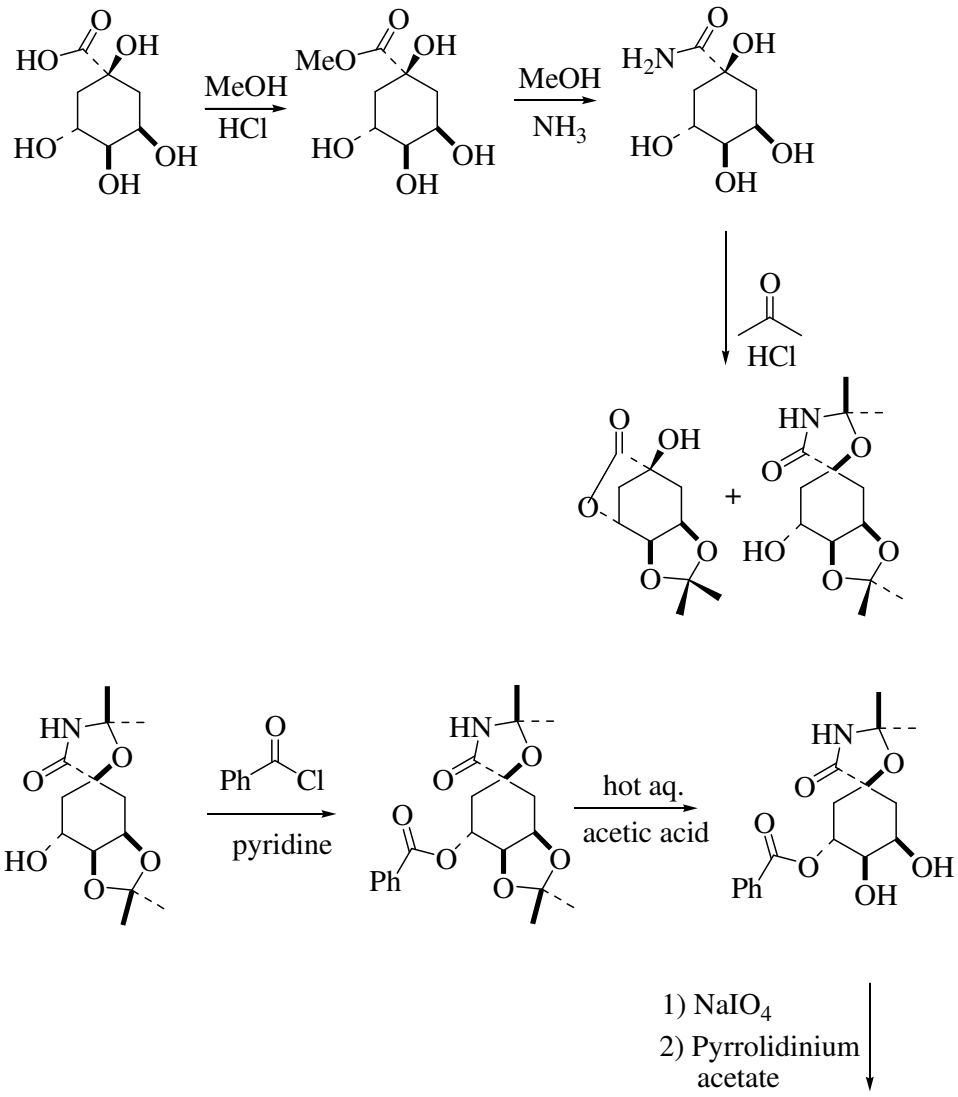


Scheme 9

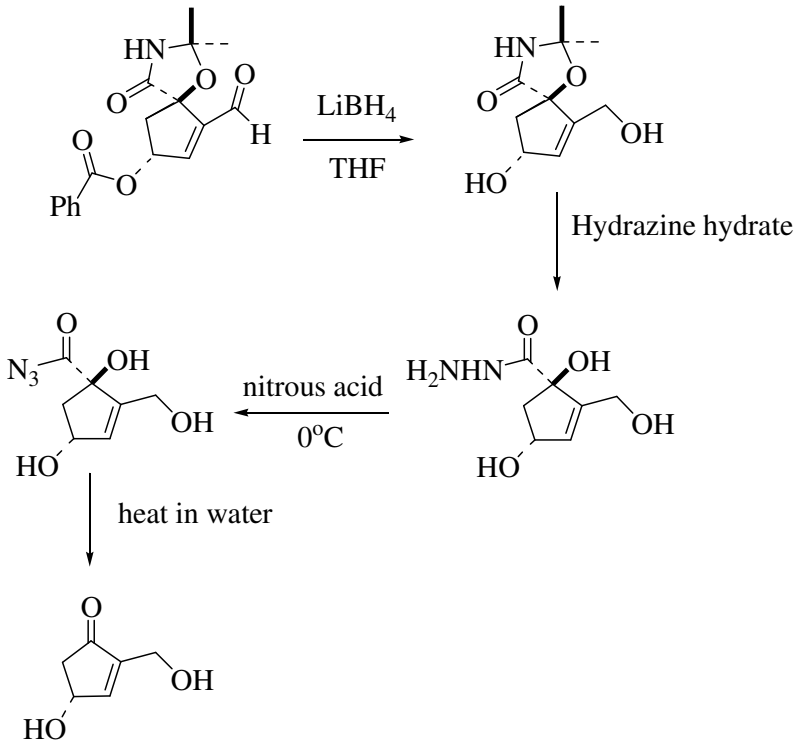
In 1980, Elliott and his coworkers performed the synthesis of 4-hydroxy-2-hydroxymethylcyclopent-2-en-1-one **27** as the racemate and as the (*4R*) isomer [21,22]. The former synthesis, which used ethyl acetoacetate and chloroacetaldehyde diethyl acetal as starting materials, involved a five-step sequence and proceeded in 14 % yield (Scheme 10). The latter synthesis, which commenced with quinic acid, required ten steps and afforded the product in 3.5 % overall yield (Scheme 11).



Scheme 10

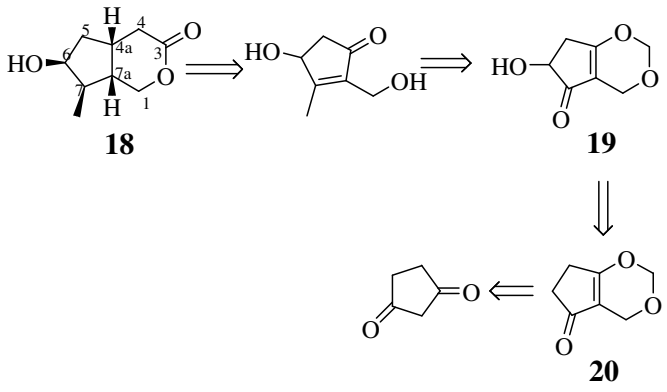

MeOH
HCl
MeOH
NH3
HCl
Ph
Cl
pyridine
hot aq.
acetic acid
1) NaIO4
2) Pyrrolidinium
acetate

Scheme 11



Scheme 11 (continued)

In designing a synthetic route to (±)-(4aα,6α,7aα)-hexahydro-6-hydroxy-7-methylcyclopenta[*c*]pyran-3(1*H*)-one **18**, the 1,3-dioxin vinylogous ester **20** which had already served successfully as a key intermediate for the syntheses of some natural products, seemed most attractive as a starting material because of the diversity of its chemical reactivity [25].



Scheme 12

Smith *et. al.* have reported that hydroxylation of the enolate, generated via treatment of **20** with LDA in THF with (+)-(10-camphorsulfonyl) oxaziridine was effected at the α´ position, giving (+)-**19** as a major product in %37 yield with 14 % ee [26].

1.5. Aim of The Work

The major aim of this research is to develop a simple and selective method for the synthesis of chiral cyclic alkenols. For this purpose, the synthesis of 4,6,7,8-tetrahydro-6-hydroxy-5*H*-1,3-benzodioxin-5-one **21** and 4,6,7,8-tetrahydro-6-acetoxy-5*H*-1,3-benzodioxin-5-one **22** and 4-hydroxy-2-hydroxymethylcyclohex-2-en-1-one **23** or 4-acetoxy-2-hydroxymethylcyclohex-2-en-1-one **24** as well as 6,7-dihydro-6-hydroxycyclopenta-1,3-dioxin-5(4*H*)-one **25** and 6,7-dihydro-6-acetoxy cyclopenta-1,3-dioxin-5(4*H*)-one **26** and 4-hydroxy-2-hydroxymethylcyclopent-2-en-1-one **27** or 4-acetoxy-2-hydroxymethylcyclo-pent-2-en-1-one **28** was chosen as a model study (Figure 12).

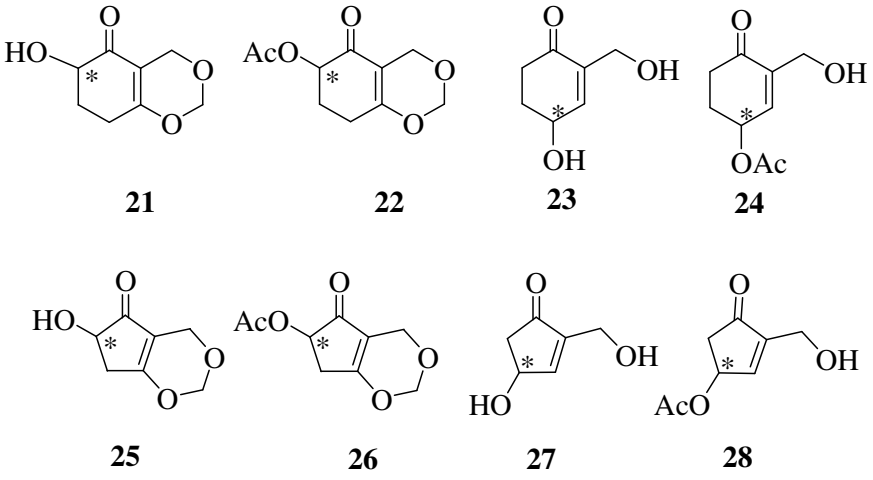
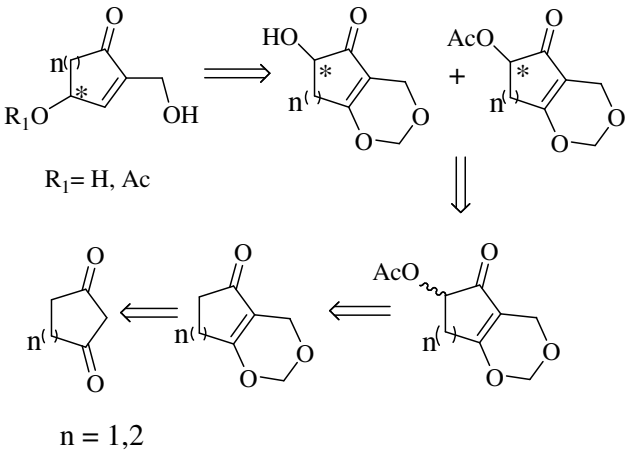


Figure 12 Chiral cyclic alkenols

As it was mentioned before racemic and chiral synthesis of the target compounds 4-hydroxy-2-hydroxymethylcyclohex-2-en-1-one **24** and 4-hydroxy-2-

hydroxymethylcyclo pent-2-en-1-one **28** required several steps and the optical and the chemical yields are very low. In this study, the aim is to develop a general applicable chemoenzymatic method for the enantioselective synthesis of these compounds. The goal of this study is shown retrosynthetically in Scheme 13.



Scheme 13

The first approach to the optically active **23** and **27** is to synthesize 4,6,7,8-tetrahydro-5*H*-1,3-benzodioxin-5-one and 6,7-dihydrocyclopenta-1,3-dioxin-5(4*H*)-one from 1,3-cyclohexanedione and 1,3-cyclopentanedione respectively. The second step is the α′-oxidation by using manganese(III) acetate. Then enzymatic bioconversion of racemic 4,6,7,8-tetrahydro-6-acetoxy-5*H*-1,3-benzodioxin-5-one and 6,7-dihydro-6-acetoxycyclopenta-1,3-dioxin-5(4*H*)-one by lipases and esterases. Finally chiral **23**, **24** and **27**, **28** can be obtained by reducing with $LiBH_4$ or $LiAlH_4$. The desired target molecules are Ac-protected hydroxyenones for further conversions (Figure 13).

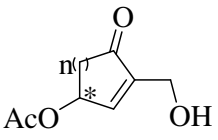


Figure 13 Chiral cyclic acetoxy enones

CHAPTER 2

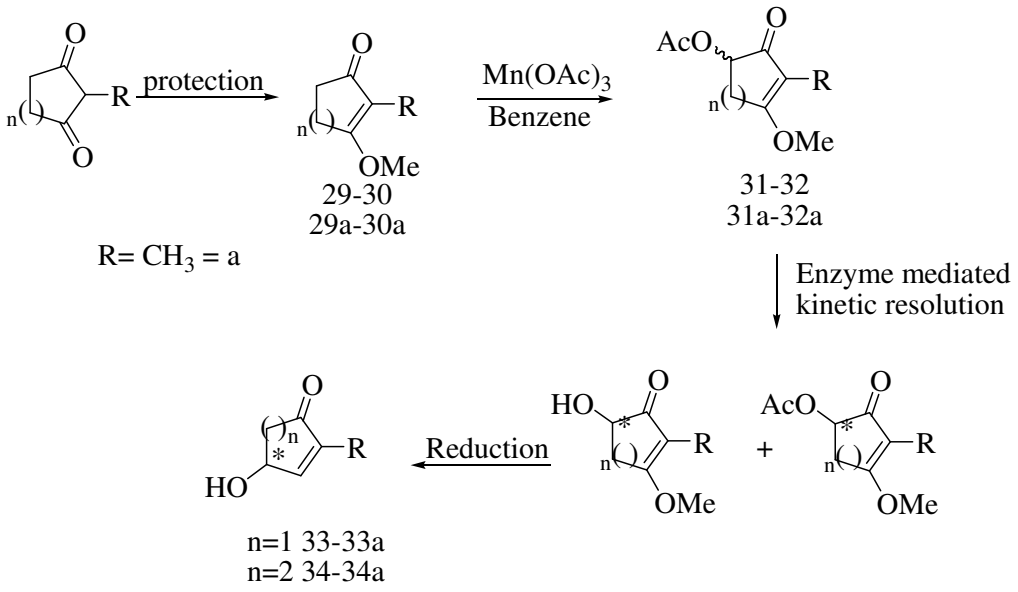
RESULTS AND DISCUSSION

2.1 Perspective of the Work

α-Hydroxymethyl-γ-hydroxy enones **23, 27** and polyoxo-ketones **21**, **22, 25, 26** are versatile chiral synthones for the construction of chiral compounds due to reactive functional groups such as carbonyl and hydroxyl groups, double bond and acetoxy group which can be transformed to other functional groups.

