STEREOSELECTIVE SYNTHESIS OF OPTICALLY ACTIVE CYCLITOL PRECURSORS VIA CHEMOENZYMATIC METHOD AND SYNTHESIS OF A NUCLEOSIDE PRECURSOR

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

BY

FUNDA OĞUZKAYA

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

JUNE 2006

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ABSTRACT

STEREOSELECTIVE SYNTHESIS OF OPTICALLY ACTIVE CYCLITOL PRECURSORS VIA CHEMOENZYMATIC METHOD
AND
SYNTHESIS OF A NUCLEOSIDE PRECURSOR

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June 2006, 99 pages

α'-acetoxylation of α,ß-unsaturated cyclic ketones was adjusted via $Mn(OAc)_3$ in regioselective manner. Then, PLE hydrolysis was carried out so as to afford enantiomerically enriched α'-acetoxylated and α'-hydroxylated cyclic compounds. From our knowledge about the literature and previous works dealing with α'-hydroxylated products which are easily racemized, protection was directly adjusted via acetylation so as to prevent this possibility. Resulting enantiomerically enriched products were subjected to Upjohn Dihydroxylation to obtain cyclitol precursors and following Luche Reduction of ketone was adjusted so as to obtain corresponding cyclitols.

In addition to such synthetic design, firstly dimethyl cyclopent-3-ene-1,1-dicarboxylate was obtained so as to reach in former manner 3-cyclopentene-1,1-dicarboxylic acid, and in latter manner cyclopent-3-enecarboxylic acid. Resulting compound was converted to 6-iodo-2-oxa-bicyclo[2.2.1]heptan-3-one and followingly to the target nucleoside precursor which is 2-oxa-bicyclo[2.2.1]hept-5-en-3-one.

Key words: $Mn(OAc)_3$, enzymatic hydrolysis, Upjohn Dihydroxylation, Luche Reduction, Nucleoside

OPTİKÇE AKTİF ÖNCÜ POLİHİDROKSİLE SİKLOHEKZAN YAPILARININ KEMOENZİMATİK YÖNTEM KULLANILARAK STEREOSEÇİCİ SENTEZLERİ
VE
ÖNCÜ BİR NÜKLEOSİT YAPISININ SENTEZİ

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Haziran 2006, 99 pages

α,ß doymamış siklik ketonlar $Mn(OAc)_3$ kullanılarak α' pozisyonundan seçici olarak asetoksilendi. Sonra enantiyomerce zenginleştirilmiş α'-asetoksilenmiş ve hidroksilenmiş maddeler elde etmek için PLE hidrolizi uygulandı. Literatür ve daha önceki çalışmalarımıza dayanarak kolayca rasemize olan α'-hidroksilenmiş ürünler bu olasılığı önlemek için asetillenme yoluyla korundu. Oluşan enantiyomerce zenginleştirilmiş ürünler Upjohn dihidroksilasyonu kullanılarak optikçe aktif öncü polihidroksile siklohekzan yapılarına dönüştürüldü ve takiben keton kısmına Luche indirgemesi uygulanarak optikçe aktif polihidroksile siklohekzan yapıları elde edildi.

Bu calısmaya ilave olarak, sırayla 3-siklopenten-1,1-dikarboksilik asit ve siklopent-3-enkarboksilik asit elde etmek için öncelikle dimetil siklopent-3-en-1,1-dikarboksilat elde edildi. Oluşan madde 6-iodo-2-okza-bisiklo-[2.2.1]heptan-3-on' a ve takiben nükleosit başlangıç maddesi olan 2-okza-bisiklo-[2.2.1]hept-5-en-3-on' a dönüştürüldü.

Anahtar kelimeler: $Mn(OAc)_3$, enzimatik hidroliz, Upjohn dihidroksilasyonu, Luche indirgemesi nükleosit

To My Family;

ACKNOWLEDGEMENT

I would like to express my greatest appreciations to my supervisor Prof. Dr. Cihangir Tanyeli for his guidance, encouragement, advices, criticism and patience.

Also, I gratefully thank to Prof. Dr. İdris Mecidoğlu Akhmedov for his valuable suggestions.

Moreover, I would like to thank to Assoc. Prof. Dr. Murat Çelik from Atatürk University for X-Ray analysis and Fatoş Polat from METU for NMR spectra.

Furthermore, I want to thank to Fadile Kapaklı for her close friendship through undergraduate and graduate education. Also, thank to Eda Kesin for her helpful manner all the time without waiting any response and for her kind friendship.

In addition, I would like to thank to all of the METU Organic Chemistry Research Group for their kind friendship.

Finally, I owe great thanks to my parents Hikmet and Semiha Oğuzkaya, my brother Okan Oğuzkaya, and my sister Yasemin Bayraktar and her family for their endless love, being wherever, whenever I want all the time, and for their great patience, , I devote this thesis to them.

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LIST OF ABBREVIATIONS

DMSO: Dimethyl Sulfoxide

PLE: Pig Liver Esterase

CCL: Lipase from *Candida Rugosa*

PPL: Porcine Pancreatic Lipase

HLE: Horse Liver Esterase

DBU: 1,8-diazabicyclo[5.4.0]undec-7-ene

THF: Tetrahydrofuran

NMO: 4-methyl morpholine N-oxide

DMAP: 4-dimethylaminopyridine

DMPU: 1,3-dimethyl-3,4,5,6-tetrahydro-2(1H)-pyrimidinone

CHAPTER 1

INTRODUCTION

1.1 Asymmetric Synthesis

Metal-mediated asymmetric synthesis is one of the most powerful methods to access to enantiopure compounds [1]. Asymmetric synthesis is described as the process for the formation of an optically active compound through reaction of an asymmetric substrate with a chiral reagent. Due to the high demand and preference for the use of enantiomerically pure drugs, there has been an upsurge of interest in the asymmetric synthesis of pharmaceutical products. In view of different pharmacological activities displayed by the individual enantiomers and differences in metabolic behaviour, the asymmetric synthesis of compounds has received growing interest in recent years [2]. The field of asymmetric synthesis evolved from the study of diastereoselectivity in the reactions of chiral compounds, through auxiliary-based methods for the synthesis of enantiomerically pure compounds, to asymmetric catalysis.

Three scientists share of 2001 Nobel Prize in Chemistry are William S. Knowles, previously at Monsanto Company, St. Louis, Missouri, USA; Ryoji Noyori, Nagoya University, Chikusa, Nagoya, Japan and K. Barry Sharpless, The Scripps Research Institute, La Jolla, California, USA. The Royal Swedish Academy of Sciences has awarded the Prize for their development of catalytic

asymmetric synthesis. The achievements of these three chemists are of great importance for academic research, for the development of new drugs and materials, and are being used in many industrial syntheses of pharmaceutical products and other biologically active substances [3].

1.2 Mirror Image Catalysis

1.2.1 Chiral Molecules

The word *chiral* comes from the Greek word *cheir,* which means hand. If, for example, the common amino acid alanine (figure 1) is concerned, it is seen that it can occur in two forms with tetrahedral arrangement: (*S*)-alanine and (*R*)-alanine, which are mirror images [4].

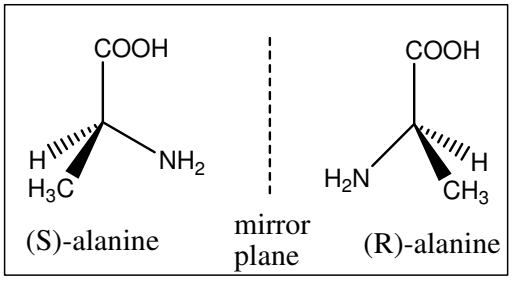


Figure 1. Chirality in the amino acid alanine is illustrated with models of its two forms, which are mirror images of each other. They are designated in (S) and (R).

It was the Dutch chemist J. H. van 't Hoff and the French chemist J. A. Le Bel who, independently of each other in 1874, discovered this tetrahedral arrangement of the groups around the central carbon atom. Thus the amino acid

alanine occurs in two forms, called *enantiomers.* When alanine is produced in a laboratory under normal conditions, a mixture is obtained, half of which is (*S*)-alanine and the other (*R*)-alanine. The synthesis is symmetrical in the sense that it produces equal amounts of both enantiomers.

Asymmetric synthesis, on the other hand, deals with the production of an excess of one of the forms. Why is this so important? The answer is in the nature. Nature is chiral. One may well think that both forms of chiral molecules ought to be equally common in nature, the reactions should be symmetrical. But when it is studied the molecules of the cells in close-up, it is evident that nature mainly uses one of the two enantiomers. That is why its concerned – and this applies to all living material, both vegetable and animal – amino acids, and therefore peptides, enzymes and other proteins, only of one of the mirror image forms. Carbohydrates and nucleic acids like DNA and RNA are other examples.

Thus the enzymes in our cells are chiral, as are other receptors that play an important part in cell machinery. This means that they prefer to bind to one of the enantiomers. In other words, the receptors are extremely selective; only one of the enantiomers fits the receptor's site like a key that fits a lock. (This metaphor comes from another Nobel Laureate in Chemistry, Emil Fischer, who was awarded the Prize in 1902.)

Since the two enantiomers of a chiral molecule often have totally different effects on cells, it is important to be able to produce each of the two forms pure. Most drugs consist of chiral molecules. And since a drug must match the molecules it should bind to itself in the cells, it is often only one of the enantiomers that is of interest. In certain cases the other form may even be harmful. This was the case, for example, with the drug thalidomide, which was sold in the 1960s to pregnant women. One of the enantiomers of thalidomide

helped against nausea, while the other one could cause foetal damage [5,6]. There are other, less dramatic examples of how differently the two enantiomers can affect our cells. *Limonene,* for example, is chiral, but the two enantiomers can be difficult to distinguish at first glance (figure 2). The receptors in our nose are more sensitive. One form certainly smells of lemons but the other of oranges [7,8].

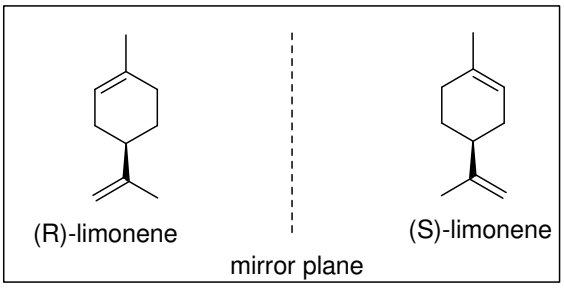


Figure 2. (R)-limonene smells of oranges while its enantiomer (S)-limonene smells of lemons

1.3 Catalytic Asymmetric Synthesis - What is it?

It is very important for industry to be able to produce products as pure as possible. It is also important to be able to manufacture large quantities of a product. For this reason the use of catalysts is very important. It is known that during long term reactions catalysts may be apparently used up [9]. A catalyst is a substance that increases the rate of the reaction without being consumed itself.

During the past few decades there has been intensive research into developing methods for producing - synthesising - one of the enantiomers rather than the

other. In a synthesis starting molecules (substrate molecules) are used to build new molecules (products) by means of various chemical reactions. The Laureates have developed chiral catalysts for two important classes of reactions in organic chemistry: hydrogenations and oxidations.

1.4 Kinetic Resolution

Kinetic resolution may be realized by chemical or enzymatic methods; in the former case the reaction may be either stoichiometric or catalytic with respect to the optically active auxiliary; from an economic standpoint catalysis is obviously preferred. According to this type of resolution, a substrate is acted on by a chiral agent to produce one enantiomer or diastereomer of the product at a much faster than the other isomer. Generally, the enantiomeric excess of the starting material increases as the reaction continues, while the enantiomeric excess of a chiral product decreases. As this is a resolution, only 50% of the substrate can be converted to the desired product unless it is meso [10]. Furthermore, it can be obviously said that enzymatic processes is more favourable than chemical one in the cases of kinetic resolutions and high enantiomeric excess or diastereomeric excess values.

1.5 Enzymes

Enzymes are biological catalysts that enable many complex reactions to be catalyzed. Enzymes take place at ordinary temperatures, because of working in complex living systems. An enzyme is capable of catalyzing a particular reaction of a particular substrate even though other isomers of that substrate and other compounds of similar structure may be present [11]. Enzymes allow many chemical reactions to occur within the homeostasis constraints of a living system. Enzymes function as organic catalysts. A catalyst can be defined as a

chemically involved in, but not changed by a chemical reaction. Many enzymes provide a function like lowering the activation energy of reactions (Figure 3). By bringing the reactants closer together, chemical bonds may be weakened and reactions will proceed faster than without the catalyst. Moreover, the efficient synthesis of biologically active compounds, either of natural or unnatural origin, frequently requires chiral synthons [12,13]. Enzymes as chiral catalysts are now widely used for their preparation, since it is often rather difficult to introduce centres of chirality or perform regiospecific transformations by the application of purely "chemical methods" [14,15].

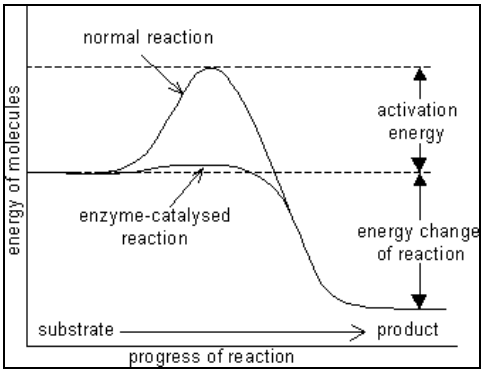


Figure 3. Enzyme Catalyzed Process

1.5.1 Factors Influencing the Enzyme Reaction Rate

The chief factors that determine the initial velocity of an enzymatic reaction are the enzyme concentration, the substrate concentration, pH, temperature, activators, inhibitors and ionic strength.

1.5.1.1 Effect of Enzyme Concentration

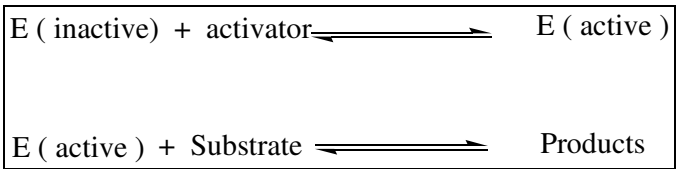
Theoratically an increase in rate should be observed for each increase in enzyme concentration. It can be sometimes found that there is a falling off from linearity at very high enzyme concentration. This does not indicate a direct decrease in the activity of enzyme but represents a limitation in the technique of measurement. In the cases of deviations from linearity observed are probably due in some measure to the presence of activators or inhibitors in the enzyme preparation. In the vast majority of cases an exact proportionality between initial velocity and enzyme concentration has been found and most of the kinetic studies have assumed such proportionality [16,17].

1.5.1.2 Effect of Substrate Concentration

The concentration of substrate is one of the most important factors that affect the rate of the enzymatic reaction. Frequently a decrease in the rate of an enzymatic reaction is observed at high substrate concentration [18].

1.5.1.3 Effect of Activators

The rate of some enzymatic reactions can be greatly increased by the addition of very small amounts of certain substances called activators. An enzymatic activator is a substance which is required for an enzyme to be an active catalyst [19,20].



Scheme 1. Enzymatic activator for an enzyme to be an active catalyst

Some activators greatly increase the efficiency of already functioning enzyme. The activity of the enzyme is increased via the addition of enough activator to activate the enzyme fully. The initial rate of the enzyme reaction is proportional to the concentration of the activator at low concentrations which provides a method for the rate determination. At high concentrations, the rate becomes independent of the amount of activator.

1.5.1.4 Effect of Inhibitors

An inhibitor is a compound that give rise to a decrease in the rate of catalytic reaction, either by reacting with the catalyst to form a catalyst-inhibitor complex or by reacting with one of the reactants. Enzymatic inhibitors are either reversible or irreversible. In reversible inhibition, the enzyme recovers its activity when the inhibitor is removed; in the case of an irreversible inhibitor it does not. With an irreversible inhibitor the inbition increases progressively with an increasing inhibitor concentration and becomes complete if enough inhibitor is present to combine with all the ezyme. With a reversible inhibitor, inhibition is progressive, but quickly reaches an equilibrium value which depends upon the inhibitor concentration. The action of the ‘nerve gases’, Sarin and Tabun, on cholinesterase is an example of irreversible inhibition; eserine, however, is a reversible inhibitor of cholinesterase [21].

1.5.1.5 Effect of Temperature

The overall enzyme reaction consists of three successive stages: the formation of enzyme-substrate complex, conversion of this complex to an enzyme-product complex and dissociation to products and free enzyme. The total effect of temperature on the reaction will be the resultant of the seperate effects of these individual steps. Increases in temperature leads to speed up the rate of nonenzyme mediated reactions, and so temperature increase speeds up enzyme mediated reactions, but only to a point. When heated too much, enzymes (since they are proteins dependent on their shape) become denatured. When the temperature drops, the enzyme regains its shape.

1.5.1.6 Effect of pH

Most enzymes are active over only a limited range of pH and in most cases a definite optimum pH can be observed. This optimum pH might be due to a number of effects:

a) an effect of pH on the stability of the enzyme, which may become
b) irreversibly destroyed on one or both sides of the optimum pH
c) an effect on the V_{max} itself
d) an effect on the affinity of the enzyme, the fall on either side of the optimum being due to a decreased saturation of the enzyme with substrate, due to a decreased affinity, and
e) an effect of pH on the indicator reaction, if one is used to monitor the progress of the enzymatic reaction by a coupled reaction sequence.

These effects may occur in combination, and can easily be distinguished experimentally.

1.5.1.7 Effect of Ionic Strength

The presence of foreign salts can affect the rate of the reaction, either by shifting the equilibrium of formation of the activated complex or by combining with reactants. So as to achieve reproducible results, one must carefully eliminate harmful foreign ions and control the ionic strength of the medium. In an ionic reaction, the rate will vary with the dielectric constant of the solvent used [22,23].

1.5.2 Pig Liver Esterase (PLE)

In contrast to the large number of readily avilable microbial lipases, less than a handful of true 'esterases' such as pig liver esterase (PLE) have been used to perform the bulk of the large number of highly selective hydrolytic reactions. Among all the esterases, PLE is clearly the champion considering its general versatility. This enzyme is constitutionally complex and consists of at least five so-called isoenzymes, which are associated as trimers of three individual proteins. However, from an organic chemist's viewpoint this crude mixture can be regarded as a single enzyme since all of the isoenzyme subunits usually possess similar stereospecifity, although some significant difference among them have been reported. Thus, the selectivity of PLE may vary, depending on the source and the pretreatment of enzyme preparation. The biological role of PLE is the hydrolysis of various esters occuring in the porcine diet, which would explain its exceptionally wide substrate tolerance. It is noteworthy that PLE has been used relatively infrequently for the resolution of racemic esters.

PLE hydrolysis of a dicarboxylate and a diacetate are often complementary to each other in terms of the selectivity. PLE catalyzed reactions afforded (S)-configured carboxylic acid. The enzyme used in catalytic level exhibited

high ee values and showed unusual versatility and diversity of the enantioselective hydrolysis of this substrate.

