CHEMOENZYMATIC SYNTHESIS OF ENANTIOMERICALLY ENRICHED 2-OXOBICYCLO[m.1.0]ALKAN-3-YL ACETATE DERİVATIVES

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Approval of the Graduate School of Natural and Applied Sciences

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ABSTRACT

CHEMOENZYMATIC SYNTHESIS OF ENANTIOMERICALLY ENRICHED 2-OXOBICYCLO[m.1.0]ALKAN-3-YL ACETATE DERIVATIVES

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α,β-Unsaturated cyclic ketones were selectively oxidized on α'- positions using $Mn(OAc)_3$ and $Pb(OAc)_4$, respectively. The resultant racemic α'-acetoxylated substrates were resolved into corresponding enantiomerically enriched α'-hydroxylated and α'-acetoxylated compounds via PLE hydrolysis. α'-Hydroxylated compounds are racemized quickly, so they were acetylated with acetyl chloride and pyridine *in situ* to give the corresponding α'-acetoxylated compounds. Resultant α'-acetoxy α,β-unsaturated cyclic ketones reacted with excess amount of diazomethane under the catalsts of $Pd(OAc)_2$ to give the resulting bicyclic diastereomeric products. At the end of the experiment, Enantiomeically enriched 2-oxobicyclo[3.1.0]hexan-3-yl acetate and 2-oxobicyclo[4.1.0]heptan-3-yl acetate were chemoenzymatically synthesized.

Key words: Enzymatic hydrolysis, manganese triacetate and kurşun tetraacetate, diazomethane, palladium(II) acetate.

ÖZ

ENANTİOMERCE ZENGİN 2-OKZOBİSİKLO[m.1.0]ALKAN-3-İL ASETAT TÜREVLERİNİN KEMOENZİMATİK SENTEZİ

Atlı, Selin
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Haziran 2005, 72 sayfa

Bu çalışmada, α,β-doymamış siklik ketonlar α' pozisyonundan $Mn(OAc)_3$ and $Pb(OAc)_4$ yardımı ile seçici bir şekilde oksitlendi. Oluşan rasemik α'-asetoksilenmiş substratlar PLE hidrolizi ile enantiyomerce zenginleşmiş α'-hidroksilenmiş ve asetoksilenmiş maddelere dönüştürüldü. α'-Hidroksilenmiş substratlar hemen rasemize oldukları için, tepkime ortamında, asetil klorür ve piridin ile asetillendi. Oluşan α'-asetoksilenmiş ürünler fazla miktarda diazometan ile $Pd(OAc)_2$ katalizörlüğünde tepkimeye girerek diastereomerik ve bisiklik ürünler oluşturdu. Bu deneyin sonunda, enantiomerce zengin 2-okzobisiklo[3.1.0]hekzan-3-il asetat ve 2-okzobisiklo[4.1.0]heptan-3-il asetat, 2-siklopentenon ve 2-siklohekzenon'dan başlayarak kemoenzimatik olarak sentezlendi.

Anahtar Kelimeler: Enzimatik hidroliz, mangan triasetat ve kurşun tetraasetat, diazometan, paladyum(II) asetat.

To my family

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CCL: Candida cylindracea

COSY: Correlation spectroscopy

Ee: Enantiomeric excess

DMSO: Dimethy sulfoxide

HETCOR: Heteronuclear chemical shift correlation

HLE: Horse liver esterase

HMBC: Heteronuclear multiple bond coherence

NOE: Nuclear overhousing effect

NOESY: Nuclear overhousing effect spectroscopy

NMR: Nuclear magnetic resonance

MCPBA: m-Chloroperoxybenzoic acid

MoOPh: Oxoperoxymolybdenum(pyridine)(hexamethyl phosphoramide)

TMSCl: Trimethylsillyl chloride

TLC: Thin layer chromatography

TPPO: Triphenylphosphite

PLE: Porchine liver esterase

PPL: Porchine pacrease esterase

CHAPTER I

INTRODUCTION

1.1 OXIDATION

Oxidation in organic chemistry generally refers to the elimination of hydrogen or the replacement of the hydrogen atom with a more electronegative element such as oxygen.

Metal-catalyzed oxidations can be divided into two types as *homolytic* and *heterolytic* [1]. Homolytic catalysis usually involves soluble transition metal salts, such as the acetates or naphthenates of Co, Mn, Fe, Cu, etc. In homolytic catalysis, the metal species recycles between several oxidation states by one equivalent changes. Free radicals are formed as intermediates from the organic substrate. In heterolytic catalysis, transition metals are coordinated to the organic substrates. It is characterized by the metal complex acting as a Lewis acid. Free radicals are not intermediates.

1.1.1 Lead (IV) Acetate Oxidations

In 1923, Dimroth and Schweizer outlined the scope of lead tetraacetate oxidations [2]. They oxidized quinizarine to quinone by using lead tetraacetate. Since then the use of lead tetraacetate as an oxidant has been increasing every year.

Many reaction mechanisms seem possible for the oxidation of organic compounds. Besides lead tetraacetate itself, acetoxy radicals, acetoxy cations, and $Pb(OAc)_3^+$ ions have been regarded as the oxidizing species [3].

In many tetraacetate oxidations, acetoxy radicals have been regarded as intermediates. They might be formed by dissociation. In the first case, the trivalent lead would have to be very short-lived so, it should have radical character.

On the other hand, many oxidations can be considered by assuming that lead tetraacetate loses an acetate ion, forming $Pb(OAc)_3^+$. An electrophilic attack of the latter forms an organolead compound with the substrate. The reaction is completed with the loss of the lead diacetate, and an AcO^+ moiety has been transferred without ever existing as a species.

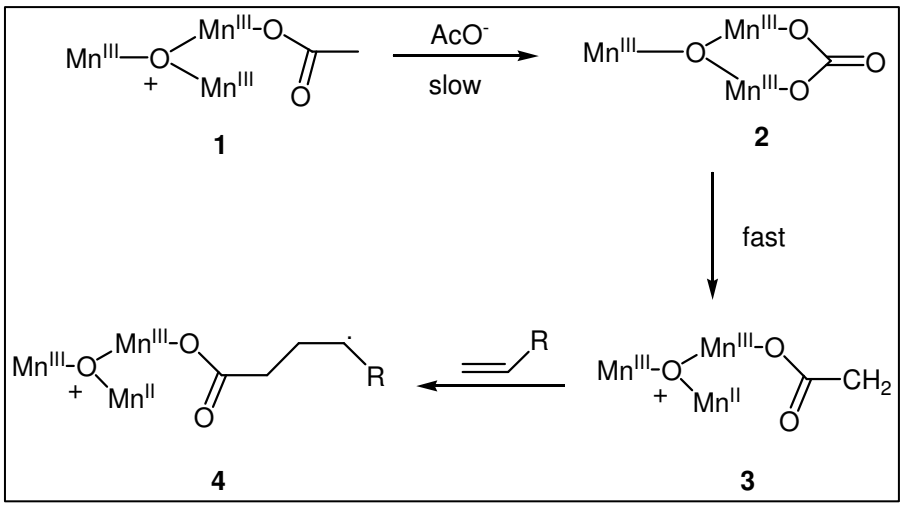
In other cases the organolead compound $R\text{-}Pb(OAc)_3$ loses $Pb(OAc)_3^-$. The electrophilic induction of $Pb(OAc)_3^+$ followed by its loss as an anion.

Different reaction mechanisms might be operative depending on substrate and reaction conditions. Acetic acid is the most frequently used solvent. It has an intermediate dielectric constant and might equally favor ionic and radical mechanisms.

1.1.2 Manganese(III) Acetate Oxidations

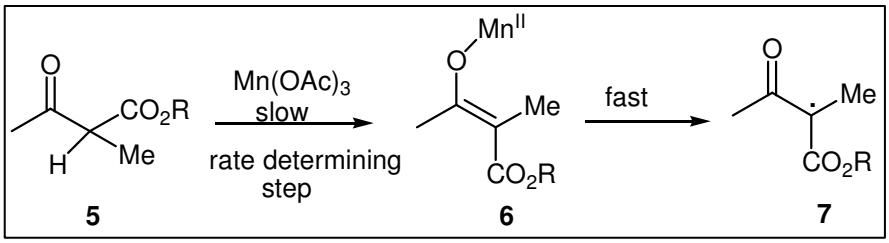
Manganese triacetate is one of the most powerful oxidizing agents and has been used for most of the oxidative reactions. In 1976, Williams and Hunter reported that the manganese(III) acetate oxidation of enones affords modest yields of α'-acetoxy enones [4]. Watt et al. reinvestigated this procedure and obtained acceptable yields [5]. $Mn(OAc)_3$ is prepared from potassium permanganate and manganeous acetate in acetic acid [6]. Anhydrous one is slightly more reactive then the dihydrate. Reaction times with the anhydrous reagent are usually somewhat shorter but the yield of products is usually comparable. Use of triflouroacetic acid as a cosolvent usually increases the rate of the reaction, but often decreases the yield of the products.

Acetate anion may accelerate enolization and act as a buffer. Acetic acid, DMSO, ethanol, methanol, dioxane, and acetonitrile are used as solvent for $Mn(OAc)_3$ reactions but among these solvents acetic acid is the most widely used one. The studies on $Mn(OAc)_3$ based oxidations help us to know the mechanism of the reaction. According to the studies of Fristad and Peterson, the rate determining step in the oxidation of acetic acid by $Mn(OAc)_3.H_2O$ which is actually an oxo-centered triangle of Mn(II) with bridging acetates [7] is the loss of a proton from a complexed acetate like **1** to give **2**, given in Scheme 1 [7, 8]. Rapid electron transfer to the oxo-centered metal system gives radical **3** which adds to the alkene to give **4**. The reaction rate is independent of alkene concentration, since the alkene is not involved in the rate determining step.



Scheme 1 Proposed mechanism of oxidation of monocarbonyl substrates by $Mn(OAc)_3$

They also studied a similar mechanism which is operative in the oxidation of α-alkyl β-keto esters [9] that is shown in Scheme 2



Scheme 2 Proposed mechanism for the oxidation of α-alkyl β-keto Esters

Electron transfer with loss of Mn(II) to give **7** is rapid and enolization to give **6** is slow. The rate of reaction is therefore independent of alkene concentration. Radical **7** reacts from the geometry shown as determined by analysis of the stereochemistry of the products as discussed below.

Comparable regio- and stereochemical results are obtained from a series of Mn(III)-based oxidative cyclizations and iodine and bromine atom-transfer cyclizations [10]

This results indicate that free radical **7** is involved in the Mn(III)-mediated oxidative cyclizations. Some differences in regiochemistry and stereochemistry between oxidative cyclizations and atom-transfer cyclizations would be expected if a Mn(III)-complexed radical was involved.

1.1.3 Oxidation of α,β- unsaturated cyclic ketones

α,β-Unsaturated ketones show diverse biological activities such as; antimicrobial, antitumor and plant growth activity. The biological activity of these compounds is attributed to the existence of the α,β-unsaturated carbonyl group. A variety of α,β-unsaturated ketones; 2-cyclohepten-1-one, 2-cyclohexen-1-one, 2-cyclopenten-1-one inhibited the urea's activity and urea's inhibitors have recently attracted the attention of scientists as new potential anti-ulcer drugs [11]. Nevertheless, α,β-unsaturated ketones are important building blocks in organic synthesis, they are frequently used as versatile and convenient intermediates in many organic reactions. α' Oxidation of α,β-unsaturated cyclic ketones possess a central position in synthetic methodology [12]. The regioselective α' oxidation of enones to α'-acetoxy enones constitutes a valuable procedure for manipulating a common functional group. Previous works on this subject involve direct oxidations with lead tetraacetate [13], mercuric acetate [14], and manganese triacetate.

1.2.1 Asymmetric Synthesis

Many natural products exist as one stereoisomer so; asymmetric synthesis is required to prepare nature-identical material. Asymmetric synthesis is the rapidly progressing field of synthetic organic chemistry in the last years.

Asymmetric synthesis was described as the process for the formation of an optically active compound through reaction of an asymmetric substrate with a chiral reagent [15]. Its original definition was coined by Marckwald in 1904. Morrison and Mosher expanded this definition in 1971 to cover a wider range of reactions. According to

their definition, asymmetric synthesis is a reaction where an achiral unit in an ensemble of substrate molecules is converted by a reactant into a chiral unit in such a manner that the stereoisomeric products are formed in unequal amounts [16].

1.2.2 Why Asymmetric Synthesis?

A wide variety of chiral compounds exist in nature. The asymmetry of these molecules arises from the inherent chirality's of the enzymes which are responsible from their production [17]. Enantioselectivity has also given a number of opportunities. Some of the arguments for use of a single enantiomer over a racemate are given in Table 1.

Table 1: Possible Benefits for Use of a Single Enantiomer for Therapeutic Uses

Properties of Racemates	Potential Benefits of Enantiomer
One enantiomer has exclusive activity.	Reduce dose and load on metabolism.
Other enantiomer is toxic.	Increased latitude in dose and broader usage.
Enantiomers have different pharmokinetics.	Better control of kinetics and dose.
Enantiomers metabolized at different rates (in one person).	Wider latitude in dose setting; less variability in patient response.
Enantiomers metabolized at different rates (different people).	Reduction in variability of patient responses; larger confidence in dose selection.
One enantiomer prone to interaction with key detoxification pathways.	Reduced interactions with other drugs.
One enantiomer is agonist, other antagonist.	Enhanced activity and reduction of dose.
Enantiomers vary in spectra of pharmacological action and tissue specificity.	Increased specificity and reduced side effects for one enantiomer; use of other enantiomer for different indication.

Physical differences between two enantiomers may seem small but, the spatial orientation of a single functional group severely affects the properties of the compound. This has strong effects for the human body.

Our senses of taste and smell are highly sensitive to stereochemical differences in molecules that stimulate them. For example, *(R)*-carvone has the odor of spearmint, whereas *(S)*-carvone smells like caraway [18, 19].

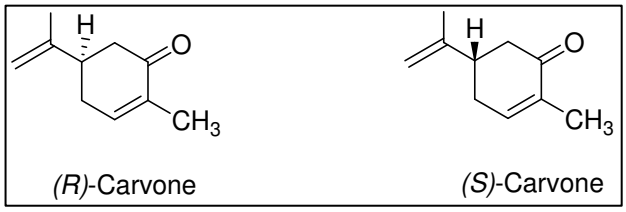


Figure 1

In the food industry, the development of an inexpensive sweet-tasting organic compound as a food additive has tremendous potential in the marketplace. Aspartame has an increasing market share as a low calorie sweetener and is used in soft drinks. Its backbone is composed of two aminoacids. Substitution of the L-phenylalanine portion of the molecule with its antipode D-phenylalanine, which in itself sweet tasting, causing the resulting dipeptideto taste bitter [20].

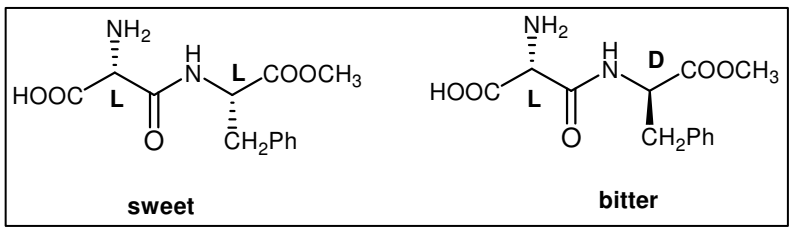


Figure 2

The question of toxicity always arises when a compound is introduced into the body. With molecules possessing one or more asymmetric centers, one enantiomer sometimes exhibit adverse toxicologic properties and the other does not [21].

The tragic consequences brought about the drug thalidomide are unforgettable. Despite the thalidomide molecule contains an asymmetric center, the drug was used in its racemic form. Its use by pregnant women resulted in a high incidence of fetal deaths, neonatal deaths, and congenital malformations. It has been found that the teratogenicity has a property of only the *(S)*-(-)-enantiomer [22].

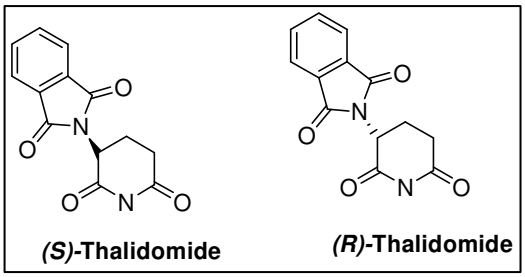


Figure 3

1.2.3 Routes to Enantiomerically Pure Compounds

The methods which is used to access enantiomeric compounds can be divided into three categories [23].

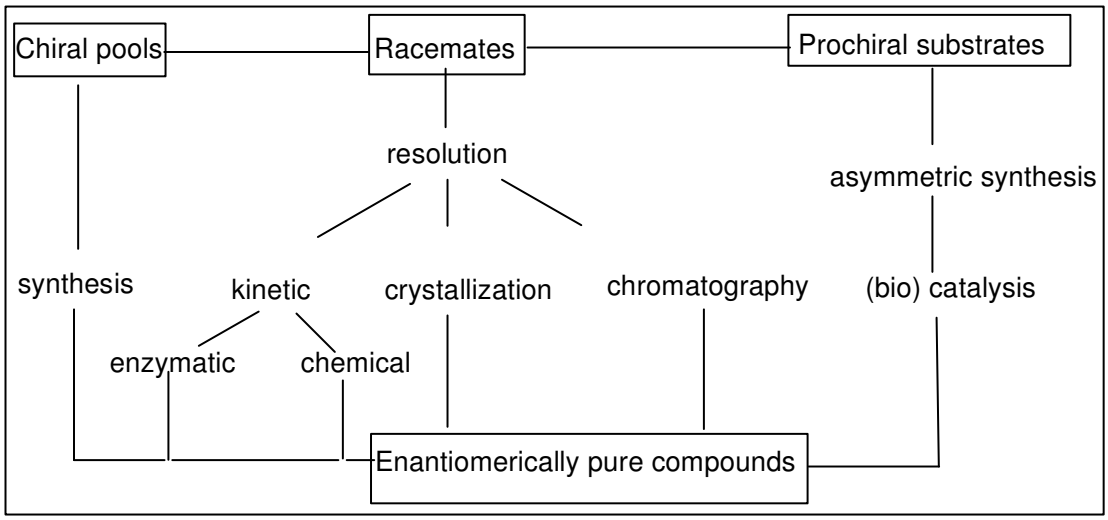
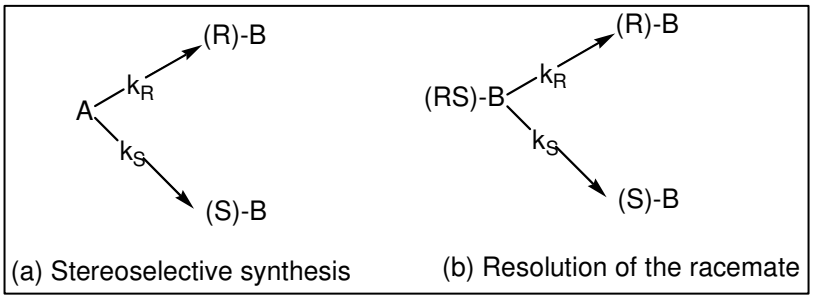


Figure 4: Methods to obtain enantiomerically pure compounds

1.2.3.1 Stereoselective synthesis

There are two methods which are used to prepare enantiomers by using enzymes [24]: (a) Stereoselective synthesis and (b) the resolution of the racemate. The resolution of the racemates is discussed below.