Demir and Sesenoglu studied on the synthesis of 4-hydroxy-2-cyclopenten-1-one **33** and 4-hydroxy-2-cyclohexene-1-one **34** [27,28]. They are similar structures which don't contain 2-hydroxymethyl substituent. In this study, first cyclic 1,3-diketones were converted to the 3-methoxy-2-cyclopenten-1-one **29** and 3-methoxy-2-cyclohexen-1-one **30** and then oxidation of enones was performed with $Mn(OAc)_3$ to obtain the desired 5- and 6-acetoxy enones **31-32**. It was followed by enzymatic kinetic resolution using lipases. The reduction of this compound with $LiAlH_4$ yielded the γ-hydroxy enones (Scheme 14).

More recently Demir, Fındık and Köse developed the synthesis of similar structures which contain 2-methyl substituent **33a** and **34a** [29]



Scheme 14

Replacement of the methyl group with hydroxymethyl functionality can give access to important structural units, which can be used as a starting material for interesting biologically active compounds.

1,3-dioxane derivative of cyclopentanone and cyclohexanone was chosen as ideal starting material for protected hydroxymethyl equivalante substances.

Based on preceding information available from the previous work on the biocatalyst-mediated reactions, a chemoenzymatic method for the synthesis of **21**, **22** and **23** by protection, $Mn(OAC)_3$ mediated acetoxylation followed by hydrolysis using lipase and esterase type enzymes and finally reduction were developed.

Lipase and esterase type enzymes are used exclusively for the synthesis of chiral 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.

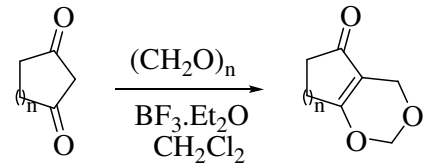
In order to achieve an appropriate experimental procedure for the enzymatic resolutions of the racemic acetoxy enone, best reaction condition that gives the best result in the enzymatic hydrolysis was determined by screening of different enzymes.

For the determination of conversion, the reactions were monitored by using TLC technique. For the determination of the ee by using various chiral HPLC columns, primarily racemic mixure was analyzed for optimization of the baseline separation.

2.2 Synthesis of α-Acetoxy Enones

2.2.1 Protection of cyclic 1,3-diketones

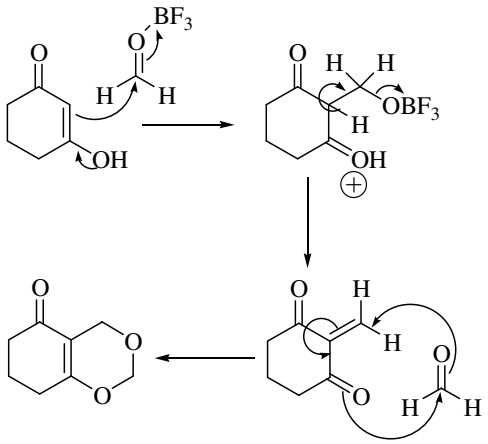
For the synthesis of chiral α′-acetoxy and α′-hydroxy enones first protection was performed according to Smith's procedure [25]. Starting materials 1,3-cyclohexanedione and 1,3-cyclopentanedione were allowed to react with 6 equivalents of paraformaldehyde in the presence of 3 equivalents of $BF_3.Et_2O$ in dry dichloromethane under argon. The reactions were monitored by TLC (Silica gel, EtOAc/Hex 1:5 and 1:3 respectively). After work-up, the products were purified by column chromatography (EtOAc/Hexane 1:5 and 1:3 respectively). According to this protocol 4,6,7,8-tetrahydro-5*H*-1,3-benzodioxin-5-one **35** was obtained as a yellow oil with 40 % yield. Similar reaction is carried out with cyclopentanedione and 6,7-dihydrocyclopenta-1,3-dioxin-5(4*H*)-one **20** was obtained with 53 % yield as white crystalline solid (Scheme 15). However better yields were obtained when trioxane was used instead of paraformaldehyde (yields are 84 % and 64 % respectively).



n = 1 20
n = 2 35

Scheme 15

To account for the formation of the 1,3-dioxins, a mechanism was proposed by Crow and his coworkers [30] wherein the electron rich enol of the 1,3-diketone attacks the BF_3-aldehyde complex in a Prins reaction. Addition of a second equivalent of aldehyde, followed by cyclization yields the dioxin as shown in Scheme 16.

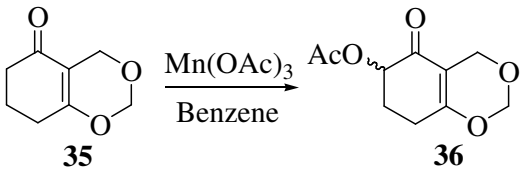


Scheme 16

The product, 4,6,7,8-tetrahydro-5*H*-1,3-benzodioxin-5-one, was identified by using NMR spectroscopy. From the ^{1}H-NMR spectrum at 5.05 and 4.34 ppm two singlets were observed, these belong to the CH_2's between two neighboring oxygens and an oxygen and a double bond respectively. Multiplets were observed at 2.35 and 2.25 ppm these belong to H_2C-CO- and CH_2-C=. And between 1.92-1.98 multiplet was observed for the ring CH_2. The results are consistent with the literature values.

2.2.2 $Mn(OAc)_3$ mediated acetoxylation of 4,6,7,8-tetrahydro-5*H*-1,3-benzodioxin-5-one

For the acetoxylation of the 1,3-dioxin vinylogous ester, 4,6,7,8-tetrahydro-5*H*-1,3-benzodioxin-5-one **35** (Scheme 17), It was allowed to reflux with 4 equivalents of $Mn(OAc)_3$ in benzene with dean-stark trap to give the racemic acetoxy derivative **36**.



Scheme 17

The reaction was monitored by TLC (Silica gel, EtOAc/Hexane 1:4). After the work-up and purification of the crude product by column chromatography (EtOAc/Hex 1:7), the desired product, racemic α'-acetoxy-4,6,7,8-tetrahydro-5*H*-1,3-benzodioxin-5-one **36** was obtained as white crystals in 87 % yield.

As stated in the literature, the source of the $Mn(OAc)_3$ is important for the yield of the reaction[17]. Commercially available $Mn(OAc)_3.2H_2O$ is used for the reaction and it is dried under high vacuum over P_2O_5 in drying pistol.

This product was identified by using NMR spectroscopy. From the ^{1}H-NMR spectrum, a singlet was observed for the $-CH_3$ at 2.07 ppm and dd at 5.19 ppm (*J*= 12.62, 5.24 Hz) for the α-proton. From the ^{13}C-NMR spectrum, CH_3 carbon was observed at 21.1 ppm and at 170.1 ppm $O\underline{C}OCH_3$ carbon. COSY, HMBC and HMQC Contoure diagrams as well as DEPT 135 spectrum verifies the structure.

Figure 14 is the x-ray of the single crystal of 6-acetoxy-4,6,7,8-Tetrahydro-5*H*-1,3-benzodioxin-5-one and Figure 15 shows the unit cell structure.

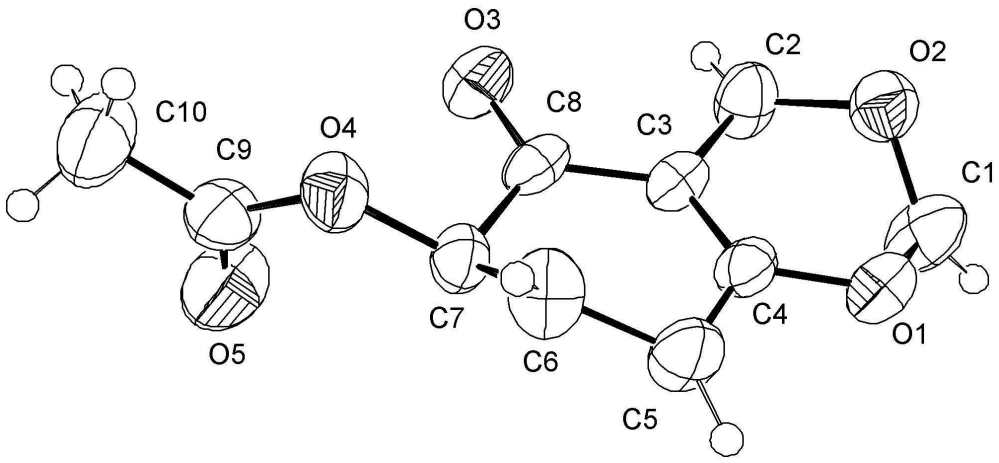


Figure 14 X-ray of 6-acetoxy-4,6,7,8-Tetrahydro-5*H*-1,3-benzodioxin-5-one

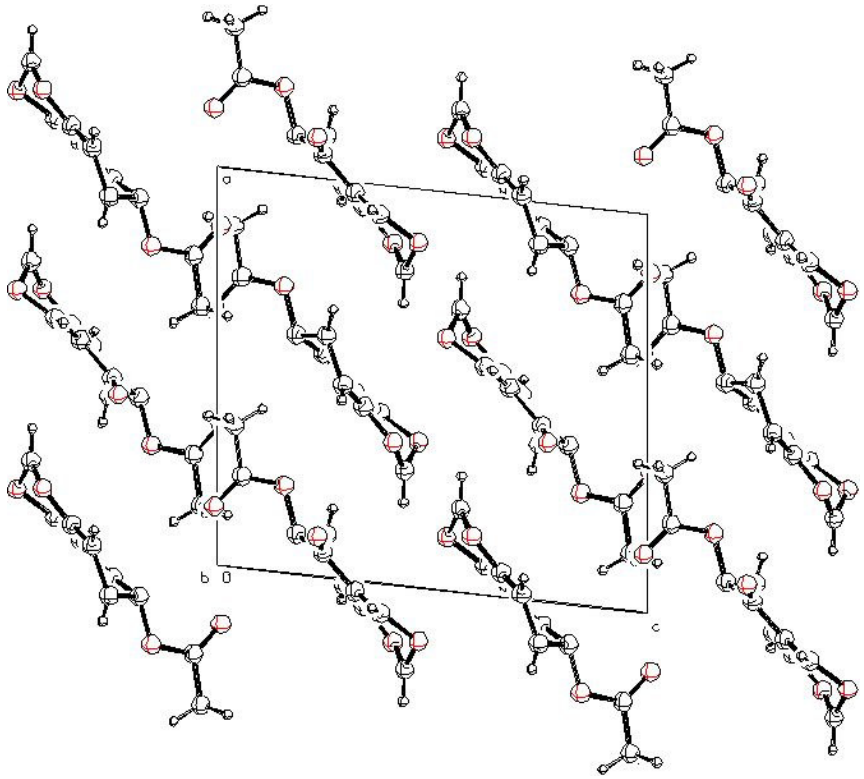
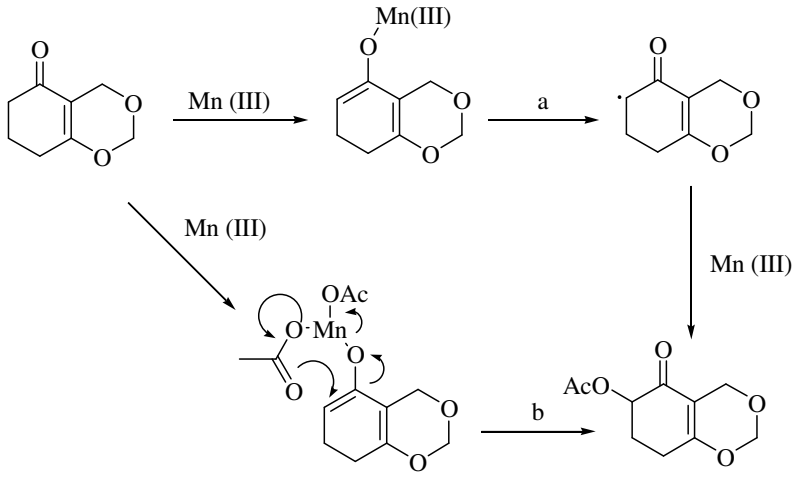


Figure 15 Unit cell structure of 6-acetoxy-4,6,7,8-Tetrahydro-5*H*-1,3-benzodioxin-5-one

For the mechanism of the reaction, both radical mechanism (route a) and ligand transfer via metal-enolate intermediate (route b) have been proposed. The suggested mechanism is as shown in Scheme 18.



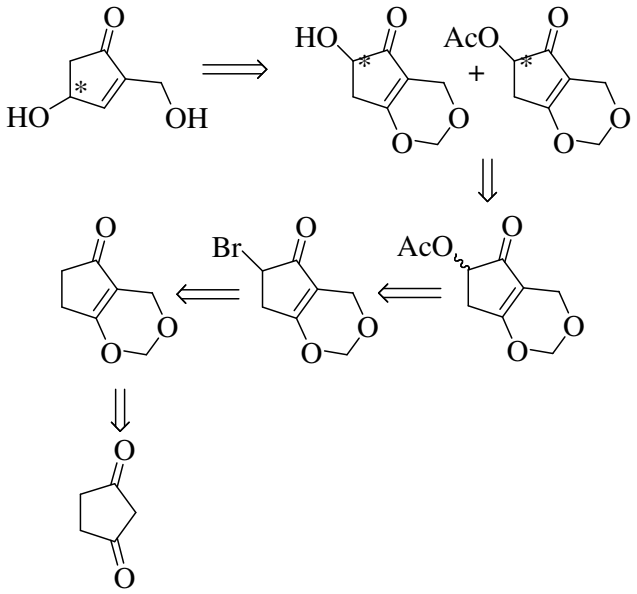
Scheme 18

2.2.3. Reactions of 6,7-dihydrocyclopenta-1,3-dioxin-5(4*H*)-one

For the acetoxylation of 6,7-dihydrocyclopenta-1,3-dioxin-5(4*H*)-one from the α′ position, several methods were tried but unfortunately none of them were successful. First the starting material was refluxed by 4 equivalents of $Mn(OAc)_3$ in benzene but no product formation was observed even the reaction was continued for about a week. Secondly different solvent systems were tried. Cyclohexane and acetonitrile was used instead of benzene but the results were the same. Then it was decided to use other oxidizing agents. The acetoxylation was tried with $Pb(OAc)_4$ under reflux with benzene and as an alterative $Hg(OAc)_2$ in acetic acid and $Bi(OAc)_3$ in benzene were also tried. Unfortunately again no α′-acetoxylated product was observed.

After these unsuccessful trials, another reaction route was designed as shown in Scheme 19 as retrosynthetic perspective.