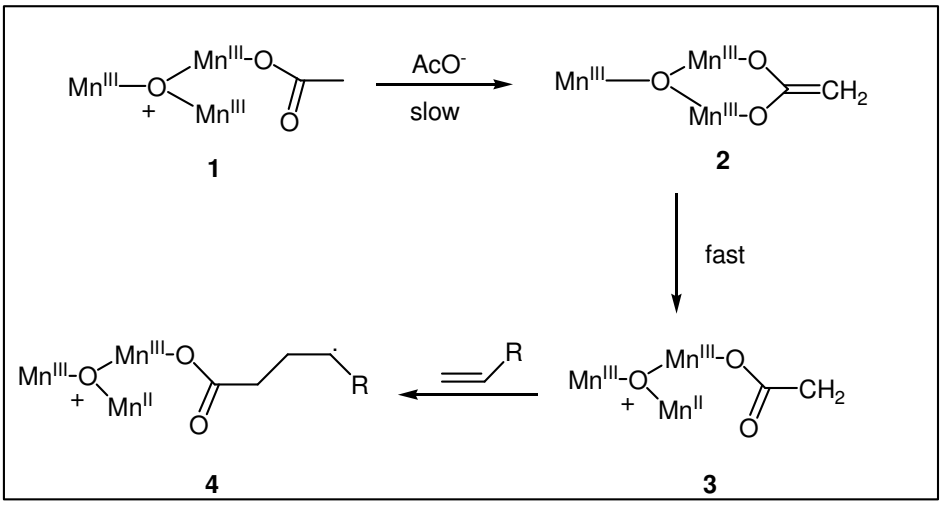
1.6 Oxidation

Transition metals and oxides occuppy a central place in the organic chemist's arsenal of oxidants. Such reagents are the most stable, inexpensive ones that are easily stored and handled. Because of these desirable properties, transition metal oxidants have been extensively studied on by synthetic organic chemistry. Through constant use has come the observation that considerable selectivity with respect to functional groups and stereochemistry can be achieved by the choice of proper oxidant and the proper conditions [24].

1.6.1 Manganese(III) Acetate Oxidations

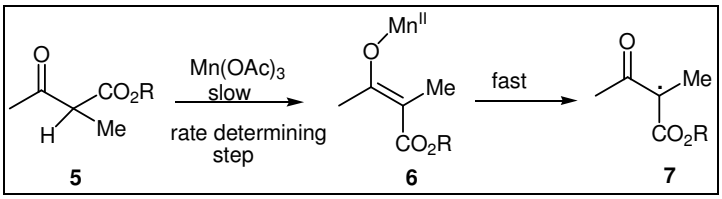
$Mn(OAc)_3$ is prepared generally from potassium permanganate and manganeous acetate in acetic acid [25]. Anhydrous $Mn(OAc)_3$ is slightly more reactive then the dihydrate. Reaction times with the anhydrous reagent are usually shorter but the yield of products are usually comparable. Triflouroacetic acid usage as a cosolvent usually increases the rate of the reaction, but often decreases the yield of the products. Manganese triacetate is one of the most powerful oxidizing agents and has been used for many years for most of the oxidative reactions. In 1976, Williams and Hunter and in the following years Watt et al. reported that the manganese(III) acetate oxidation of enones provides modest yields of α'-acetoxy enones [26]. 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 [27] is the loss of a proton from a complexed acetate like **1** to give **2**, given in Scheme 2. [27,28]. 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 2. Proposed mechanism of oxidation of monocarbonyl substrates by $Mn(OAc)_3$

Similar mechanism was also studied for the oxidation of α-alkyl β-keto esters that is shown in Scheme 3.



Scheme 3. 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. 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.

1.7 Dihydroxylation

The oxidative functionalization of olefins is so important for both organic synthesis and the industrial production of bulk and chemicals. Among the different oxidation products of olefins, 1,2-diols are used in wide variety of applications [29]. The osmium-catalyzed dihydroxylation of olefins is one of the most powerful methods for the enantioselective introduction of chiral centers in organic substrates. As combinatorial chemistry gains momentum in the chemical community, osmium-catalyzed dihydroxylation of olefins retains its status for the preparation of vicinal diols as the most reliable method. The

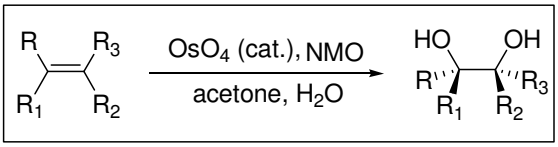
power of this transformation is achieved by the wide variety of applicaple substrates, providing quick entry into highly functionalized molecules from commercial sources. However, for many reasons the use of polyenes as substrates for dihydroxylation is a relatively undeveloped area [30].

Osmium tetroxide (OsO_4) is the most reliable reagent that is available for the production of cis-diols from olefins [31]. Despite of widely usage of these reactions in the synthesis of pharmaceuticals, fine chemicals, etc., there are few large-scale industrial applications due to the high cost of osmium as well as the high toxicity and volatility of the osmium component [32].

The syn-selective dihydroxylation of alkenes by osmium tetroxide has been known for almost 100 years. More recently, the reaction has been developed into a highly efficient process using catalytic osmium tetroxide and a reoxidant, most commonly N-methylmorpholine N-oxide (NMO) or potassium hexacyanoferrate. Moreover, the oxidation can also be accompanied by high levels of enantioselectivity in the product, and this variant of the reaction, the asymmetric dihydroxylation (AD), is one of the most powerful tools available to synthetic chemists [33].

1.7.1 Upjohn Dihydroxylation

The classical Upjohn catalytic osmium tetroxide oxidation of olefins is a very useful methodology to provide vicinal cis-diols [34,35]. This methodology has been widely applied to stereoselective dihydroxylation of olefins and, by using chiral ligands, has been employed, successfully, in enantioselective dihydroxylation of olefins [36,37,38].

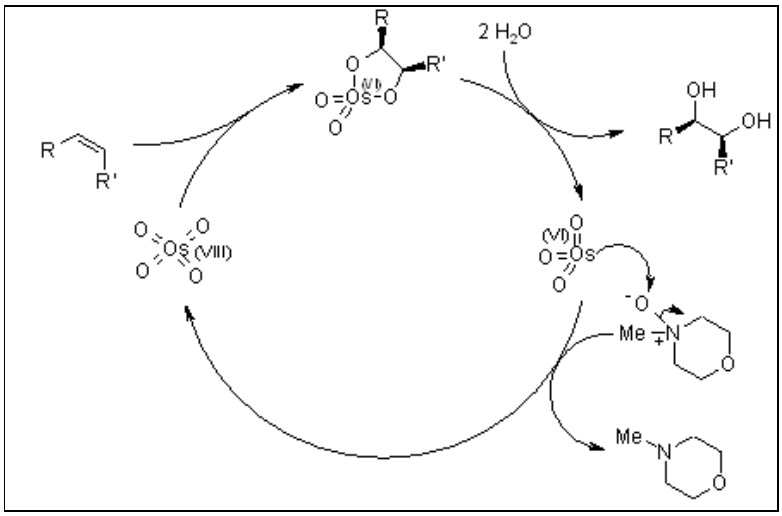


Scheme 4. Upjohn dihydroxylation

In this process, osmium (VIII) is reduced to osmium (VI) via reaction with the olefin, forming an osmium (VI) glycolate. A catalytic amount of osmium tetroxide is used if a suitable reoxidant is present, which oxidizes osmium (VI) back to active reagent. Synthetically useful reoxidant for Upjohn Dihydroxylation is N-methylmorpholine N-oxide (NMO) [39].

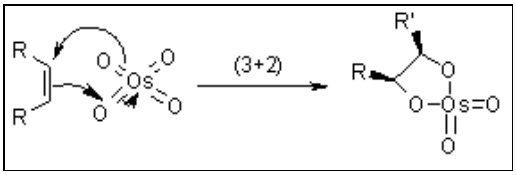
1.7.1.1 Mechanism

The toxic and volatile OsO_4 can also be prepared *in situ* by the oxidation of $K_2OsO_2(OH)_4$ with NMO. NMO is the cooxidant that enables the use of a catalytic amount of OsO_4, because this reagent is able to reoxidize an Os(VI) species to an Os(VIII) species.

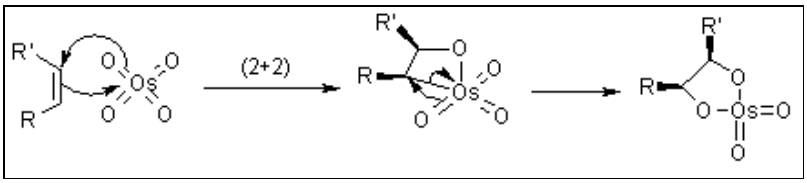


Scheme 5. Mechanism for Upjohn Dihydroxylation

The key step is the cycloaddition of OsO_4 to the olefin. There has been some speculation regarding the actual addition step, for which experimental data suggest the possible involvement of two separate steps. Thus, the question arises during these discussions of whether the key step takes place via an initial (3+2)-addition (1,3-dipolar cycloaddition), or by a (2+2)-addition followed by expansion of the metallacycle.

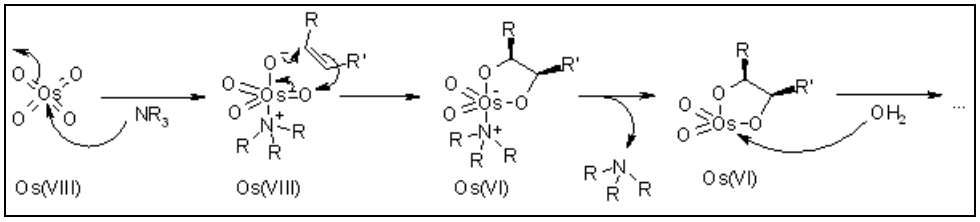


Scheme 6. (3+2) cycloaddition



Scheme 7. (2+2) cycloaddition

Quantum chemical calculations have shown an initial (3+2)-addition of OsO_4 to be energetically more favorable. However, this energy difference is substantially smaller in the related Re(VII) oxide additions, for example.



Scheme 8. Tertiary amines such as DMAP and pyridine accelerate the addition reaction

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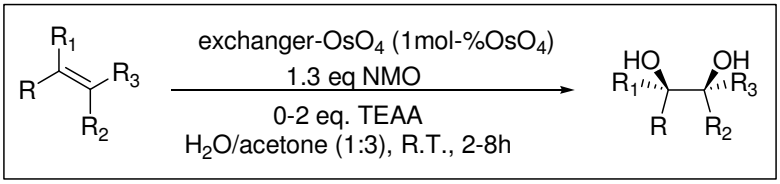
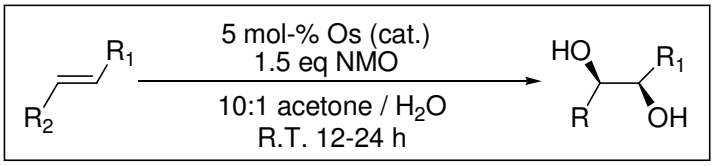
1.7.2 Different types of dihydroxylation



Scheme 9. Catalytic Asymmetric Dihydroxylation of Olefins with Reusable OsO_4^{2-} on Ion-Exchangers



Scheme 10. An *in situ* polymerization gave osmium tetroxide encapsulated in a polyurea matrix 1 (The use of these microcapsules as reusable catalyst in dihydroxylation and oxidative cleavage of olefins is described)

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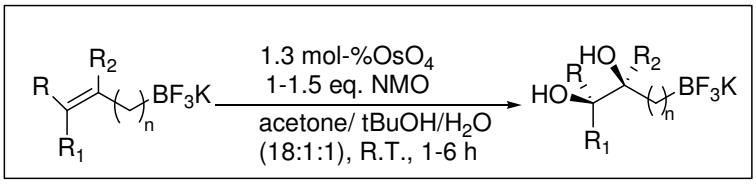


Scheme 11- An *in situ* polymerization gave osmium tetroxide encapsulated in a polyurea matrix 2 (The use of these microcapsules as reusable catalyst in dihydroxylation and oxidative cleavage of olefins is described.)

The cis-dihydroxylation of olefin-containing potassium alkyl- and aryltrifluoroborates proceeds readily in moderate to excellent yields. The resulting diols are efficient coupling partners in Suzuki-Miyaura-type reactions with both alkenyl and aryl bromides.

1.8 Cyclitols

Cyclitols are cycloalkanes containing one hydroxyl group on each of three or more ring atoms. These compounds, and others closely related to them, possess features of relative and absolute configuration that are characteristic of their class and have been extensively studied; but these features are not clearly displayed by general methods of stereochemical nomenclature, so that special methods of specifying their configuration are justified and have long been used. In other than stereochemical respects, their nomenclature should follow the general rules of organic chemistry.

Cyclitols have attracted a great deal of attention from synthetic chemists due to their diverse biological activity and their versatility as synthetic intermediates. Although many methods are available for the synthesis of cyclitol derivatives, there still remains a need for new methodology starting from simple starting materials since cyclitol derivatives are structurally very diverse and so new methodology could be applied for the synthesis of other highly functionalized cyclic compounds.

Cyclitols are also valuable due to their physiological activity as an insulin mediator and its limited availability.

Polyhydroxy cyclohexanes, such as inositols and quercitols, and polyhydroxy cyclohexenes, belong to the family of cyclitols. These compounds can exist in a

number of stereoisomers; inositols, quercitols, and condiritols have 9, 16, and 6 possible stereoisomers, respectively. Among them, the inositols have been studied mostly because of their important biological properties [40].

Various methadologies have been developed for the synthesis of enantiopure cyclitols and their derivatives. Recently, the synthesis of enantiopure cyclitols was achieved via the transformation of other cyclitols by some groups [41]. The microbial oxidation of halobenzenes was employed by Hudlickly et al. in the preparation of inositols [42]. The (RCM) ring-closing metathesis reaction has also been applied in asymmetric synthesis of cyclitols using sugars, tartrates, and polyhydroxyl allylsilanes as chiral building blocks. The Ferrier-II rearrangement [43] and the free radical cycliztion [44] of sugar derivatives are also the other useful methods developed by Ikegami and Yadav et al., respectively. Furthermore, the reduction of allylsilanes in combination with the asymmetric dihydroxylation reported by Landais and co-workers provides an easy access to cyclitols [45]. Even though there are a great deal of number of methods available for the asymmetric synthesis of cyclitols, they are generally target-specific and limited in applications. In this regard, there still remains a need so as to develop a method that can also be applied to synthesis of other functionalized cyclic compounds such as five-membered cyclopentitols and amino group-containing aminocyclitols. Cyclitols are known as a diverse group of cyclic, polyhydroxylated compounds which usually have a cyclohexane skeleton. Their biological activities vary according to their structures and many of them occur in phosphorylated form. Phosphorylated inositols have been shown to act as second messengers in many intracellular signal transduction processes via mediating the release of calcium from non-mitochondrial stores. Aminodeoxyinositols and conduritols occur in the aminocyclitol antibiotics, and a number of conduritol derivatives have important physiological actions, such as glycosidase inhibition, antifeedant, antibiotic, tumourstatic and growth

regulating activities. The synthetic activity is also boosted by the fact that various hydroxylated cyclohexene derivatives act as glycoside inhibitors [46,47].

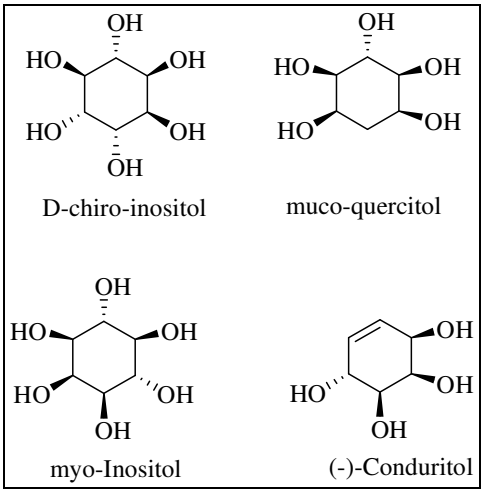


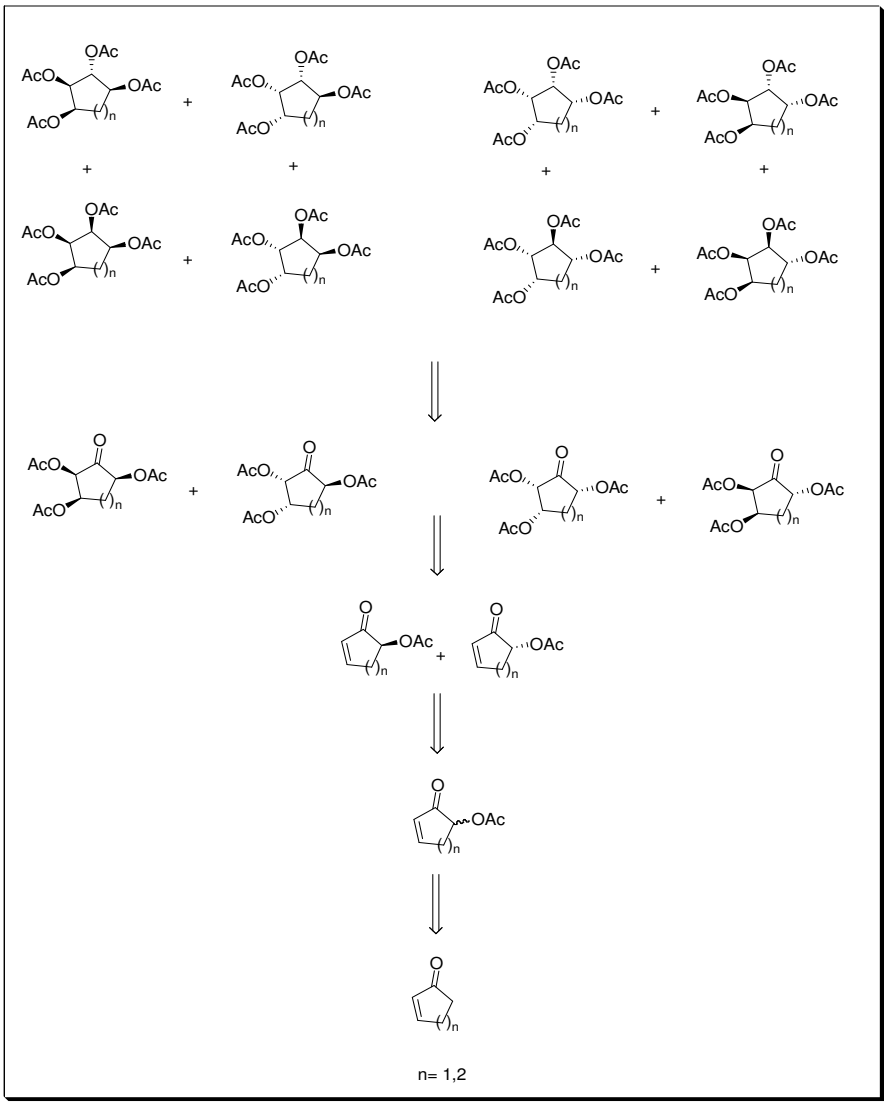
Figure 4- Different types of cyclitols

During the past few years synthesis of the cyclitols has seen a renaissance via the rapid development of novel strategies for the construction of polyhydroxylated systems in a stereochemically homogeneous manner. An interesting development is the utilization of the microbial oxidation of benzene and its derivatives [48,49].

1.9 The Aim of the Work

The objective of the work is to synthesize optically active cyclitol precursors stereoselectively via chemoenzymatic method. In our approach, α,ß-unsaturated cyclic enones, 2-cyclopenten-1-one and 2-cyclohexen-1-one, are regioselectively oxidized by using $Mn(OAc)_3$. The corresponding α'-acetoxylated compounds are subjected to enzymatic resolution by means of PLE hydrolysis to attain enantiomerically enriched α'-acetoxylated and α'-hydroxylated cyclic ketones. α'-hydroxylated cyclic ketones are protected via *in situ* acetylation in order to provide resistance against racemization. Upjohn dihydroxylation and Luche reduction are applied followingly to enantiomerically enriched compounds so as to reach target cyclitol precursors.