Scheme 3: Stereoselective synthesis versus resolution of the racemate

1.2.3.2 Resolution of Racemates

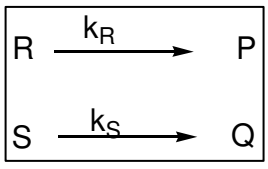
There are four methods for the resolution of enantiomers. These are; a) direct preferential crystallization, b) crystallization of diastereomeric salts c) chromatography and d) kinetic resolution.

1.2.3.2.1 Diastereomer crystallization.

True racemate mixtures can not be separated by preferential crystallization, but can be resolved using the diastereomer crystallization developed by Pasteur in 1848. In this method, a solution of racemic mixture in methanol or water is allowed to react with a pure enantiomer, thereby forming a mixture of diastereomers that can be separated by crystallization [25].

1.2.3.2.2 Kinetic resolution catalyzed by lipases

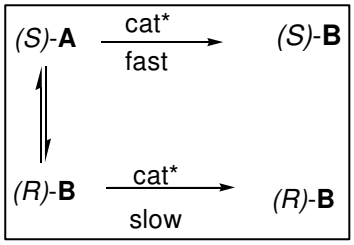
In this method, the two enantiomers react at different rates with a chiral entity. The chiral entity may be a biocatalyst (enzyme or a microorganism) or a chemocatalyst (chiral acid or base or even a chiral metal complex) and it must be in catalytic amounts. In the kinetic resolution, one enantiomer reacts faster than the other ($k_R > k_S$) [26].



Scheme 4: Catalytic kinetic resolution

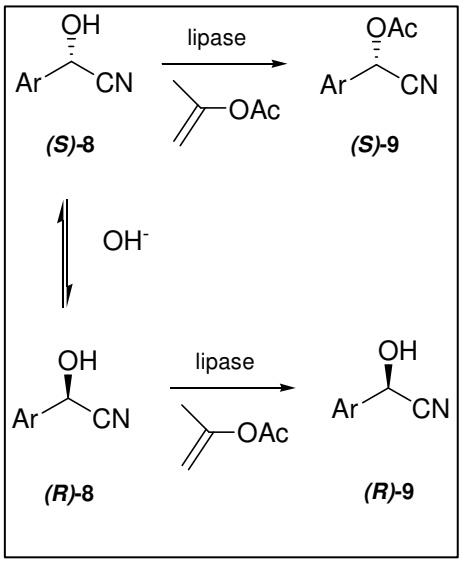
1.2.3.2.3 Dynamic kinetic resolution

In Dynamic kinetic resolution method, the R and S enantiomers react at different rates. In conventional kinetic resolution the *(S)*-enantiomer substrate is left behind as an unreacted starting material while in the case of dynamic kinetic resolution, the substrate is continuously isomerized during the resolution process, thus the *(R)*- and *(S)*- substrates are in equilibrium.



Scheme 5

The simplest process of this type of resolution is the enzyme-catalyzed acylation of a racemic cyanohydrin (Scheme 8) [27].



Scheme 6

Rapid interconversion of the *(R)*- and *(S)*-isomers of the cyanohydrin occurs leading to a high yield of one enantiomer of the product when this reaction is carried out in the presence of a basic anion exchange resin.

1.2.4 Introduction to Enzymes

Enzymes are biological catalysis. They are chemically involved in, but not changed by a chemical reaction. Enzymes are proteins, and their function is determined by their complex structure. The reaction takes place in the active site, which is the small part of the enzyme.

In an enzyme-catalyzed reaction, firstly the substrate binds to the active site of the enzyme to form an enzyme-substrate (ES) complex. While attached to the enzyme, substrate is converted into product and finally product is released. The mechanism of the enzyme-substrate relation is shown below.

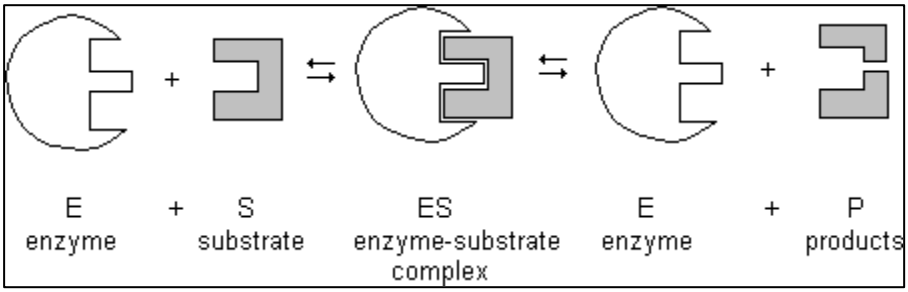


Figure 5

As can be seen from the figure, there is a key-lock relation between the substrate molecule and the enzyme [27].

Considering the energy changes that take place during a chemical reaction, the way how the enzymes work can be seen. Enzymes reduce the activation energy of the

reaction, so most molecules get over the activation energy barrier and turn into product.

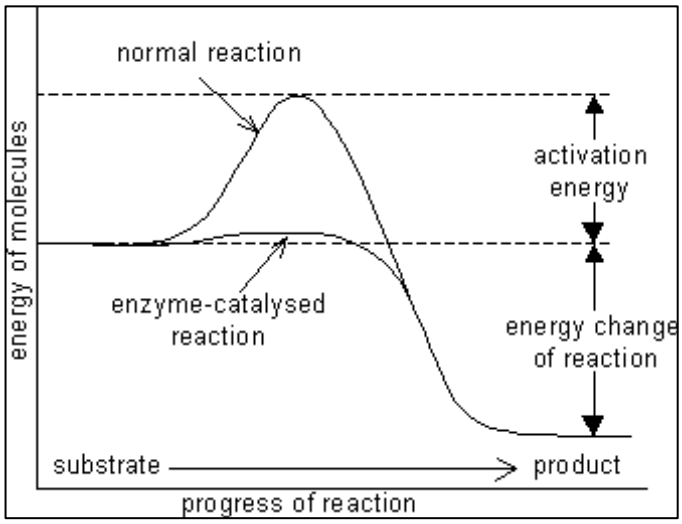


Figure 6

The energy which is required to form the transition state is called the activation energy. Enzymes lower the activation energy by stabilizing the transition state, and they do this by changing the active site. Rate of enzyme reactions are affected by several factors such as, temperature, pH, enzyme concentration, substrate concentration, covalent modifications and inhibitors.

1.2.5 Enzymatic Hydrolyses in Organic Synthesis

It has been known for many years that the enzymes act as chiral catalysts in organic synthetic reactions [28]. Hydrolytic enzymes, in particular lipases and hydrolases are among the most widely used enzymes, especially in asymmetric synthesis. Use of lipases in enantioselective hydrolyses is an important way to obtain chiral building blocks as intermediates in asymmetric synthesis [29]. Pig liver esterase (PLE) is the most widely used one among the used hydrolyses.

1.2.5.1 Pig Liver Esterase

In asymmetric synthesis, the first application of PLE is reported in 1903 [30]. PLE is a serine protease type hydrolase that catalyzes the hydrolysis of a wide range of ester structure with considerable specificity [31]. PLE-stereoselectivity is apparently fickle, such as changing from R center to S center ester preference within structurally similar series of substrates that were triggered by apparently trivial changes in substrate structure and size [32].

Another disadvantage of PLE-catalyzed generation of chiral synthons has been that the ee's of the products are too low for asymmetric synthetic purposes.

An active-site model of the enzyme that would permit all of the enzyme's specificity properties to be interpreted and predicted was developed to prompt the synthetic uses of PLE.

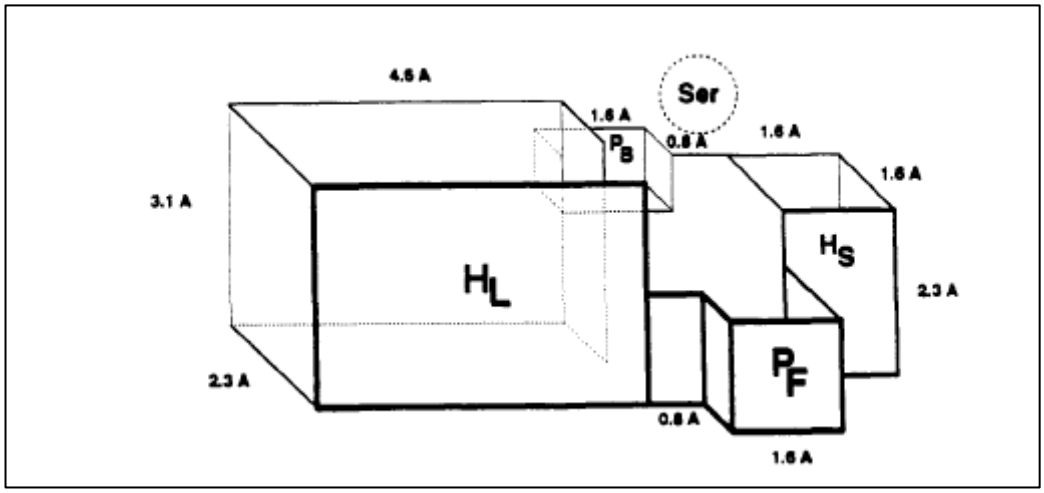


Figure 7

Four pockets which are shown in figure 7, two P_F and P_B are polar in nature, the other two are hydrophobic, and one H_L is bigger than the other H_S. An ester must be able to fit into these regions appropriately, with polar and hydrophobic moieties binding into complementary sites in order to be a substrate. The stereoselectivity-determining factor depends whether or not a hydrophobic group fits into H_S or H_L. If a group swing from H_S-binding to H_L-binding, it will rotate the substrate orientation and change from an R to S (or vice versa) preference [33]. This is the basis of PLE's stereoselectivity changes.

1.3 CYCLOPROPANATION

1.3.1 Reactions of Diazo Compounds in Natural Product Synthesis

Transition metal-catalyzed diazo decomposition for the formation of carbenoids is a general method in the synthetic organic chemistry. Carbenoids are metal-complexed intermediates formed from the decomposition of diazocompounds in the presence of a transition metal. Ranging from cyclopropanation, insertions, ylide generation and β-Hydride eliminations diazocompounds can undergo many transformations, which have synthetic means towards preparing natural products [34].

Metal-catalyzed decomposition of diazo compounds has been known for more than 80 years, the first catalysts were copper powder, copper bronze, copper chloride, copper oxide, and cupric sulfate. In the early 1970's, Teyssié introduced the more versatile $Rh_2(OAc)_4$ catalyst and $Pd(OAc)_2$ [35]. These catalysts have found great synthetic utility in transformations leading to natural products and are still widely used today.

There are three important reactions of metal carbenes. These are: C-H insertions, intramolecular cyclopropanation and intermolecular cyclopropanation.

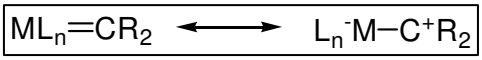
1.3.2 Intramolecular Insertion Reactions

Free carbenes have been known to insert into C-H bonds both intermolecularly and intramolecularly. Because of low selectivity and competing intramolecular reactions, intermolecular C-H insertion is not useful. From the intramolecular insertion of α-diazocarbonyl compounds into unactivated C-H bonds, various carbocycles and heterocycles have been obtained. A variety of ring sizes such as three-, four-, five- and six- membered ring can be constructed by C-H insertion; but five-membered ring construction has been favored. The type of diazo functionality, the degree of substitution of the carbon where C-H insertion takes place, steric and electronic factors affect the regioselectivity [36].

In general, tertiary C-H sites are more reactive for insertion or elimination than secondary C-H sites, which in turn more reactive than primary C-H sites and a benzylic site is less reactive than an aliphatic site. This observed trend is based on the availability of electron density in the C-H bond. Alkyl groups are inductively electron-donating, thereby increasing the electron density of the C-H bond, making it more susceptible to attack by the electophilic metal-carbene species. Similarly, electron withdrawing groups such as vinyl and phenyl groups decrease the reactivity of the adjacent C-H bond. Formed metal-carbene complex has an electron- deficient carbon; therefore electron-withdrawing ligands destabilize this complex [37]. It could be generalized that electron-withdrawing ligands favor β-hydride elimination and electron-donating ligands favor cyclizations.

1.3.3 Carbenoid Transformations

Transition-metal catalysts react with diazo compounds to generate electrophilic metal carbenes.



Scheme 7

The catalytic activity of transition-metal compounds depends on the oxidation state of the metal which allows them to react as electrophiles with diazocompounds. Electrophilic addition causes the loss of dinitrogen and the production of a metal-stabilized carbene [38]. Transfer of the carbene entity to an olefinic substrate completes the catalytic cycle:

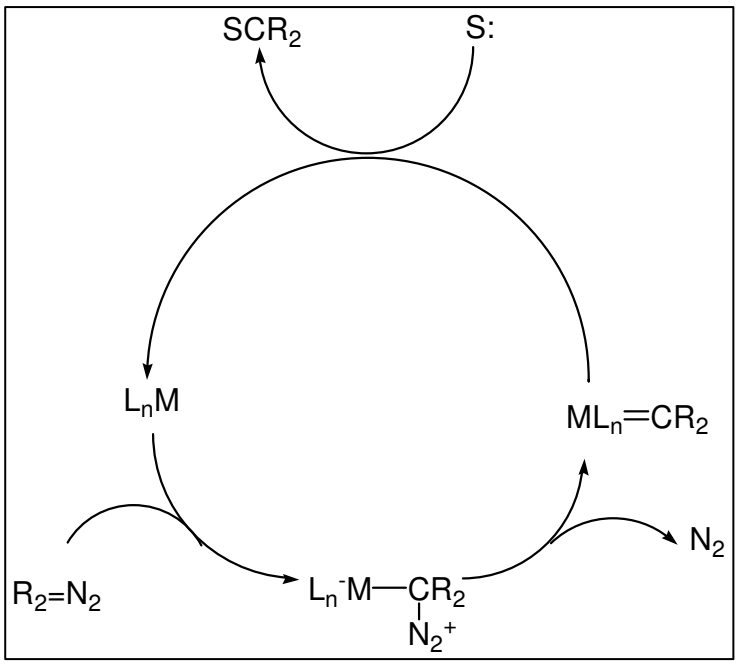


Figure 8

1.3.4 Cyclopropanation and Related Reactions

Due to their biological significance, synthetic utility and occurrence in natural products cyclopropanes have received considerable attention during the past several decades.

In cyclopropanation reactions, there is simultaneous bond formation to both carbons of the C=C double bonds without charge build up; but in the C-H insertion, electrophilic addition of the metal-bound carbene occurs followed by 1,2-migration reaction [39].

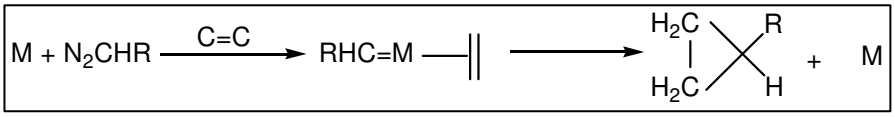
There are two types of mechanisms which have been proposed for carbene formation, possibly as competitive pathways. These are the metal-olefin coordination mechanism and the carbenoid mechanism. There are several conditions in the evaluation of these mechanistic possibilities [40]. These are:

1- The coordination capability of the transition metal compound with olefins.

2- The nucleophilicity of the diazocompound.

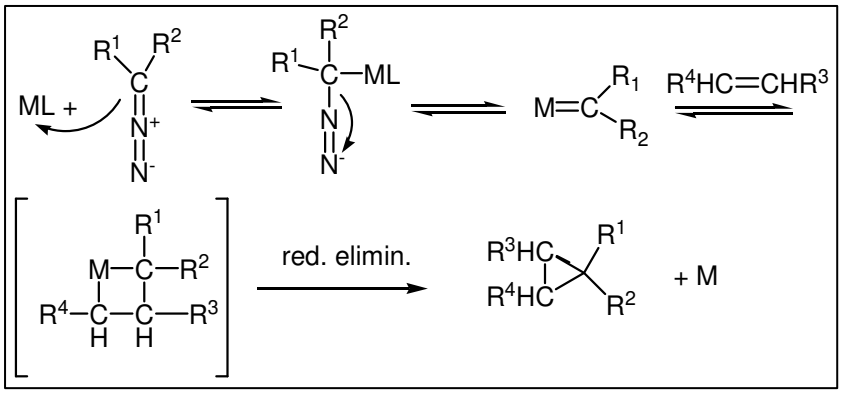
3- Comparative selectivities in cyclopropanation and other typically metal carbene transformations.

Coordination mechanism:



Scheme 8

Carbenoid mechanism:



Scheme 9

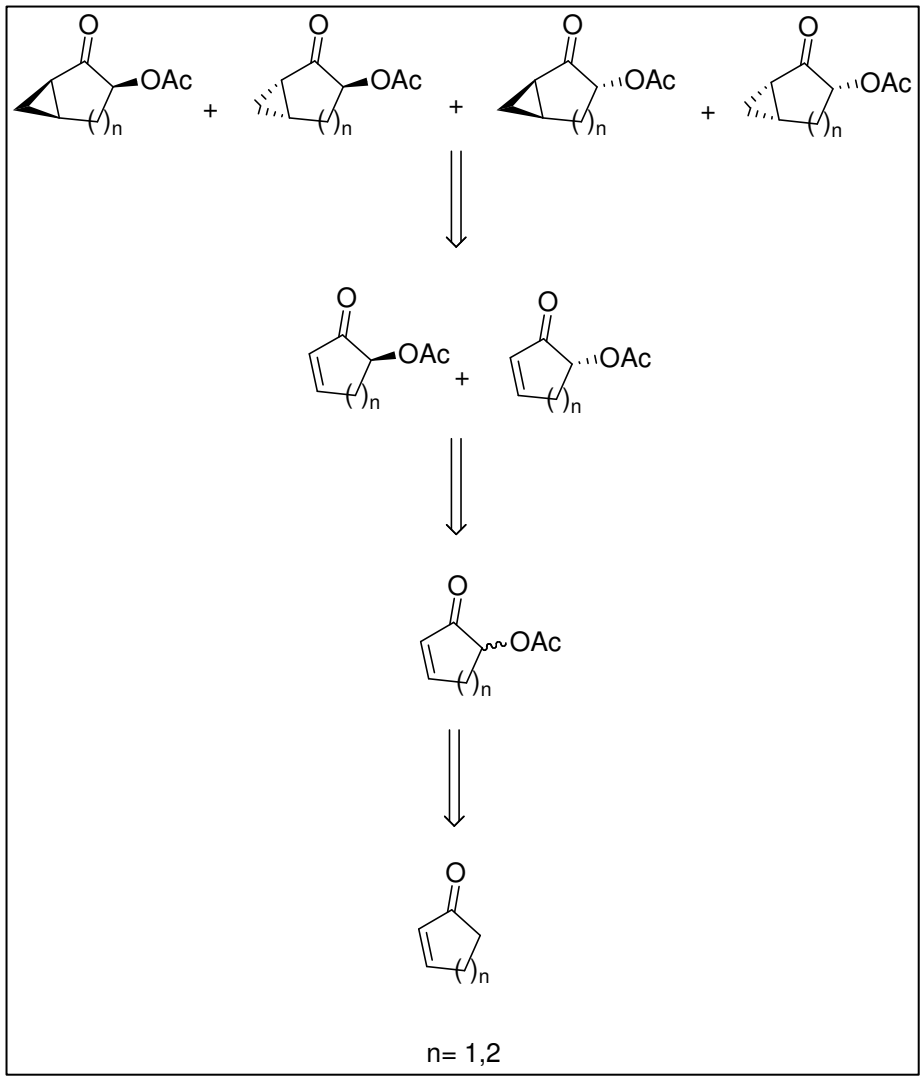
There are three major classes of catalysts; those possessing one vacant metal coordination site, more than one coordination site and those that fall into the borderline case. Catalysts which have one coordination site per metal such as Rh(II) carboxylates favor carbenoid reactions.