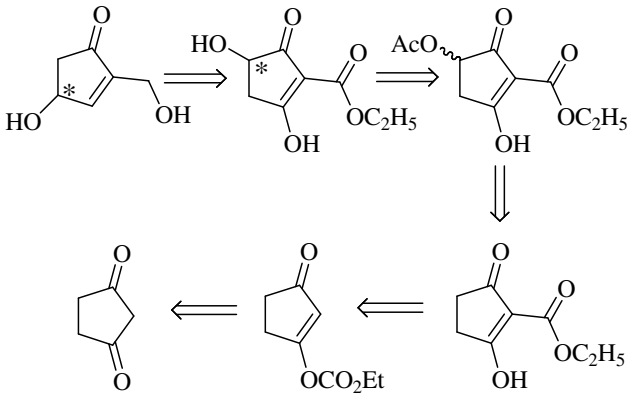
For the formation of acetoxyenone neither α-radical formation nor enolate formation followed by ligand transfer occured. The rigid structure of this compound could be the reason for this negative result.



Scheme 19

In this method, functionalization of the α′ position by bromination was tried. First Br_2 in CCl_4 was used but several bromination products were observed then alternative bromination methods were checked and bromination with $CuBr_2$ in ethyl acetate and NBS in CCl_4 were applied but they all gave the similar results and no α′-Br-6,7-Dihydrocyclopenta-1,3-dioxin-5(4*H*)-one formation is observed.

In order to obtain the target molecule 4-hydroxy-2-(hydroxymethyl)-2-cyclopentenone **27**, another route was suggested as shown in Scheme 20.



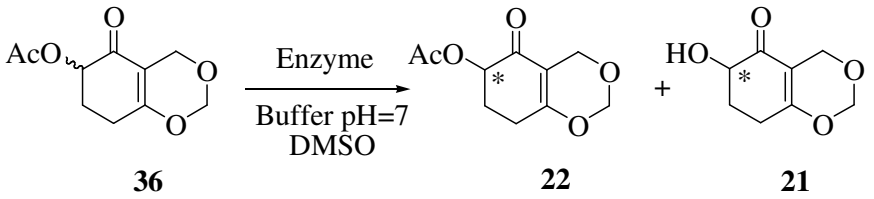
Scheme 20

In order to reach the expected target molecule first 1,3-cyclopentanedione was reacted with chloro ethyl formate in the presence of pyridine in $CHCl_3$ starting from 0^oC then allowing it to reach 20^oC. Ethyl-3-oxocyclopent-1-enyl carbonate was obtained successfully but rearrangement in the presence of 3 equivalents of the Lewis acid $AlCl_3$ in $CHCl_3$ failed and ethyl-2-hydroxy-5-oxocyclopent-1-ene carboxylate did not form so further procedure could not be applied.

2.3. Enzyme Catalyzed Kinetic Resolution of α′-Acetoxy Enone

Among the biocatalysts used in organic synthesis, lipases are the most frequently used biocatalysts. Lipases are able to discriminate between enantiotopic groups and between the enantiomers of a racemate. This type of enzyme is very easy to handle and stable at higher temperatures (up to 100 ^oC) and toward organic solvents. Most of the lipases used are able to accept a broad range of substrates due to their ability to change their conformation depending on the substrate structure (induced fit enzyme). This type of biocatalysts can be used to perform enantioselective hydrolytic reactions and the formation of ester and amide bonds [31].

Racemic α'-acetoxy-4,6,7,8-Tetrahydro-5*H*-1,3-benzodioxin-5-one **36** was used in the enzymatic hydrolysis (Scheme 21).



36 **22** **21**

Scheme 21

Initially in order to obtain optimum conditions for the enzymatic hydrolysis of acetoxy enone **36,** analytical screening was done. For this purpose, the reactions were performed in analytical scale. About 45 mg of enone **36** was dissolved in minimum amount of DMSO because of the poor solubility of the substrate in aqueous medium and 300 µl phosphate buffer was added. Then catalytic amount of enzyme was included. The reactions were performed at room temperature at pH=7. They were monitored by TLC and when % 50 conversion was observed, the reactions were terminated by the addition of 500 µl chloroform. After workup, HPLC analysis were performed for the determination of ee values by using racemic acetoxy enone as a reference.

Table 1. Enzymatic hydrolysis of 6-acetoxy-4,6,7,8-Tetrahydro-5*H*-1,3-benzodioxin-5-one

Enzyme	***Reaction time (h)***	***ee % (Acetoxy)***
PLE *(Pig Liver Esterase)*	*1,5*	*95*
CAL (Candida Antarctica Lipase)	528	88
MML (Mucor Miehei Lipase)	96	78
PCL (Pseudomonas Cepacia Lipase)	5	68
SL (Pseudomonas Burkholderia Cepacia SL-25)	16	60
Amano (Amano PS)	4	57
PFL (Pseudomonas Fluorescens Lipase)	8	54
PPL (Porcine Pancreatic Lipase)	118	50
HPL (Hog Pancreas Lipase)	336	49
CCL (Candida Cylindracea Lipase)	312	46
RAL (Rhizopus Arrhizus Lipase)	336	36
Aspergillus *(Aspergillus Niger)*	*8*	*35* *(reverse)*
RNL (Rhizopus Niveus Lipase)	528	34
QLM (Burkholderia Graduloi)	16	28
PL (Alcaligenes)	118	5
UL (Rhizopus sp.)	213	~4
TL (Pseudomonas Stutzeri PL-836)	118	~4
AL (Acromobacter sp)	245	No selectivity

As it can be seen from the table, best result was obtained from the PLE (*Pig Liver Esterase*) enzyme giving 6-acetoxy-4,6,7,8-tetrahydro-5*H*-1,3-benzodioxin-5-one with 95 % ee. Various chiral HPLC columns were tried for the best separation of enantiomers. Chiralpak OD column was able to resolve the acetoxy enantiomers whereas hydroxy enantiomers were not seperated with the used column and solvent system so the ee's were determined by using the peak area %'s of the resolved acetoxy peaks.

For the determination of R_f values of enantiomers, first racemic acetoxyenone was analyzed by HPLC (Figure 16).

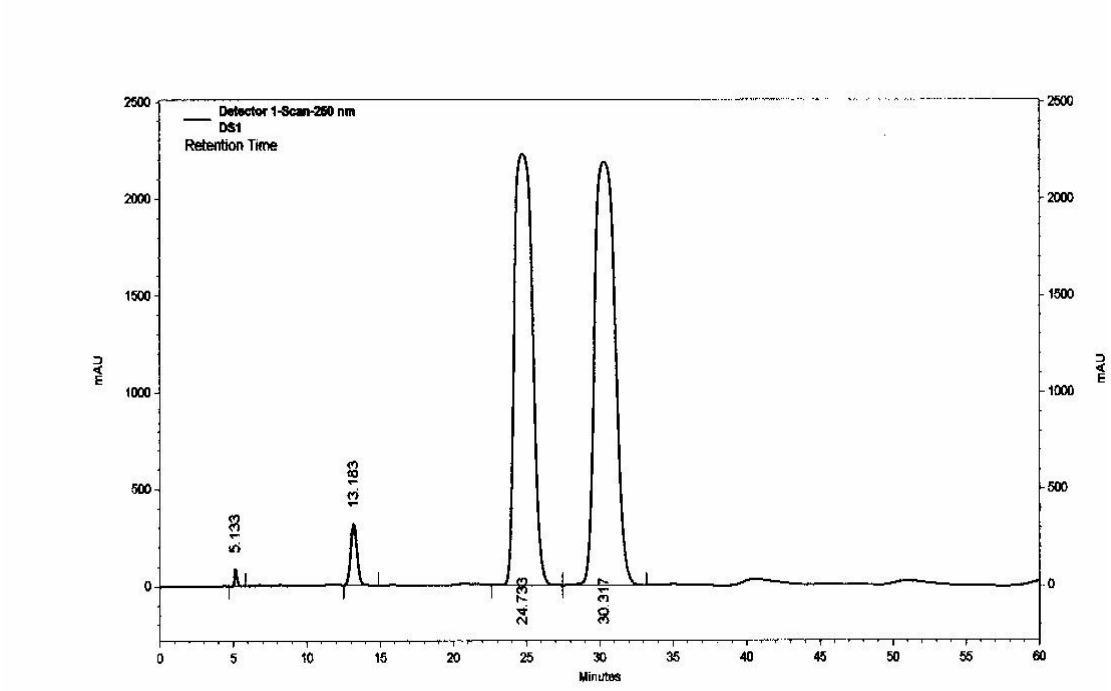


Figure 16 HPLC Chromatogram of racemic 6-acetoxy-4,6,7,8-Tetrahydro-5*H*-1,3-benzodioxin-5-one

As can be seen, retention time of the first enantiomer is 24,7 min. and the other enantiomer is 30,3 min. with the used column, solvent system and flow rate (Chiralpak OD column, eluent: hexane/2-propanal=90:10, flow rate= 0.80 mL min^{-1}).

Figure 17 shows the HPLC spectrum of PLE catalyzed reaction in analytical scale. All the other enzymes gave similar spectrums with differing enantiomeric excesses. Among them only *Aspergillus* showed reverse selectivity. It gave the other enantiomer (Figure 18).

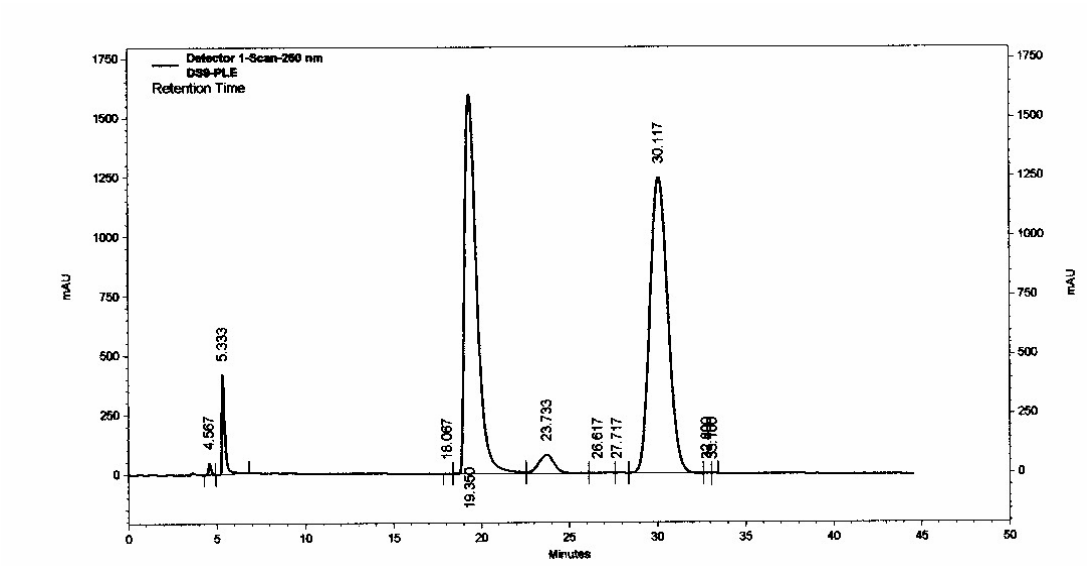


Figure 17 HPLC Chromatogram of *PLE* catalyzed hydrolysis

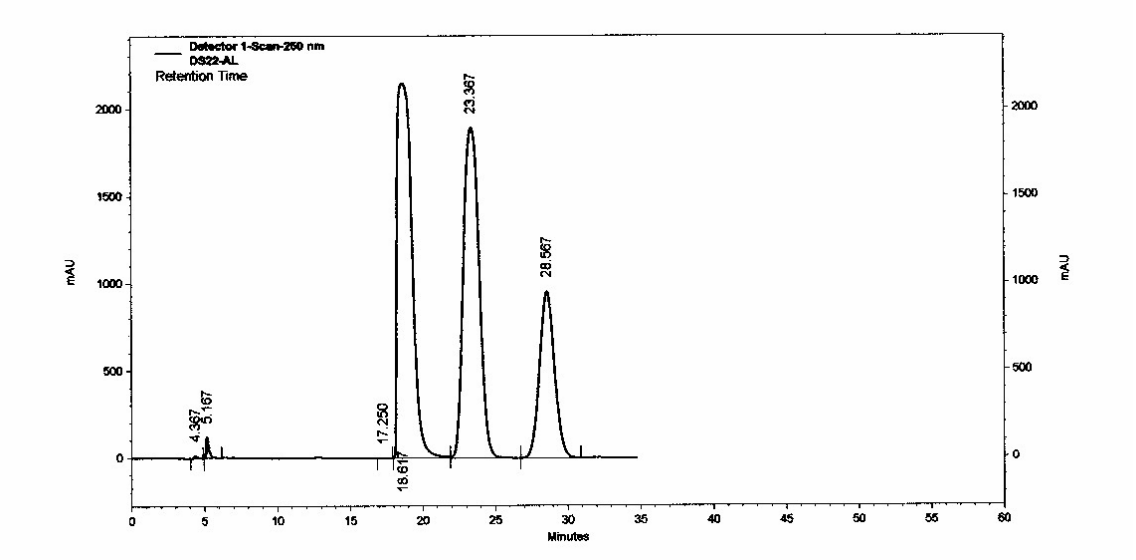


Figure 18 HPLC Chromatogram of *Aspergillus* catalyzed hydrolysis

Once it was found that PLE was the best enzyme for enantioselective hydrolysis of the substrate 6-acetoxy-4,6,7,8-tetrahydro-5*H*-1,3-benzodioxin-5-one, PLE was used in further enzymatic kinetic resolution reactions in preperative synthesis.

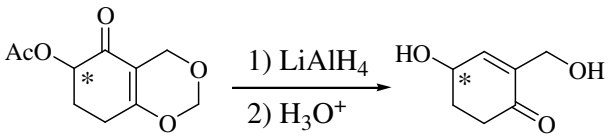
The products were identified by using NMR spectroscopy. From the ^{1}H-NMR spectrum, a broad singlet at 3.58 ppm for the –OH proton was observed, and a multiplet between 3.95-4.05 ppm for the α-proton. From the ^{13}C-NMR spectrum a peak at 71.2 ppm was seen for –CH-OH carbon.

Enantiomeric excess values were determined with HPLC (Chiralpak OD column, UV detection at 254 nm, eluent: hexane/2-propanol= 90:10, flow 0.80 mL min^{-1} 20 ° C) using racemic compound as the reference.

The α value for the 6-hydroxy-4,6,7,8-tetrahydro-5*H*-1,3-benzodioxin-5-one is $[\alpha]_D^{25}$ = + 219.23 (c 0.13, CH_3OH) and for 6-acetoxy-4,6,7,8-Tetrahydro-5*H*-1,3-benzodioxin-5-one is $[\alpha]_D^{25}$ = - 163.53 (c 1.67, CH_3OH)

2.4. Conversion of α-Acetoxy Enone to γ-Acetoxy Enone

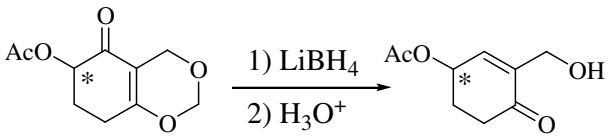
As described before the hydroxymethylated 4-hydroxy (or 4-acetoxy) enone is an important synthon in organic chemistry. For the generation of chiral α-hydroxymethyl-γ-hydroxy enone, reduction procedure as described in the literature was applied with slight modifications [26]. Scheme 22 shows the conversion of α′-acetoxy enone to γ-hydroxy enone. Firstly, reduction with $LiAlH_4$ was tried as similar structures in the literature were reduced by using this reagent but with our molecule, very low yields were observed.