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



Scheme **12**. Retrosynthesis of the work

CHAPTER 2

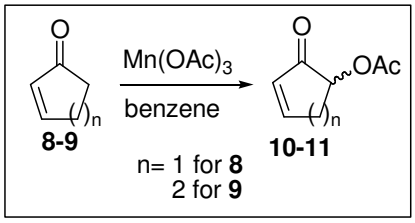
RESULTS AND DISCUSSIONS

2.1 Oxidation of α,β-unsaturated cyclic ketones

Synthetic methods for the selective oxidation of common functional groups generally occupy a central position in the syntheses of various complex natural products. α'-acetoxy-α,ß-unsaturated cyclic ketones are also important in synthetic methodologies. As the protective group for the hydroxyl functions in the α-hydroxy ketones acetate group is used [50,51].

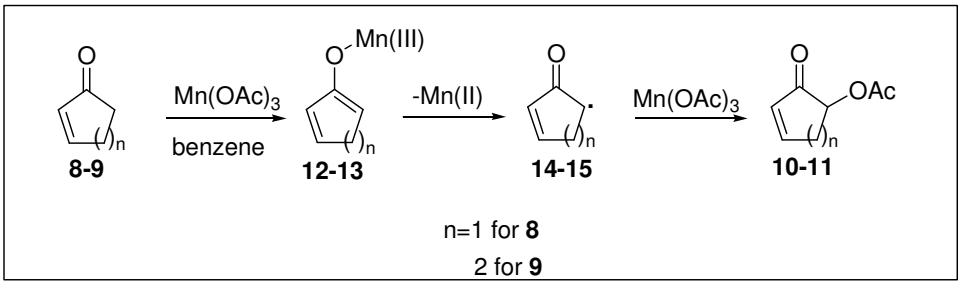
Manganese(III) acetate mediated acetoxylation of α,β-unsaturated ketones selectively at the α'-position is well documented. The other processes which provide regioselective oxidation of enones to α'-acetoxy enones are lead(IV) tetraacetate [52,53], MoOPh [54,55], triphenylphosphite ozonide (TPPO) [56,57], MCPBA [58,59] mediated oxidations.

In this method as shown in Scheme 13, α'-acetoxylation of specific α,β-unsaturated cyclic ketones, 2-cyclohexen-1-one and 2-cyclopenten-1-one, were carried out by using dry manganese triacetate and as the solvent dry benzene.



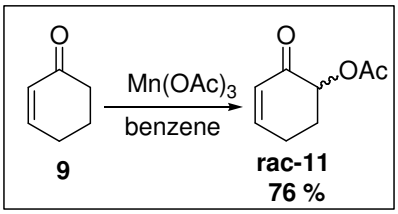
Scheme 13- $Mn(OAc)_3$ mediated acetoxylation

Suggested mechanism for this type of acetoxylation occurs via the formation of the Mn(III) enolate **12**,**13**, which loses Mn(II) to give α'-keto radical **14**,**15** [60] (Scheme 14). Oxidation of the intermediate **14-15** by another equivalent of $Mn(OAc)_3$ provides α'-acetoxy cyclic ketones.



Scheme 14- Mechanistic Representation of $Mn(OAc)_3$ mediated acetoxylation

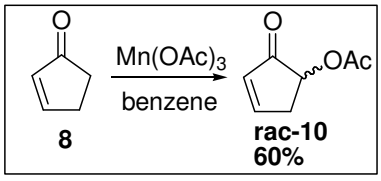
2.1.1 Synthesis of (±)-6-Acetoxy-2-cyclohexenone (rac-11)



Scheme 15-$Mn(OAc)_3$ mediated acetoxylation of 2-cyclohexen-1-one **(9)**

Regioselective oxidation of α,ß-unsaturated cyclic enone **(9)** was adjusted via $Mn(OAc)_3$ mediated acetoxylation. At the end of this process, racemic α'-acetoxy-2-cyclohexenone **(rac-11)** was obtained with 76 % yield. The product was characterized by ^{1}H-NMR and ^{13}C-NMR spectra. The spectra in accordance with the literature [60] are given in appendix. (Figure 11 and 12)

2.1.2 Synthesis of (±)-5-Acetoxy-2-cyclopentenone (rac-10)



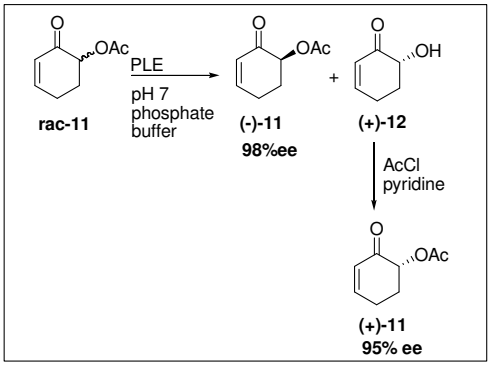
Scheme 16-$Mn(OAc)_3$ mediated acetoxylation of 2-cyclopenten-1one **(8)**

α'-acetoxy-2-cyclopenten-1-one **(rac-10)** was synthesized from the treatment of 2-cyclopentenone **(8)** with $Mn(OAc)_3$ in benzene as in cyclohexenone **(9)** case. (±)-5-Acetoxy-2-cyclopentenone **(rac-10)** was obtained with 60 % yield. Structure elucidation was done by using ^{1}H-NMR and ^{13}C-NMR spectra which are in accordance with the literature [61] was given in the appendix. (Figure 13 and 14)

2.2 Enzymatic resolution of α'-acetoxylated cyclic enones

Racemic α'-acetoxylated compounds were converted to enantiomerically enriched products via enzymatic hydrolysis. Throughout various studies in the literature [60,61], many ezymes such as PLE, CCL, HLE and PPL using a substrate: enzyme ratio from 1:1 to 1:0.5 were tested and PLE proved to be the most suitable one among all the enzymes for the enantioselective hydrolysis of the substrates. Absolute configuration determination was achieved by transforming enantiomerically enriched α'-acetoxylated compounds to the corresponding saturated α-acetoxy cyclic ketones, the configurations of which are known in the literature [62].

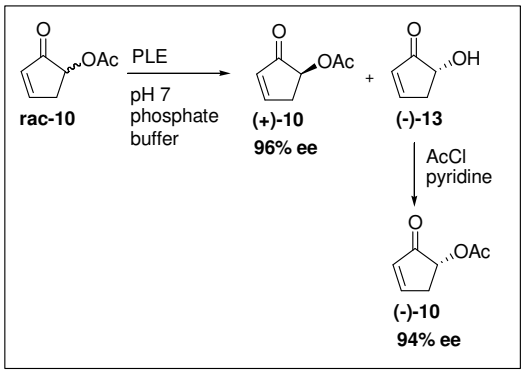
2.2.1 Enzymatic Resolution of (±)-6-Acetoxy-2-cyclohexenone (rac-11)



Scheme 17. Chemoenzymatic Resolution of **rac-11**

Enzymatic hydrolysis of (±)-6-acetoxy-2-cyclohexenone **(rac-11)** was carried out in pH 7 phosphate buffer in the presence of PLE. The resolution was followed via TLC. When 50 % conversion was monitored the reaction was ended to afford (-)-6-acetoxy-2-cyclohexenone **((-)-11)** with 49 % chemical yield. Enantiomeric excess value was determined by using HPLC with OD-H as a chiral column, as 98% ee. (+)-6-hydroxy-2-cyclohexenone **((+)-12)** was directly protected after purification through acetylation so as to prevent expected racemization. Compound **(+)-11** was obtained just as the enantiomer of the compound **(-)-11** after the protection procedure with 95% ee.

2.2.2 Enzymatic Resolution of (±)-5-Acetoxy-2-cyclopentenone (rac-10)



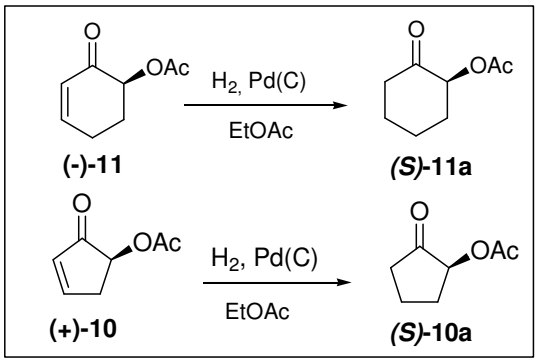
Scheme 18. Chemoenzymatic Resolution of **rac-10**

Enrichment of (±)-5-acetoxy-2-cyclopentenone **(rac-10)** was carried out through resolution by PLE in the same condition case mentioned above for the resolution of (±)-6-acetoxy-2-cyclohexenone **(rac-11)** to obtain compound **(+)-10** with 45% chemical yield, 96% ee. Due to the same racemization possibility, (-)-5-hydroxy-2-cyclopentenone **((-)-13)** was acetylated to afford compound **(-)-10** with 94% ee.

2.3 Absolute Configuration Determination

Absolute configurations of 2-acetoxycyclopentanone and 2-acetoxycyclohexanone, which are the saturated forms of our enantiomericaly enriched α'-acetoxylated cyclic enone products **(-)-11** and **(+)-10**, are known in the literature.[68] Hence, (+)-5-acetoxy-2-cyclopentenone **((+)-10)** and (-)-6-acetoxy-2-cyclohexenone **((-)-11)** were transformed into the corresponding saturated cyclic ketones which are (+)-2-acetoxycyclopentanone **((*S*)-10a)** and

(-)-2-acetoxycyclohexanone **(*(S)*-11a)** through hydrogenation by using Pd(C) catalysts. The specific rotation signs of each were compared with the literature value [62] and both were found in S absolute configuration.



Scheme 19. Absolute configuration determination of **(-)-11 and (+)-10**

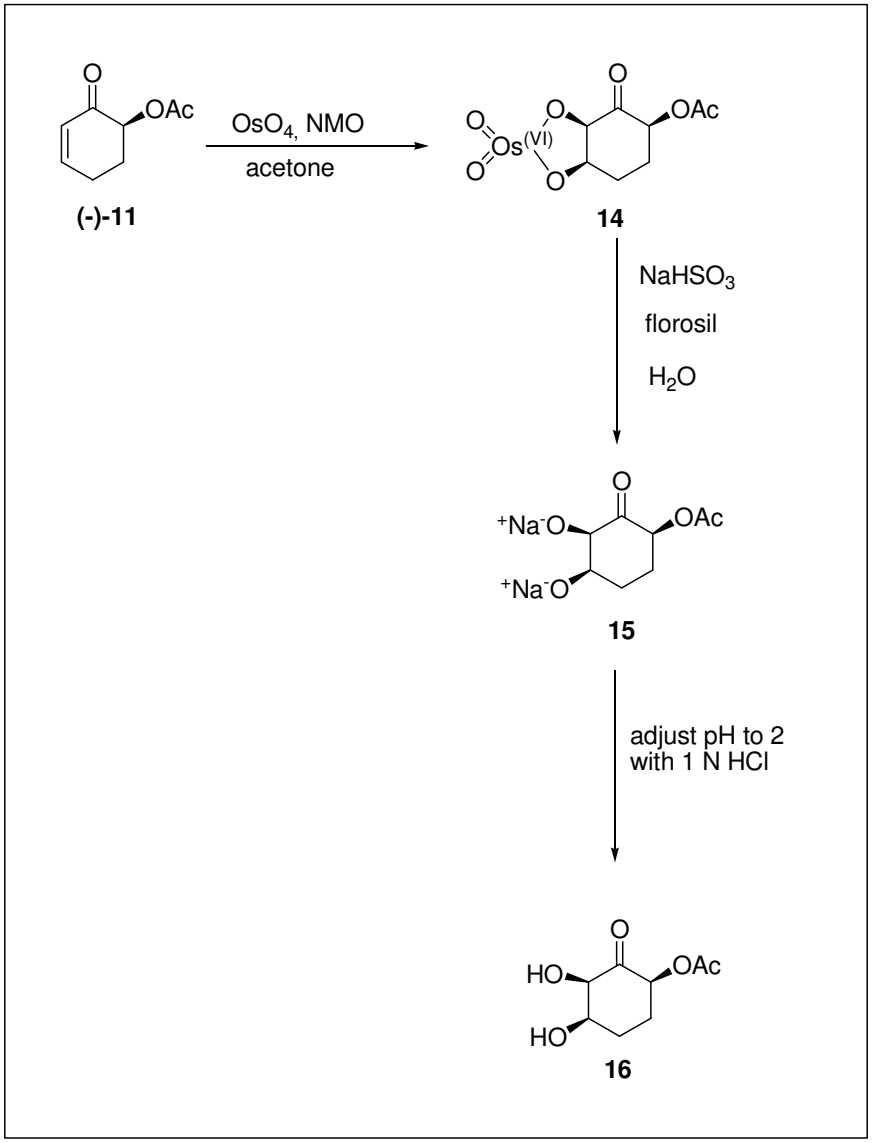
2.4 Dihydroxylation

Osmium-catalyzed oxidation reaction is one of the most useful methods for dihydroxylation of olefins so as to give the corresponding diols. The oxidation proceeds in the presence of catalytic amount of OsO_4 with a cooxidant such as N-methylmorpholine N-oxide (Upjohn procedure), potassium ferricyanide, hydrogen peroxide, molecular oxygen or hydrogen peroxide-flavin base [63]. Although these reactions have widespread applications in organic synthesis, there have been few large scale industrial applications due to toxicity, high cost performance, and volatility of the reagent. So as to overcome these problems, several immobilized osmium catalysts have been developed.

2.4.1 Upjohn Dihydroxylation

The classical Upjohn catalytic osmium tetroxide oxidation of olefins is a very powerful methodology to provide vicinal cis-diols. This methodology has been widely applied to stereoselective dihydroxylation of olefins.

2.4.2 Synthesis of (S)-6-acetoxy-(2R,3R)-dihydroxy-cyclohexanone (16)



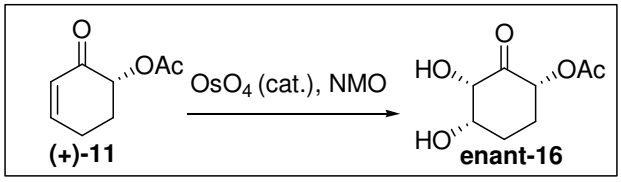
Scheme 20. Synthesis of (S)-6-acetoxy-(2R,3R)-dihydroxy-cyclohexanone **(16)**

In dihydroxylation approach, NMO was dissolved in H_2O under argon atmosphere as a first manner to perform as a cooxidant. The temperature of the medium was attained to -5 ^oC. Under inert atmosphere, OsO_4 stock solution was added to the medium including (S)-(-)-6-acetoxy-2-cyclohexen-1-one **((-)-11)** in acetone and NMO solution. The reaction was followed via TLC for 26 hours. As soon as the required conversion was obtained, that is, when the corresponding complex was considered to be formed, $NaHSO_3$, florosil and H_2O were added so as to break of the bonds attached to the Os (VI) and to form required salt. Resulting solution was allowed to stir for additional 12 hours. In order to obtain compound **16**, pH of the reaction mixture was adjusted to 2 by using 1N HCl. Because of the difficulty of the isolation of the compound **16**, only some amount of diol obtained was used to characterize the structure of the compound **16**. Remaining amount was subjected to *in situ* acetylation for protection.

Structure characterization of the corresponding diol **16** was identified by ^{1}H-NMR spectrum.

From the ^{1}H-NMR spectrum of the target compound **16**, we observed multiplet between 1.80-1.86 ppm for H_b and H_c, singlet at 2.11 for methyl protons of the acetoxy group, multiplet between 2.14-2.25 ppm for H_d and H_e, broad singlets at 2.6 ppm for proton of hydroxy group, 3.72 ppm for proton of other hydroxy group, 4.23 ppm for H_f, and 4.32 ppm for H_g and doublet of doublet at 5.19 ppm for H_a (J=12.2 and 6.8), as they were expected. (Figure 15)

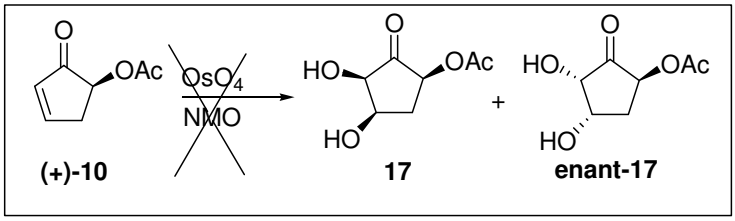
2.4.3 Synthesis of (R)-6-acetoxy-(2S,3S)-dihydroxy-cyclohexanone (enant-16)



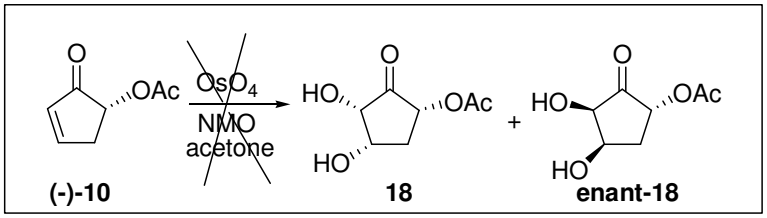
Scheme 21. Synthesis of (R)-6-acetoxy-(2S,3S)-dihydroxy-cyclohexanone **(enant-16)**

In such synthetic design, same dihydroxylation process was applied to afford (R)-6-acetoxy-(2S,3S)-dihydroxy-cyclohexanone **(enant-16)** just as the enantiomer of the compound **16**. Due to same racemization possibility was under consideration, except some amount purified for the characterization, compound **enant-16** was protected through acetylation. Characterization via ^{1}H-NMR spectrum proved that corresponding compounds **16** and **enant-16** are in enantiomeric relation.

2.4.4 Synthesis of (S)-5-acetoxy-2,3-dihydroxycyclopentanone (17, enant-17) and (R)-5-acetoxy-2,3-dihydroxycyclopentanone (18, enant-18)



Scheme 22. Dihydroxylation of (S)-(+)-5-acetoxy-2-cyclopentenone **((+)-10)**



Scheme 23-Dihydroxylation of (R)-(-)-5-acetoxy-2-cyclopentenone **((-)-10)**

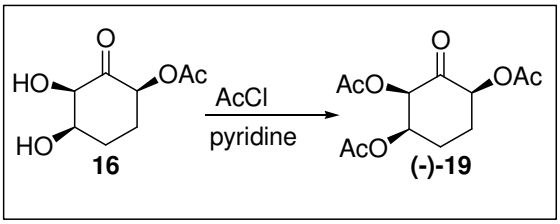
In such synthetic concern, dihydroxylation process was performed in the same manner with the cyclohexenone type derivative of both (S)-(+)-5-acetoxy-2-cyclopentenone **((+)-10)** and (R)-(-)-5-acetoxy-2-cyclopentenone **((-)-10)**. Although a new product formation was monitored for both via TLC, after purification procedure no isolation was attempted. The reaction temperature was increased then to room temperature so as to estimate whether the fault was because of the kinetic reasons or not, but no change was observed. There can be many factors for such decomposition. First off all, the structure of the

compound was so rigid and when it was thought in three dimensional form it was seen that osmium coordination was rather difficult than cyclohexenone type derivative because of steric factors. Furthermore, racemization could have occured during reaction. Also, osmium correlation may have been occured, but the bulkiness of the structure could have caused to stretch of the molecule and so could have caused some bond deformations and at the end decomposition of the compound.