Catalysts which have several sites for coordination promote coordination reactions. Palladium(II) is an example of this type of catalysts. Copper is a borderline case. Copper catalysts show carbenoid behavior; but when they complexed with weak ligands such as triflates, they show coordination reaction.

1.4 The Aim of the Work

The aim of this work is to synthesize enantiomerically enriched 2-oxobicyclo [3.1.0]hexan-3-yl acetate and 2-oxobicyclo[4.1.0]heptan-3-yl acetate. 2-Cyclopenten-1-one and 2-cyclohexen-1-one are chosen as starting materials since their biological activity. In our synthetic design, 2-cyclopentenone and 2-cyclohexenone will be regioselectively oxidized to corresponding α'-acetoxylated cyclic ketones with $Mn(OAc)_3$ and they will be subjected to enzymatic resolution by using PLE. From the literature and our previous works it is known that α'-hydroxylated products can easily be racemized. In order to prevent that, they will be protected by simple acetylation procedure. At the end, enantiomerically enriched products are going to be subjected to cyclopropanation to get our target compounds given above.

The aim of this work is shown retrosynthetically in Scheme 12.



Scheme 10. Retrosynthesis of the Work

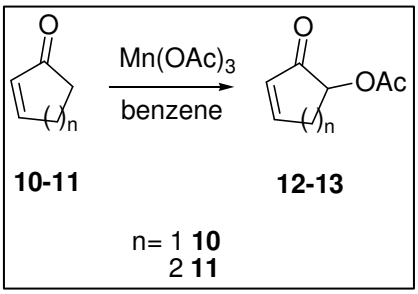
CHAPTER 2

RESULTS AND DISCUSSIONS

2.1 Oxidation of α,β-unsaturated cyclic ketones

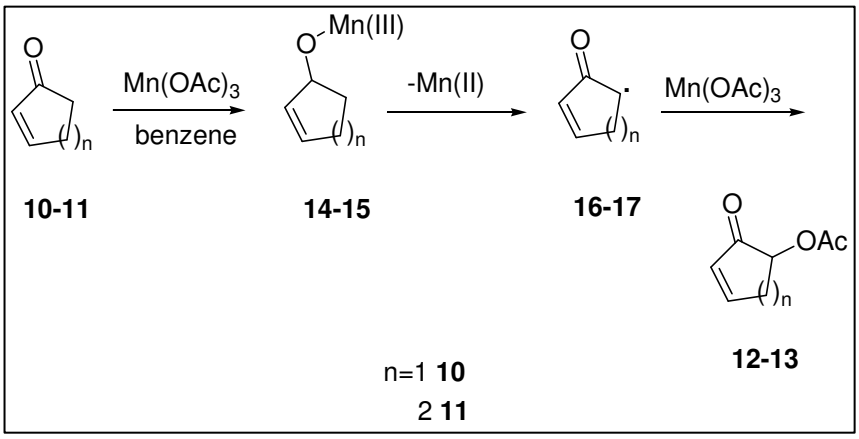
Selective α'-acetoxylation of α,β-unsaturated ketones occupy a central position in the syntheses of various complex natural products. Enones can be regioselectively oxidized to α'-acetoxy enones by using lead(IV) tetraacetate [41, 42], MoOPh [43, 45], triphenylphosphite ozonide (TPPO) [46, 47], MCPBA [48, 49] and manganese(III) acetate [50, 51]

Manganese(III) acetate mediated acetoxylation is the most commonly used procedure for the synthesis of α'-acetoxy α,β-unsaturated ketones. In this method as shown in Scheme 11, dried manganese triacetate is added to benzene solutions of various enones in portions to give the resultant α'-acetoxy enones in good yields.



Scheme 11

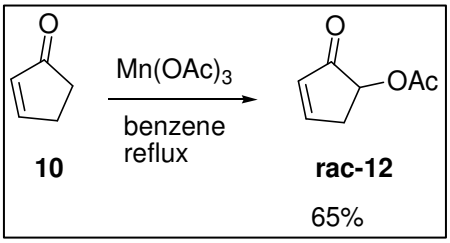
The reaction proceeds via the formation of the Mn(III) enolate **14-15,** which loses Mn(II) upon one-electron oxidation to give α'-keto radical **16-17** [52] (Scheme 12). Oxidation of intermediate **16-17** by another equivalent of $Mn(OAc)_3$ provides α'-acetoxy cyclic ketones.



Scheme 12

2.1.1 Synthesis of α'-acetoxy-2-cyclopenten-1-one

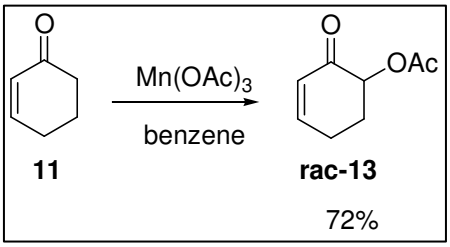
Direct oxidation of α,β-unsaturated cyclic ketones were done by using $Mn(OAc)_3$. 2 Equivalent of $Mn(OAc)_3$ is introduced into the benzene solution of 2-cyclopenten-1-one. At the end of this process, racemic α'-acetoxy-2-cyclopenten-1-one was obtained with 65 % yield. The product was characterized by ^{1}H-NMR and ^{13}C-NMR spectra. The spectra are given in appendix. NMR spectra are in accordance with the literature [50].



Scheme 13: $Mn(OAc)_3$ mediated acetoxylation of **10**

2.1.2 Synthesis of α'-acetoxy-2-cyclohexen-1-one

In the regioselective oxidation of 2-cyclohexen-1-one, the same procedure was applied as in cyclopentenone case. Rac-6-acetoxy-2-cyclohexenone was obtained with 72% yield. Structure elucidation was done by using ^{1}H-NMR and ^{13}C-NMR spectra which are in accordance with the literature data [51].



Scheme 14: $Mn(OAc)_3$ mediated acetoxylation of **11**

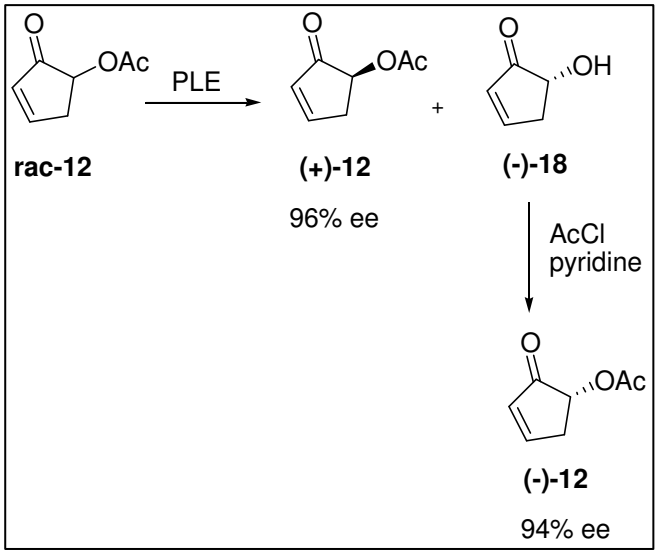
Both 2-cyclopentenone and 2-cyclohexenone were oxidized by using $Pb(OAc)_4$ in hexane to afford α'-acetoxylated products with almost the same chemical yield.

2.2 Enzymatic resolution of α'-acetoxylated cyclic ketones

Enrichment of racemic α'-acetoxylated compounds was done by enzymatic hydrolysis. Throughout this study, various hydrolases include PLE, CCL, HLE and PPL using a substrate: enzyme ratio from 1:1 to 1:0.5 was tested. Among the hydrolyses studied, PLE proved to be the most suitable one for the enantioselective hydrolysis of the substrates. To determine the absolute configuration of these α'-acetoxylated compounds, we transformed them into the corresponding saturated α-acetoxy saturated cyclic ketones.

2.2.1 Enzymatic resolution of 5-acetoxy-2-cyclopenten-1-one

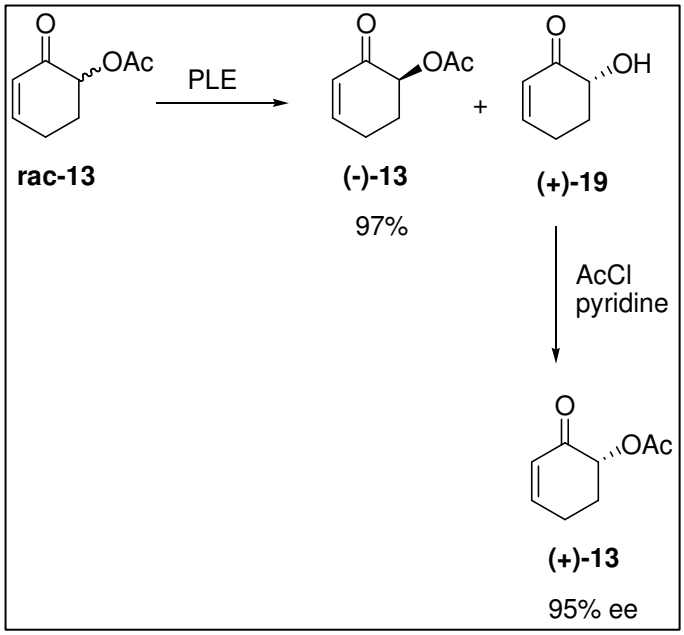
Resolution of (±)-5-acetoxycyclopentenone was successfully done by PLE which was added to a mixture of racemic substrate **(11a)** in pH 7.00 phosphate buffer in one portion. The resolution was monitored by TLC and stopped after 50% conversion. Enantiomeric excess value was determined by HPLC with ODH chiral column as 96%. In the biotransformation α'-hydroxy-2-cyclopentenone was also obtained as hydrolysis product which is quickly racemized. In order to prevent the racemization, after purification step, it was readily subjected to acetylation to afford the opposite enantiomer.



Scheme 15 Chemoenzymatic resolution of rac-**12**

2.2.2 Enzymatic resolution of 6-acetoxy-2-cyclohexen-1-one

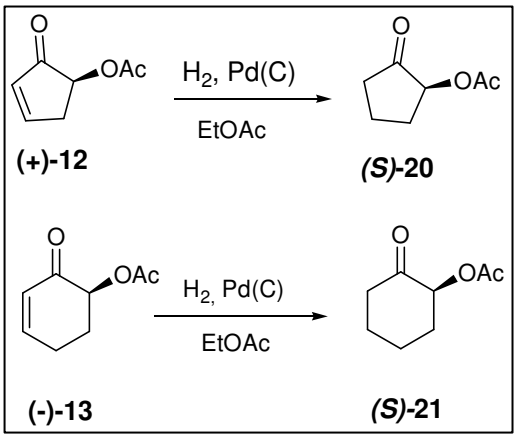
In (±)-6-acetoxy-2-cyclohexenone resolution, PLE was used again as the biocatalysts under the same condition given above. Enantiomerically enriched (-)-acetoxylated one isolated in 46% chemical yield and 97% ee. Similar racemization problem was observed for α'-hydroxy-2-cyclohexenone, therefore it was subjected to acetylation to afford (+)-6-acetoxy-2-cyclohexenone.



Scheme 16 Chemoenzymatic resolution of rac-**13**

2.3 Absolute Configuration Determination

In the literature, the absolute configuration of 2-acetoxycyclopentanone and 2-acetoxycyclohexanone are known which are the saturated form of our target α'-acetoxylated products. Therefore, (+)-5-acetoxy-2-cyclopentenone and (-)-6-acetoxy-2-cyclohexenone were transformed into the corresponding saturated cyclic ketones (+)-2-acetoxycyclopentanone and (-)-2-acetoxycyclohexanone, respectively by hydrogenation with Pd(C) catalysts [53, 54]. The specific rotation signs of each were compared with the literature value and both have the S absolute configuration.



Scheme 17 Absolute configuration determination of **12-13**

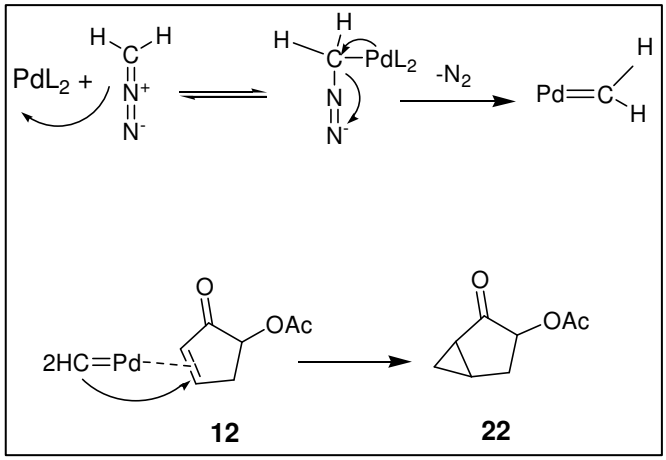
2.4 Cyclopropanation of α,β-unsaturated cyclic ketones

The importance of three-membered ring compounds has drawn the attention of scientists in diverse areas of organic chemistry. Cyclopropanes occur as structural subunits in biologically active natural and unnatural products [39]. They are increasingly valuable as synthetic intermediates. Many biomolecules including fatty acids and sterols possess a cyclopropane ring.

Generally cyclopropanation reaction occurs by the addition of a carbene to an olefin [55]. Transition metal catalyzed cyclopropanation evolved until the late of 1960s, although the origins of it extend back to 1906. A number of transitiom metal species including Cu, Ru, Rh, Pd, Pt, V, W, Cr, Ni are used for the cyclopropanation with diazomethane; but among these transitiom metals Pd is the most effective carbene source [56].

2.4.1 Cyclopropanation of alkenes with diazomethane and $Pd(OAc)_2$

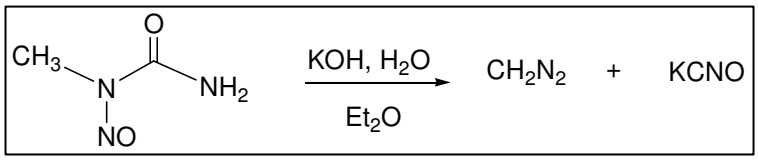
Palladium-catalyzed reactions between diazomethane and olefinic substrates have been used as an efficient method for the cyclopropanation of α,β-unsaturated carbonyl compounds. In the generally accepted mechanism of the reaction, formation of a metal-carbene complex occur which adds to the C=C bonds [57]. Firstly, double bond of the diazomethane attacks to $Pd(OAc)_2$, after the removal of dinitrogen Pd-carbene complex occurs. Pd-carbene complex coordinates to the double bond of the α,β-unsaturated cyclic ketone, Pd leaves the medium and a cyclopropane ring occurs.



Scheme 18

Mechanism of the Cyclopropanation of alkenes with diazomethane and $Pd(OAc)_2$

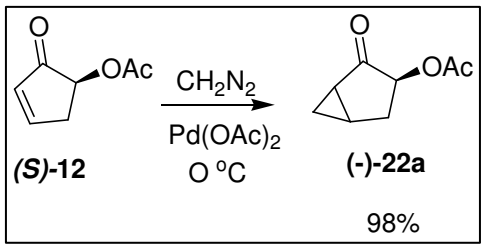
Diazomethane is generally prepared from N-nitroso-N-methylurea salt with KOH and diethyl ether as given in Scheme 22.



Scheme 19: Preparation of diazomethane

2.4.2 Cyclopropanation of (S)-5-acetoxycyclopentenone

As it was indicated in "the aim of the work", the target enantiomerically enriched 2-oxobicyclo[3.1.0]hexan-3-yl acetate was synthesized via the $Pd(OAc)_2$ diazomethane cyclopropanation of *(S)*-5-acetoxy-2-cyclopentenone in a stereoselective manner. The following general procedure was applied in which diazomethane was prepared *in situ*. To mixture of *(S)*-5-acetoxy-acetoxy-2-cyclopentenone and $Pd(OAc)_2$ in ether at 0 ^{o}C, diazomethane was passed and then the resulting mixture was stirred for four hour. TLC controlling was done until the absence of the starting compound.



Scheme 20: cyclopropanation of ***(S)-***12 with $Pd(OAc)_2$ by using diazomethane

In this cyclopropanation there are theoretically two possible diastereomers but, the crude NMR showed just one stereoisomer.The structure elucidation and the absolute configuration of the product was determined by applying NMR techniques.