22 **23**

Scheme 22

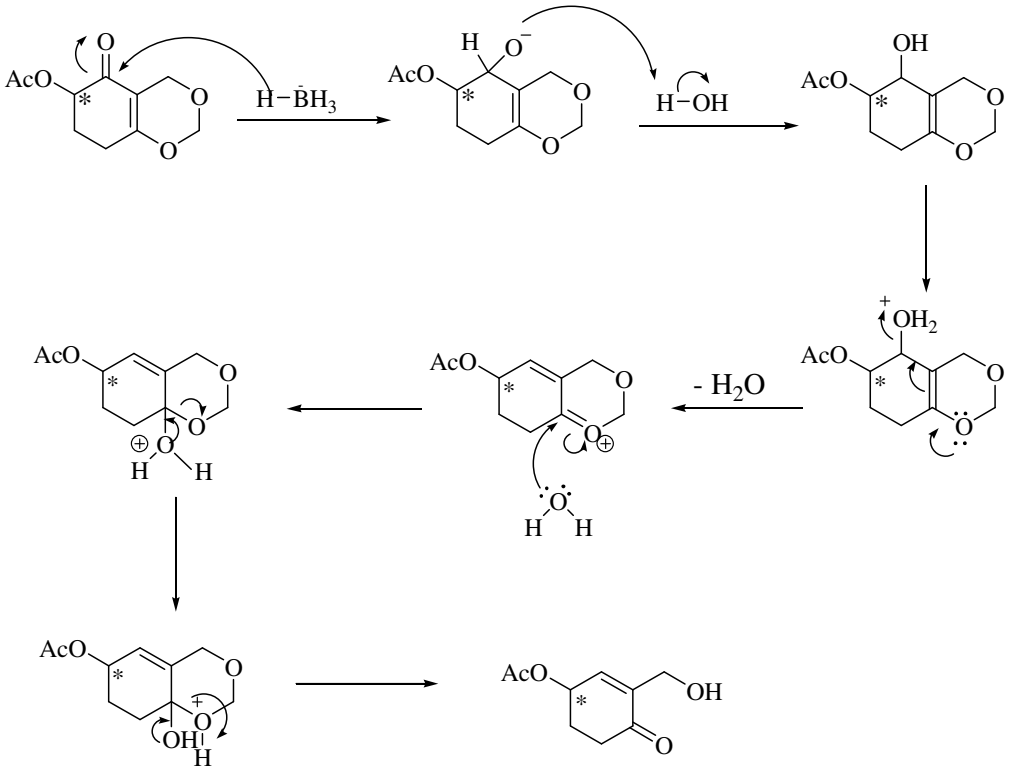
Since the reduction and hydrolysis of either α´-acetoxy- or α´-hydroxy- α,β-unsaturated enones **21** and **22** provide access to γ-hydroxy-α,β-unsaturated enone, [28,32] the acetoxy analogue was chosen for the reduction. After obtaining low yield from $LiAlH_4$ reduction, another reducing agent $LiBH_4$ was used instead. The reaction of **22** with $LiBH_4$ followed by elimination furnished the product **24** in 78 % yield. The advantage of this step over $LiAlH_4$ reduction is to prevent the cleavage of the acetoxy group. Scheme 23 shows the reduction of **22** to **24**.



22 **24**

Scheme 23

The suggested mechanism for the formation of **24** is shown in scheme 24



Scheme 24

In this process it is important to conserve the chirality and the OAc group at the α′ position. α'-Acetoxy-4,6,7,8-tetrahydro-5*H*-1,3-benzodioxin-5-one was first treated with $LiBH_4$ in THF and then hydrolyzed with 2 N HCl.

The product was characterized by using NMR spectroscopy. From ^{1}H NMR spectrum, doublet was observed at 6.72 ppm (*J*= 1.25 Hz) for the olefinic proton. And a singlet was observed at 4.20 ppm for the CH_2 protons in the hyroxymethyl functionality and a singlet for $OCOCH_3$ at 2.04 ppm. From the ^{13}C NMR a peak for $O\underline{C}OCH_3$ is observed at 170.15 ppm and peaks for the olefinic carbons are observed at 139.95 and 143.04 ppm. At 61.45 ppm a peak for $\underline{C}H_2OH$ is observed.

The α value for the (R/S) 4-acetoxy-2-hydroxymethyl-cyclohexenone is $[\alpha]_D^{25} = -71.4$ (c 0.42, CH_3Cl).

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

In summary, first and efficient chemoenzymatic synthesis of both enantiomers of α′-acetoxy **22**, α′-hydroxy **21** and γ-acetoxy enone **24** is described. This is a model study for the synthesis of chiral cyclic alkenols.

In this study, in order to reach the target compounds first protection of 1,3-cyclohexanedione and 1,3-cyclopentanedione was done as stated in the literature to obtain 4,6,7,8-tetrahydro-5*H*-1,3-benzodioxin-5-one **35** and 6,7-dihydrocyclopenta-1,3-dioxin-5(4*H*)-one **20** respectively. Then oxidation of 4,6,7,8-tetrahydro-5*H*-1,3-benzodioxin-5-one with four equivalents of manganese (III) acetate in benzene gave the desired acetoxy enone whereas attempts to obtain α′-acetoxy-6,7-dihydrocyclopenta-1,3-dioxin-5(4*H*)-one failed. The research is then continued with the six membered analogue. The third step was the enzymatic kinetic resolution. In order to find the best enzyme giving the best result, screening with 18 different enzymes was done by the usage of DMSO as the organic solvent and phosphate buffer at pH=7. The results showed that the highest ee was obtained from *PLE* (95 % ee) enzyme. All the other enzymes hydrolyzed the same enantiomer whereas only *Aspergillus* hydrolyzed the other enantiomer selectively. Finally obtained acetoxy enone was reduced to 4-acetoxy-2-(hydroxymethyl)-2-cyclohexenone **24** with $LiBH_4$.

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.

Column chromatographies were done for the purification of the products by using Merck Silica Gel 60 (partical size 40-63 μm).

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

3.2 General Procedures

3.2.1 Synthesis of 4,6,7,8-tetrahydro-5*H*-1,3-benzodioxin-5-one (35)

A mixture of 1,3-cyclohexanedione (2.30 g, 20 mmol), paraformaldehyde (3.60 g, 120 mmol), $BF_3.Et_20$ (7.38 mL, 60 mmol) and methylene chloride (150 mL) was stirred at room temperature for 38 h. The reaction mixture was then quenched with saturated $NaHCO_3$ (20 mL) and the aqueous phase extracted with methylene chloride (3 X 20 mL). The combined organic phases were washed with brine, dried over $MgSO_4$, and concentrated in vacuo to afford a yellow oil. Purification via

column chromatography (gradient elution, hexanes/EtOAc, 4:l to 3:l) afforded 4,6,7,8-tetrahydro-5H-1,3-benzodioxin-5-one.

^{1}H-NMR (400 MHz, $CDCl_3$+CCl_4)

δ (ppm): 1.92-1.98 (m, 2H)

2.27-2.30 (m, 2H)

2.34-2.37 (m, 2H)

4.34 (bs, 2H)

5.05 (s, 2H)

^{13}C-NMR (100 MHz, $CDCl_3$+CCl_4)

δ (ppm): 195.7, 170.1, 112.0, 91.6, 62.9, 36.6, 27.8, 20.9

3.2.2 Synthesis of 6,7-dihydrocyclopenta-1,3-dioxin-5(4*H*)-one (20)

A mixture of 1,3-cyclopentanedione (3.24 g, 33.0 mmol), paraformaldehyde (6.00 g, 200 mmol), $BF_3.Et_2O$ (12.6 mL, 100 mmol) and methylene chloride (200 mL) was stirred vigorously at room temperature for 38 h. The mixture was then filtered and the filtrate added to 10% NaOH (130 mL) and ice (50 g). The resultant aqueous layer was extracted with methylene chloride (3 X 75 mL), and the combined organic phases were washed with brine and dried over $MgSO_4$. Removal of the solvent in vacuo and purification of the residue via column chromatography (silica; hexane/EtOAc, 3:l) afforded 6,7-Dihydrocyclopenta-1,3-dioxin-5(4H)-one.

^{1}H-NMR (400 MHz, $CDCl_3$+CCl_4)

δ (ppm): 2.32-2.35 (m, 2H, CH_2)
2.56-2.58 (m, 2H, CH_2)
4.39 (t, J: 2.07 Hz, 2H)
5.16 (s, 2H)

^{13}C-NMR (100 MHz, $CDCl_3$+CCl_4)

δ (ppm): 205.8, 181.8, 115.3, 93.0, 63.4, 32.9, 26.8

3.2.3 Synthesis of 6-acetoxy-4,6,7,8-Tetrahydro-5*H*-1,3-benzodioxin-5-one (39)

1,3-dioxin vinylogous ester **38** (1.20 g, 7.8 mmol) and $Mn(OAc)_3$ (8.36 g, 31.2 mmol) was heated to reflux in benzene for 4 days. The reaction was monitored by TLC. After cooling, the reaction mixture was first filtered then washed with saturated $NaHCO_3$ solution and with brine afterwards. The solution was then dried over $MgSO_4$, concentrated and purified by column chromatography to yield 87 % desired racemic acetoxy enone.

^{1}H-NMR (400 MHz, $CDCl_3$+CCl_4)

δ (ppm): 2.04-2.10 (m, 1H, CH_2)
2.07 (s, 3H, CH_3)
2.12-2.17 (m, 1H, CH_2)
2.42-2.48 (m, 1H, CH_2)
2.56-2.61 (m,1H,CH_2)
4.24 (dd, J= 14.6, 2.0 Hz, 1H, CH_2)
4.39 (dt, J= 14.6, 2.0 Hz, 1H, CH_2)
4.98 (d, J=5.5 Hz, 1H, CH_2)

5.14 (d, J= 5.5 Hz, 1H, CH_2)

5.19 (dd, J= 12.6, 5.3 Hz, 1H, CH)

^{13}C-NMR (100 MHz, $CDCl_3$+CCl_4)

δ (ppm): 190.0, 170.1, 169.1, 110.9, 91.6, 72.1, 62.9, 26.9, 26.7, 21.1

3.2.4 Enzyme-Catalyzed Kinetic Resolution

To a stirred solution of (±)-6-acetoxy-4,6,7,8-Tetrahydro-5*H*-1,3-benzodioxin-5-one (300 mg, 1.76 mmol) in DMSO (2 mL) and phosphate buffer (pH 7.0, 75 mL) enzyme (PLE 300 μL) was added in one portion and the reaction mixture was stirred at rt. Conversion was monitored by TLC and when 50 % conversion was attained, the reaction was terminated by the addition of excess organic solvent, chloroform. After filtration, the filtrate was extracted with dichloromethane, dried over $MgSO_4$, concentrated and the unreacted acetate and the product is separated by column chromatography (2:1 EtOAc:Hex) to obtain 6-acetoxy-4,6,7,8-Tetrahydro-5*H*-1,3-benzodioxin-5-one and 6-hydroxy-4,6,7,8-Tetrahydro-5*H*-1,3-benzodioxin-5-one respectively.

HPLC: Chiralpak OD column, UV detection at 254 nm, eluent: hexane/2-propanol= 90:10, flow 0.80 mL min^{-}1 20 ° C using racemic compound as the reference. The α value for the 6-hydroxy-4,6,7,8-Tetrahydro-5*H*-1,3-benzodioxin-5-one is $[\alpha]_D^{25} = +219.23$ (c 0.0013, CH_3OH).

^{1}H-NMR (400 MHz, $CDCl_3$+CCl_4)

δ (ppm): 1.80 (ddd, J=25.4, 12.7, 5.4 Hz, 1H, CH_2)

2.29-2.34 (m, 1H, CH_2)

2.39-2.45 (m, 1H, CH_2)

2.50-2.70 (m, 1H, CH_2)

3.58 (bs, 1H, OH)

3.95-4.05 (m,1H, CH)

4.28 (dd, J= 14.5, 2.1 Hz, 1H, CH_2)

4.48 (d, J= 14.5 Hz, 1H, CH_2)

4.99 (d, J= 5.5 Hz, 1H, CH_2)

5.17 (d, J= 5.5 Hz, 1H)

^{13}C-NMR (100 MHz, $CDCl_3$+CCl_4)

δ (ppm): 196.1, 170.6, 109.4, 91.9, 71.2, 62.7, 29.3, 27.1

3.2.5 Synthesis of 4-acetoxy-2-(hydroxymethyl)-2-cyclohexenone

To a stirred solution of 4,6,7,8-tetrahydro-6-acetoxy-5H-l,3-benzo-dioxin-5-one (0.0408 g, 0.240 mmol) in 5 mL of dry ether cooled to 0 ^{o}C was added 0.360 mmol of $LiBH_4$. The reaction mixture was allowed to warm to room temperature, stirred for 2 h, cooled back to 0 ^{o}C, and then quenched by the addition of saturated aqueous Na_2SO_4 (0.5 mL). The insoluble salts were removed by filtration, and the filtrate was concentrated in vacuo. The resultant oil was then dissolved in 2 mL of THF and 0.2 mL of 2 N HC1 added. After 15 min, the reaction was neutralized with aqueous K_2CO_3, diluted with ether, dried over $MgSO_4$, filtered, and evaporated. Column chromatography (2:1 EtOAc:Hex) provided 4-acetoxy-2-(hydroxymethyl)-2-cyclohexenone as a colorless oil.

^{1}H-NMR (400 MHz, $CDCl_3$+CCl_4)

δ (ppm): 1.41 (bs, 1H, OH)

1.97-2.06 (m, 1H)

2.04 (s, 3H)

2.27 -2.31 (m, 1H)

2.35 -2.44 (m, 1H)

2.58 (dt,J: 16.95, 5.26 Hz, 1H)

4.20 (s, 2H)

5.51 (m, 1H)

6.71 (d, J= 1.25 Hz, 1H)

^{13}C-NMR (100 MHz, $CDCl_3$+CCl_4)

δ (ppm): 198.4, 170.2, 143.0, 139.9, 68.2, 61.5, 35.6, 29.1, 21.3

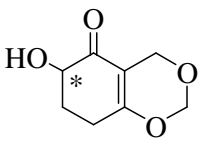
CHAPTER 4

CONCLUSION

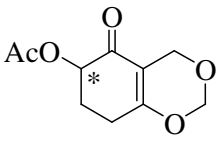
α-hydroxymethyl-γ-hydroxy enones **23, 27**and polyoxo-ketones **21**, **22, 25, 26** are important intermediates due to their reactive functional groups. They can be used in the construction of chiral biologically active natural compounds that contain these structures such as; RES-1149-2 showing antiviral activity, prostaglandins, pentenomycins, sarkomycin, sesquiterpene antiviral agents, etc.