2.5 Protection of diols (16 and enant-16)

The corresponding diols obtained **16 and enant-16** were directly protected via simple acetylation so as to prevent decomposition.

2.5.1 Synthesis of (2R,3R,6S)-triacetoxy-cyclohexanone ((-)-19)



Scheme 24-Acetylation of (S)-6-acetoxy-(2R,3R)-dihydroxy-cyclohexanone **(16)**

The corresponding diol obtained **16** was acetylated *in situ* to attain (2R,3R,6S)-triacetoxy-cyclohexanone **((-)-19)** with 85% chemical yield.

Product characterization was identified by ^{1}H-NMR and ^{13}C-NMR spectra.

From the ^{1}H-NMR spectrum, we observed multiplet between 1.91-2.16 ppm for H_c and H_d, multiplet between 2.17-2.24 ppm for H_e and H_g, singlet for the methyl protons of three different acetoxy groups at 2.10 ppm, at 2.16 ppm, at 2.19 ppm, and doublet of doublet (J=6.8 and 4.9) at 5.34 ppm for H_f proton, doublet (J=3.3) at 5.41 ppm for H_b, broad singlet at 5.65 ppm for H_a, as they were expected. (Figure 16)

From the ^{13}C-NMR spectrum, at 195.8 ppm signal of ketone carbon, at 169.8 ppm, 169.5 ppm, 169.4 ppm signals of carbonyl carbons belong to acetoxy groups, at 75.4 ppm, 74.5 ppm, 72.3 ppm signals of carbons attached to three acetoxy groups, at 26.8 ppm and 25.0 pm signals for methylene carbons, and lastly at 20.8 ppm, 20.5 ppm and 20.4 ppm, signals for methyl carbons were observed as they were expected. (Figure 17)

The corresponding compound **(-)-19** was obtained as solid and we were concerned that it could have been directly recrystallized so as to determine the absolute configuration of the product clearly. Among many recrystallization techniques, the one which provided us the required crystals was, dissolving of the compound **(-)-19** in diethyl ether and then, forming a layer with dichloromethane. As a result of this approach, X-Ray diffraction of the corresponding crystals was examined and absolute configuration of the target compound **(-)-19** was determined as (2R,3R,6S)-triacetoxy-cyclohexanone **((-)-19)**. So as to identify the absolute configuration of the product we used the knowledge of the absolute configuration of the chiral center C_4 before. Then, we determined the absolute configurations of new chiral centers formed, C_1 and C_2, relatively by taking the C_4 center as the reference point.

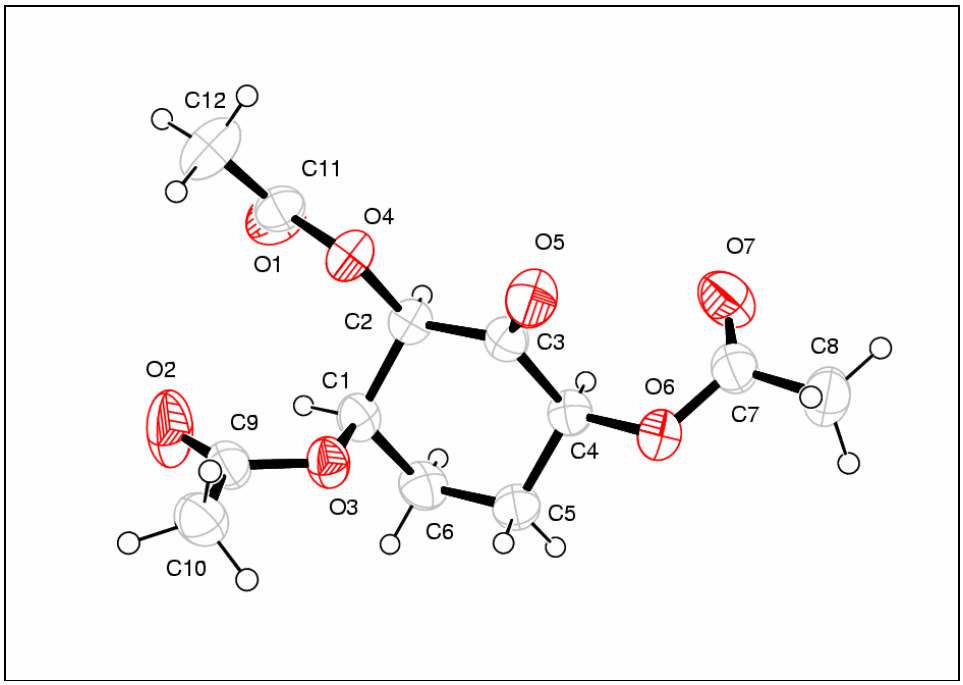


Figure 5. Ortep structure of (2R,3R,6S)-triacetoxy-cyclohexanone **((-)-19)**

ORTEP view of the molecule with displacement ellipsoids drawn at the 50% probability level is estimated in Figure 5.

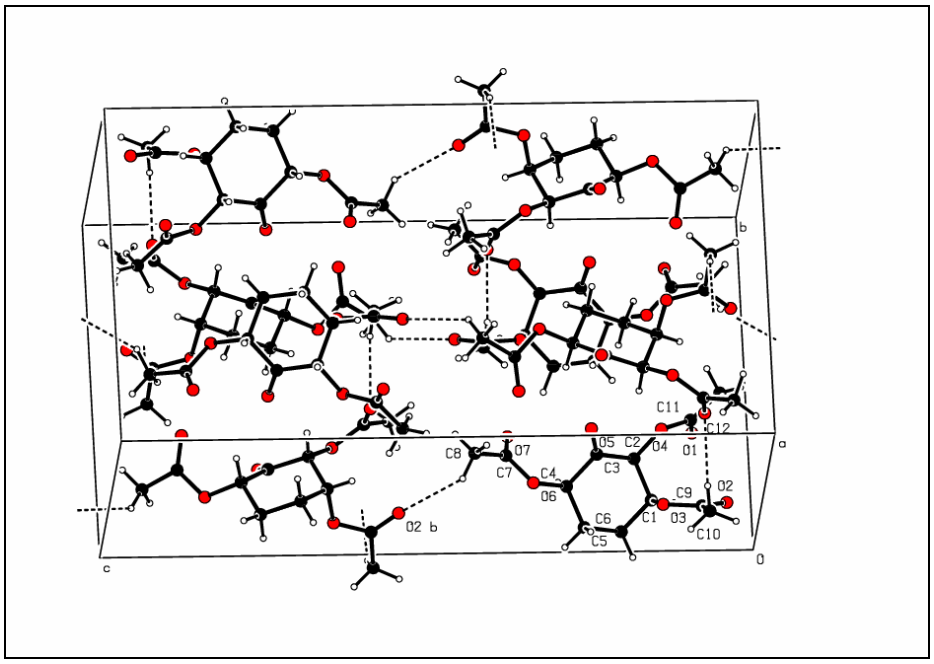
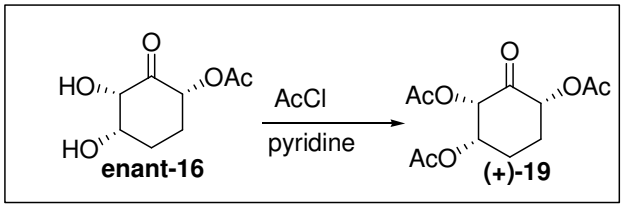


Figure 6. View of H-bonds and unit cells through a-axis

2.5.2 Synthesis of (2S,3S,6R)-triacetoxy-cyclohexanone ((+)-19)



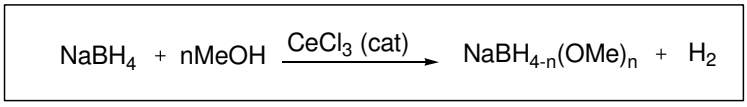
Scheme 25. Acetylation of (R)-6-acetoxy-(2S,3S)-dihydroxy-cyclohexanone **(enant-16)**

In the same manner mentioned above for compound **16**, compound **enant-16** was subjected to direct acetylation due to decomposition and isolation problems. Characterization of the product was performed via ^{1}H-NMR and ^{13}C-NMR spectra. Since the compound **(+)-19** was just the enantiomer of the compound **(-)-19**, both spectra of the products correlated each other absolutely.

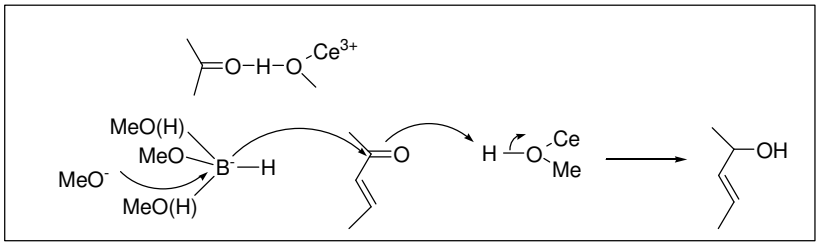
Only difference for (2R,3R,6S)-triacetoxy-cyclohexanone **((-)-19)** and (2S,3S,6R)-triacetoxy-cyclohexanone **((+)-19)** was specific rotation values in sign, which was monitored for (2S,3S,6R)-triacetoxy-cyclohexanone **((+)-19)** as (+) whereas for (2R,3R,6S)-triacetoxy-cyclohexanone **((-)-19)** as (-).

2.6 Reduction of ketone group via $NaBH_4$

The selective 1,2-reduction of enones with sodium borohydride is achieved in combination with $CeCl_3$ and $CeCl_3$ is a selective Lewis acid catalyst for the methanolysis of sodium borohydride. The resulting reagents, various sodium methoxyborohydrides, are harder reducing agents and therefore effect 1,2-reduction with higher selectivity. Furthermore, $CeCl_3$ activates methanol [64].

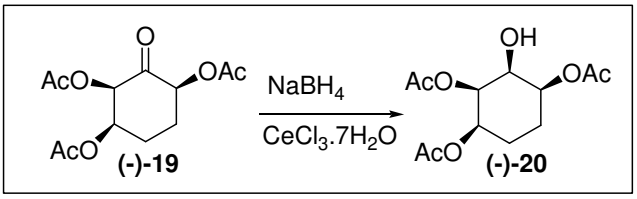


Scheme 26. Luche Reduction



Scheme 27- Suggested Mechanism for Luche Reduction

2.6.1 Reduction of (2R,3R,6S)-triacetoxy-cyclohexanone ((-)-19)

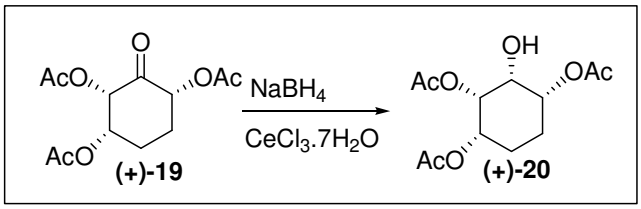


Scheme 28. Synthesis of (2R,3R,6S)-triacetoxy-cyclohexan-1S-ol **((-)-20)**

In this consideration, (2R,3R,6S)-triacetoxy-cyclohexanone **((-)-19)** was treated with $NaBH_4$, $CeCl_3.7H_2O$ in MeOH to afford (2R,3R,6S)-triacetoxy-cyclohexan-1S-ol **((-)-20)**, with 82% chemical yield.

Because the resolution of the ^{1}H-NMR spectra of the target compound **((-)-20)** was not so good, it was directly protected by simple acetylation technique so as to identify the absolute configuration of the reducing part more clearly.

2.6.2 Reduction of (2S,3S,6R)-triacetoxy-cyclohexanone ((+)-19)

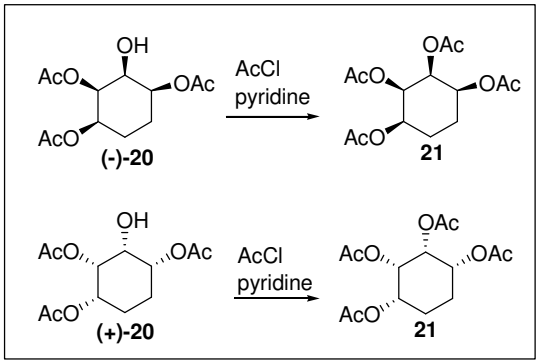


Scheme 29-Synthesis of (2S,3S,6R)-triacetoxy-cyclohexan-1R-ol **((+)-20)**

Same approach was applied for the reduction of (2S,3S,6R)-triacetoxy-cyclohexanone **((+)-19)** so as to obtain (2S,3S,6R)-triacetoxy-cyclohexan-1R-ol **((+)-20)** with 80 % chemical yield as a single diastereomer as it was expected.

Protection was also applied through acetylation to the compound **((+)-20)** so as to identify the absolute configuration of the chiral center newly formed.

2.7 Synthesis of meso-tetracetoxy-cyclohexanes (21)



Scheme 30. Synthesis of meso-tetracetoxy-cyclohexanes **(21)**

The corresponding alcohol **(-)-20** was directly protected via acetylation so as to clarify the absolute configuration of the target compound **(-)-20**. In the result, meso-tetracetoxy-cyclohexane **21** was obtained with 87 % chemical yield. Same process was applied for the compound **(+)-20** to afford meso-tetracetoxy-cyclohexane **21** with 84 % chemical yield. Characterization was applied by ^{1}H-NMR and ^{13}C-NMR spectra.

For ^{1}H-NMR, we observed singlet at 2.07 ppm, singlet at 2.08 ppm, triplet (J=6.7) at 5.22 ppm for H_b of the proton, and broad singlet at 5.97 ppm for H_a proton (Figure 18), as correlated with the expectations.

For ^{13}C-NMR, we observed at 169.9 ppm carbonyl carbon, at 69.0 ppm for C_1, at 68.4 ppm for C_2, at 22.9 ppm methylene carbon, at 20.9 ppm one of the methyl carbon, and at 20.7 ppm the other type of methyl carbon. (Figure 19)

Specific rotation value of the compound **21** was measured to be $[\alpha]^{25}_{D}$=0 (c 0.02, $CHCl_3$), and this value was provided us to estimate one more time after NMR spectra, the structure was absolutely meso. Therefore we could have estimated clearly that the absolute configuration of the hydroxy group of compound **(-)-20** as in (S) configuration, and the absolute configuration of the hydroxy group of compound **(+)-20** as in (R) configuration.

CHAPTER 3

INTRODUCTION

3.1 Carbasugars

Recent glycobiology studies have estimated the versatile and critical roles of cell surface-carbohydrates in the recognition processes of cells via carbohydrate-protein interactions, which eventually result in cell adhesion, cell growth, immune response, fertilization, viral infection, inflammation, metastasis of cancer,etc. [65]. The most important issues in glycobiology now are the understanding at the molecular level of the carbohydrate-protein interactions in pathophysiologically important processes, and how to efficiently develop molecular tools that can regulate such carbohydrate-protein interactions, thus leading to therapies. Most of the researches on this subject have been focused on developing glycomimetic molecules both as biochemical probes and potential drug candidates [66]. These researches have shown the requirement for the diverse structures of carbohydrate mimetics, among which non-hydrolyzable analogues [67] which are highly desirable because of their stability in vivo.

Carbasugars are suitable building blocks for nonhydrolayzable carbohydrate mimetics due to their inherent stability of to glycosidases and also

their structural resemblance to cyclic monosaccharides. Ogawa et al. have developed this area by synthesizing a number of carbasugars, including disaccaride and trisaccaride analogues as glycosidase inhibitors or glycosyltransferase inhibitors [68]. They also synthesized some major carbasugar analogues of glycosylceramide as potential ceramide glycosyltransferase inhibitors.

Application of carbasugars to diverse mimetics is certainly still limited because of the pausity of readily available carbasugars as building block. So as to have a widely usage as carbohydrate mimetics, all required carbasugars such as enantiopure stereoisomers of carbasugars as glycosyl acceptor mimics and their 1,2-epoxide derivatives as glycosyl donor mimics ought to be made available by practical routes. Futhermore, the available builing blocks of 16(32) of possible stereoisomers [69] and 8(16) of possible 1,2-epoxides should be appropriately protected in order to be used for further synthetic elaborations. Despite of being a variety of synthetic methods since the pioneering work by McCasland et al. in 1966, there are no general and practical routes to be defined for ready access to the carbasugar building blocks.

The term carbasugar is recently used to define structures in which the endocyclic oxygen atom in a carbohydrate has been substituted by a methylene group. Two synthetic strategies based on radical cyclization of modified carbohydrates have been currently developed [70, 71].

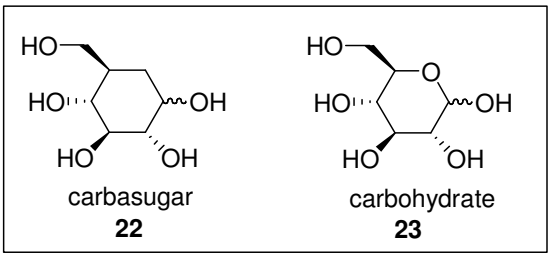


Figure 7- carbasugar **22** and carbohydrate **23**

The first approach is based on a 6-*endo-trigonal* radical cyclization and the second one features a 6-*exo-digonal* radical ring closure. The implementation of these methods have allowed the preparation of seven different carbasugars and some derivatives of them.

These studies have also provided a synthetic method which permits a *one pot* correlation between terminal alkynes and 1,2-diols [72].

3.2 Carbocyclic Nucleosides

Infectious diseases are unfortunately posing increasingly severe health risks, as evidenced by the recent SARS flu epidemic and the rapid spread of AIDS in developing countries. Accordingly, extensive researches have been directed at finding effective therapeutic agents for the treatment of viral infectious as well as cancers. Therefore, carbocyclic nucleosides have gained considerable attention [73].

The natural carbocyclic nucleosides, neplanocin **24** and aristeromycin **25** have potent antivirial activities.

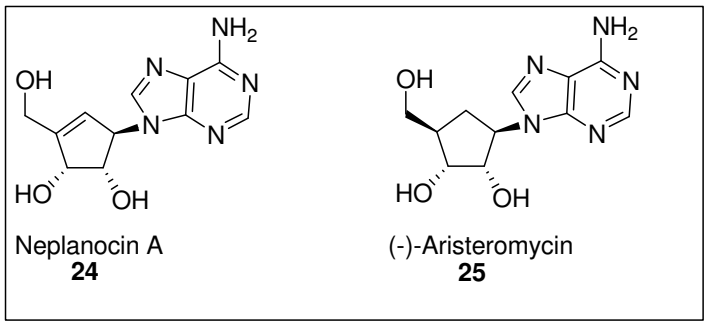


Figure 8- Neplanocin A **24** and (-)-Aristeromycin **25**

(-)-Aristeromycin **25** is a carbocyclic analogue of adenosine which terminates viral growth by inhibiting S-adenosyl-$_L$-homocycsteine hydrolase [74]. However, the high cyctotoxicity of aristeromycin, presumably caused by the metabolism of **24**, to its 5'-phosphates, has greatly hindered its therapeutic application [75]. In the search for a less toxic analogue of aristeromycin, Schneller and co-workers found that the 5'-nor compound **(±)-26** had improved antivirial activity with no cytotoxicity [76]. Recently, the same workers found that the (-)-enantiomer of **26** was more active than the (+)-enantiomer [77].