2.4.3 Characterization of (-)-2-oxobicyclo[3.1.0]hexan-3-yl acetate 22a

In ^{1}H-NMR spectrum of the compound, H-1 attached to C-1 gives triplet due to the interaction with methylene protons on C-2. H-2 protons give doublet of doublet of doublet due to the interaction with H-1 proton and methine proton of cyclopropane ring. Acetoxy group methyl protons give a sharp singlet at 2.13 ppm. H-3 proton gives multiplet between 2.05 and 2.16 ppm. Methine proton of cyclopropane ring next to the carbonyl group of the cyclopentanone ring gives multiplet between 1.87 and 1.93 ppm. Finally, one of the methylene protons of the cyclopropane ring gives multiplet between 1.25 and 1.33 ppm and the other methylene proton shows multiplet between 1.16 and 1.20 ppm. (Figure 9)

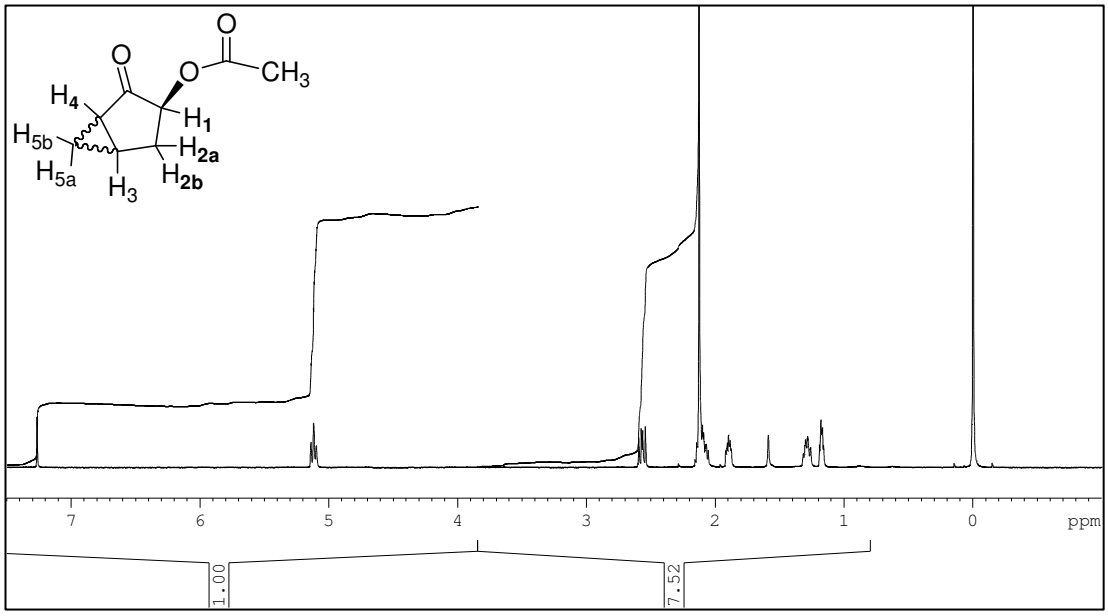


Figure 9: ^{1}H-NMR of (-)-2-oxobicyclo[3.1.0]hexan-3-yl acetate **22a**

In ^{13}C-NMR spectrum of (-)-2-oxobicyclo[3.1.0]-hexan-3-yl acetate **22a**, carbonyl carbon of cyclopentanone ring and acetoxy group show the signals at 208.0 and 170.0 ppm respectively. Acetoxy bearing carbon C-1 is shifted to 70.10 ppm due to the electronegative oxygen atom. Methylene carbons (C-2) resonate at 29.00 ppm whereas methyl carbon of acetoxy group gives the signal of 24.6 ppm. Cyclopropane ring carbons C-4, C-5, C-3 give the signals at 20.7, 20.0 and 14.9 ppm, respectively.

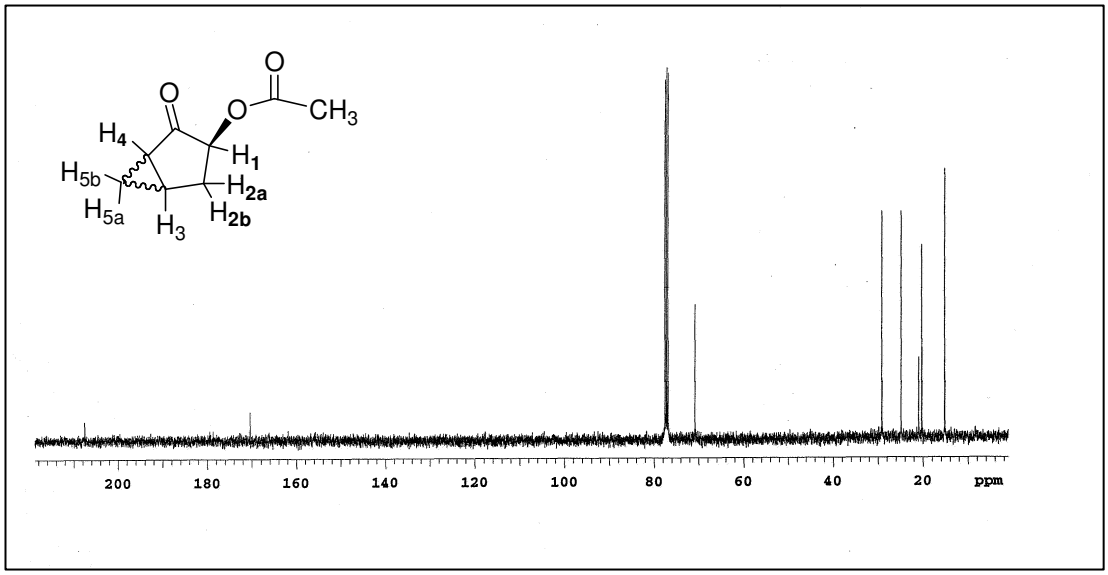


Figure 10: ^{13}C-NMR spectrum of **22a**

The analysis done with ^{1}H-NMR and ^{13}C-NMR does not give any information regarding the stereochemistry of the products. In order to elucidate the stereochemistry of the products, first of all each signal must definitely be assigned. For this purpose, double resonance experiments were done as given in Figure 11 and 12.

In the first double resonance experiment, the characteristic H-1 proton attached to the carbon bearing acetoxy group was irradiated. It was observed that there was a change in the splitting pattern of diastereotopic methylene protons attached to C-2 as doublet of doublet instead of doublet of doublet of doublet, because ignoring of H-1 proton caused the only coupling of C-2 methylene protons with methine proton of cyclopropane ring C-3 as shown in Figure 11. In order to confirm this relation, one of the methylene protons of C-2 was irradiated and a drastic change was observed on H-1 proton as doublet of doublet.

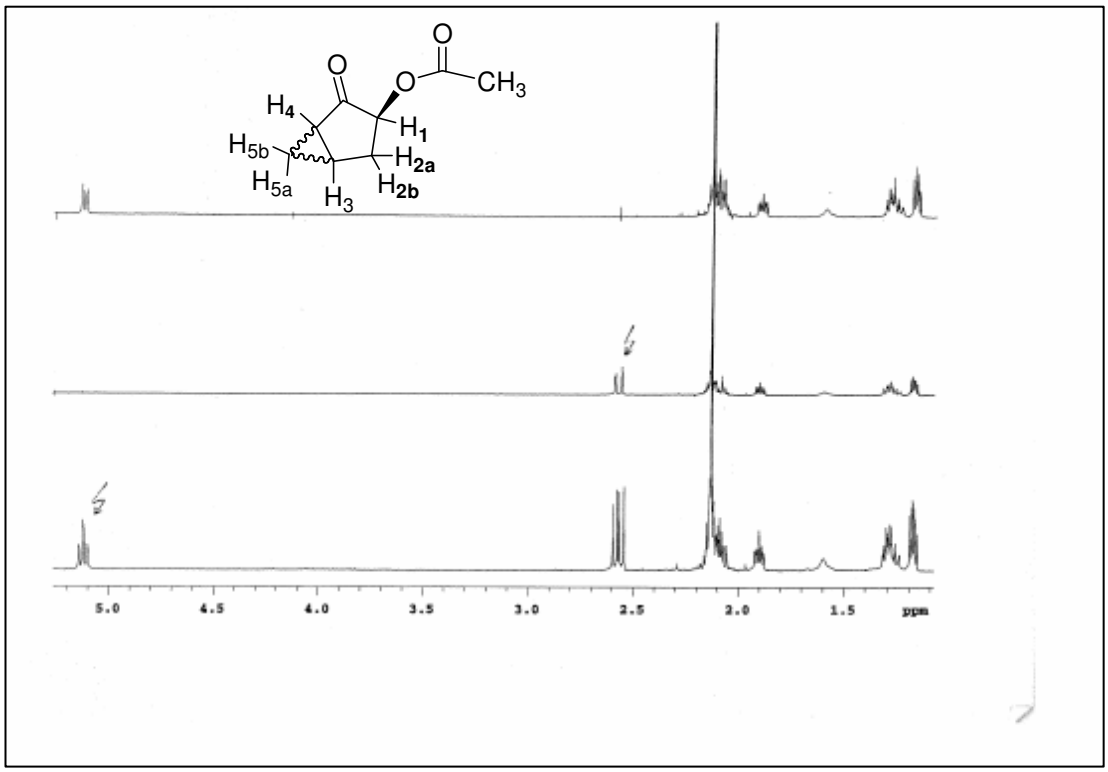


Figure 11: Double resonance experiment of **22a**

In the second double resonance experiment shown in Figure 12, irradiation of one of methylene protons of cyclopropane ring, a change in the splitting pattern of the other methylene proton and later on irradiation of the second methylene proton caused a drastic change in the splitting pattern of the other. Both irradiations showed a change on methine protons of cyclopropane ring and the H-3 resonated at 1.9 ppm. This also informs us to predict the exact position of C-3 methine proton.

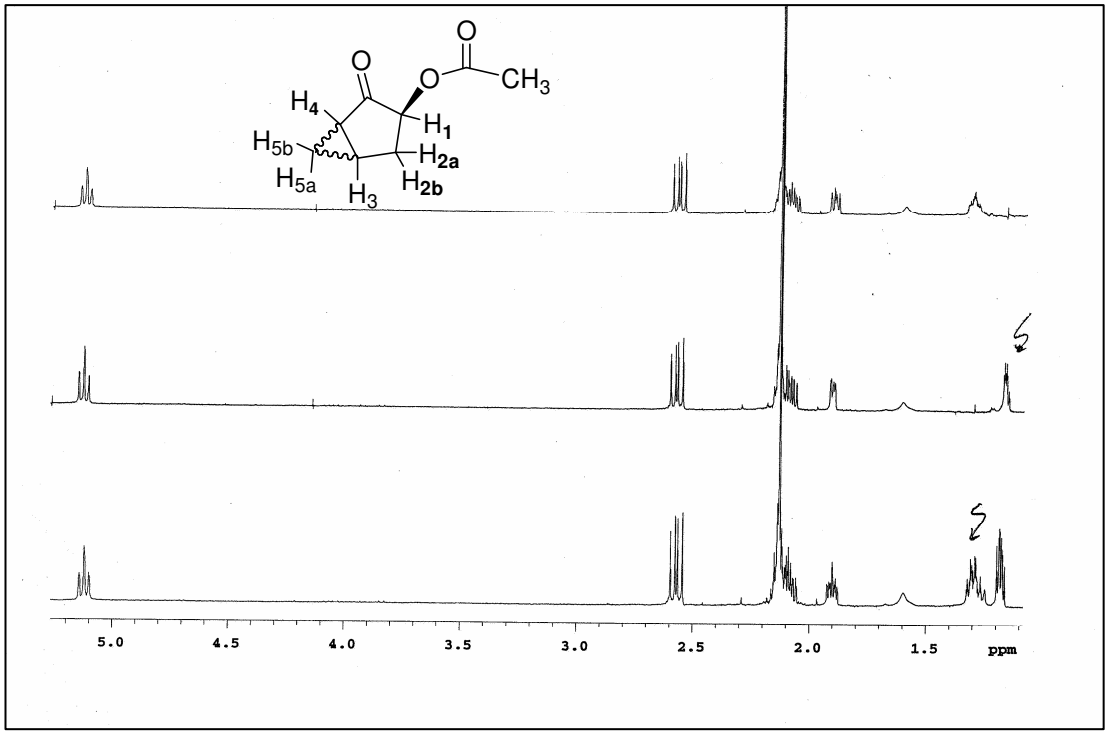


Figure 12: Double Resonance experiment of **22a**

As a result of double resonance experiment, the position of each proton was exactly determined. Subsequently, NOE experiments were applied to find the configuration of cyclopropane ring with respect to acetoxy attached chiral center with a known stereochemistry. In Figure 13, irradiation of methylene protons of cyclopropane ring, separately showed NOE relation with H-1 proton of cyclopropane ring. This finding strongly supports the position of cyclopropane ring and H-1 proton as on the same space of the cyclopropane ring system.

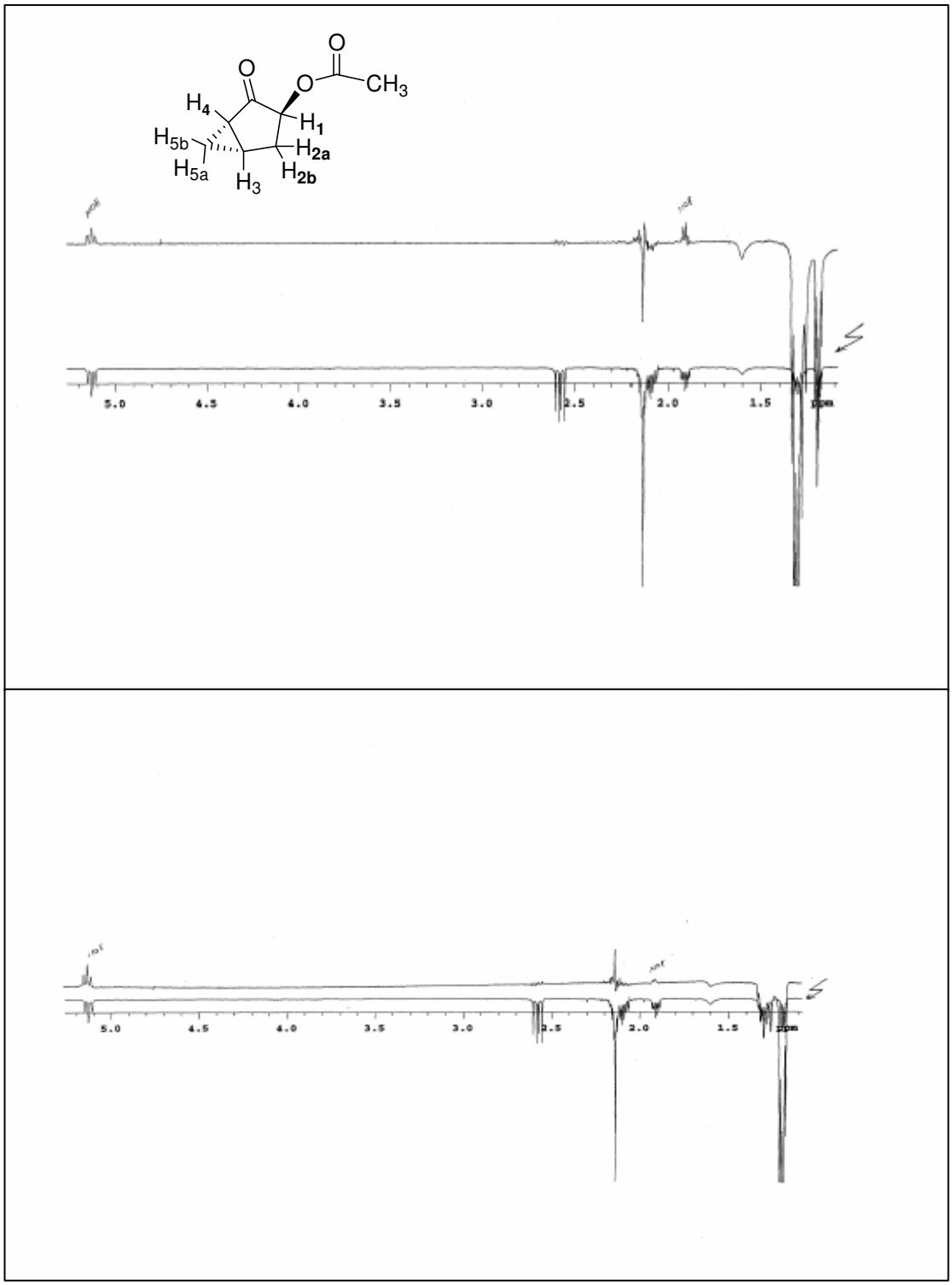


Figure 13: NOE spectra of **22a**

In order to confirm the syn relation of cyclopropane ring and H-1 proton as shown in Figure 14 as a result of irradiation of H-1 proton, NOE relations were observed among the one of the methylene protons on C-2 and one of the methylene protons of cyclopropane ring.

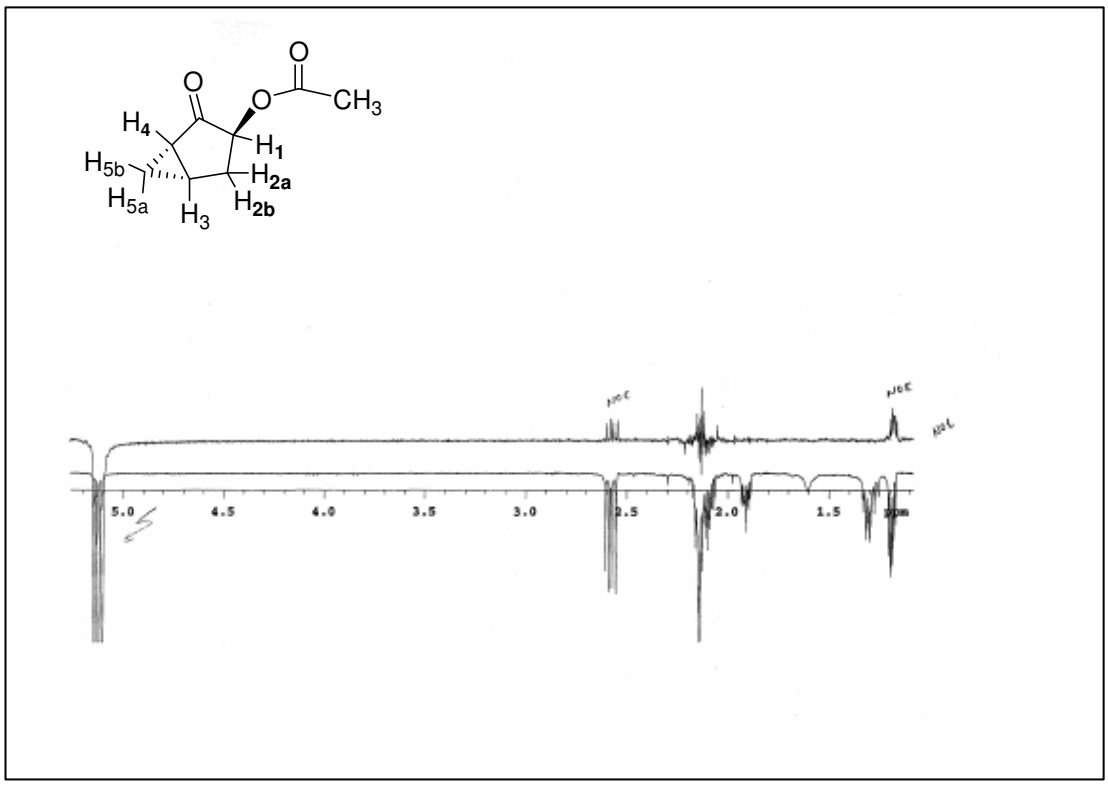
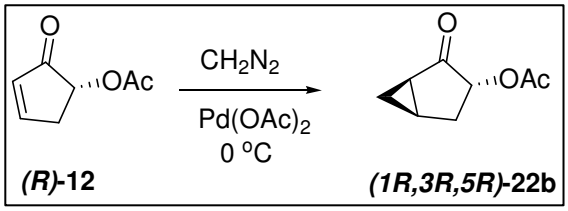


Figure 14: NOE experiment of structure **22a**

Depending upon all of these NOE diff. experiments, it was determined that, acetoxy group and cyclopropane ring are in tarns relation and the absolute configuration of the product was determined as *(1S,3S,5S).*

2.4.4 Cyclopropanation of (R)-5-acetoxycyclopentenone

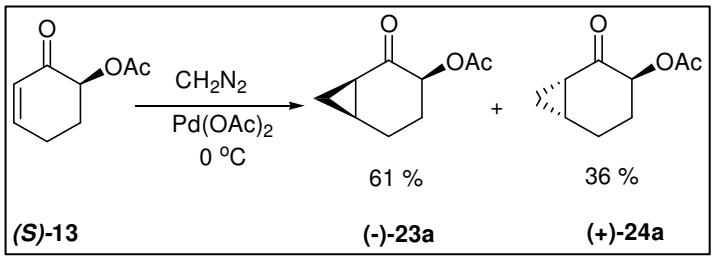
(R)-5-acetoxy-2-cyclopentenone was also subjected to cyclopropanation under the same condition given aboveto confirm the yields of the reaction and configuration assignment of the resultant oppositely configured product. Comparison of specific rotation value of the product with (-)-*(1S,3S,5S)*-2-oxobicyclo[3.1.0]hexan-3-yl acetate showed opposite sign which proves the enantiomeric relation.