In this study a new and efficient chemoenzymatic route was developed for the synthesis of optically active polyoxo cyclohexenones and γ-hydroxy enone. Primarily 1,3-cyclohexanedione was protected with paraformaldehyde by lewis acid catalyzation. Then this protected 1,3-dioxin vinylogous ester was acetoxylated from the α′ position by using $Mn(OAc)_3$. Then it is hydrolyzed selectively by lipase and esterase type enzymes. It was found that *PLE* was the best enzyme among them giving 95 % ee. Finally, chiral α-acetoxy enone is converted into chiral γ-hydroxy enone by reduction with $LiBH_4$.

Unfortunately attempts for the acetoxylation of the five membered analogue didn't give promising results. First oxidation with $Mn(OAc)_3$ was tried but it didn't work then oxidation with $Pb(OAc)_4$ and with $Hg(OAc)_2$ as well as with $Bi(OAc)_3$ was tried but again no satisfactory results were obtained then functionalization of the α′ position by bromination was tried but this gave many bromination products that were not isolated.



21



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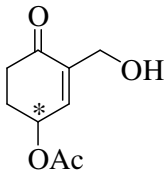

24

As a conclusion, this is a model study for the chemoenzymatic synthesis of chiral cyclic alkenols. In addition, it provides a simple new method for the synthesis of chiral hydroxy and acetoxy enones **21** and **22** as well as chiral cyclic 4-acetoxy-2-hydroxymethyl enones **24**.

As a representative example we showed that 1,3-dioxin vinylogous esters can be converted to α′-acetoxy and to α′-hydroxy analogues. According to the literature procedures α´-hydroxy enones can be converted to its acetoxy derivatives. This is giving the possibility to obtain opposite enantiomer of (-) **24**.

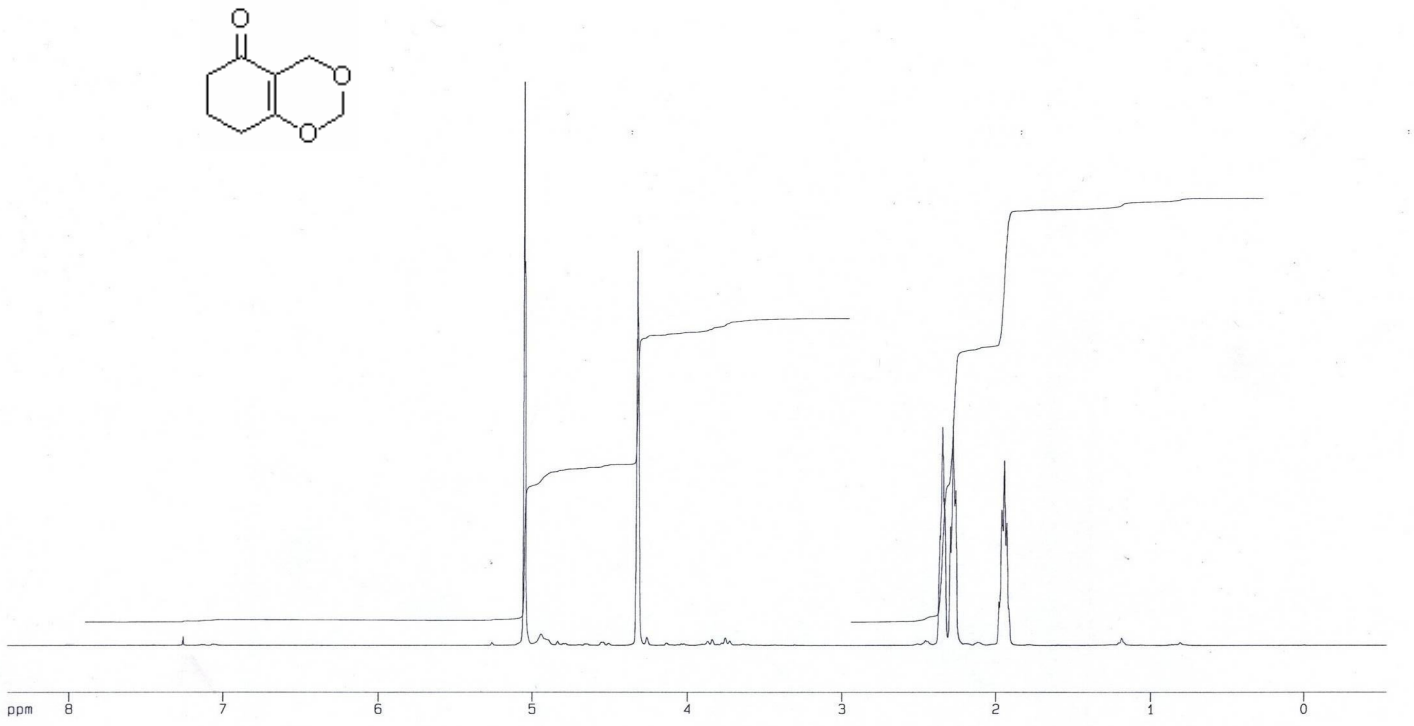

Figure 19: ^{1}H-NMR Spectrum of 4,6,7,8-Tetrahydro-5*H*-1,3-benzodioxin-5-one **35**

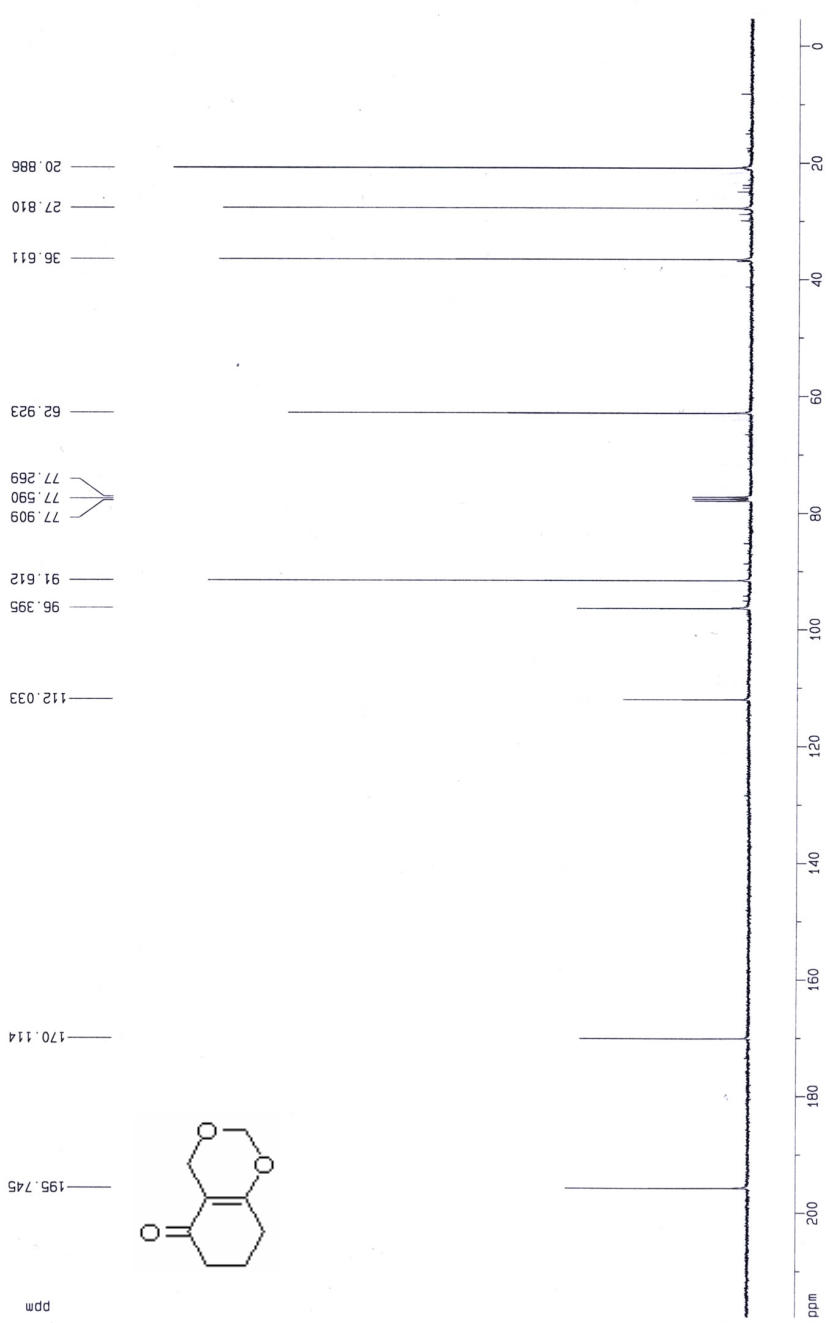

Figure20: ^{13}C-NMR Spectrum of 4,6,7,8-tetrahydro-5*H*-1,3-benzodioxin-5-one **35**

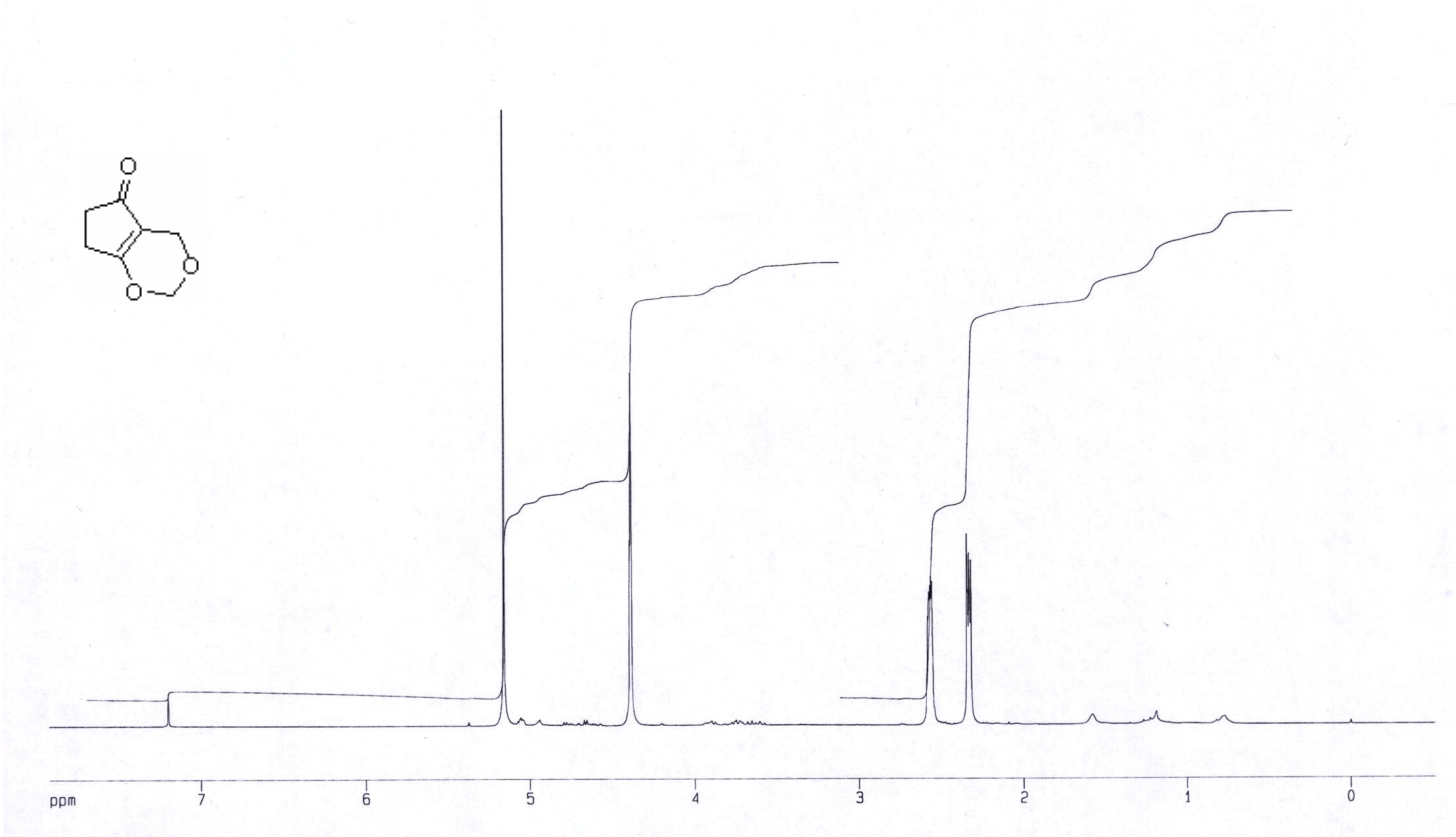

Figure 21: ^{1}H-NMR Spectrum of 6,7-dihydrocyclopenta-1,3-dioxin-5(4*H*)-one **20**