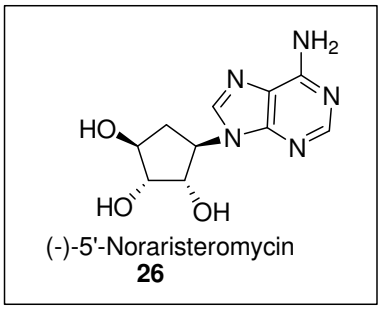


Figure 9- (-)-5'-Noraristeromycin **26**

Sinefungin **27** is an other natural nucleoside firsly isolated from Streptomyces griseolus in 1973 and S. İncarnatus in 1976 [78]. Preliminary works showed that sinefungin inhibited the growth of several fungi and viruses and that it showed significant antiparasitic [79] activity in vitro.

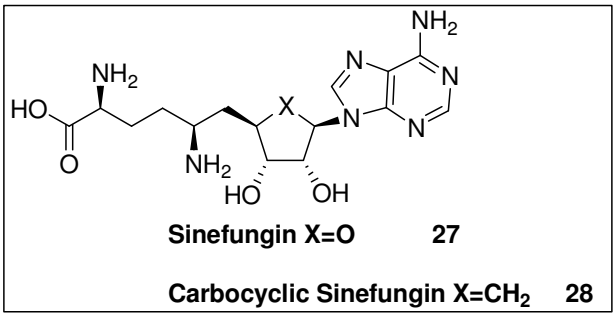


Figure 10- Sinefungin 27 and Carbocyclic Sinefungin **28**

Despite of sinefungin **27** showing strong bioactivity against viruses and parasites, it caused fatalities, probably resulting from its nephrotoxic side effects, when tested in vivo with larger mammals [80]. Methyltransferases are important for many biological functions in humans and also other animals. Inhibition of this important enzyme to affect some desirable therapeutic activity can be difficult due to its toxicity. Thus the development of selective methyltransferase inhibitors continues to be with considerable interest [81].

3.3 Ribonucleosides

The synthesis of new ribonucleosides is an essential research area in the investigation of new therapeutically useful reagents [82]. It is well known that

natural and synthetic nucleosides are potent chemotherapeutic compounds using in the treatment of HIV infection.

The study of the conformations of nucleosides is the important target because the conformational profile may have direct implications in their drug activity, being an important target for new drug design. When the conformational properties of drug are known which play an important role in establishing its therapeutic value, any newly synthesizing analogue must have similar conformational properties to enhance the probability that it will bind to the receptor target.

3.4 Aim of The Work

The aim of the work is to develop a new methodology to prepare a target precursor for synthesizing carbocyclic nucleosides and ribonucleosides which have very important roles in drug usage for the treatment of many diseaes such as AIDS and HIV infection. Dimethyl malonate is chosen as the starting material. In our synthetic design, firstly dimethyl cyclopent-3-ene-1,1-dicarboxylate **(29)** was obtained so as to reach in former manner 3-cyclopentene-1,1-dicarboxylic acid **(30)**, and in latter manner cyclopent-3-enecarboxylic acid **(31)**. As a result, 6-iodo-2-oxa-bicyclo[2.2.1]heptan-3-one **(32)** is synthesized and converted into to the target precursor which is 2-oxa-bicyclo[2.2.1]hept-5-en-3-one **(33)**.

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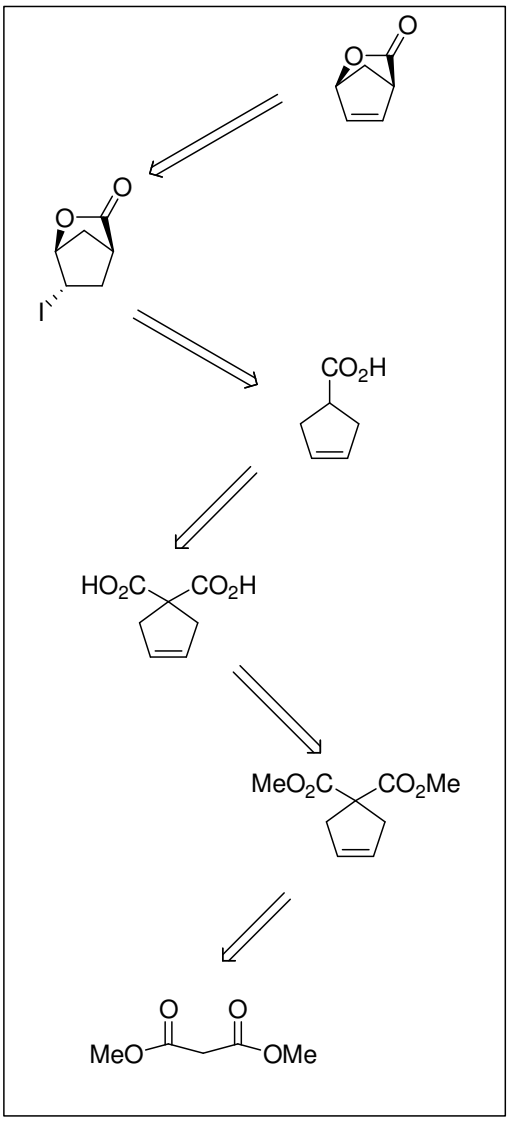
The aim of this work is shown retrosynthetically in Scheme 31.



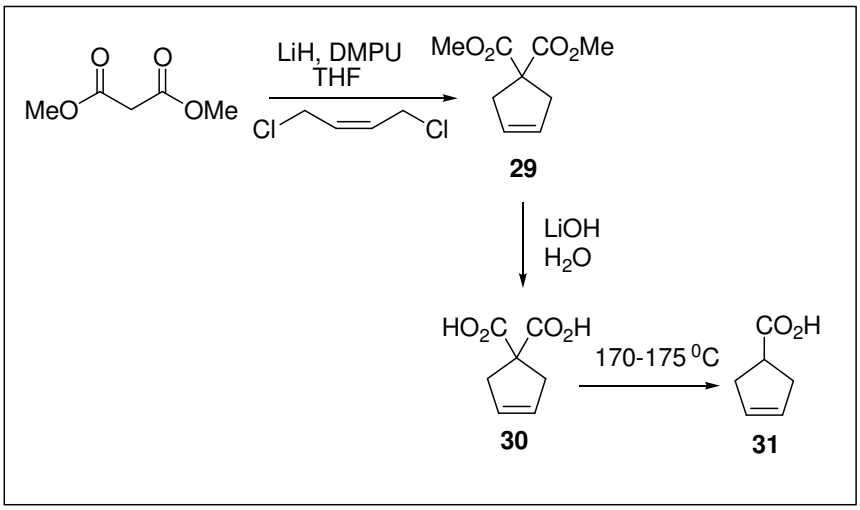
Scheme 31- Retrosynthesis of the nucleoside precursor

CHAPTER 4

RESULTS AND DISCUSSIONS

4.1 Synthesis of cyclopent-3-ene-1,1-dicarboxylate (29), 3-cyclopentene-1,1-dicarboxylic acid (30), and cyclopent-3-enecarboxylic acid (31)

In this synthetic design, dimethyl cyclopent-3-ene-1,1-dicarboxylate **(29)**, in the appearence of brown oil were adjusted via the reaction between dimethyl malonate and cis-1,4-dichloro-2-butene, providing basic condition. *In situ*, compound **29** was transformed into 3-cyclopentene-1,1-dicarboxylic acid **(30)** as an off-white solid by using lithium hydroxide monohydrate. The resulting compound, 3-cyclopentene-1,1-dicarboxylic acid **(30)**, was directly adjusted to decarboxylation process by increasing the temperature of the medium between 170-175 $^{\circ}$C so as to afford cyclopent-3-enecarboxylic acid **(31)**, with 89 % yield.



Scheme 32- Synthesis of cyclopent-3-ene-1,1-dicarboxylate **(29)**, 3-cyclopentene-1,1-dicarboxylic acid **(30)**, and cyclopent-3-enecarboxylic acid **(31)**

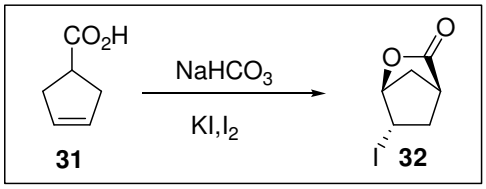
The product was characterized by ^{1}H-NMR and ^{13}C-NMR spectra. The spectra are given in appendix (Figure 20,21). NMR spectra are in accordance with the literature [83].

4.2. Iodolactonization

Iodenol lactone derivatives have been studied extensively due to their usefulness as synthetic intermediates and their biological activities [84]. Halolactonizations are useful chemical transformations for the construction of lactones from olefinic carboxylic acids, carboxylic esters, and amides [85]. In extensive studies on the stereoselectivity of these reactions, it has been proved that the stereochemistry of the halolactonized product can be controlled by

substrates or reagents. While the substrate controlled method has been studied in some detail [86] and applied in the synthesis of natural products [87], there is lack of understanding of how the stereochemistry of the lactones is influenced under reagent-controlled reactions.

4.2.1 Synthesis of 6-iodo-2-oxa-bicyclo[2.2.1]heptan-3-one (32)



Scheme 33- Synthesis of 6-iodo-2-oxa-bicyclo[2.2.1]heptan-3-one **(32)**

Corresponding cyclopent-3-enecarboxylic acid **(31)** was treated with $NaHCO_3$, KI and I_2 so as to apply iodolactonization approach. In such consideration, cyclopent-3-enecarboxylic acid **(31)** was converted to 6-iodo-2-oxa-bicyclo[2.2.1]heptan-3-one **(32)**, with 81 % chemical yield, as an off white solid.

The product was characterized via ^{1}H-NMR and ^{13}C-NMR spectra.

For ^{1}H-NMR spectrum, we observed doublet (d, J=11.2 Hz) at 2.35 ppm for H_e, doublet of triplet (dt, J=14.4 and 4.0 Hz) at 2.40 ppm for H_c, doublet (d, J=11.1 Hz) for H_f at 2.47 ppm, doublet of doublet (dd, J=14.2 and 8 Hz) for H_b at 2.60 ppm, broad singlet at 2.90 ppm for H_g, singlet at 4.97 ppm for H_a, broad singlet at 4.09 ppm for H_d. (Figure 22)

For ^{13}C-NMR, we observed carbonyl carbon (C_3) at 176.2 ppm, carbon attached to the oxygen (C_2) at 84.9 ppm, C_4 carbon at 43.1 ppm, C_5 at 36.8 ppm, C_6 at 36.2 ppm, and C_1 at 15.9 ppm correlated with the expectations. (Figure 23)

Apart from characterization via ^{1}H-NMR and ^{13}C-NMR spectra, the target compound 32 was also identified by full analysis including DEPT (Figure24), HMQC (Figure 26), HMBC (Figure 27), and COSY (Figure 25).

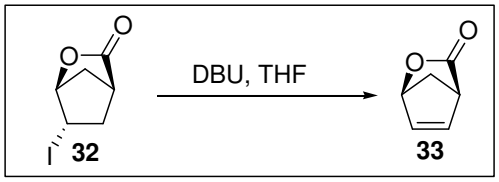
DEPT (Distortonless Enhanced by Polarization Transfer) correlates which carbons for which signals. For DEPT spectrum of compound 32, methylene carbons were directly estimated as the down signals of the base-line at 36.2 ppm for C_6 and at 36.8 ppm for C_5. Moreover other carbons were also observed as, at 15.9 ppm for C_1, at 43.1 ppm for C_4, at 84.9 ppm for C_2, and at 176.2 ppm for C_3. (Figure 24)

COSY (Correlation Spectroscopy) provides to estimate is there any interaction between two protons. For COSY spectrum of compound **32**, diagonal correlation attaintment estimated the correction of the structure with itself. All the protons correlation with themselves were observed. By means of other signals, couplings were identified. H_a proton was observed to be affected by H_c proton, means there is M or W coupling. For bicyclic systems, couplings over four bonds called M or W coupling. H_g was seen to be coupled by H_c proton through J^3 vicinal coupling. Furthermore, H_a proton was estimated to give coupling with H_e or H_f proton again over M or W coupling (J^4). H_d was also attained to give coupling with H_b and H_c through J^3 coupling. Moreover, Hg coupling was seen to couple with H_c over vicinal coupling. (Figure 25)

HMQC includes correlation of ^{13}C-NMR and ^{1}H-NMR spectra. For HMQC of compound **32**, H_a proton correlates with C_2, H_d proton with C_1, H_g proton with C_4, H_b, H_c protons with C_6 and H_e and H_f protons with C_5. (Figure 26)

For HMBC (Heteronuclear Multi Bond Coherence), ^{13}C-NMR and ^{1}H-NMR spectra of the molecule is compared to inform about the correlations of the couplings over two or three bonds, correlations over one bond is not observed on HMBC spectra. By means of such spectrum, H_a was observed to be affected by C_1, C_6, C_4, C_3; H_d by C_6 and C2; Hg by C_1 and C_2; H_b by C_2, C_5, C_4, C_3; H_f by C_6, C_4, C_1; H_c by C_5, C_1, C_3, and H_e by C_2, C_5, C_3, C_1. (Figure 27)

4.3 Synthesis of 2-oxa-bicyclo[2.2.1]hept-5-en-3-one (33)



Scheme 34- Synthesis of 2-oxa-bicyclo[2.2.1]hept-5-en-3-one **(33)**

In such synthetic design, corresponding 6-iodo-2-oxa-bicyclo[2.2.1]heptan-3-one **(32)** was transformed into unsaturated lactone, 2-oxa-bicyclo[2.2.1]hept-5-en-3-one **(33)** with 47% yield, as a brown liquid, via elimination of iodine using DBU.

The product was identified by ^{1}H-NMR and ^{13}C-NMR spectra.

For ^{1}H-NMR spectrum, we observed multiplet between 2.35-2.45 ppm, multiplet between 2.51-2.52 ppm, multiplet for the proton close to carbonyl carbon between 2.87-2.92 ppm, multiplet for the proton close to oxygen between 4.08-4.14 ppm, singlet at 5.01 ppm for one of the olefinic protons, and singlet for the other olefinic proton at 5.70 ppm. Due to elucidation problem, although ^{13}C-NMR spectrum of the compound **33** was clear, ^{1}H-NMR spectrum includes some dirtiness caused by solvent, so it is not estimated in Appendix.

For ^{13}C-NMR spectrum, at 181.9 ppm carbonyl carbon, at 127.9 ppm olefinic carbons, at 83.9 ppm carbon close to oxygen, at 40.4 ppm carbon close to carbonyl group and at 35.2 ppm bridged carbon were observed. (Figure 28)

CHAPTER 5

EXPERIMENTAL

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

^{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), bs (broad singlet), d (doublet), dd (doublet of doublet), dt (doublet of triplet), t (triplet), p (pentet), sxt (sextet), m (multiplet).

Flash column chromatography was applied 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.

5.1 General Procedure for the Synthesis of (±)-6-Acetoxy-2-cyclohexenone (rac-11) and (±)-5-Acetoxy-2-cyclopentenone (rac-10)

A mixture of $Mn(OAc)_3$ (8.36g, 31.1 mmol) in benzene (160 mL) was refluxed for 45 min using a Dean-Stark trap. Then the mixture was allowed to cool to room temperature and 2-cyclohexenone **(9)** (1.5 g, 15 mmol) was gradually added. The mixture was allowed to reflux until the dark brown color disappeared and also monitored by TLC. The reaction mixture was diluted with an 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 seperated by flash column chromatography using ethyl acetate/hexane(1:2) as eluent to afford the (±)-6-acetoxy-2-cyclohexenone **(rac-11)** (1.80 g, 76%).

(±)-11. 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): 194.0, 169.4, 150.1, 128.4, 73.9, 28.5, 25.5, 20.7

The same procedure which was applied for the acetoxylation of 2-cyclohexenone was used for the acetoxylation of 2-cyclopentenone to afford the (±)-5-acetoxy-2-cyclopentenone **(rac-10)** (1.08 g, 60 %).

(±)-(10). 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): 202.5, 169.9, 160.3, 131.7, 70.4, 34.5, 19.7

5.2 General Procedure for the Enzymatic Resolution of rac-10 and rac-11

The (±)-6-acetoxy-2-cyclohexenone **(rac-11)** (1.4 g, 9.1 mmole) 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. The unreacted (*S*)-(-)-6-acetoxy-2-cyclohexenone **((-)-11)** (0.690g, 49%) and (*R*)-(+)-6-hydroxy-2-cyclohexenone **((+)-12)** were separated by flash column chromatography.

(S)-(-)-(11): colorless oil, 98% ee, $[\alpha]_D$=-64.199 (c 0.02, MeOH)

The same process was adjusted for (±)-5-acetoxy-2-cyclopentenone **(rac-10)** so as to obtain (S)-(+)-5-acetoxy-cyclopentenone **((+)-10)** (0.41 g, 45%) and (R)-(-)-5-hydroxy-cyclopentenone **((-)-13)** via flash coloumn chromatography.

(S)-(+)-(10): colorless oil, 96% ee, $[\alpha]_D$=+60.3 (c 0.56, $CHCl_3$)

5.3 Hydrogenation of *(S)*-11 and *(S)*-10

To a stirred solution of the compound **((-)-11)** (20 mg) in EtOAc (20 mL), Pd(C) (10 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)*-11a**. The same procedure was applied for the transformation of **(+)-10** into ***(S)*-10a**. All spectroscopic data of the products are in accordance with ***(S)*-11a** and ***(S)*-10a**, respectively.

5.4 General Procedure for Acetylation of (R)-12 and (R)-13

(R)-6-hydroxy-2-cyclohexenone **((+)-12)** (0.191 g, 1.71 mmole), was mixed with (0.268g, 3.39 mmole) dry pyridine in 25 ml CH_2Cl_2 under inert atmosphere, at 0 ^oC for $^1/_2$ hours. Then, acetylchloride (0.202g, 2.56mmole) was added and mixed for 12 hours at room temperature. The organic phase was extracted with 0.1 N HCl, $NaHCO_3$, brine respectively. Dried over $MgSO_4$, filtrated and evaporated. The crude product was seperated by flash coloumn chromatography using ethyl acetate/hexane (1:2) as eluent to afford the R-(+)-6-acetoxy-2-cyclohexenone **((+)-11)** (0.250g, 95%). The same general procedure was applied for the acetylation of (R)-5-hydoxy-2-cyclopentenone **((-)-13)** to obtain (R)-5-acetoxy-2-cyclopentenone ((-)-10) (0.48 g, 97%)

5.5 General Procedure for Dihydroxylation of α'-acetoxylated cyclic enones

5.5.1 OsO_4 stock solution

Firstly, acetone was allowed to reflux for four hours and after one hour reflux $KMnO_4$ in solid form was added so as to purify acetone. Then distillation was applied in order to obtain dry acetone as solvent for OsO_4 stock solution. The required volume of distillate was measured and placed into a dark bottle under argon atmosphere. Then, OsO_4 capsule was broken carefully and left into the bottle again under argon atmosphere, and so the stock solution (100mg OsO_4/40 ml acetone) was ready for the freshly usage.