Scheme 21: Cyclopropanation of *(R)*-5-acetoxycylopentenone

2.4.5 Cyclopropanation of *(S)*-6-acetoxycyclohexenone

The effectiveness and the stereochemical behaviour of cyclopropanation depending upon chiral center having acetoxy group were tested with 6-membered ring derivative *(S)*-6-acetoxy-2-cyclohexenone **(13)**. The same cyclopropanation procedure was applied as previous case. The chemical yield of the reaction as high as 5-membered ring case (97%). Hovewer, ^{1}H-NMR spectrum of the product showed two stereoisomers in contrast to *(S)*-5-acetoxy-2-cyclopentenone cyclopropanation.



Scheme 22: Cyclopropantion of ***(S)*-13**

Resultant stereoisomers were characterized by looking their ^{1}H-NMR spectra. The protons of acetoxy attached carbon have resolvable sets of signals at 4.81 ppm as the major and at 5.05 ppm as the minor product, respectively. Fortunately, ın crude ^{1}H-NMR spectrum, both sets of protons give doublet of doublet with the same coupling constant (J=6.4 Hz). By measuring the integral values of these signals in ^{1}H-NMR spectrum, the distereomeric ratio was determined as 63:37. ^{1}H-NMR spectrum of the diastereomeric mixture and the each separated products are given below in Figure 15.

Great effort was made by using flash column chromatography to separate the diastereomers. The structure elucidation and the absolute configuration determination for separated diastereomers were done by applying NMR techniques.

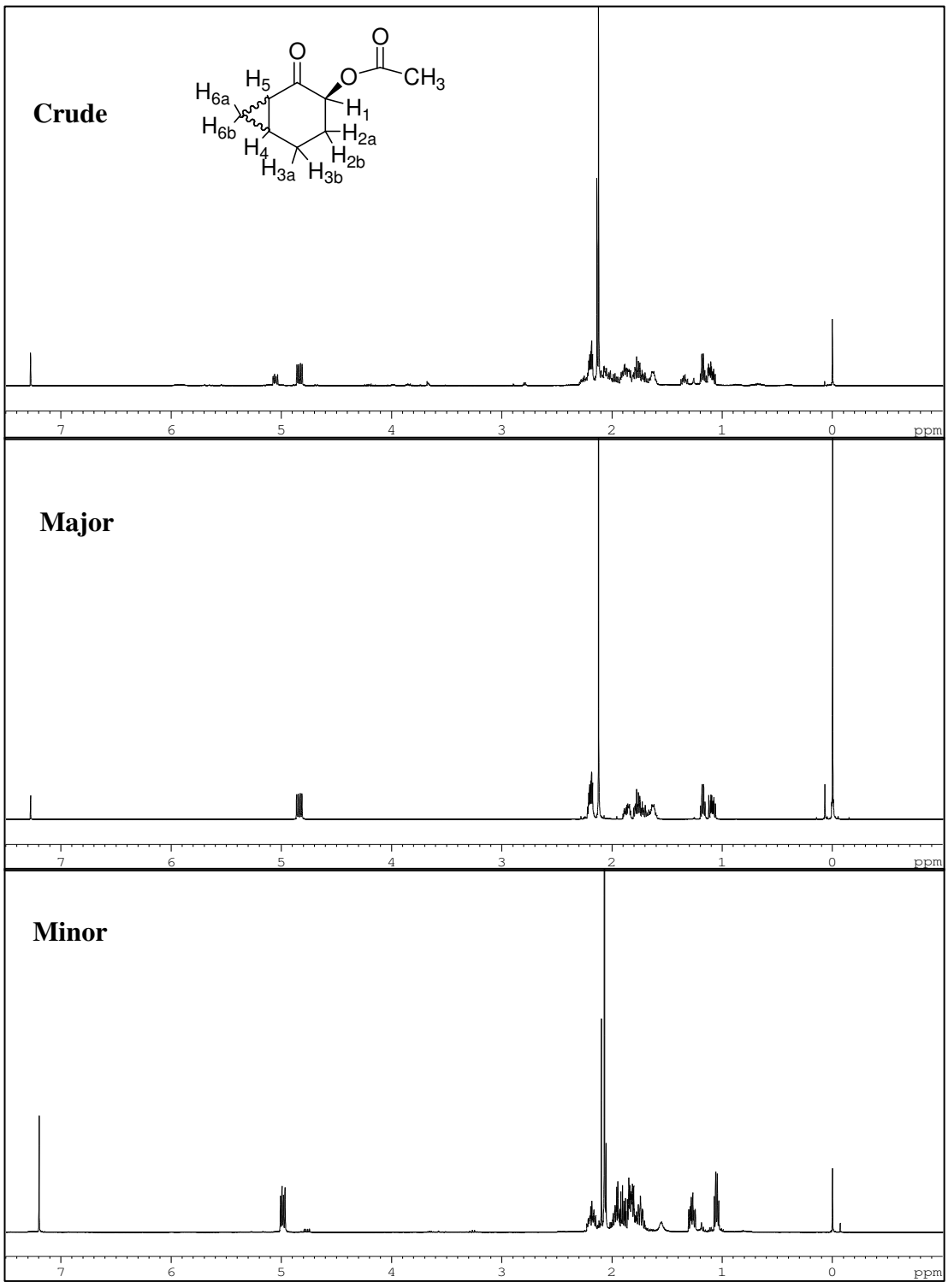


Figure 15: ^{1}H-NMR spectra of diastereomeric mixture and separated isomers

2.4.6 Characterization of (-)-2-oxobicyclo[4.1.0]heptan-3-yl acetate

In ^{1}H-NMR spectrum of the major product, H-1 attached to C-1 splits into doublet of doublet at 4.81 ppm due to the interaction of two methylene protons next to it. Methylene protons (H-3) which are next to the cyclopropane ring give multiplet between 2.16 and 2.21 ppm and the acetoxy group methyl protons give a sharp singlet at 2.08 ppm. Methylene protons (H-2) which are next to the acetoxy attached carbon splits into multiplet between 1.82 and 1.89 ppm and the methine proton (H-5) of the cyclopropane which is next to the carbonyl group of the cyclohexane ring gives multiplet between 1.68 and 1.80 ppm and the other methine proton (H-4) of the cyclopropane ring gives multiplet between 1.58 and 1.65 ppm. Finally one of the methylene protons (H_6) of the cyclopropane ring gives doublet of doublet at 2.17 ppm and the other methylene proton splits into multiplet between 1.05 and 1.12 ppm.

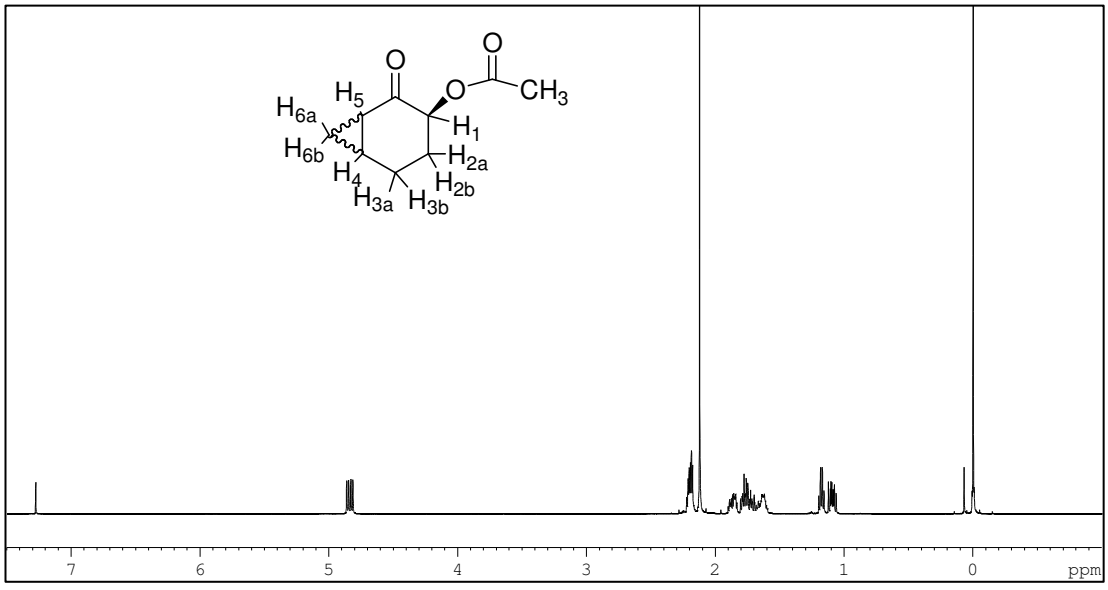


Figure 16: ^{1}H-NMR spectrum of **(-)-23a**

In ^{13}C-NMR spectrum of (-)-2-oxobicyclo[4.1.0]heptan-3-yl acetate, carbonyl carbon of the cyclohexane ring and acetoxy group show the signals at 202.0 ppm and 170.0 ppm, respectively. Acetoxy attached carbon (C-1) is shifted to 74.2 ppm due to the electronegative oxygen atom. Methylene group carbon (C-3) which is next to the cyclopropane ring gives the signal of 24.40 ppm and the methylene group carbon (C-2) which is next to the acetoxy bonded carbon is shifted to 21.5 ppm. β-position methine carbon (C-4) of the cyclopropane ring gives the signal of 21.2 ppm and the acetoxy group carbon gives the signal at 20.56 ppm. Methine carbon of the cyclopropane ring which is next to the carbonyl carbon of the cyclohexane ring shows the signal at 15 ppm. Finally, methylene carbon of the cyclopropane ring is shifted to 9.0 ppm

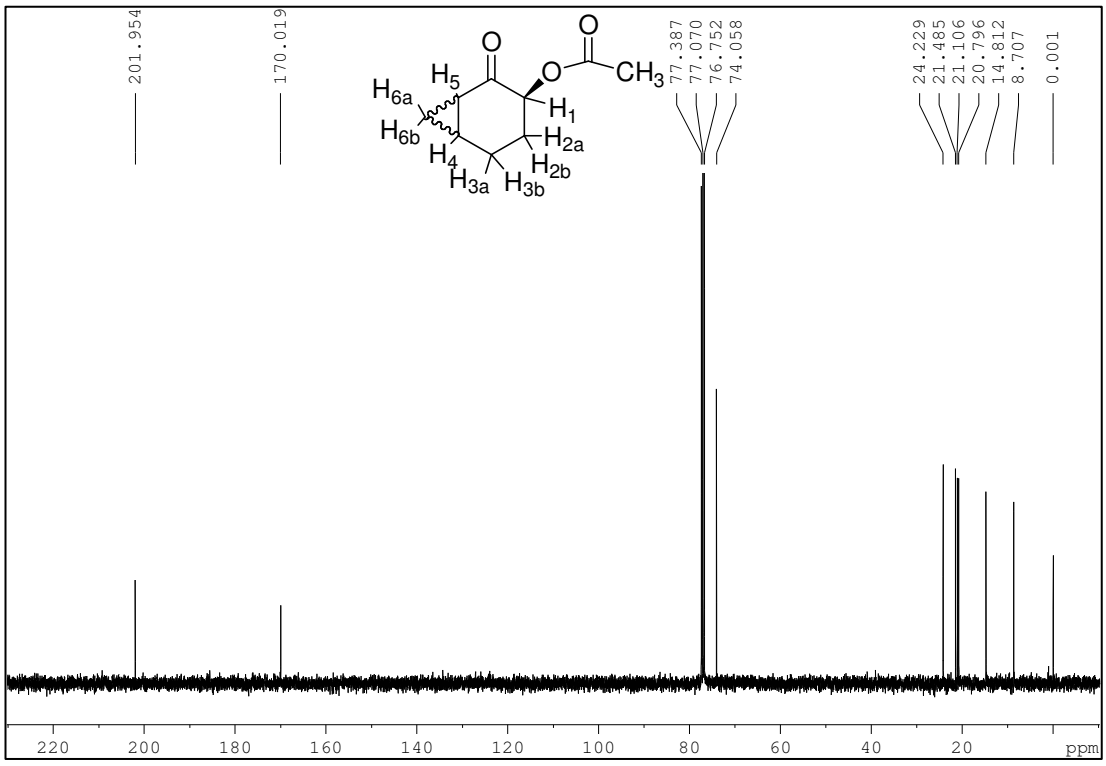


Figure 17: ^{13}C-NMR spectrum of **(-)-23a**

Each proton signal must definitely be assigned in order to elucidate the stereochemistry of the products. For this purpose, double resonance, 2D-COSY, 2D-HETCOR, 2D-HMBC experiments were done.

In the first double resonance experiment, H-1 proton attached to the acetoxy bearing carbon was irradiated. A change on the splitting pattern of diastereotopic methylene protons attached to C-2 was observed due to their interaction. When the methylene protons which are next to the cyclopropane ring were irradiated, a change on the splitting pattern of the diastereotopic methylene protons (H-2) and the methine proton (H-4) of the cyclopropane ring were observed. Finally, irradiation of methine proton of the cyclopropane ring caused a change in the splitting pattern of the methylene protons next to it and methylene protons of the cyclopropane ring.

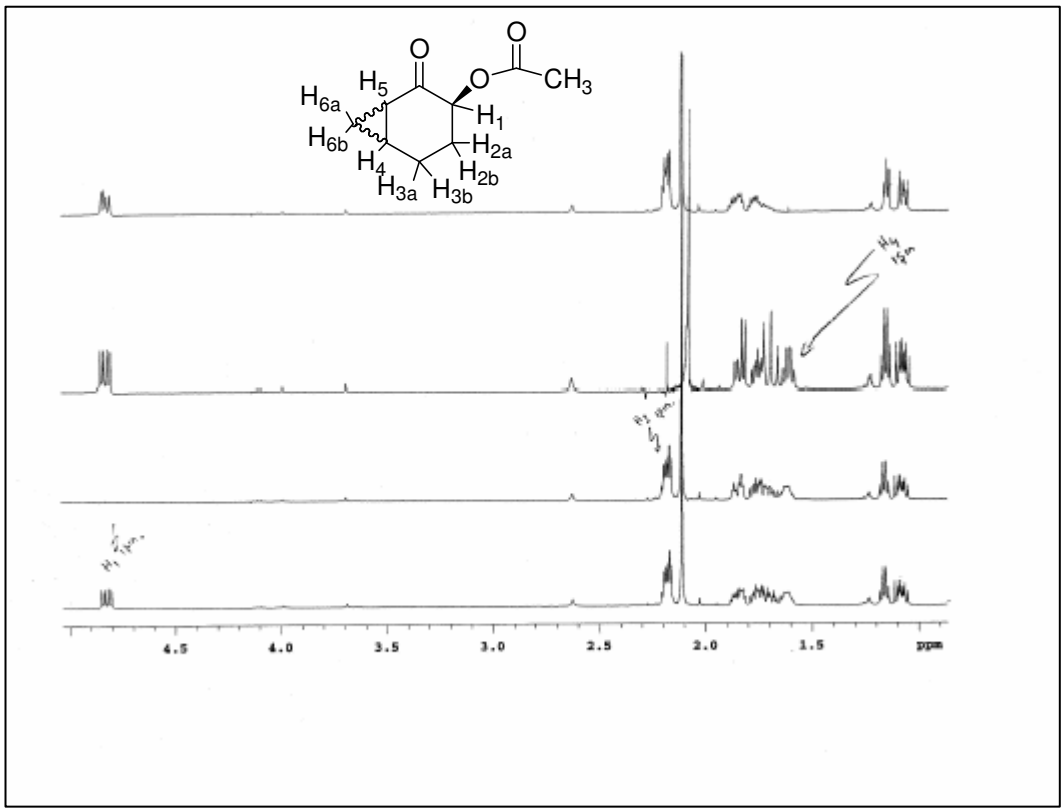


Figure 18: Double Resonance Experiment of **(-)-23a**

In the second double resonance experiment shown in Figure 19, irradiation of the methylene protons of the cyclopropane ring caused a drastic change in the splitting pattern of the methine protons of the cyclopropane ring.

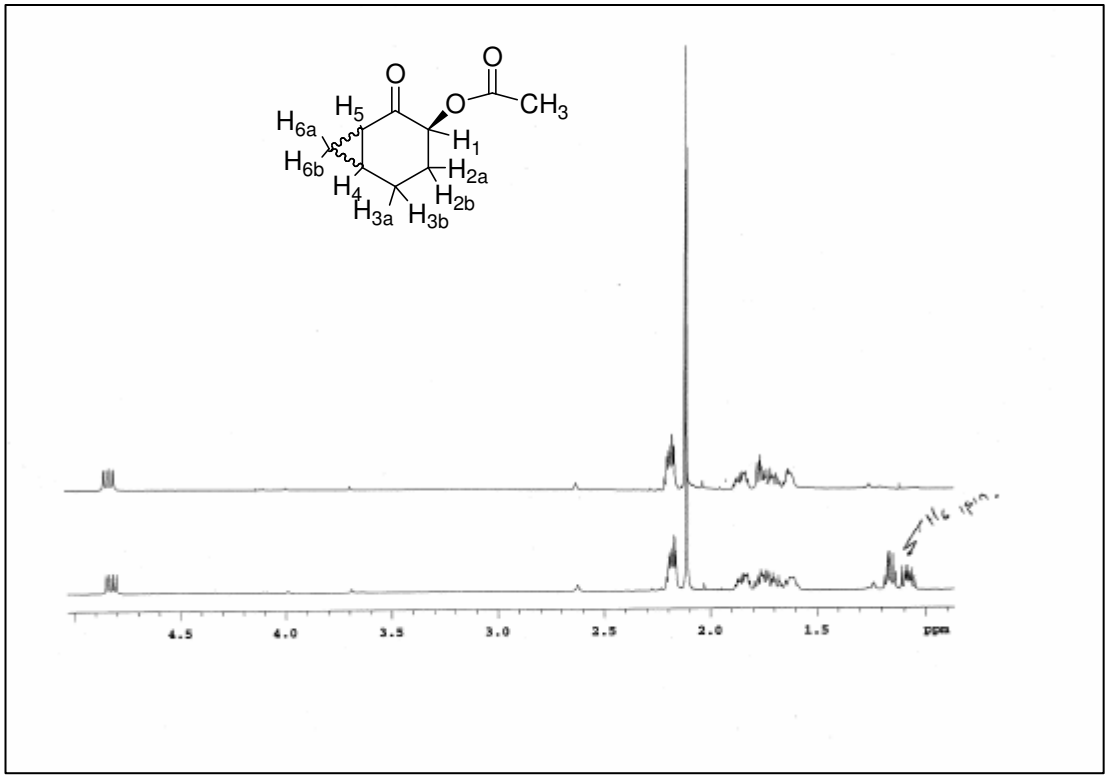


Figure 19: Double Resonance experiment of **(-)-23a**

COSY experiment is used to predict the interactions between the protons in a given molecule. In spectrum two frequency axis F_1 and F_2, are given and for each axis 1D-NMR spectrum of the compound has been drawn. 1D-spectrums are given to make easier interpretation of the spectrum. There exist diagonal peaks on the diagonal which has been drawn between two corners. Symmetrically dispersed peaks were seen out of the diagonal peaks. These peaks give information about the spin-spin interaction between protons.