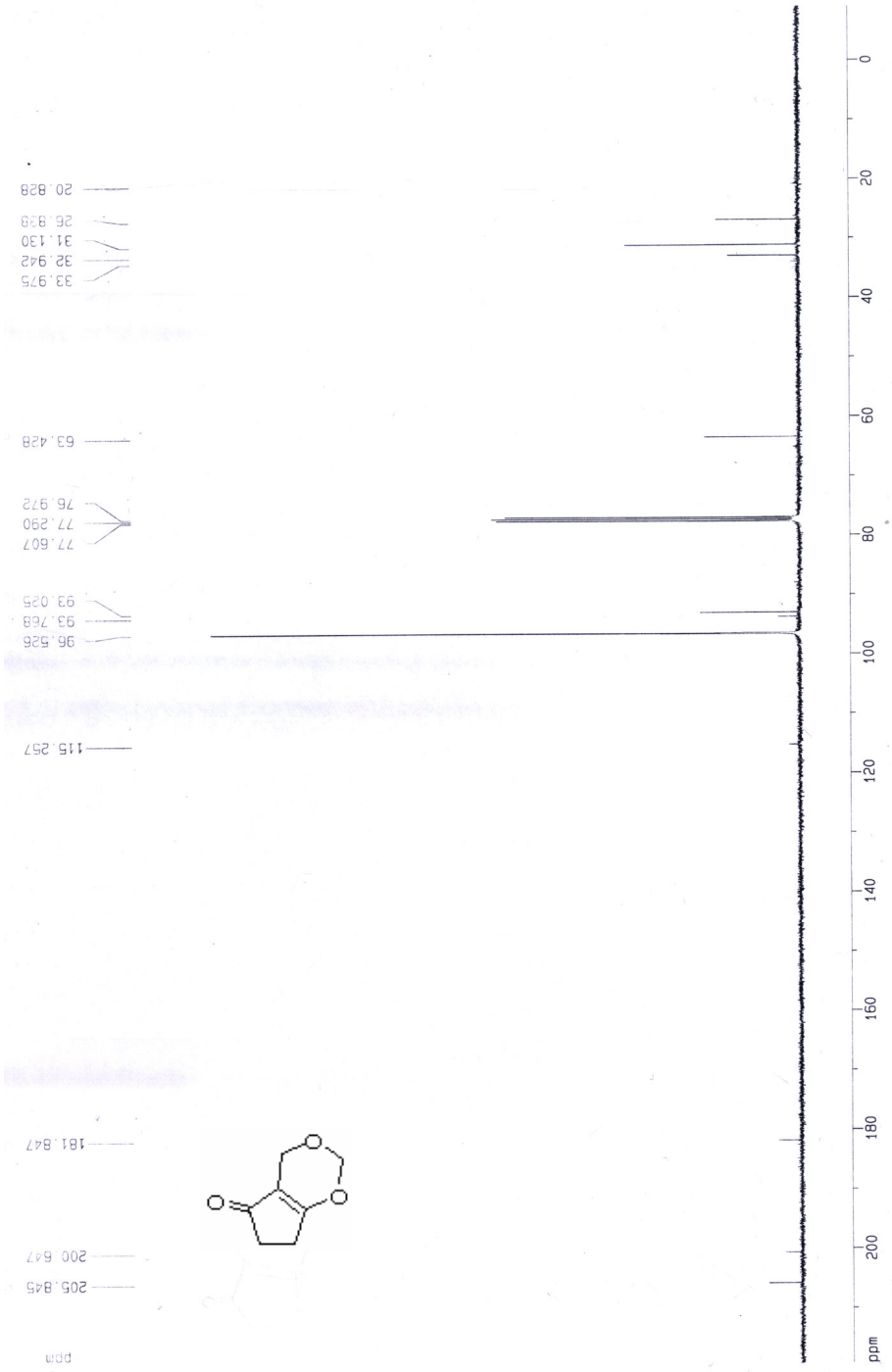

Figure 22: ^{13}C-NMR Spectrum of 6,7-dihydrocyclopenta-1,3-dioxin-5(4*H*)-one **20**

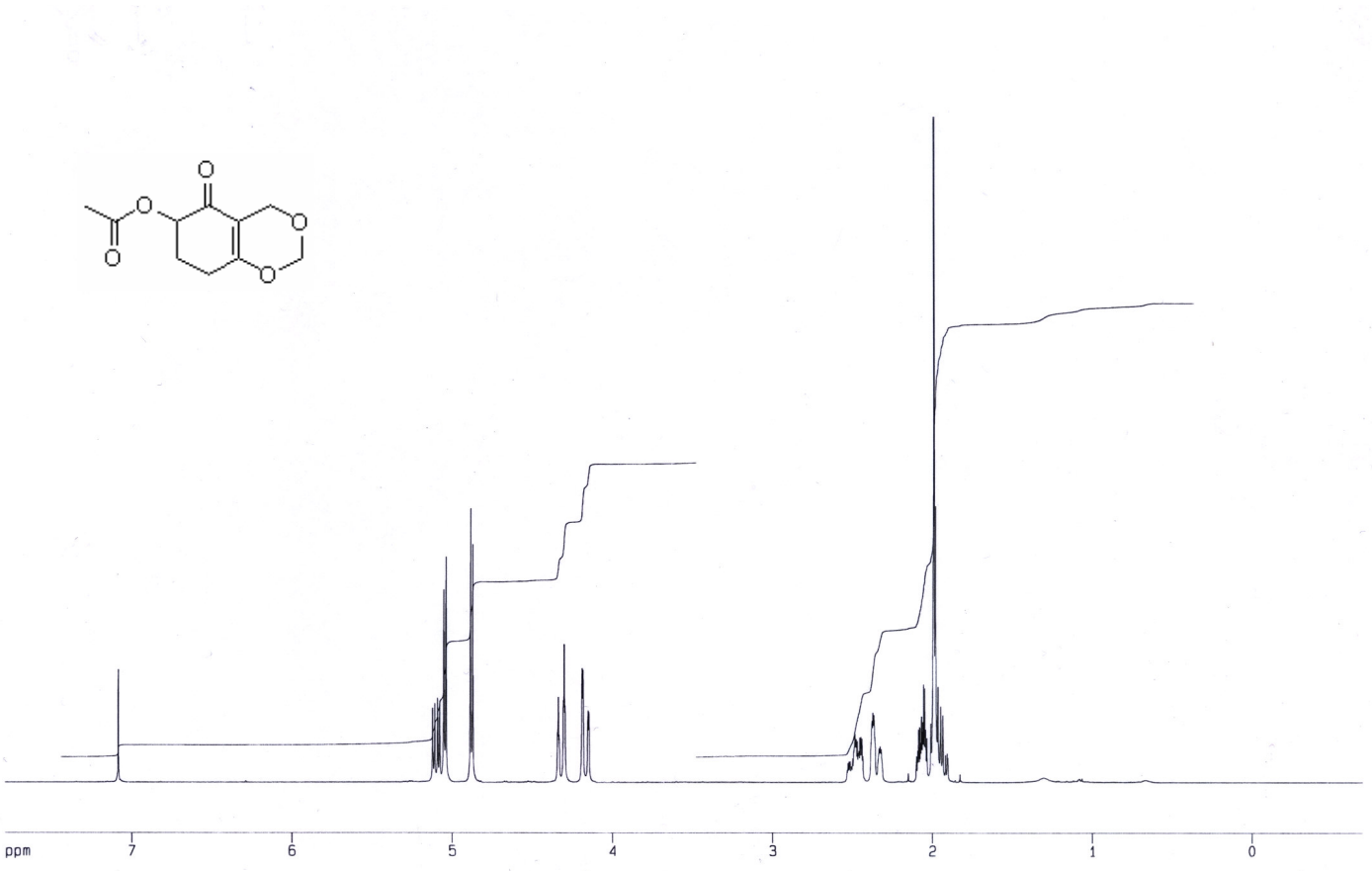

Figure 23: ^{1}H-NMR Spectrum of 6-acetoxy-4,6,7,8-tetrahydro-5*H*-1,3-benzodioxin-5-one **36**

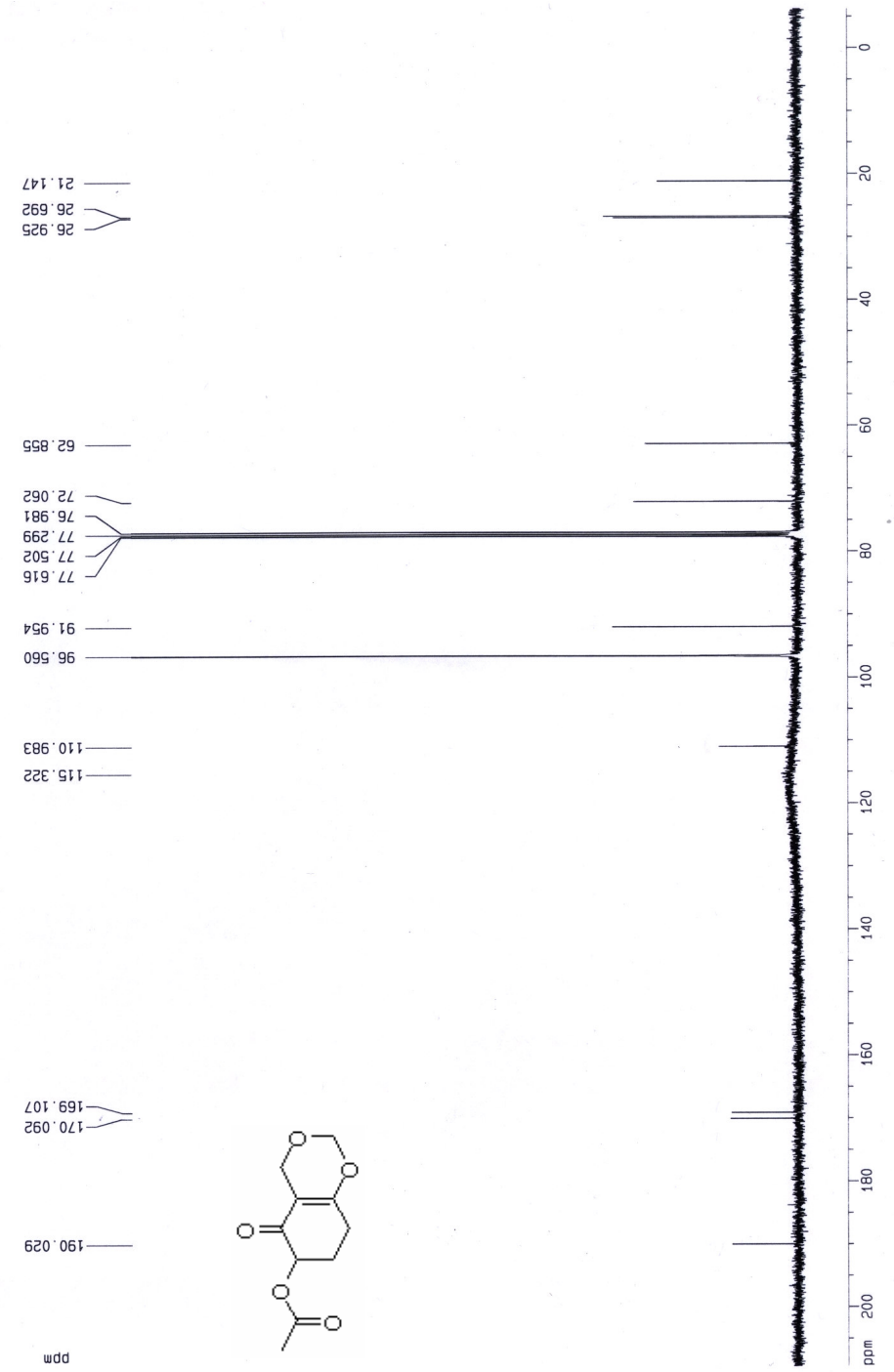

Figure 24: ^{13}C-NMR Spectrum of 6-acetoxy-4,6,7,8-tetrahydro-5*H*-1,3-benzodioxin-5-one **36**

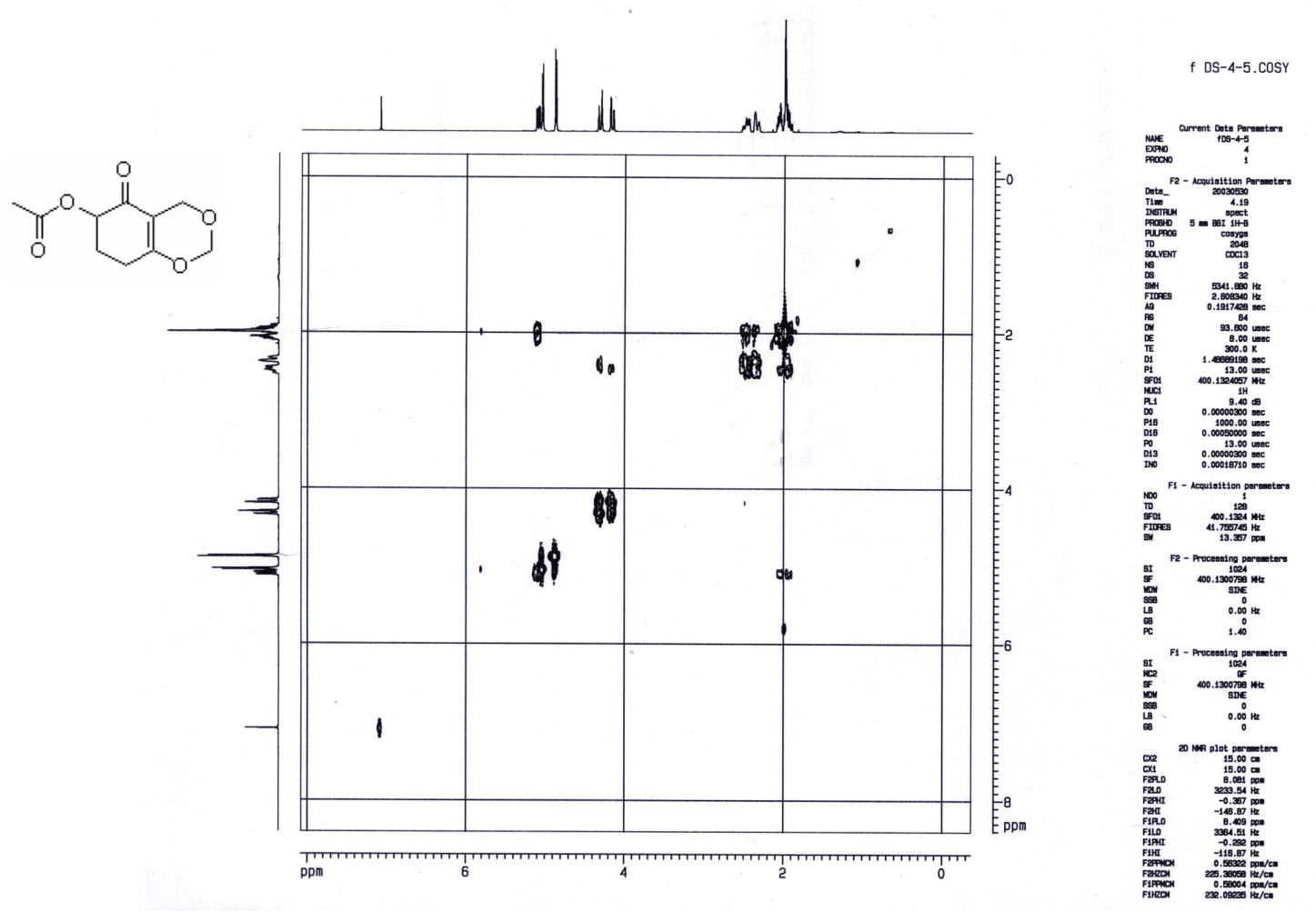

Figure 25: COSY Conture Diagram of 6-acetoxy-4,6,7,8-tetrahydro-5*H*-1,3-benzodioxin-5-one **36**