5.5.2 Synthesis of (S)-6-acetoxy-(2R,3R)-dihydroxy-cyclohexanone (16) and (R)-6-acetoxy-(2S,3S)-dihydroxy-cyclohexanone (enant-16)

Firstly, 4-Methylmorpholine-N-Oxide monohydrate 97% (0.668g, 4.94 mmole) was dissolved in H_2O (4.19 ml, 0.23 mole) under argon atmosphere. At that time, the temperature of the flask in which there is (S)-(-)-6-acetoxy-2-cyclohexen-1-one **((-)-11)** (0.690 g, 4.46 mmole) was adjusted to -5 ^oC and onto the compound dry acetone (9.9 g, 0.062 mole) was added. Under argon atmosphere for a while the compound in acetone was stirred until NMO was completely dissolved in water. Then firstly 14.07 ml OsO_4 (100mg / 40ml) stock solution was added to the medium including (S)-(-)-6-acetoxy-2-cyclohexen-1-one **((-)-11)** in acetone and lastly, dissolved NMO solution was slowly added. The mixture was allowed to stir for 26 hours at -5 ^oC until all the starting material had finished by controlling via TLC. After the required conversion was obtained, $NaHSO_3$ (0.690g, 6.64 mmole), florosil (4.29g, 0.076 mole) and H_2O (8.37g, 0.465mole) were also added and allowed the resulting solution to stir for 12 hours. Then the pH of the reaction mixture was adjusted to 2 by using 1N HCl. The solution obtained was diluted with an equal amount of ethyl acetate and the organic phase was dried over $MgSO_4$ and evaporated in vacuo and the corresponding diol **(16)** was obtained. (0.630g / 75%).

By adjusting the same process to (R)-(+)-6-acetoxy-2-cyclohexen-1-one **((+)-12)**, corresponding diol **(enant-16)** (0.175g / 72%) was obtained.

Compound **16** and **enant-16;**

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

δ (ppm): 1.80-1.86 (m, 2H)
2.11 (s, 3H)

2.14-2.15 (m, 2H)

2.6 (bs, 1H)

3.72 (bs, 1H)

4.23 (bs, 1H)

4.32 (bs, 1H)

5.19 (dd, J=12.2 and 6.8, 1H)

5.6 Synthesis of (2R,3R,6S)-triacetoxy-cyclohexanone ((-)-19) and (2S,3S,6R)-triacetoxy-cyclohexanone ((+)-19)

Obtained diol **(16)** (0.630 g, 3.35 mmole), was mixed with (1.32g, 13.4 mole) dry pyridine in 25 ml CH_2Cl_2 under inert atmosphere, at 0 ^oC for $^1/_2$ hours. Then, acetylchloride (0.79g, 10.1 mole) was added and mixed for 16 hours at room temperature. The organic phase was extracted with 0.1 N HCl, $NaHCO_3$, brine respectively. Dried over $MgSO_4$, filtrated and evaporated. The crude product was seperated by flash coloumn chromatography using ethyl acetate/hexane (1:2) as eluent to afford the (2R,3R,6S)-triacetoxy-cyclohexanone **((-)-19)** (0.780g, 85%). $[\alpha]^{25}_D$= -7.25 (c 0.02, $CHCl_3$), mp: 104-106°C

X-RAY;

A white crystal of dimensions 0.21x0.19x0.18 mm was mounted on a glass fiber. X-ray diffraction intensity data collection and cell refinement were performed on Rigaku R-AXIS RAPID IP diffractometer equipped with a graphite monochromator. A total of 4004 unique reflections were collected using MoKα (λ = 0.71073 Å) radiation by the oscillation scan technique at 291(2) K, of which 3908 reflections had I>2σ(I) and were used in the structure

solution and refinements. The corrections for Lp factors and empirical absorption were applied to the intensity data. The structure was solved by direct methods and refined on F^2 using a full-matrix least-squares technique (SHELXS-97 and SHELXL-97)[8]. The non-hydrogen atoms were also refined by a full-matrix least-squares technique, anisotropically, and hydrogen atoms were included but not refined. The final cycle of full-matrix least-squares refinement was based on 3908 observed reflections and 178 parameters. Convergence with unweighted and weighted agreement factors was achieved at R = 0.086 and Rw = 0.114 ($w = 1/[\sigma(F_o^2) + (0.0084P)^2 + 2.9387P]$ where $P = (F_o^2 + 2F_c^2)/3$, The maximum and minimum peaks on the final difference Fourier map correspond to 0.223 and -0.185 eÅ^3.

Crystal data for 4: empirical formula, $C_{24}H_{32}O_{14}$; formula weight, 544.5; calculated density, 1.36 g/cm3; volume (V), 2652.6(2) Å^3; crystal system, ortorombik; space group, Pcab ; Z = 4; unit cell dimensions, a = 9.684(2), b = 12.328(5), c = 22.217(5), absorption coefficient , 0.113 mm^{-1}; index ranges, $-12 \leq h \leq 13$, $-17 \leq k \leq 17$, $-31 \leq l \leq 31$; F(000), 1152; θ_{max}=30.6; GOF, 1.32.

(2S,3S,6R)-triacetoxy-cyclohexanone **((+)-19)** (0.205 g, 81%) was also obtained by applying the same procedure. $[\alpha]^{25}_D$= +6.75 (c 0.02, $CHCl_3$).

Compounds **(-)-19** and **(+)-19**;

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

δ (ppm):	1.91-2.16 (m, 2H)
	2.17-2.24 (m, 2H)
	2.10 (s, 3H)
	2.16 (s, 3H)

2.19 (s, 3H)

5.34 (dd, J=6.8 and 4.9, 1H)

5.41 (d, J=3.3, 1H)

5.65 (bs, 1H)

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

δ (ppm): 195.8, 169.8, 160.5, 169.4, 75.4, 74.5, 72.3, 26.8, 25.0, 20.8, 20.5, 20.4

5.7 Synthesis of (2R,3R,6S)-triacetoxy-cyclohexan-1S-ol ((-)-20) and (2S,3S,6R)-triacetoxy-cyclohexan-1R-ol ((+)-20)

To a cooled solution (which is -78 ^{o}C, providing via dry ice and acetone) of (2R,3R,6S)-triacetoxy-cyclohexanone **((-)-19)** (0.109 g, 0.4 mmole) in methanol (4.04 ml, 3.19g, 0.1 mole), $NaBH_4$ (0.016 g, 0.4 mmole) and $CeCl_3.7H_2O$ (0.156 g, 0.4 mmole) were added and allowed to stir. When it was observed that all the starting material had finished after 3 hours by controlling via TLC, the reaction was quenched with H_2O (3 ml, 0.17 mole). Then diluted with the equal amount of Et_2O, washed with brine and dried over $MgSO_4$, filtrated and evaporated. The crude product was seperated by flash coloumn chromatography using ethyl acetate/hexane (1:2) to afford (2R,3R,6S)-triacetoxy-cyclohexan-1S-ol **((-)-20)** (0.089 g, 82%). $[\alpha]^{25}_D$=-1.3 (c 0.02, $CHCl_3$)

By applying the same procedure (2S,3S,6R)-triacetoxy-cyclohexan-1R-ol **((+)-20)** (164 mg, 80%) was obtained.. $[\alpha]_D$=+1.1 (c 0.02, $CHCl_3$)

5.8 Synthesis of meso compounds 21

Obtained (2R,3R,6S)-triacetoxy-cyclohexan-1S-ol ((-)-20) (0.089 g, 0.3 mmole) was mixed with dry pyridine (0.048 g, 0.6 mmole) in 25 ml CH_2Cl_2 under inert atmosphere, at 0 ^oC for $^1/_2$ hours. Then, acetylchloride (0.036 g, 0.5 mole) was added and mixed for 5 hours at room temperature. The organic phase was extracted with 0.1 N HCl, $NaHCO_3$, brine respectively. Dried over $MgSO_4$, filtrated and evaporated. The crude product was seperated by flash coloumn chromatography using ethyl acetate/hexane (1:2) as eluent to afford the (2,3,5,6)-tetraacetoxycyclohexane **(21)** (0.088 g, 87%).

Compound **21**;

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

δ (ppm): 2.07 (s, 8H)
2.08 (s, 8H)
5.22 (bs, 1H)
5.97 (bs, 1H)

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

δ (ppm): 169.9, 69.0, 68.4, 22.9, 20.9, 20.7

5.9 Synthesis of cyclopent-3-ene-1,1-dicarboxylate (29), 3-cyclopentene-1,1-dicarboxylic acid (30), and cyclopent-3-enecarboxylic acid (31)

Under inert atmosphere dimethyl malonate (1.87 g, 0.014 mole) in THF (25 ml) was treated with DMPU (2.97 g, 0.0232 mole) and mixed at room temperature for 10 minutes and then the resulting solution was cooled by means of an ice bath and LiH (0.279 g, 0.0352 mole) addition in one portion was adjusted. Nitrogen flow was discontinued since hydrogen evaluation was expected. Then the flask capped with septum and connected to a Nujol-filled bubbler. After 15 minutes, cooling bath was removed but stirring was continued until hydrogen evaluation was completed approximately for 2 hours. Then cis-1,4-dichloro-2-butene (1.875 g, 0.015 mole) was rapidly added and the reaction mixture was heated by means of an oil bath to 40-45 $^{\circ}$C for 24 hours. After TLC controlling (silica gel EtOAc/Hex 1:1) it was understood that all the starting materials had finished and corresponding spot newly formed was for dimethyl cyclopent-3-ene-1,1-dicarboxylate **(29)**, in the appearence of brown oil. Then, mixture was cooled to 20 $^{\circ}$C and water addition was adjusted dropwisely following by lithium hydroxide monohydrate addition. After that, the reaction mixture was allowed to stir at 20 $^{\circ}$C for an additional 24 hours, and when TLC controlling (silica gel EtOAc/Hex 1:1) was applied it was observed that the new product formation and all dimethyl cyclopent-3-ene-1,1-dicarboxylate **(29)** had finished. Then the resulting solution was diluted with an equal amount of EtOAc, and washed with 3 N HCl, 6 N HCl, and then brine, and dried over $MgSO_4$. After flash coloumn chromatography (silica gel, EtOAc/Hex 1:2) 3-cyclopentene-1,1-dicarboxylic acid **(30)** was obtained as an off white solid. Then the resulting compound which was 3-cyclopentene-1,1-dicarboxylic acid **(30)** was directly adjusted to decarboxylation process by increasing the temperature of the medium between 170-175 $^{\circ}$C. The process was allowed to continue until carbondioxide evaluation was complete, approximately 4 hours. Following TLC

controlling (silica gel EtOAc/Hex 1:1) and observing all the starting material had finished and new spot formation, resulting solution was allowed to cool at room temperature. Then purification process was applied via work-up and flash coloumn chromotography, and cyclopent-3-enecarboxylic acid **(31)** was obtained as brown oil with 89 % yield.

Compound **31**;

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

δ (ppm): 2.67 (s, 2H)
2.69 (s, 2H)
3.11-3.20 (m, 1H)
5.68 (s, 2H)
11.78 (bs, 1H)

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

δ (ppm): 182.9, 128.9, 41.5, 36.2

5.10 Synthesis of 6-iodo-2-oxa-bicyclo[2.2.1]heptan-3-one (32)

Obtained cyclopent-3-ene-carboxylic acid **(31)** (0.224 g, 2.0 mmole) was treated with $NaHCO_3$ (0.504 g, 6.0 mmole), KI (1.99 g, 0.012 mole) and I_2 (0.532 g, 2.1 mmole) in H_2O (15 ml). The reaction stirred for 20 h at room temperature. The reaction was followed via TLC (silica gel, EtOAc/Hex 1:2), and when it was observed all cyclopent-3-enecarboxylic acid **(31)** had finished and new product formed, the reaction was quenched with H_2O. The resulting suspension was treated with $CHCl_3$ and then washed with $Na_2S_2O_3$, and then dried over $MgSO_4$.

Then flash coloumn chromotography was adjusted (silica gel, EtOAc/Hex 1:2). 6-iodo-2-oxa-bicyclo[2.2.1]heptan-3-one **(32)** was obtained as an off white solid. (0.386g, 81%)

Compound **32**;

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

δ (ppm): 2.34 (d, J=11.2 Hz,1H)
2.41 (dt, J=14.2 and 3.9 Hz, 1H)
2.47 (d, J=11.2 Hz, 1H)
2.60 (dd, J=14.2 and 7.9 Hz, 1H)
4.09 (bs, 1H)
4.97 (s, 1H)

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

δ (ppm): 176.2, 84.9, 43.1, 36.8, 36.2, 15.9

5.11 Synthesis of 2-oxa-bicyclo[2.2.1]hept-5-en-3-one (33)

To a solution of 6-iodo-2-oxa-bicyclo[2.2.1]heptan-3-one **(32)** (0.230 g, 0.97 mmole) in 20 ml THF, DBU (0.22 ml, 1.46 mmol) was added and allowed to reflux for 8 hours. Upon being cooled to room temperature, the resulting solution was diluted with an equal amount of EtOAc and then washed with 0.5 N HCl and brine, and then dried over $MgSO_4$, concentrated under reduced pressure. Purification was adjusted by flash coloumn chromotography (silica gel

EtOAC/Hex 1:2) to obtain 2-oxa-bicyclo[2.2.1]hept-5-en-3-one **(33)** (0.051 g, 47%).

Compound **33**;

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

δ (ppm): 2.35-2.45 (m, 1H)
2.51-2.52 (m, 1H)
2.87-2.92 (m, 1H)
4.08-4.19 (m, 1H)
5.01 (s, 1H)
5.70 (s, 1H)

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

δ (ppm): 181.9, 127.9, 83.9, 40.4, 35.2

CHAPTER 6

CONCLUSION

α'-acetoxylation of α,ß-unsaturated cyclic ketones was adjusted via $Mn(OAc)_3$ in regioselective manner. Resolution was carried out via PLE hydrolysis after selective oxidation so as to afford enantiomerically enriched α'-acetoxylated and α'-hydroxylated cyclic compounds. According to the literature and previous works dealing with α'-hydroxylated products which are easily racemized, protection was directly adjusted via acetylation so as to prevent this possibility. Corresponding enantiomerically enriched products were subjected to Upjohn Dihydroxylation to obtain cyclitol precursors **((-)-19, (+)-19)** and following Luche Reduction of ketone was adjusted so as to attain target cyclitols **((-)-20, (+)-20)**.

As a different synthetic design, dimethyl cyclopent-3-ene-1,1-dicarboxylate **(29)** was obtained so as to reach in former manner 3-cyclopentene-1,1-dicarboxylic acid **(30)**, and in latter manner cyclopent-3-enecarboxylic acid **(31)**. Iodolactonization of compound **31** was adjusted to afford 6-iodo-2-oxa-bicyclo[2.2.1]heptan-3-one **(32)** and via elimination by using DBU, the target nucleoside precursor, 2-oxa-bicyclo[2.2.1]hept-5-en-3-one **(33)**, was obtained.