In 2D-COSY spectrum of the (-)-2-oxobicyclo[4.1.0]heptan-3-yl acetate **(23a)**. Starting from the peak resonated at 4.85 ppm a parallel line through F_1 axis and a perpendicular line through F_2 axis was drawn. Because of the symmetrical property of the spectrum it was observed that, these lines intersect with same cross peaks. When the line which is parallel to F_1 line was examined, it was observed that this line intersects with three different cross peaks. This means that proton which resonates at 4.85 ppm interacts with three different protons. A parallel line through F_2 axis was drawn to find which protons these were and it was observed that the proton (H-1) resonated at 4.81 ppm correlates with the protons that resonate at 1.63 (H-4), 1.75 (H-5), 1.85 (H-2) ppm. A parallel line through F_1 axis was drawn from the signal of the proton (H-3) that resonates at 2.1 ppm and it was observed that, this parallel line intersects with three different cross peaks. Then, parallel line through F_2 axis was drawn and it was observed that, this proton correlate with the protons 1.63 (H-4), 1.75 (H-5), 1.85 (H-2) ppm. The same method was followed for the proton (H-5) that resonance at 1.75 ppm and it was observed that this proton correlates with the proton (H-6) that resonates at 1.16 ppm and proton (H-4) that resonates at 1.63 ppm correlate with the same proton (H-6).

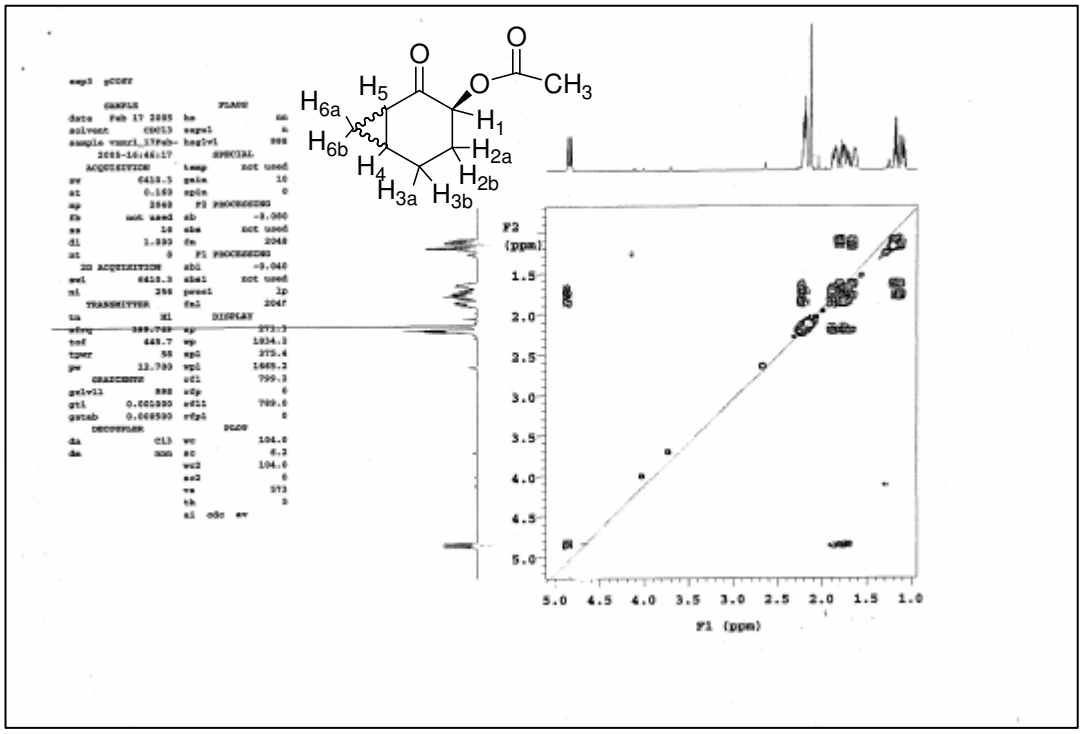


Figure 20: 2D-COSY spectrum of **(-)-23a**

2D-HETCOR method is used to determine the interactions between two different nuclei. By using HETCOR spectrum it was determined which proton is directly bonded to which carbon atom. In HETCOR spectrum there are not diagonal peaks that observed in COSY spectrum and spectrum is not symmetrical. Compared with the interpretation of 2D-COSY spectrum, interpretation of 2D-HETCOR spectrum is easier.

In the 2D-HETCOR spectrum of **(-)-23** as shown in Figure 21, a parallel line through F_2 axis was drawn for H-1 proton resonated at 4.8 ppm and found its cross peaks. A parallel line through F_1 axis was drawn starting from the cross peak. It was determined that H-1 proton resonated at 4.8 ppm is attached to the C-1 resonated at 74.00 ppm. The same procedure was followed for the other protons and following results were found;

H-3 is bonded to C-3 at 21.5 ppm.

H-7 is bonded to C-7 at 21.2 ppm.

H-2 is bonded to C-2 at 20.56 ppm.

H-5 is bonded to C-5 at 24.4 ppm.

H-4 is bonded to C-4 at 15.0 ppm.

H-6 is bonded to C-6 at 9.0 ppm.

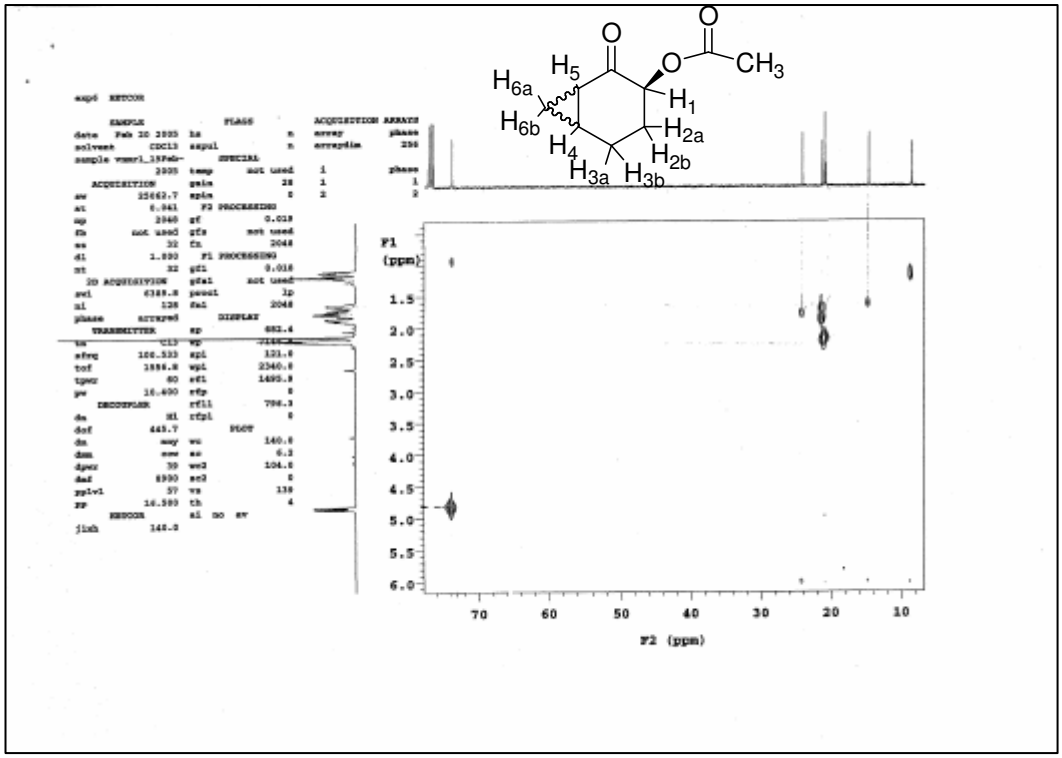


Figure 21: 2D-HETCOR spectrum of **(-)-23a**

In 2D-HETCOR spectrum, one bond correlations between carbon and proton atoms were investigated. Because of two or three bond interactions have been eliminated, they were not observed in 2D-HETCOR spectrum. In 2D-HMBC experiment, correlations over one bond are eliminated and correlations only over two and three bonds are shown.

As shown in Figure 22, 2D-HMBC spectrum of **(-)-23a** are in accordance with the structure.

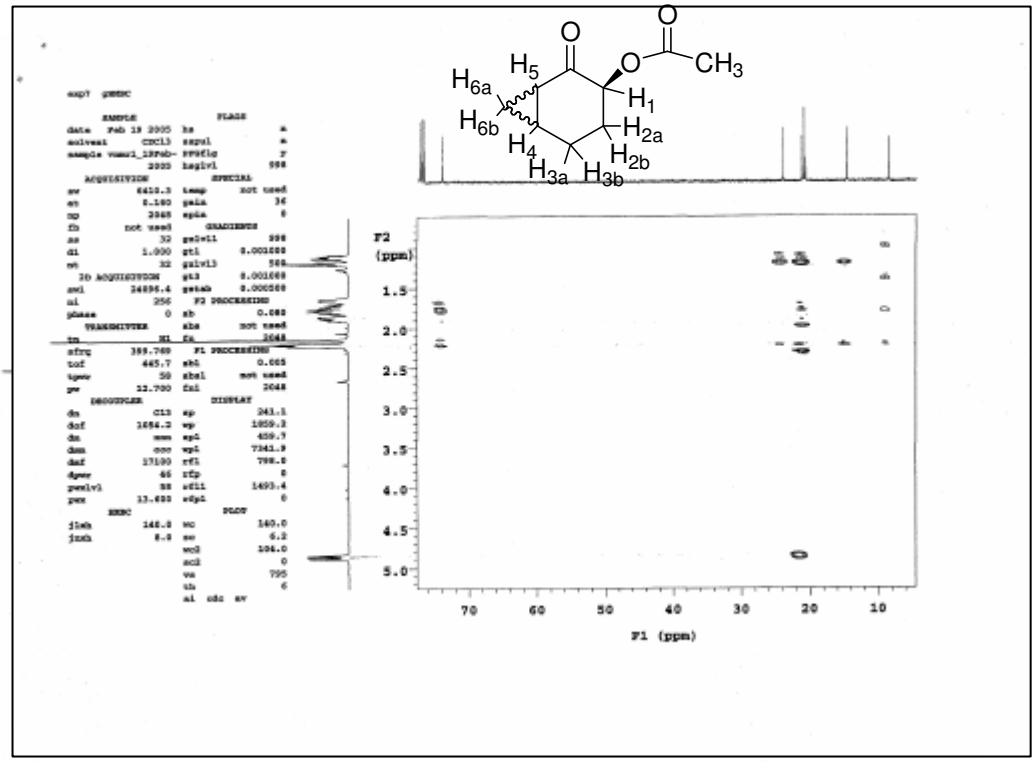


Figure 22: g-HMBC spectrum of **(-)-23a**

1D-NOE experiment gives valuable information about the conformation of the molecule. In order to prove the stereochemistry of the **(-)-23a**, 1D-NOE experiment was applied. NOE relation was not observed on H-1 proton when the methylene proton of the cyclopropane ring was irradiated. This means that, there is not an interaction between the proton (H-1) of acetoxy attached carbon and the methylene protons (H-6) of the cyclopropane ring, so they are a trans relation between these protons.

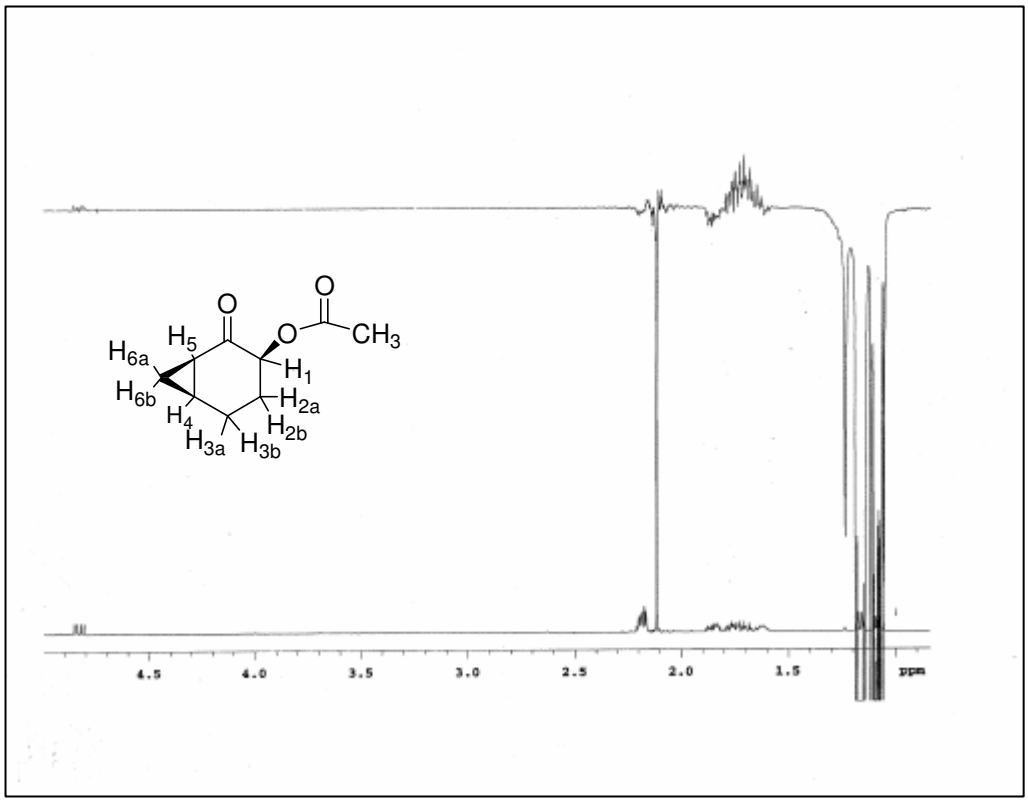


Figure 23: 1D-NOE spectrum of **(-)-23a**

In 2D-NOESY spectrum it is possible to investigate all NOE interactions in a given molecule. In 2D-NOESY spectrum there are diagonal peaks and cross peaks are observed. NOE relations between the protons are displayed with the analysis of these cross peaks. As it is seen from Figure 23, NOE relation was not observed for H-1 with the methylene protons (H-6) of the cyclopropane ring. This relation may prove the fact that, H-1 proton and the methylene protons of the cyclopropane ring are trans to each others.

Depending upon all of these, 2D-HMBC, 1D-NOE and 2D-NOESY experiments, it acetoxy group and cyclopropane ring may be in cis relation for major diastereomer and the absolute configuration of this isomer is *(1R,3S,6S)*-2-oxobicyclo[4.1.0]heptan-3-yl acetate.

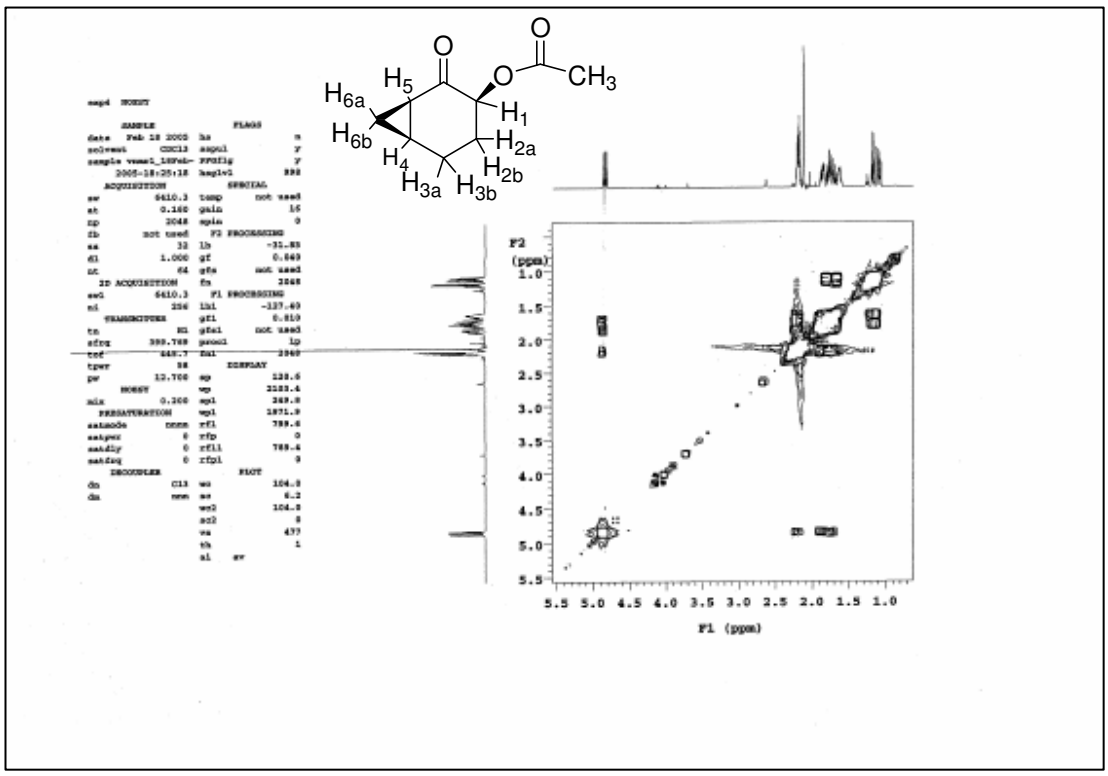


Figure 24: 2D-NOESY experiment of **(-)-23a**

The stereoselective behavior of cyclopropanation depending the chiral center having acetoxy group were tested with (+)-2-oxobicyclo[4.1.0]heptan-3-yl acetate **(+)-23a**.

2.4.7 Characterization of (+)-2-oxobicyclo[4.1.0]heptan-3-yl acetate

In the ^{1}H-NMR spectrum of **(+)-24a** as shown in Figure 25, H-1 attached to C-1 gives doublet of doublet at 5.05 ppm due to the interaction with methylene protons next to it. Methylene protons (H-3) which is next to the cyclopropane ring splits into multiplet between 2.21 and 2.29 ppm. Acetoxy group methyl protons give a sharp singlet at 2.07 ppm. Methylene protons (H-2) next to the acetoxy group splits into multiplet between 1.95 and 2.05 ppm and the methine proton (H-5) of the cyclopropane ring next to the carbonyl group of the cyclohexane ring gives multiplet between 1.86 and 1.93 ppm. Methine (H-4) proton of the cyclopropane ring gives

multiplet between 1.78 and 1.84 ppm and finally, one of the methylene protons of the cyclopropane ring splits into multiplet between 1.31 and 1.38 ppm and the other methylene proton gives doublet of doublet at 1.02 ppm.