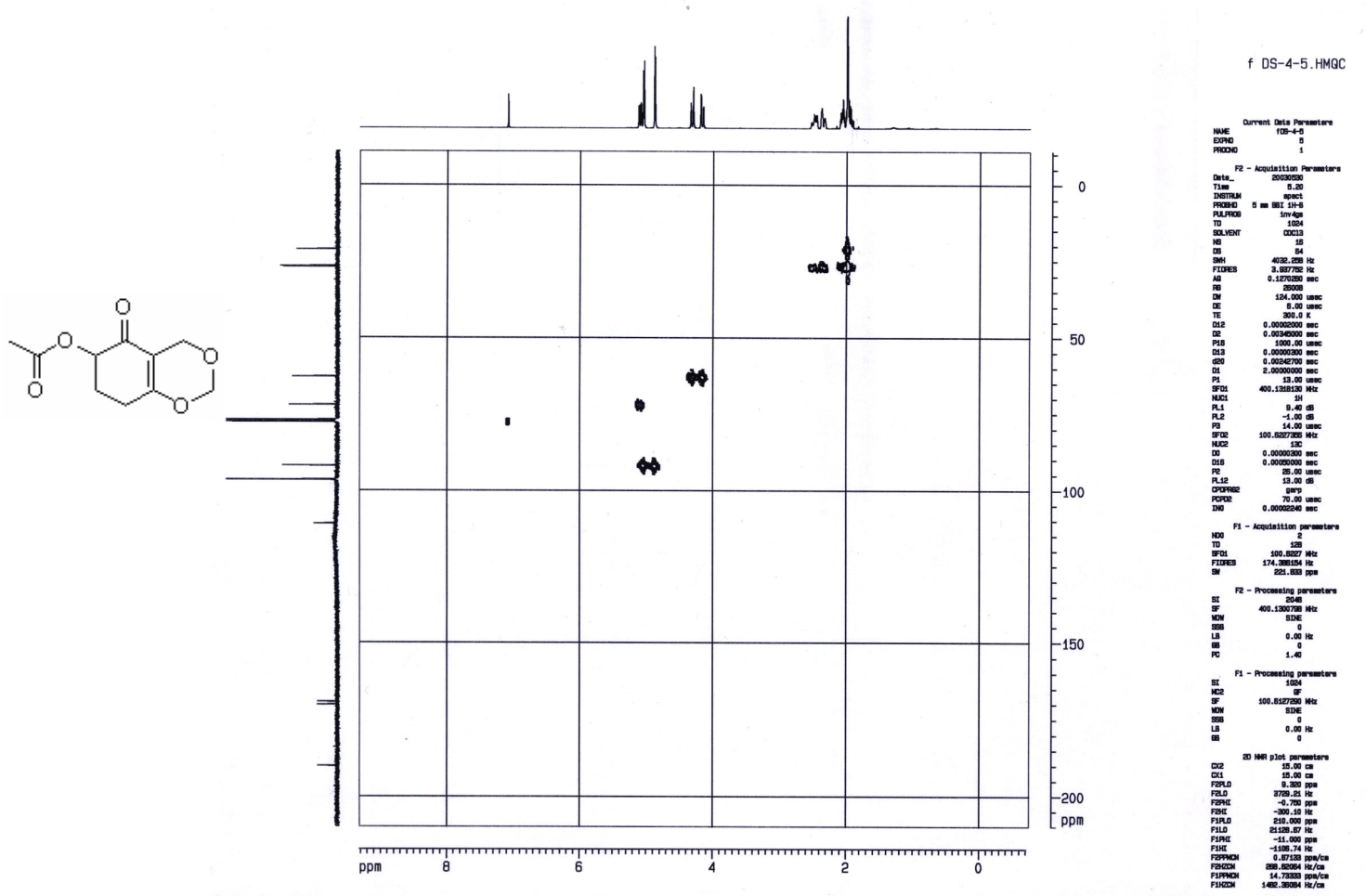

Figure 26: HMQC Conture Diagram of 6-acetoxy-4,6,7,8-tetrahydro-5*H*-1,3-benzodioxin-5-one **36**

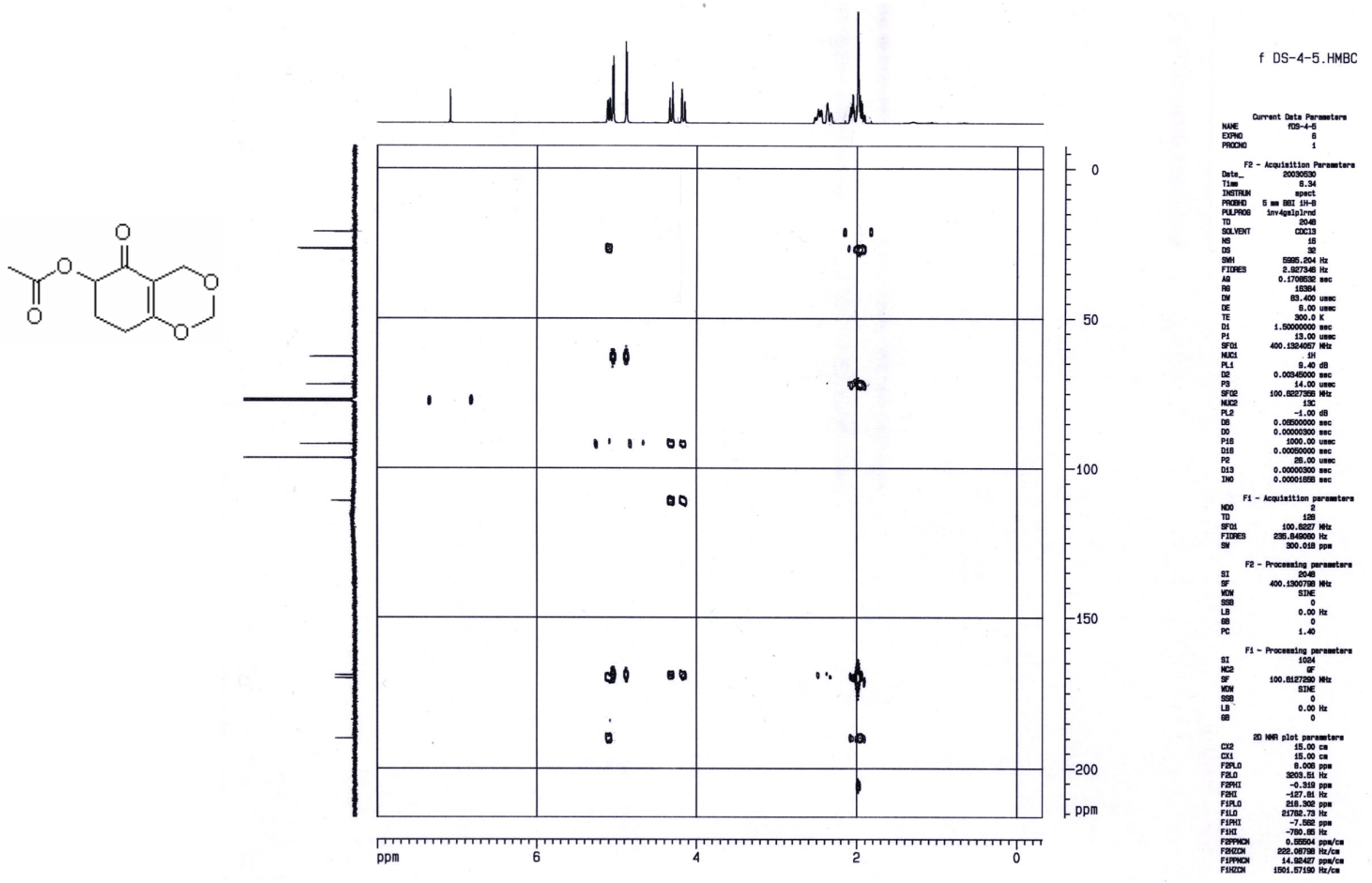

Figure 27: HMBC Conture Diagram of 6-acetoxy-4,6,7,8-tetrahydro-5*H*-1,3-benzodioxin-5-one **36**

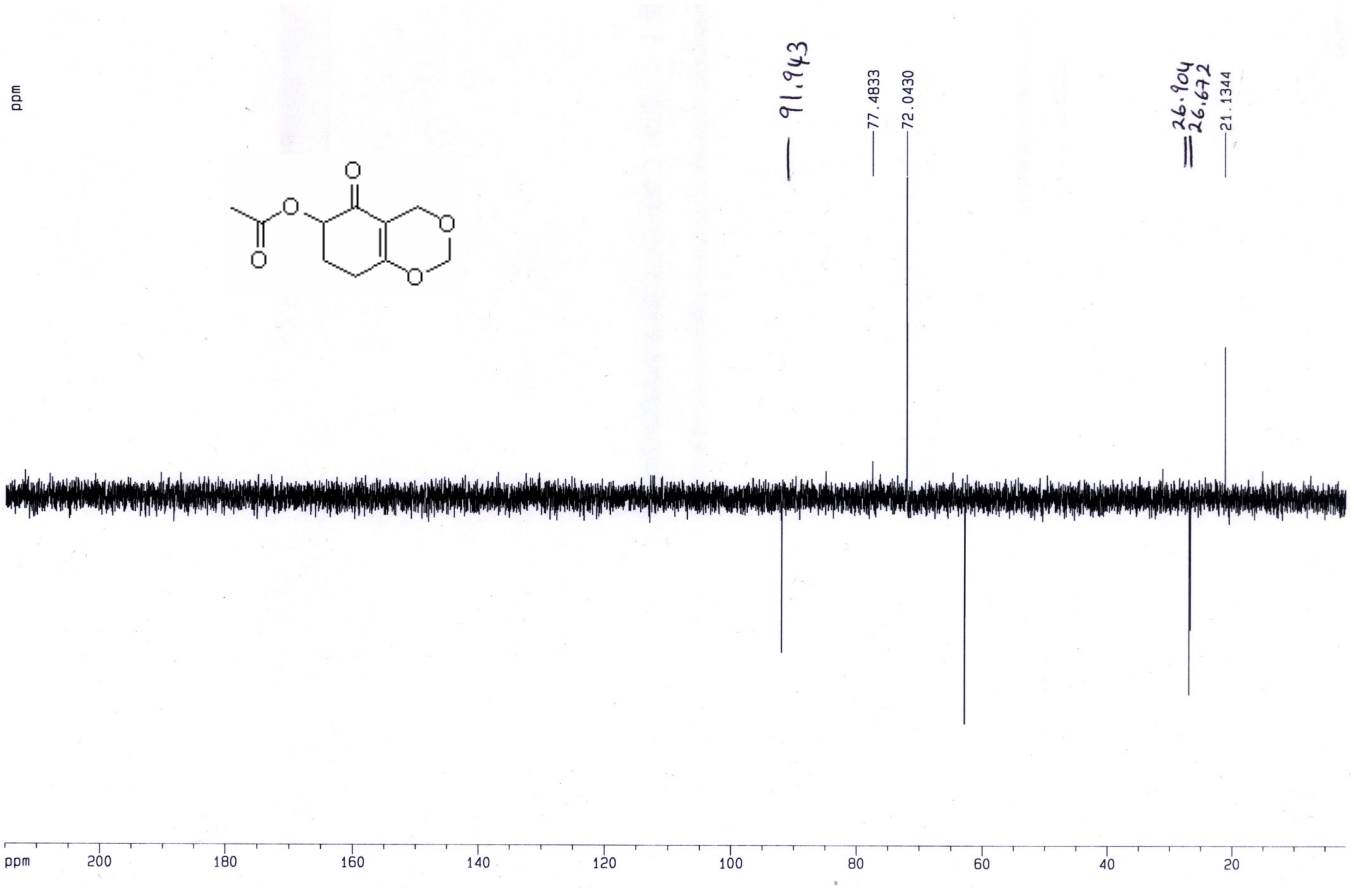

Figure 28: DEPT 135 spectrum of 6-acetoxy-4,6,7,8-tetrahydro-5*H*-1,3-benzodioxin-5-one **36**

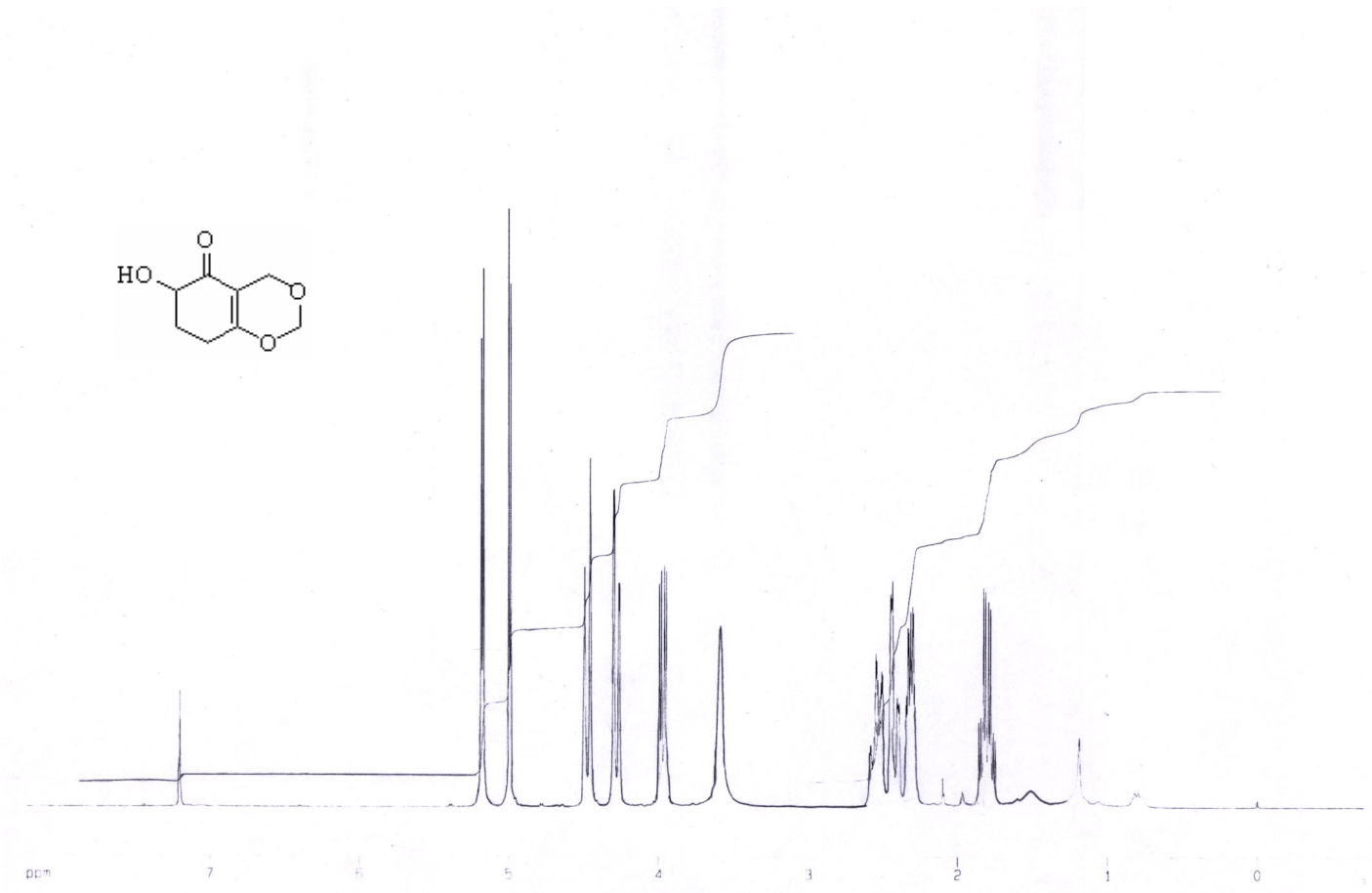

Figure 29: ^{1}H-NMR of 6-hydroxy-4,6,7,8-tetrahydro-5*H*-1,3-benzodioxin-5-one **21**