APPENDIX A

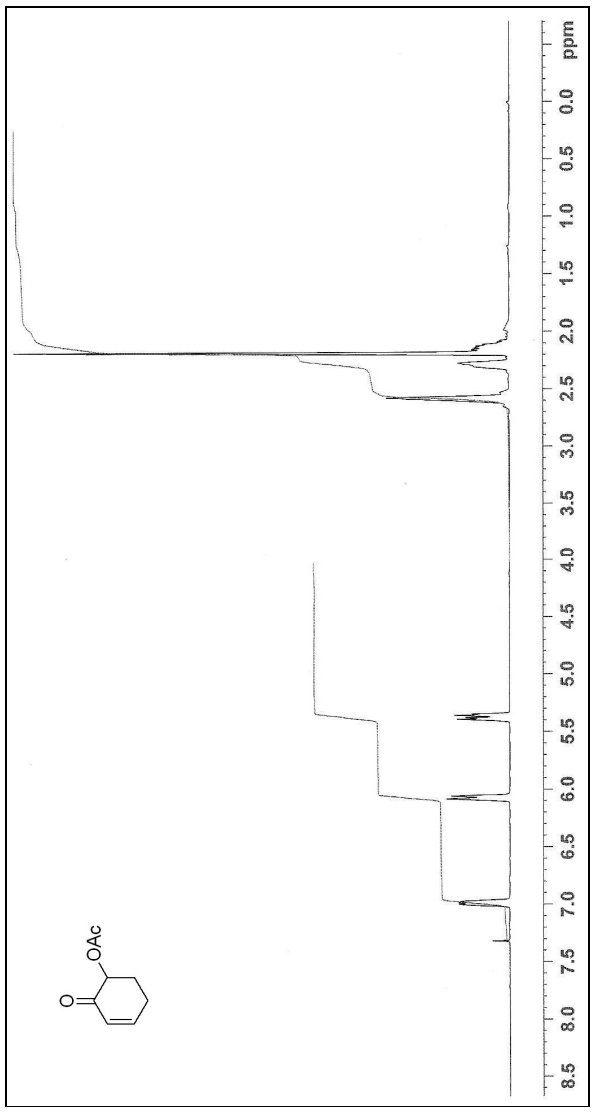


Figure 11. ^{1}H-NMR of **rac-11**

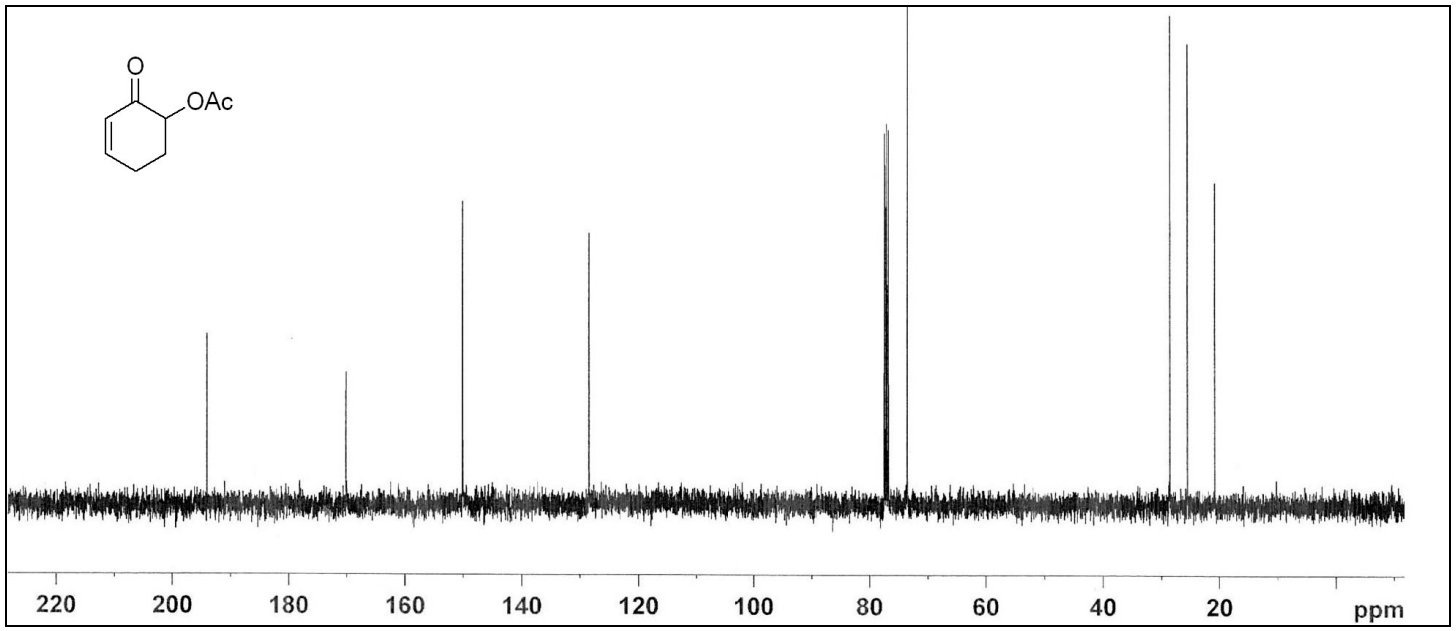


Figure 12. ^{13}C-NMR of **rac-11**

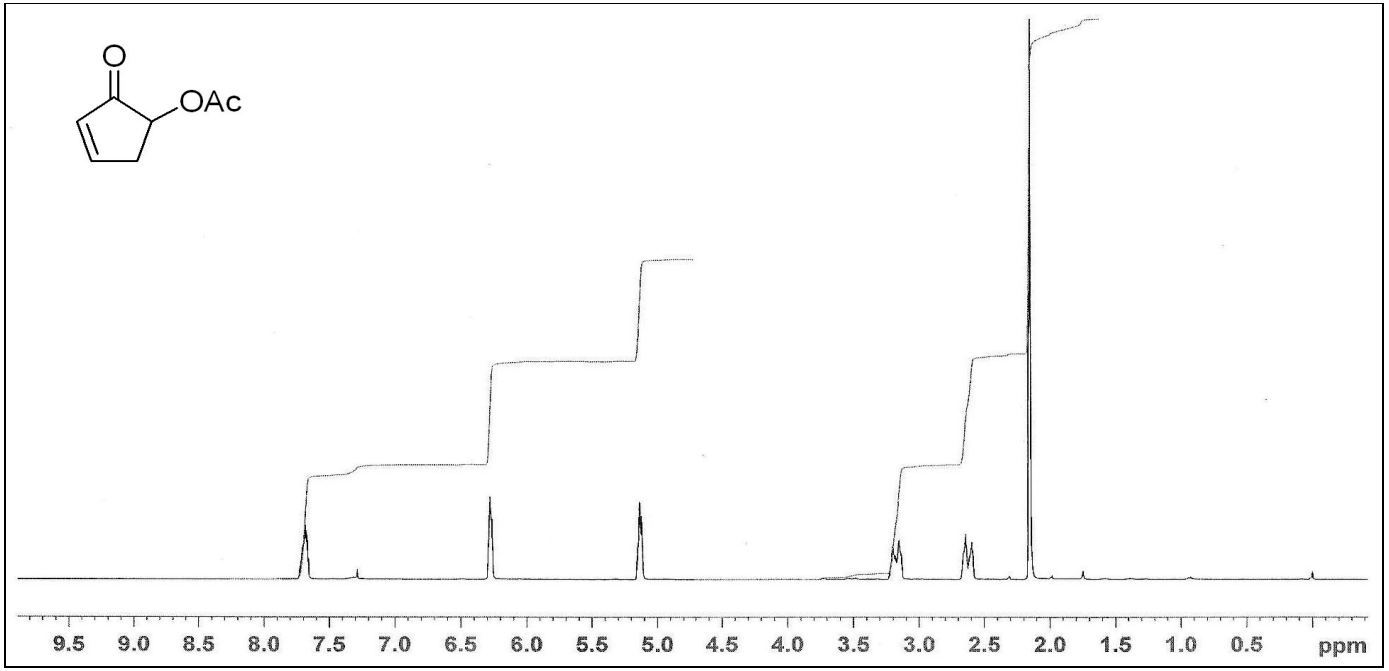


Figure 13. ^{1}H-NMR of **rac-10**

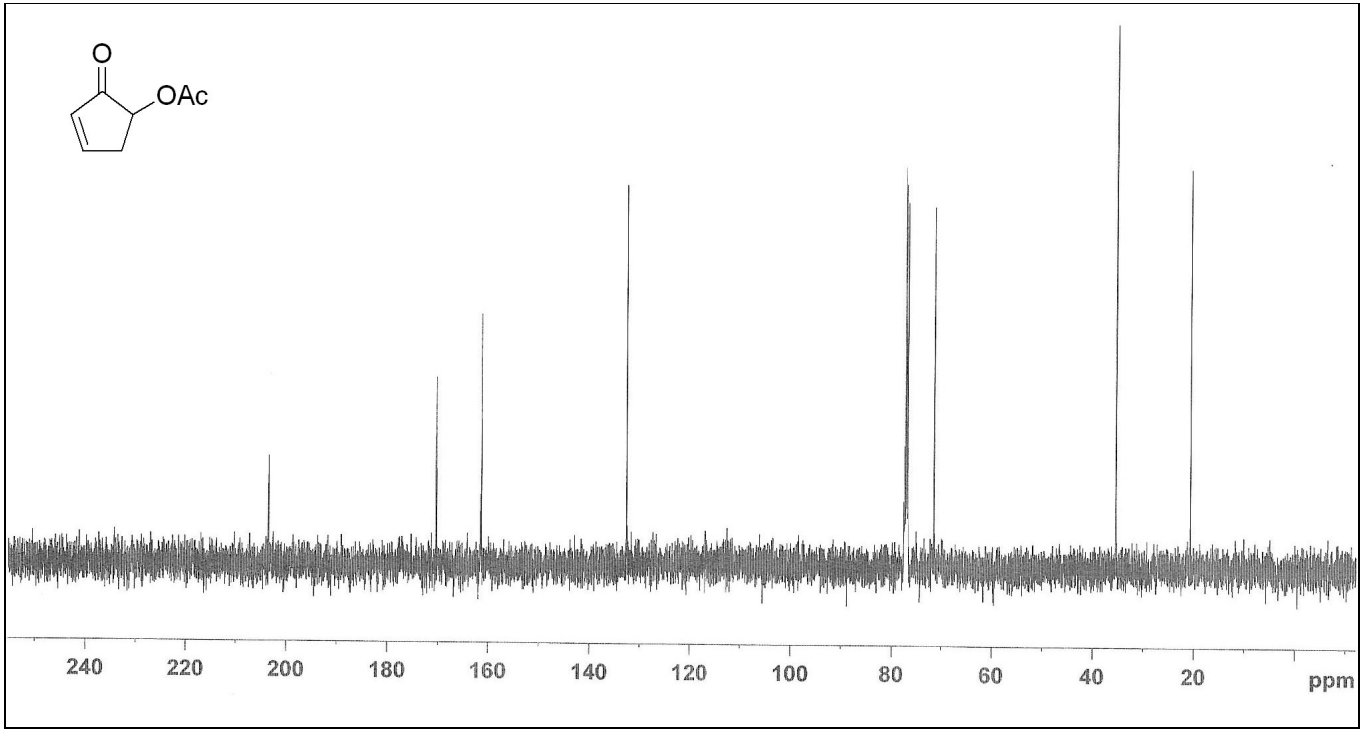


Figure 14. ^{13}C-NMR of **rac-10**

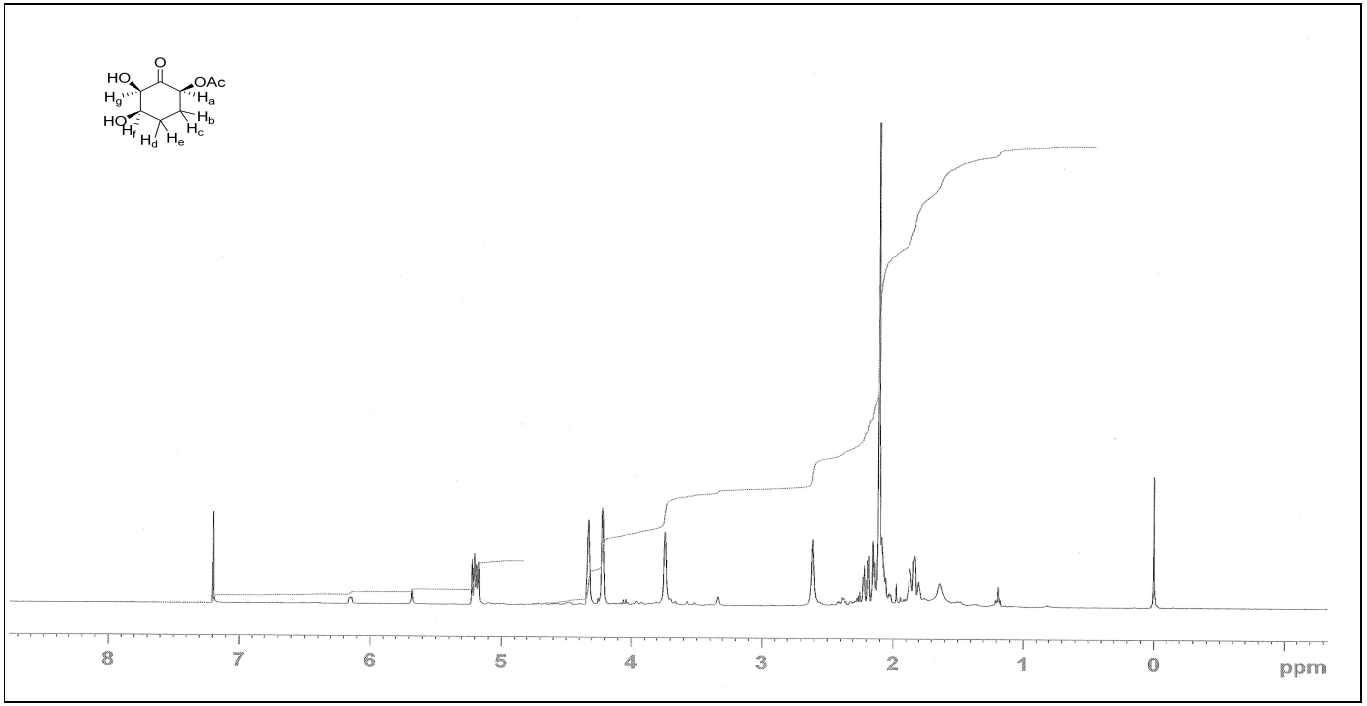


Figure 15. ^{1}H-NMR of **16**

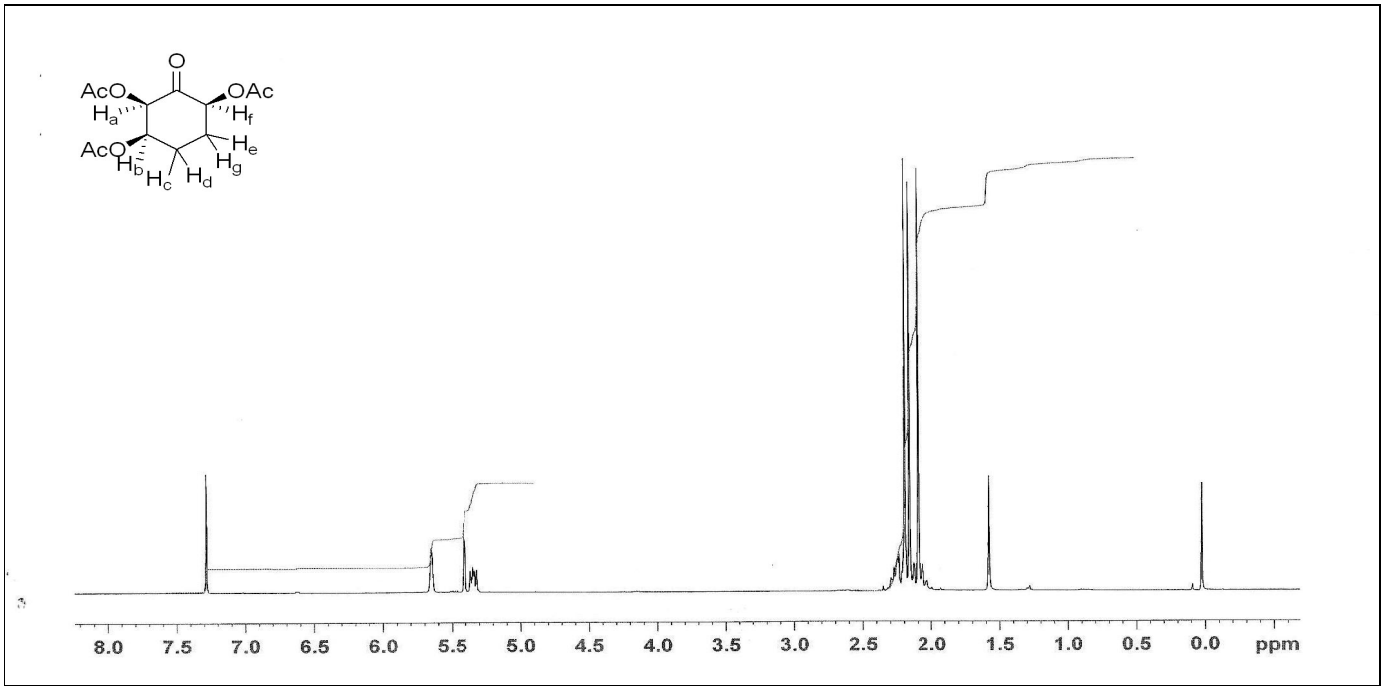


Figure 16. ^{1}H-NMR of **(-)-19)**

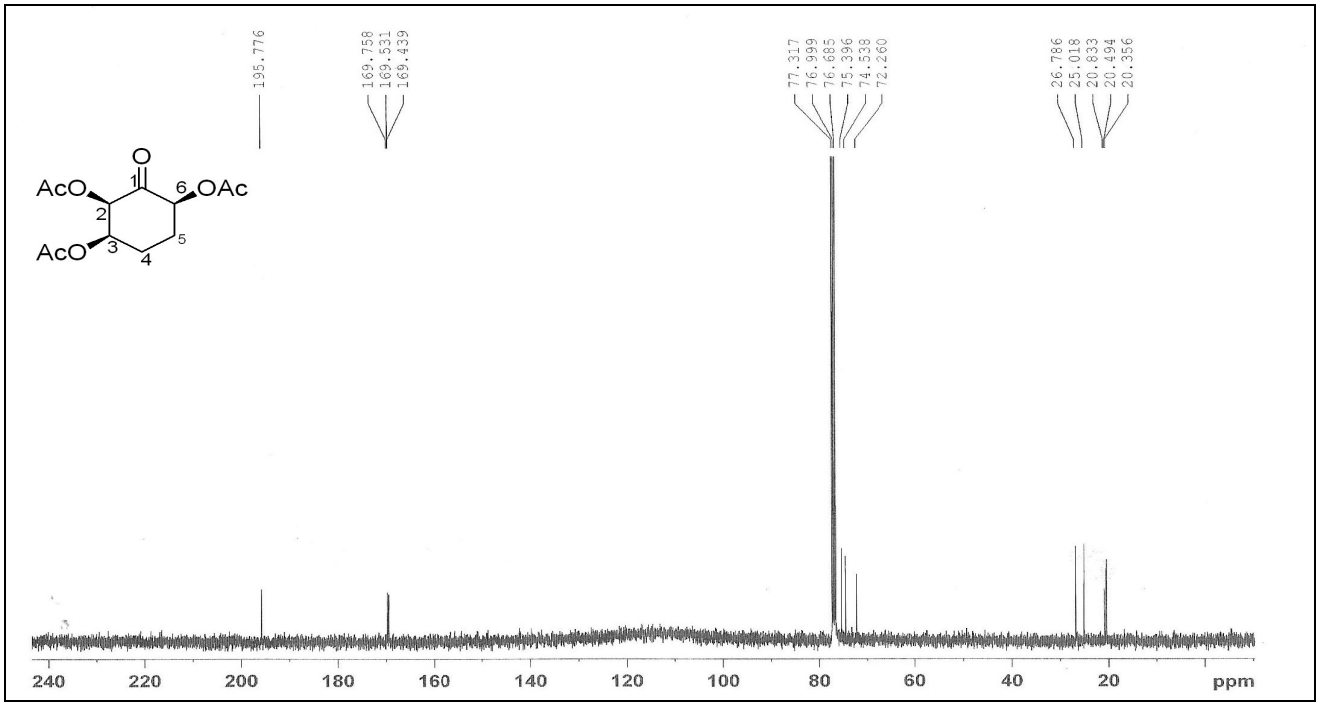


Figure 17. ^{13}C-NMR of **(-)-19)**