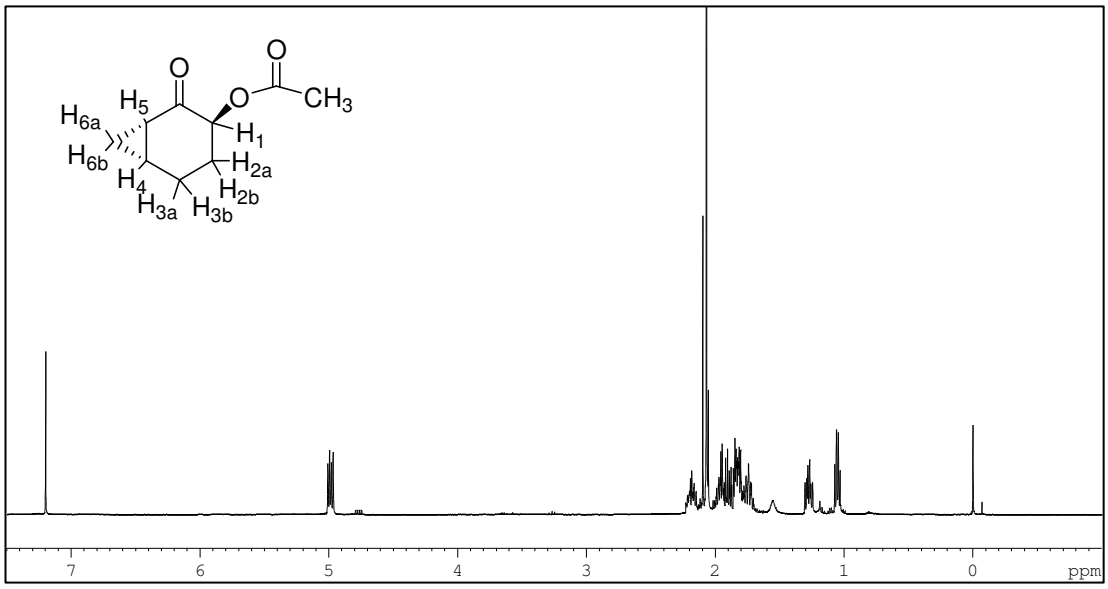


Figure 25: ^{1}H-NMR spectrum of **(+)-24a**

In Figure 26, ^{13}C-NMR spectrum of compound **(+)-24a** is shown. According to this spectrum, the carbonyl group carbon of the cyclohexane ring and acetoxy group is shifted to 216.0 ppm and 170.0 ppm, respectively. Acetoxy attached carbon (C-1) gives the signal of 72 ppm due to electronegative oxygen atom. Methylene carbon (C-3) which is next to the cyclopropane ring and methylene carbon which is next to acetoxy bonded carbon (C-2) show the signals at 32.02 ppm and 26.02 ppm, respectively. The methine carbon (C-4) of the cyclopropane ring is shifted to 21.01 ppm and the methyl carbon of acetoxy group is shifted to 22.00 ppm. Finally the methine carbon and the methylene carbon of the cyclopropane ring give the signals at 21.00 and 18.02 ppm, respectively.

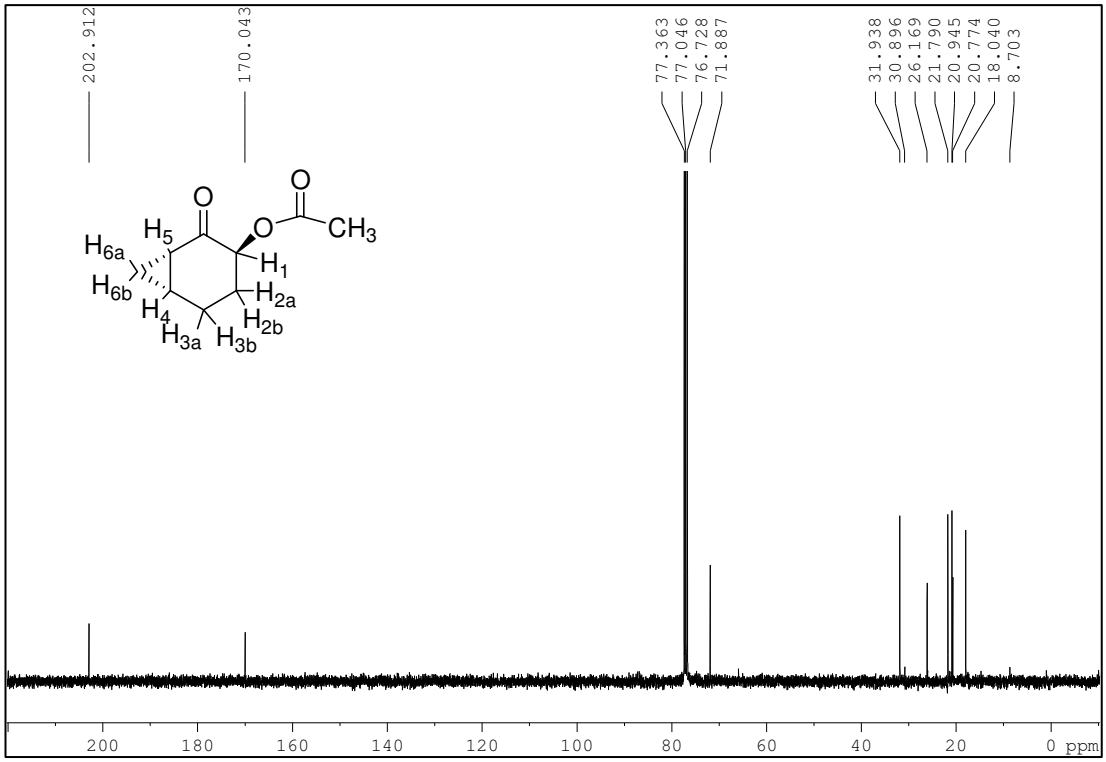


Figure 26: ^{13}C-NMR spectrum of **(+)-24a**

The interactions between different protons were determined by using 2D-COSY experiment. According to the results of 2D-COSY experiment of **(+)-24a**, it was observed that, H-1 resonated at 5.05 ppm interacts with the methylene protons (H-2) next to it and the methine proton the cyclopropane ring interacts with the methylene protons of cyclopropane.

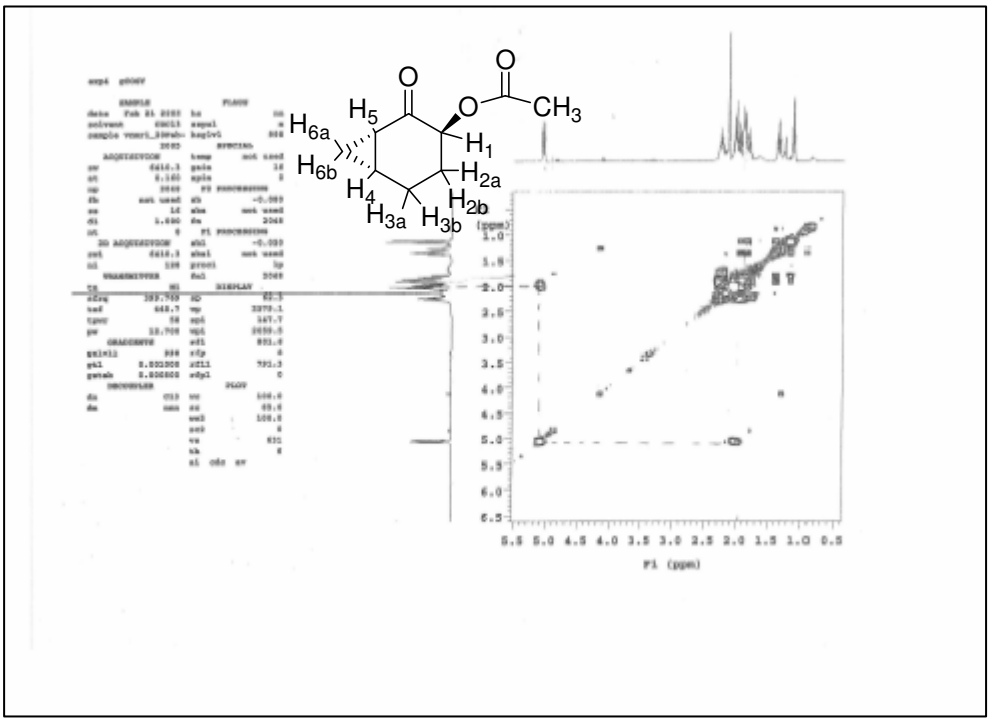


Figure 27: 2D-COSY spectrum **(+)-24a**

In Figure 28, 2D-HETCOR spectrum of **(+)-24a** is shown. Which proton is attached to which carbon was determined by looking to 2D-HETCOR spectrum. The results of this are in accordance with our structure **(+)-24a.**

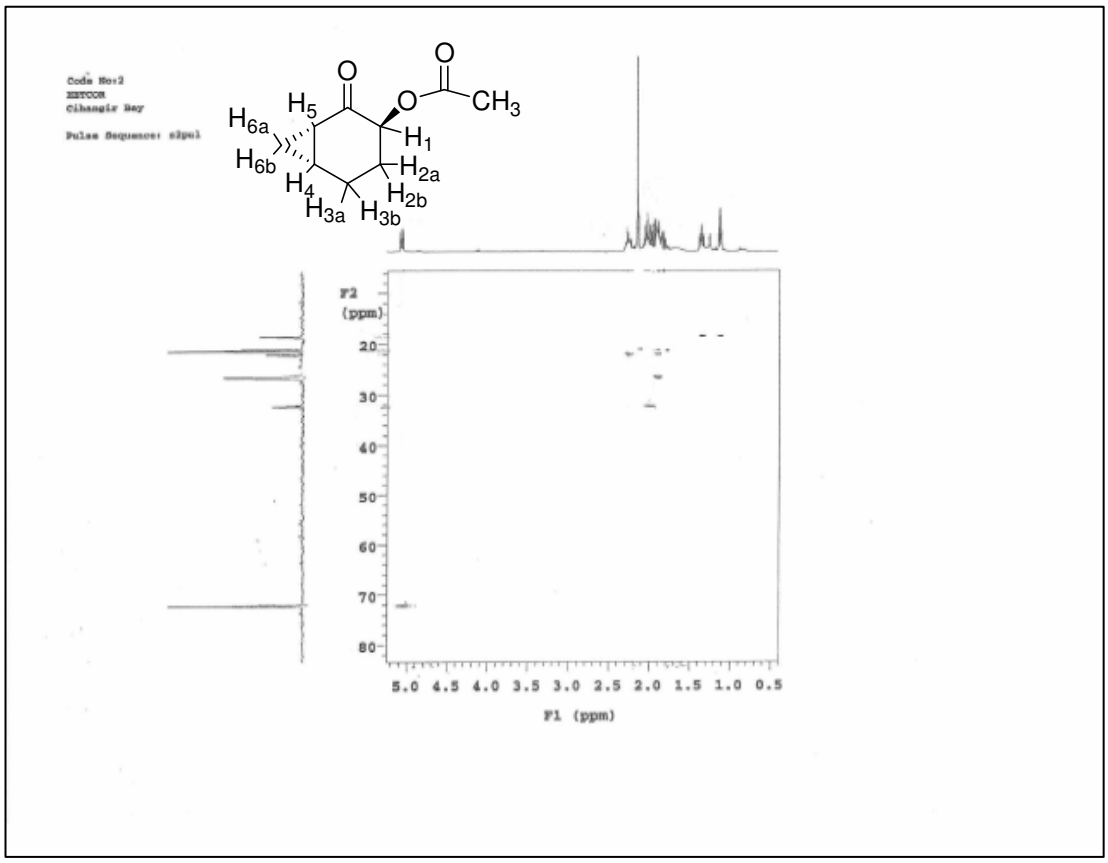


Figure 28: HETCOR spectrum of **(+)-24a**

In order to prove the conformation of the **(+)-24a**, NOE experiment was applied. As shown in Figure 29, when the proton of acetoxy attached carbon was irradiated, NOE relation was observed H-1 and the methylene protons of the cyclopropane ring. This result explains the facts that, the protons of the cyclopropane ring and the proton of acetoxy bonded carbon are on the same space of the molecule. This indicates that, cyclopropane ring and acetoxy group are trans to each others. Depending upon this finding **(+)-24** can be assigned as *(1S,3R,6S)*-2-oxobicyclo[4.1.0]heptan-3-yl acetate.

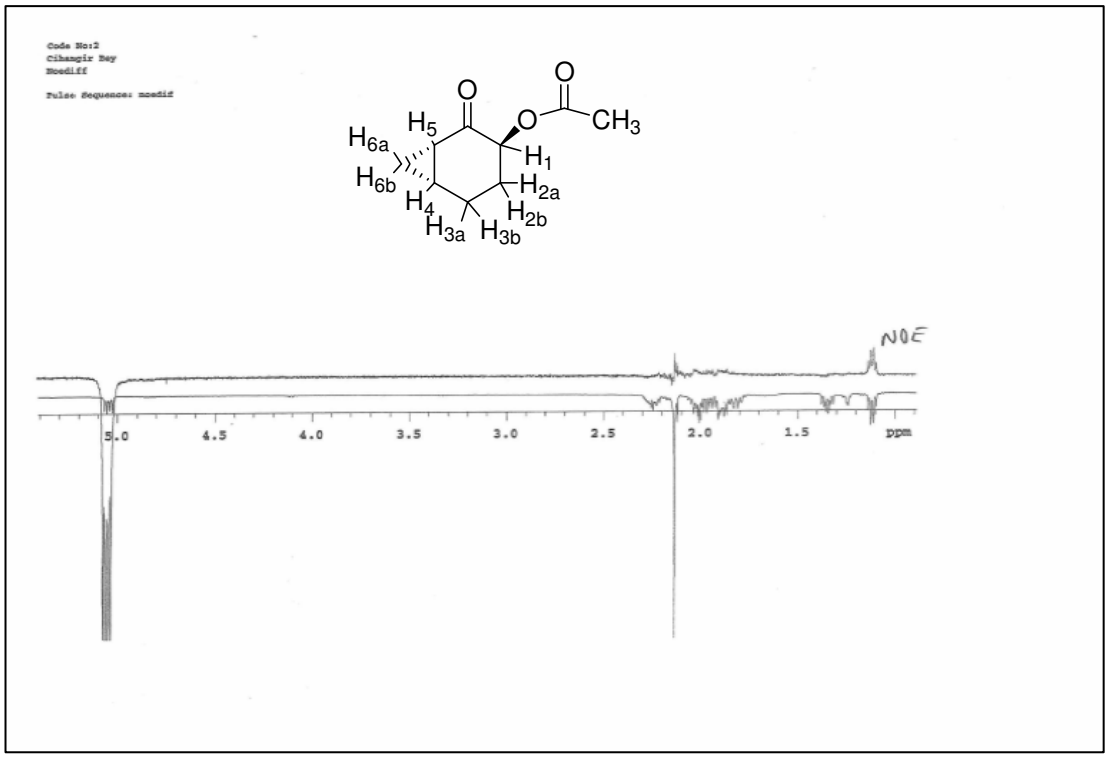
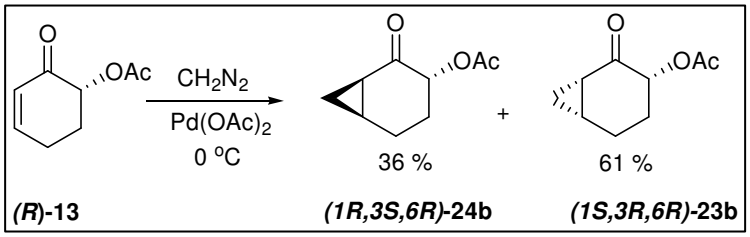


Figure 29: 1D-NOE experiment of **(+)-24a**

2.4.8 Cyclopropanation of *(R)*-6-acetoxycyclohexenone

(R)-6-acetoxy-2-cyclohexenone ***(R)*-13** was also subjected to cyclopropanation under the same conditions given above to confirm the yields of the reaction and configuration assignment of the resultant oppositely configured product. Comparison of the specific rotation value of the products with *(S)*-6-acetoxy-2-cyclohexenone cyclopropanation products showed opposite signs which proves the enantiomeric relation.



Scheme 23: Cyclopropanation of ***(R)*-13**

CHAPTER 3

EXPERIMENTAL

In this study we used the instruments which are written below for the structure characterization of the compounds.

^{1}H-NMR and ^{13}C-NMR spectra were recorded in $CDCl_3$ on Bruker Spectrospin Avance DPX 400 spectrometer. Chemical shifts are given in ppm from tetramethylsilane. Spin multiplicities are mentioned as: s (singlet), br s (broad singlet), d (doublet), dd (doublet of doublet), dt (doublet of triplet), t (triplet), p (pentet), sxt (sextet), m (multiplet).

Flash column chromatography was performed by using thick-walled glass columns with a flash grade (Merck Silica Gel 60). Reactions were monitored by thin layer chromatography using precoated silica gel plates (Merck Silica Gel PF-254), visualized by UV-light and polymolybden phosphoric acid, in ethanol as appropriate.

All extractions were dried over anhydrous magnesium sulphate and solutions were concentrated under vacuum by using rotary evaporator.

3.1 General Procedure for the Synthesis of (±)-5-Acetoxy-2-cyclopentenone and (±)-6-Acetocy-2-cyclohexenone

A mixture of $Mn(OAc)_3$ (36.4 mmol) in benzene (150 mL) was refluxed for 45 min using a Dean-Stark trap. Then the mixture was cooled to room temperature and the 2-cyclopentenone **(10)** (1.50 g, 18.2 mmol) was gradually added. The mixture was allowed to reflux until the dark brown color of the solution disappeared and also monitored by TLC. The reaction mixture was diluted with equal amount of ethyl acetate and the organic phase was washed with 1N HCl followed by saturated $NaHCO_3$ and brine. The organic phase was dried over $MgSO_4$ and evaporated in vacuo. The crude product was separated by flash column chromatography using ethyl acetate/hexane (1:3) as eluent to afford the **(±)**-5-Acetoxy-2-cyclopentenone **(12)** (65 %).

(±)-(12): Colorless oil; R_f (EtOAc/Hexane 1:3) 0.38; ν_{max} (neat) 1743, 1635 cm^{-1}

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

δ (ppm): 1.95 (s, 3H)
2.35-2.47 (m, 1H)
2.91-3.04 (m, 1H)
4.93 (dd, 3.8 Hz, 1H)
6.02-6.11 (m, 1H)
7.44-7.53 (m, 1H)

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

δ (ppm): 203.4, 170.7, 161.8, 132.9, 71.8, 35.8, 21.0

The same procedure which is used for the acetoxylation of 2-cyclopentenone was used for the acetoxylation of 2-cyclohexenone to afford the (±)-6-acetoxy-2-cyclohexenone **(13)** (1.08 g, 72 %).