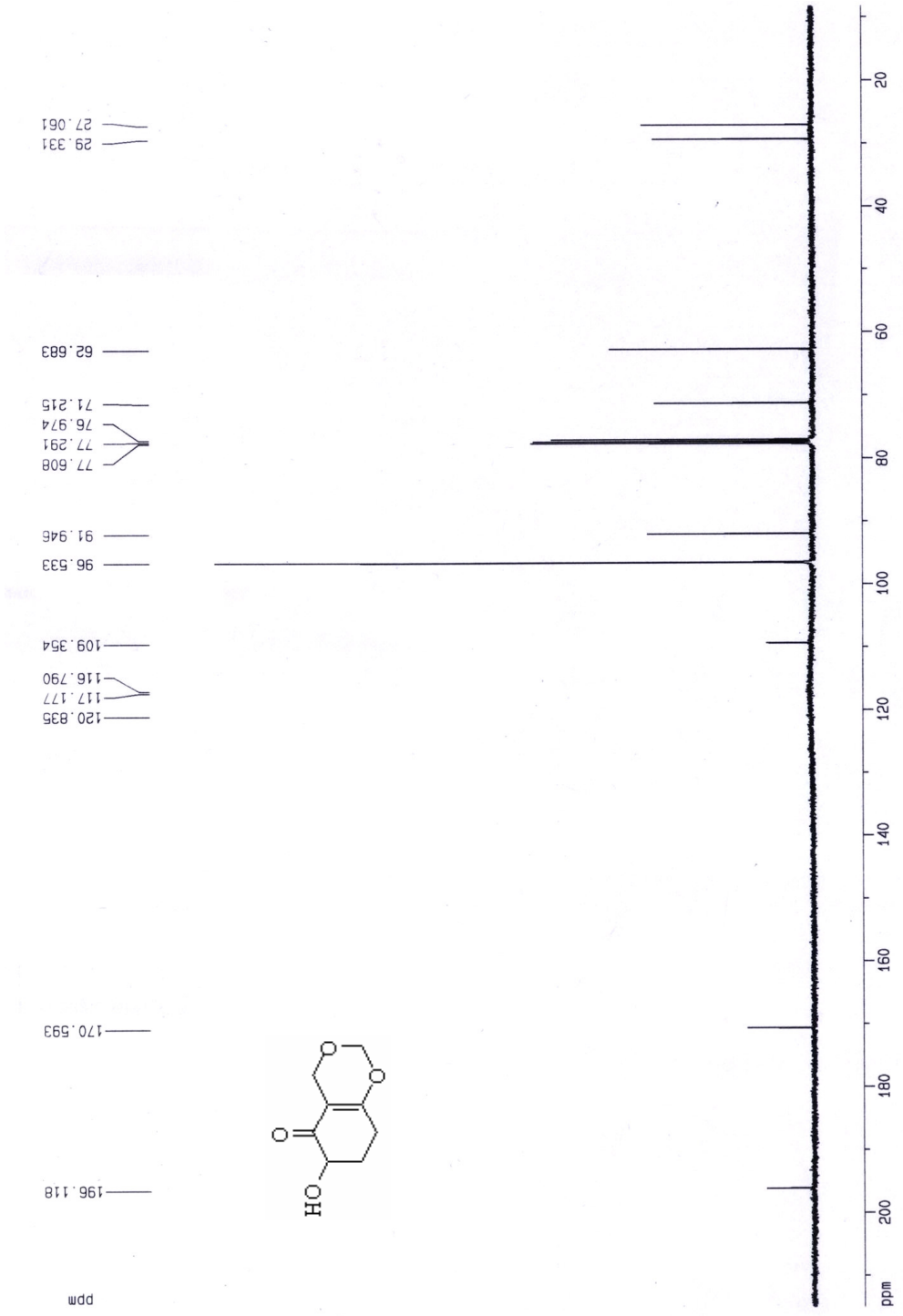

Figure 30: ^{13}C-NMR Spectrum of 6-Hydroxy-4,6,7,8-tetrahydro-5*H*-1,3-benzodioxin-5-one **21**

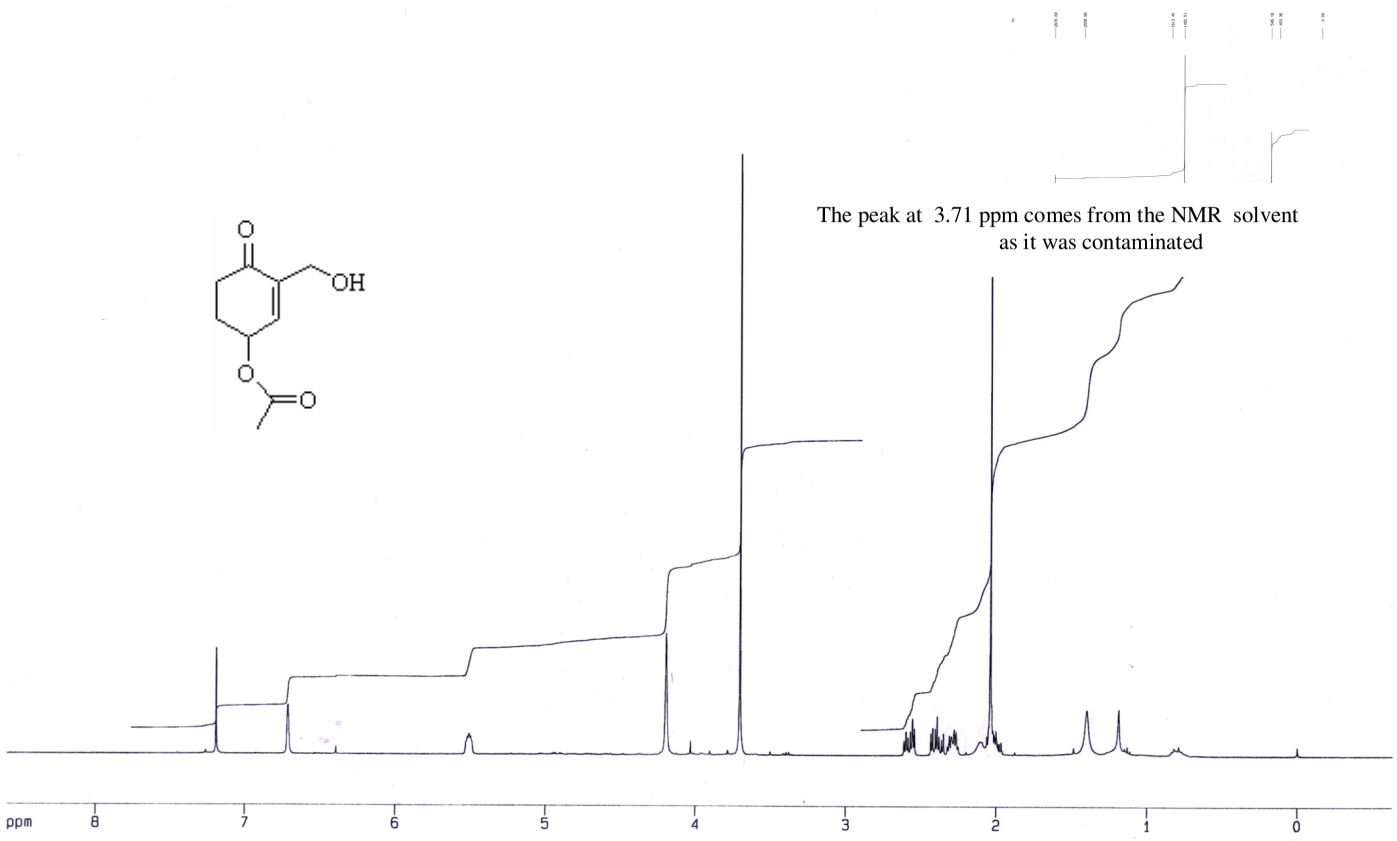

Figure 31 ^{1}H-NMR Spectrum of 4-Acetoxy-2-Hydroxymethyl-cyclohexenone **24**

APPENDIX

X-RAY DATA OF 6-ACETOXY-4,6,7,8-TETRAHYDRO -5*H*-1,3-BENZODIOXIN-5-ONE

Atom Coordinates:

Atom	x	y	z	U_{eq}
C1	1.2089(4)	-0.0489(6)	0.5649(4)	0.0629(12)
H1	1.2910	-0.0535	0.5683	0.075
C2	1.0737(4)	-0.2587(5)	0.6218(3)	0.0542(10)
H2	1.0650	-0.3814	0.6406	0.065
C3	1.0267(3)	-0.0985(5)	0.6803(3)	0.0411(9)
C4	1.0609(3)	0.0736(5)	0.6599(3)	0.0432(9)
C5	1.0134(4)	0.2415(5)	0.7074(3)	0.0552(11)
H5	1.0518	0.3546	0.7121	0.066
C6	0.8936(4)	0.2050(5)	0.7473(3)	0.0540(10)
H6	0.8266	0.2779	0.7314	0.065
C7	0.8993(3)	0.0324(5)	0.8168(3)	0.0450(9)
C8	0.9393(3)	-0.1314(5)	0.7551(3)	0.0416(9)
C9	0.7701(4)	-0.0747(6)	0.9439(3)	0.0548(10)
C10	0.6444(4)	-0.0946(8)	0.9607(4)	0.0772(14)
H10A	0.5957	-0.0530	0.8969	0.116
H10B	0.6281	-0.0224	1.0224	0.116
H10C	0.6275	-0.2216	0.9738	0.116
O1	1.1439(2)	0.1093(4)	0.5919(2)	0.0531(8)
O2	1.1342(2)	-0.1956(4)	0.5327(2)	0.0561(8)
O3	0.9000(3)	-0.2856(4)	0.7679(2)	0.0608(9)
O4	0.7817(2)	0.0013(4)	0.84540(19)	0.0528(8)
O5	0.8533(3)	-0.1128(6)	1.0080(3)	0.0853(12)

Bond Length and Angles:

C1	O2	1.393(5)
C1	O1	1.426(5)
C2	O2	1.435(5)
C2	C3	1.499(5)
C3	C4	1.342(5)
C3	C8	1.449(5)
C4	O1	1.355(4)
C4	C5	1.482(5)
C5	C6	1.521(5)
C6	C7	1.517(5)
C7	O4	1.437(4)
C7	C8	1.511(5)
C8	O3	1.223(4)
C9	O5	1.195(5)
C9	O4	1.355(5)
C9	C10	1.473(6)

O2	C1	O1	111.7(3)
O2	C2	C3	110.3(3)
C4	C3	C8	120.4(3)
C4	C3	C2	120.5(3)
C8	C3	C2	119.0(3)
C3	C4	O1	122.0(3)
C3	C4	C5	124.7(3)
O1	C4	C5	113.3(3)
C4	C5	C6	111.3(3)
C7	C6	C5	110.1(3)
O4	C7	C8	109.9(3)
O4	C7	C6	106.7(3)
C8	C7	C6	111.3(3)
O3	C8	C3	121.3(3)
O3	C8	C7	121.5(3)
C3	C8	C7	117.2(3)
O5	C9	O4	122.6(4)
O5	C9	C10	126.3(4)
O4	C9	C10	111.0(4)
C4	O1	C1	113.8(3)
C1	O2	C2	110.8(3)
C9	O4	C7	118.1(3)

Crystallographic Data:

data_ds4

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'C10 H7 O5'	
_chemical_formula_weight	207.16
_symmetry_cell_setting	Monoclinik (Crystal system)
_symmetry_space_group_name_H-M	P 21/a
_cell_length_a	11.3615(12)
_cell_length_b	7.2705(11)
_cell_length_c	12.3396(13)
_cell_angle_alpha	90.00
_cell_angle_beta	96.383(4)
_cell_angle_gamma	90.00
_cell_volume	1013.0(2)
_cell_formula_units_Z	4
_cell_measurement_temperature	293(2)
_exptl_crystal_size_max	0.40
_exptl_crystal_size_mid	0.30
_exptl_crystal_size_min	0.20
_exptl_crystal_density_diffrn	1.358
_exptl_crystal_density_method	'not measured'
_exptl_crystal_F_000	428
_exptl_absorpt_coefficient_mu	0.111
_exptl_absorpt_correction_T_min	0.9568
_exptl_absorpt_correction_T_max	0.9781
_diffrn_ambient_temperature	293(2)
_diffrn_radiation_wavelength	0.71073
_diffrn_radiation_type	MoK\a
_diffrn_radiation_source	'fine-focus sealed tube'
_diffrn_radiation_monochromator	graphite
_diffrn_reflns_number	2039
_diffrn_reflns_av_R_equivalents	0.0196
_diffrn_reflns_av_sigmaI/netI	0.0275
_diffrn_reflns_limit_h_min	-13
_diffrn_reflns_limit_h_max	0
_diffrn_reflns_limit_k_min	-8
_diffrn_reflns_limit_k_max	0
_diffrn_reflns_limit_l_min	-15
_diffrn_reflns_limit_l_max	15
_diffrn_reflns_theta_min	3.26
_diffrn_reflns_theta_max	26.23
_reflns_number_total	1933
_reflns_number_gt	1374
_reflns_threshold_expression	>2sigma(I)
_computing_structure_refinement	'SHELXL-97 (Sheldrick, 1997)'

_refine_ls_structure_factor_coef Fsqd
_refine_ls_matrix_type full
_refine_ls_weighting_scheme calc
_refine_ls_weighting_details
'calc w=1/[\s^2^(Fo^2^)+(0.1907P)^2^+0.6482P] where P=(Fo^2^+2Fc^2^)/3'
_atom_sites_solution_primary direct
_atom_sites_solution_secondary difmap
_atom_sites_solution_hydrogens geom
_refine_ls_hydrogen_treatment mixed
_refine_ls_extinction_method SHELXL
_refine_ls_extinction_coef 0.013(8)
_refine_ls_extinction_expression
'Fc^*^=kFc[1+0.001xFc^2^\l^3^/sin(2\q)]^-1/4^'
_refine_ls_number_reflns 1933
_refine_ls_number_parameters 137
_refine_ls_number_restraints 0
_refine_ls_R_factor_all 0.1102
_refine_ls_R_factor_gt 0.0790
_refine_ls_wR_factor_ref 0.2827
_refine_ls_wR_factor_gt 0.2502
_refine_ls_goodness_of_fit_ref 1.043
_refine_ls_restrained_S_all 1.043
_refine_ls_shift/su_max 0.001
_refine_ls_shift/su_mean 0.000
_diffrn_measured_fraction_theta_max 0.950
_diffrn_reflns_theta_full 26.23
_diffrn_measured_fraction_theta_full 0.950
_refine_diff_density_max 0.675
_refine_diff_density_min -0.464
_refine_diff_density_rms 0.080

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