(±)-13: Colorless oil; R_f (EtOAc/Hexane 1:2) 0.26; ν_{max} (neat) 1732, 1677, 1608 cm^{-1}

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

δ (ppm): 2.02-2.09 (m, 1H)
2.11 (s, 3H)
2.19-2.23 (m, 1H)
2.47-2.51 (m, 2H)
5.30 (dd, J=5.3 and 8.2 Hz, 1H)
5.98-6.02 (m, 1H)
6.87-6.92 (m, 1H)

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

δ (ppm): 194.4, 170.5, 150.3, 128.9, 73.9, 28.9, 25.9, 21.2

3.2 General Procedure for the Enzymatic Resolution of 12 and 13

The (±)-α'-acetoxylated cyclic ketone (900 mg) was added to the solution of potassium phosphate buffer (pH 7, 50 mL) containing esterase (100 µL). The reaction mixture was stirred at room temperature and monitored by TLC. When maximum conversion was reached, the reaction was ended by extraction with EtOAc. For five-membered ring, the unreacted *(S)*-(+)-5-acetoxycylopentenone **(+)-12** (0.41 g, 45%) and *(R)*-5-hydroxy-2-cyclopentenone **(-)-13** were separated by flash column chromatography. For six-membered ring, (-)-6-acetoxy-2-cyclohexenone **(-)-**

12 (0.46 g, 46 %) and (+)-6-hydroxy-2-cyclohexenone **(+)-19** (0.56 g, 56 %) were obtained.

(S)-(+)-**(11a):** Colorless oil; 96% ee $[\alpha]^{20}_{D}$=+60.3 (*c* 0.2, $CHCl_3$)

3.3 Hydrogenation of (S)-11a and (S)-11b

To a stirred solution of (+)-**12** (10 mg) in EtOAc (10 mL), Pd(C) (5 mg) was added and stirred at room temperature under hydrogen atmosphere for 3 h. The filtration of the mixture followed by evaporation of solvent in vacuo afforded quantitatively *(S)*-**20**. The same procedure was applied for the transformation of (-)-**12** into *(S)*-**20**. All spectroscopic data of the products are in accordance with *(S)*-**13** and *(S)*-**21**, respectively.

3.4 General Procedure for Acetylation of *(R)*-14a and *(R)*-14b

The *(R)*-5-Hydoxycyclopentenone **(18)** (0.49 g, 5 mmol) was dissolved in 50 mL CH_2Cl_2 and pyridine (0.6 mL, 8.5 mmol) was added at 0 ^{o}C under argon atmosphere. 45 min later, acetyl chloride (0.7 mL, 8.5 mmol) was added and refluxed at room temperature and monitored by TLC. After the reaction was completed, the reaction was ended by extraction. The reaction mixture was diluted with equal amount of ethyl acetate and the organic phase was washed with 1N HCl followed by saturated $NaHCO_3$ and brine. The organic phase was dried over $MgSO_4$ and evaporated in vacuo. The crude product was separated by flash column chromatography using ethyl acetate/hexane (1:3) as eluent to afford the (R)-5-acetoxy-2-cyclopentenone **(R)-12** (0.47 g, 95%). The same general procedure was applied for the acetylation of *(R)*-6-hydroxycyclohexenone **19** to afford the *(R)*-6-acetoxycyclohexenone ***(R)*-13** (0.53 g, 95%).

3.5 General Procedure for the Cyclopropanation of α'-acetoxylated Cyclic Ketones

Palladium diacetate (13.32 mg, 0.059 mmol) was added to an ice-cooled solution of *(S)*-5-acetoxycyclopentenone **(S)-12** (300 mg, 2.14 mmol) in ether (30 mL). On the resultant suspension was distilled an etheral solution of diazomethane prepared from N-methyl-N-nitroso-urea (7.2 g, 60 mmol) and KOH (20g, 356 mmol). The mixture was stirred at 0 ^{o}C for 4 h, and then filtered through *celite*, evaporated in vacuo. The crude product was separated by flash chromatograph with ethyl acetate/hexane (1:3) solvent system to afford *(1S,3S,5S)*-2-oxobicyclo[3.1.0]hexan-3-yl acetate **22** (294 mg, 98%).

(1S, 3S, 5S)-22: Colorless oil; R_f (EtOAc/Hexane 1:2), $[\alpha]^{20}_D$= -22,03; ν_{max} (neat) 1732, 1639, 2870, 2925, 2956, 2989 cm^{-1}

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

δ (ppm): 5.13 (t, 1H)
2.56 (dd, J= 4.2 Hz, 2H)
2.13 (s, 3H)
2.05-2.16 (m, 1H)
1.87-1.93 (m, 1H)
1.25-1.33 (m, 1H)
1.16-1.20 (m, 1H)

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

δ (ppm): 208, 170, 70.05, 29.0, 24.6, 20.7, 20.0, 14.9

The same cyclopropanation procedure was applied for the *(R)*-6-acetoxycyclopentenone to afford *(1R,3R,5R)*-2-oxobicyclo[3.1.0]hexan-3-yl acetate (294 mg, 98%).

For *(S)*- and *(R)*-6-acetoxycyclohexenone, the same cyclopropanation procedure which is given above was applied to afford *(1R,3S,6S)*-2-oxobicyclo[4.1.0]heptan-3-yl acetate (36%)**,** (1S,3S,6R)-2-oxobicyclo[4.1.0]heptan-3-yl acetate (61%) and *(1R,3R,6S)*-2-oxobicyclo[4.1.0]heptan-3-yl acetate (36%) and *(1S,3R,6R)*-2-oxobicyclo[4.1.0]heptan-3-yl acetate (61%), respectively.

(1R, 3S, 6S)- 23a: Colorless oil; R_f (EtOAc/Hexane 1:2), $[\alpha]^{20}_D$= -22.05; ν_{max} (neat) 1746, 1712, 2870, 2922, 2955, 2989 cm^{-1}

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

δ (ppm): 4.81 (dd, J=13.0 and 6.4 Hz, 1H)
2.16-2.21 (m, 2H)
2.08 (s, 3H)
1.82-1.85 (m, 2H)
1.68-1.80 (m, 1H)
1.58-1.65 (m, 1H)
2.17 (dd, J=10.05and 5.05 Hz, 1H)
1.05-1.12 (m, 1H)

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

δ (ppm): 202, 170, 74.2, 24.4, 21.5, 21.2, 20.56, 15.0, 9.00

(1S, 3S, 6R)-24a: Colorless oil; R_f (EtOAc/Hexane 1:2), $[\alpha]^{20}_D$= +5.04; ν_{max} (neat) 1720, 1754, 2854, 2886, 2947, 2989 cm^{-1}

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

δ (ppm): 5.05 (dd, J=13.0 and 6.4 Hz, 1H)
2.21-2.29 (m, 2H)
2.07 (s, 3H)
1.95-2.05 (m, 1H)
1.86-1.93 (m, 1H)
1.78-1.84 (m, 1H)
1.31-1.38 (m, 1H)
1.02 (dd, J=10.05and 5.05 Hz, 1H)

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

δ (ppm): 216, 170, 72.0, 32.01, 26.02, 22.00, 21.01, 21.00, 18.02

CHAPTER 4

CONCLUSION

In this study, enantiomerically enriched 2-oxobicyclo[3.1.0]hexan-3-yl acetate and 2-oxobicyclo[4.1.0]heptan-yl acetate were chemoenzymatically synthesized starting from 2-cyclopenten-1-one and 2-cyclohexen-1-one.

2-cyclopenten-1-one and 2-cyclohexen-1-one were oxidized to α'-acetoxy α,β-unsaturated cyclic ketones by using $Mn(OAc)_3$ and enzymatic resolution of these cyclic ketones were done by using PLE. After the enzymatic resolution of these α'-acetoxy α,β-unsaturated cyclic ketones; *(S)*-acetoxylated and *(R)*-hydroxylated products were obtained. Hydoxylated products are racemized quickly, so they were acetylated in situ. At the end of the acetylation process, *(R)*-acetoxylated products were obtained. Obtained enantiomers were cyclopropanated by using $Pd(OAc)_2$ as the catalysts with excess amount of diazomethane. At the end of the cyclopropanation process; *(1S,3S,5S)*-2-oxobicyclo[3.1.0]hexan-3-yl acetate and *(1R,3R,5R)*-2-oxobicyclo[3.1.0]hexan-3-yl acetate were obtained with 98% chemical yield. *(1S,3R,6S)*-2-oxobicyclo[4.1.0]heptan-3-yl acetate (36%), *(1R,3S,6S)*-2-oxobicyclo[4.1.0]heptan-3-yl acetate (61%) and *(1R,3S,6R)*-2-oxobicyclo[4.1.0]heptan-3-yl acetate (36%), *(1S,3R,6R)*-2-oxobicyclo[4.1.0]heptan-3-yl acetate (61%) were obtained. All of the products were obtained with high ee's. At the end of the cyclopropanation reaction for 5-acetoxycyclopentenone, only one was obtained isomer because of the rigid structure of 5-membered ring. For 6-acetoxycyclohexenone at the end of the cyclopropanation reaction, cis- and trans-products were obtained because 6-membered ring is more flexible than the 5-membered ring, so lower stereoselectivity is observed for 6-membered ring.

APPENDIX A

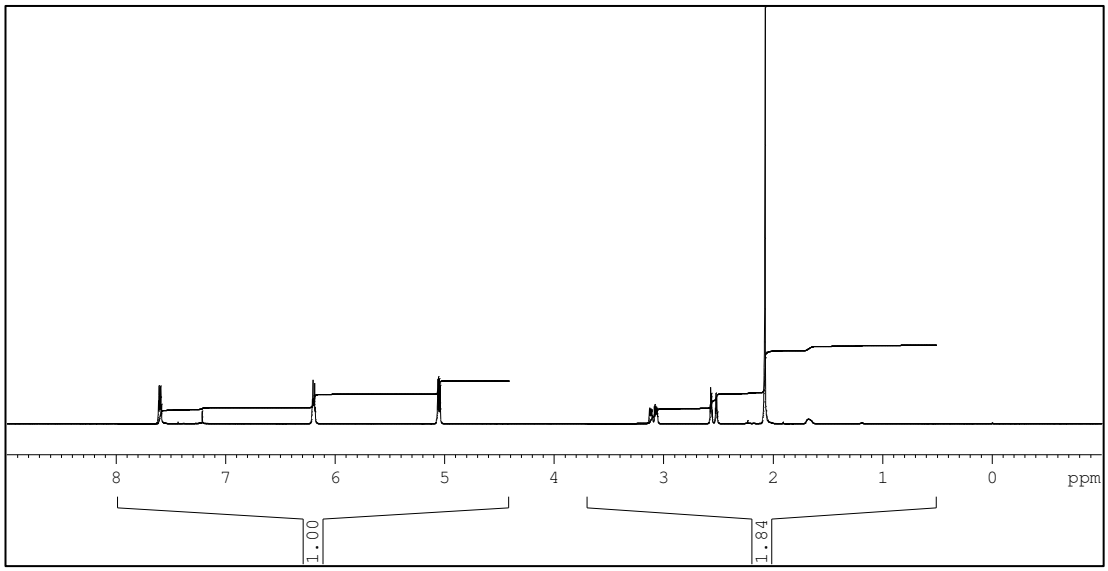


Figure 30: ^{1}H-NMR spectrum of **12**

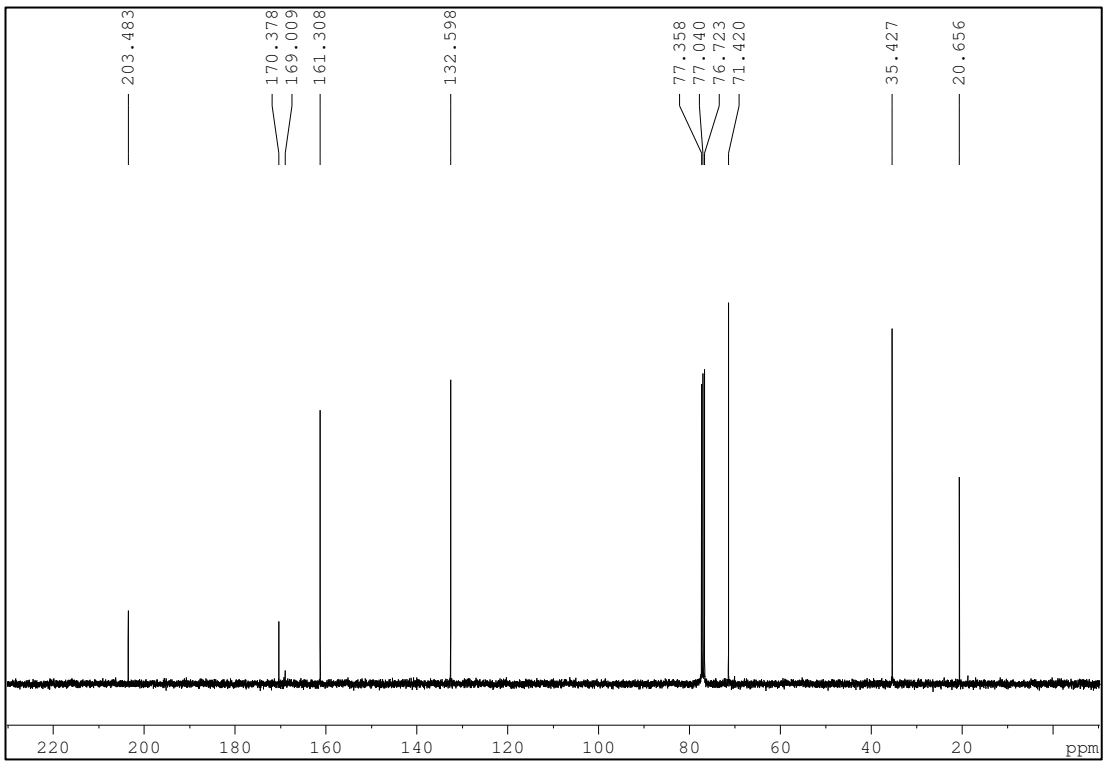


Figure 31: ^{13}C-NMR spectrum of **12**

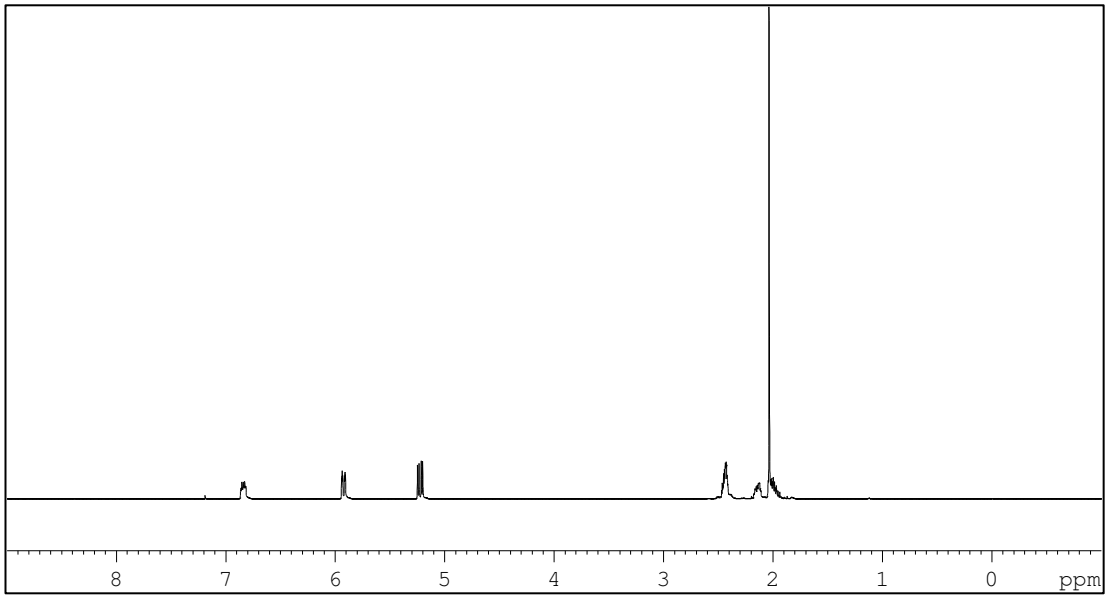


Figure 32: ^{1}H-NMR spectrum of **13**

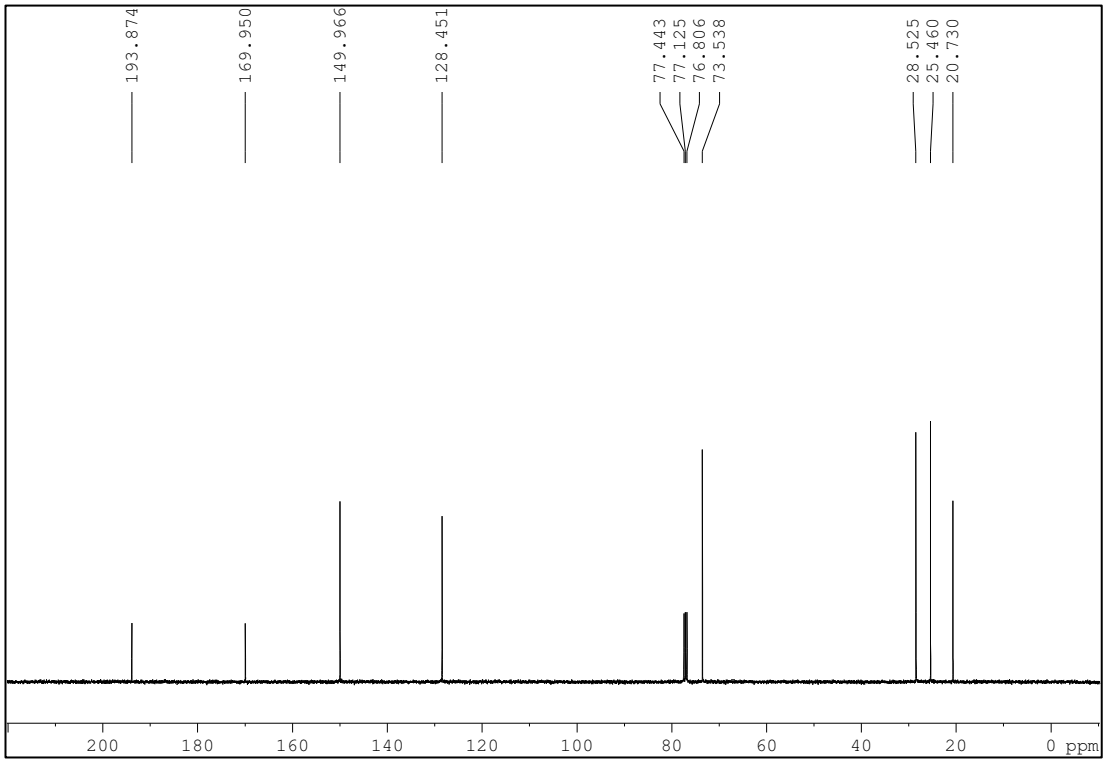


Figure 33: ^{13}C-NMR spectrum of **13**

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