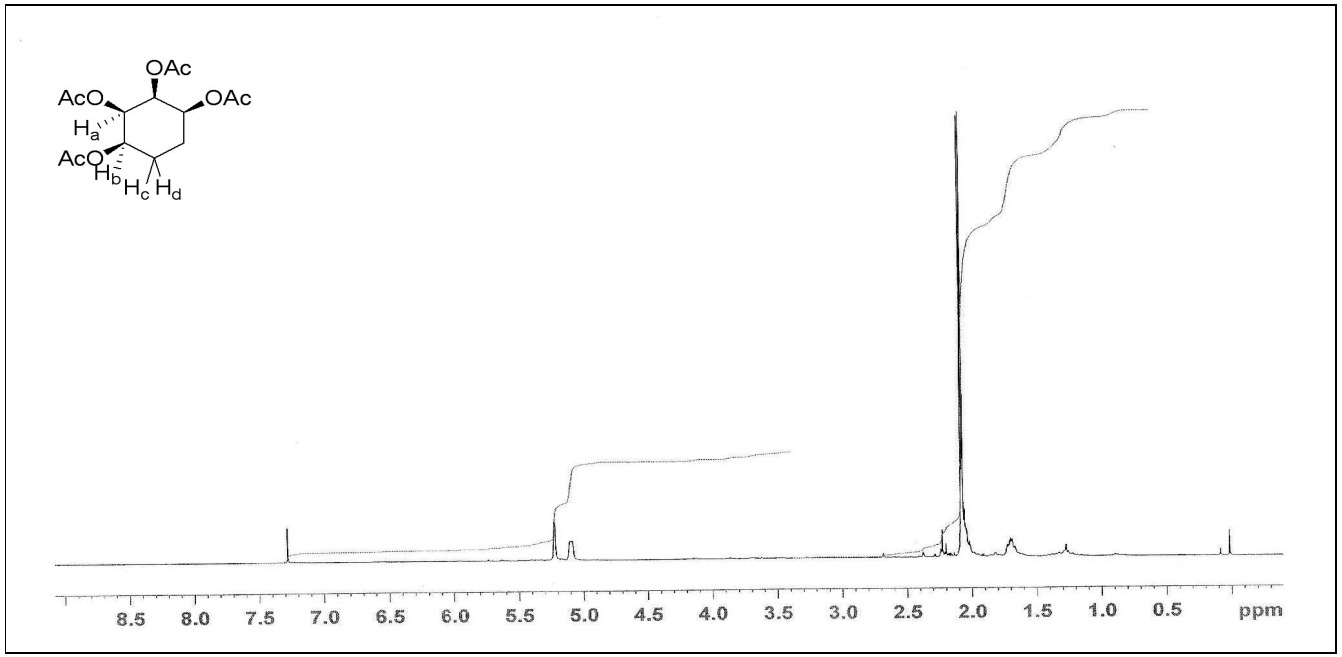


Figure 18. ^{1}H-NMR of **21**

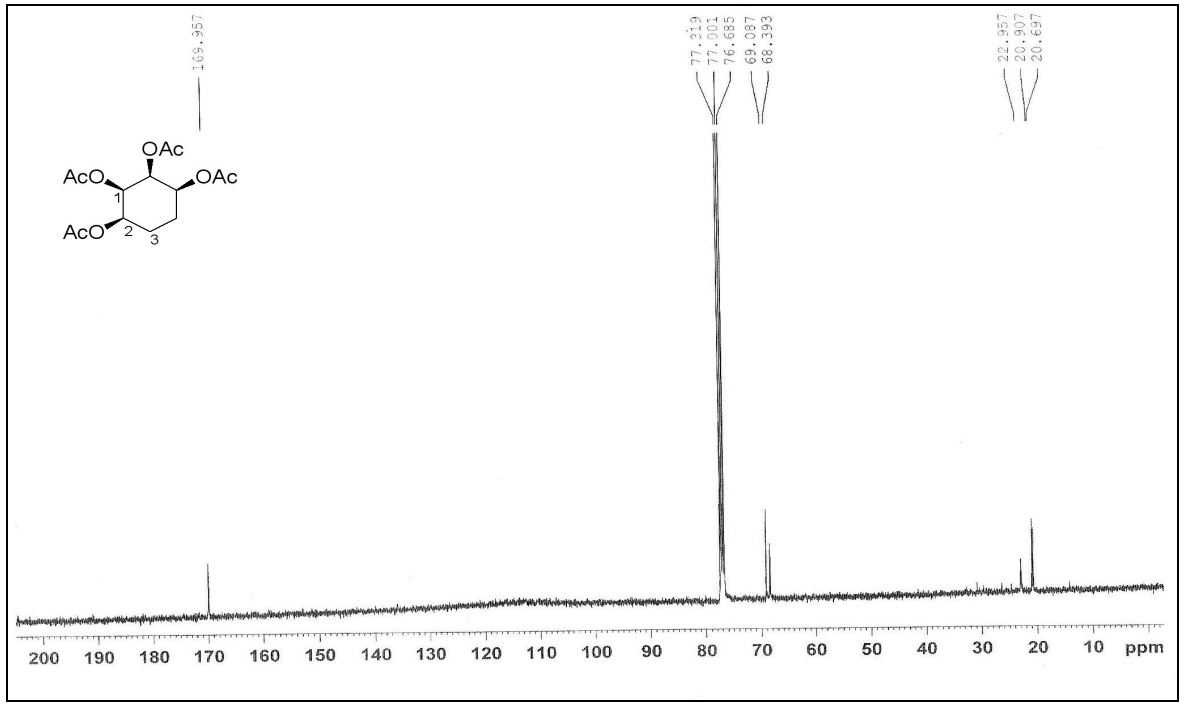


Figure 19. ^{13}C-NMR of **21**

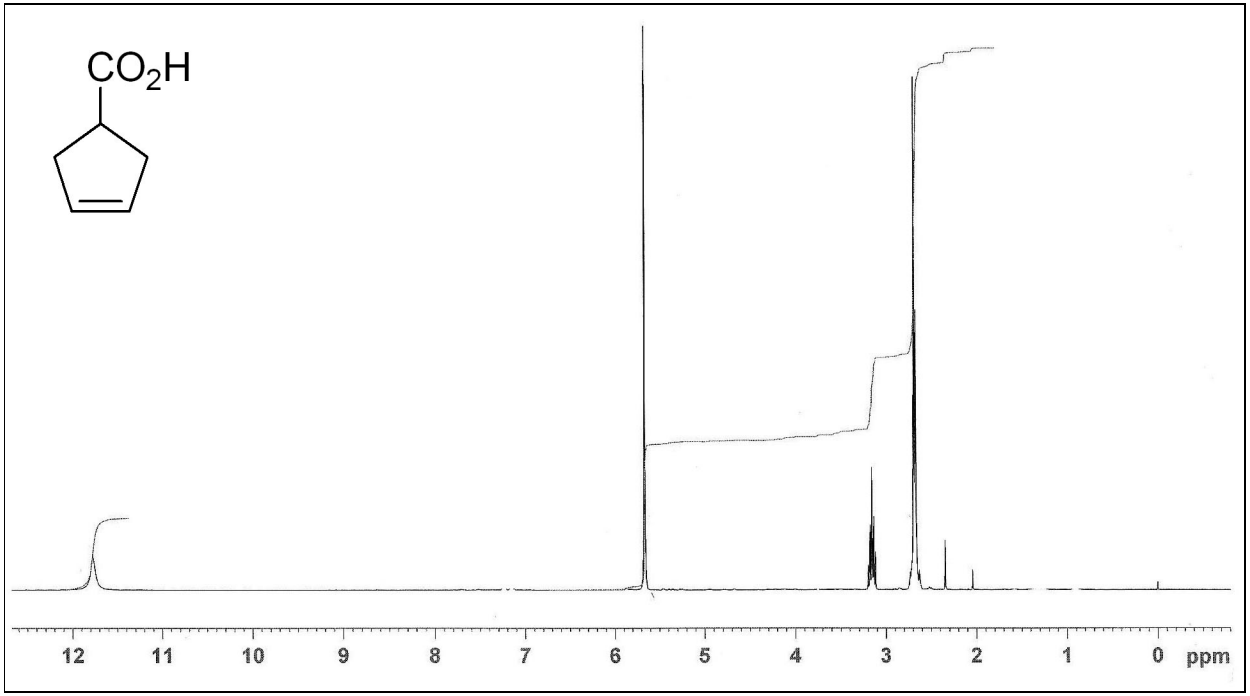


Figure 20. ^{1}H-NMR of **31**

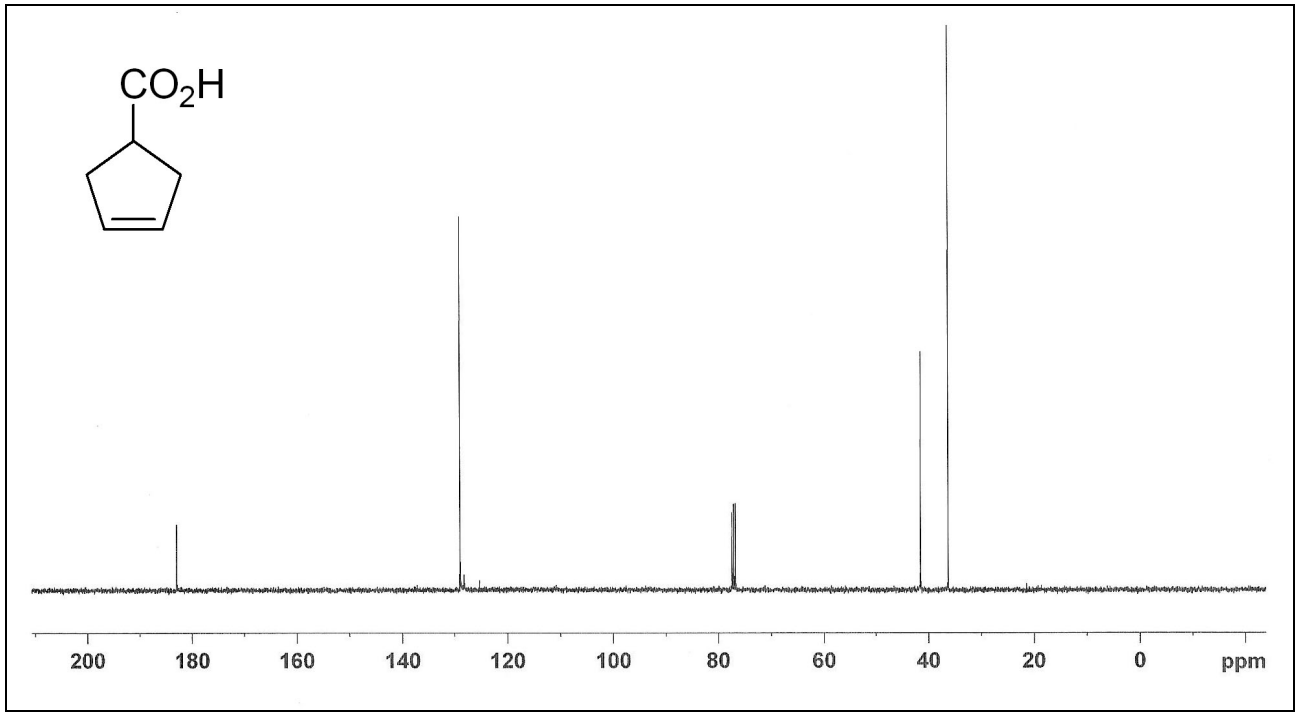


Figure 21. ^{13}C-NMR of **31**

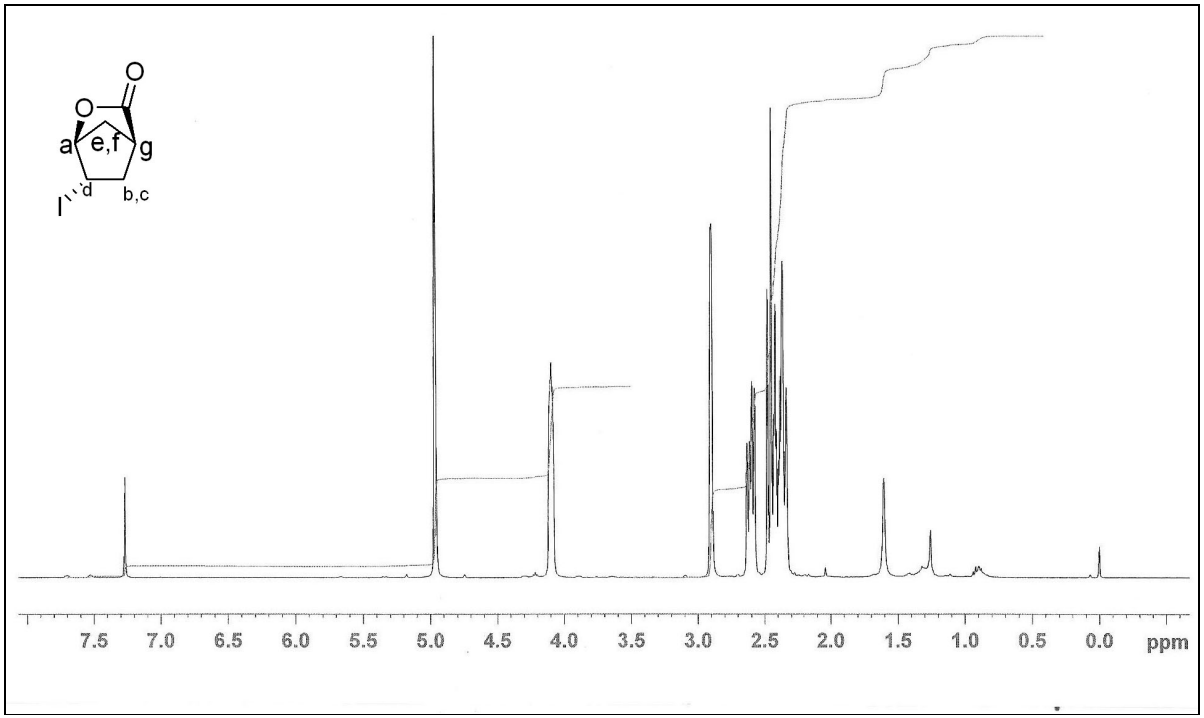


Figure 22. ^{1}H-NMR of **32**

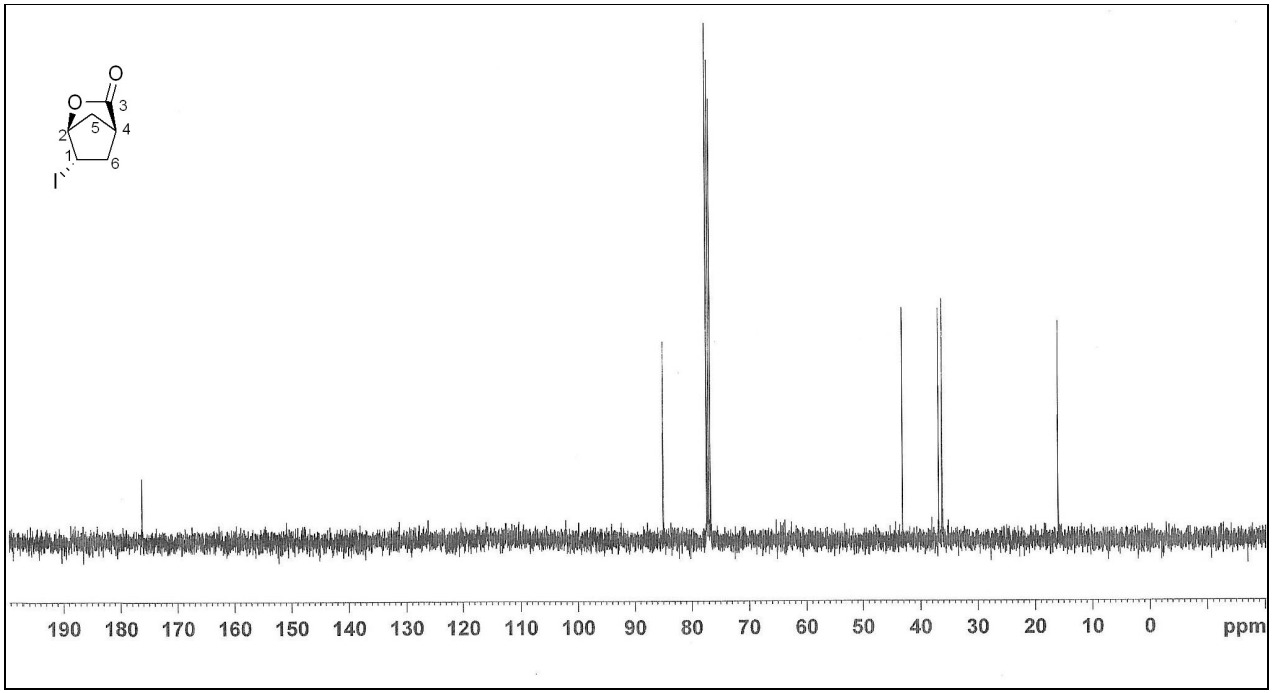


Figure 23. ^{13}C-NMR of **32**

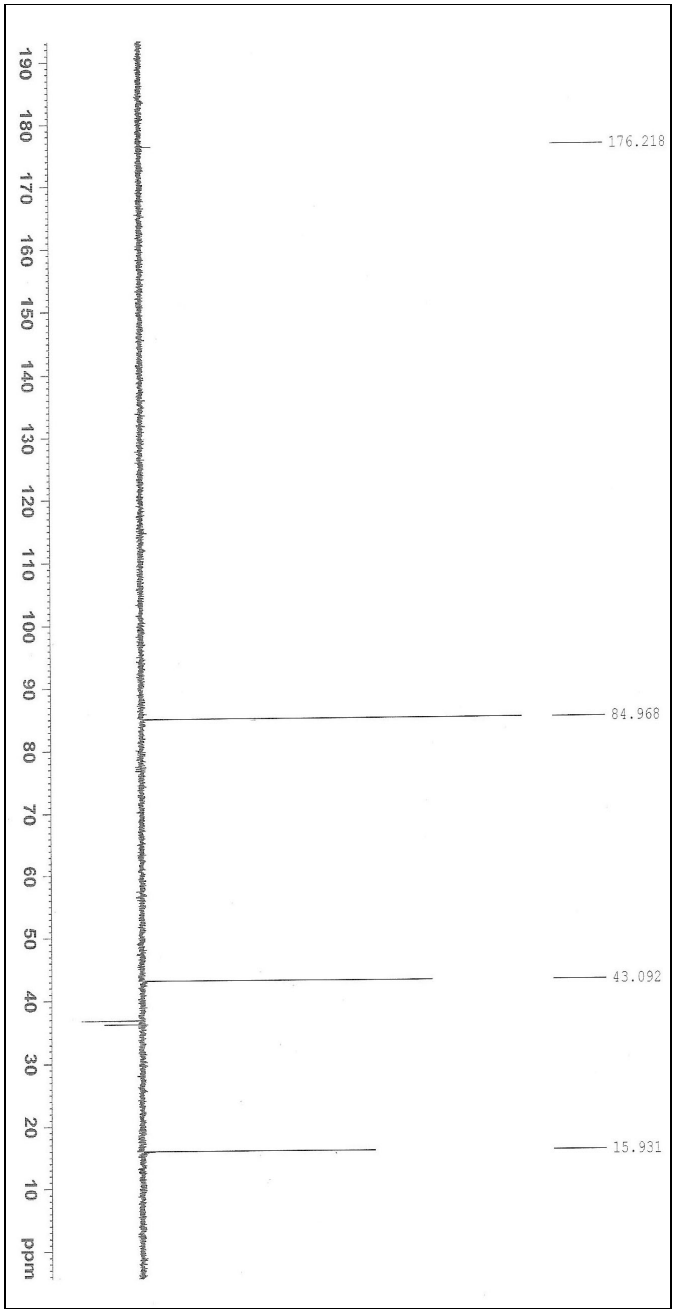


Figure 24. DEPT of 32

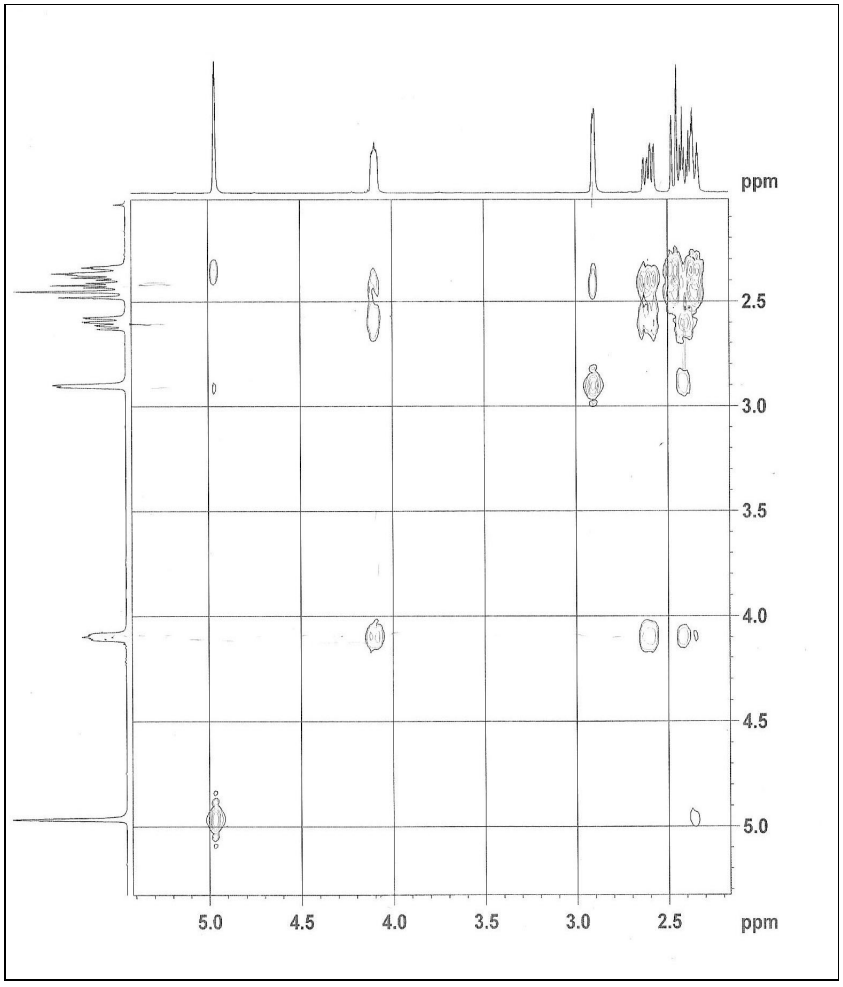


Figure **25**. COSY of **32**

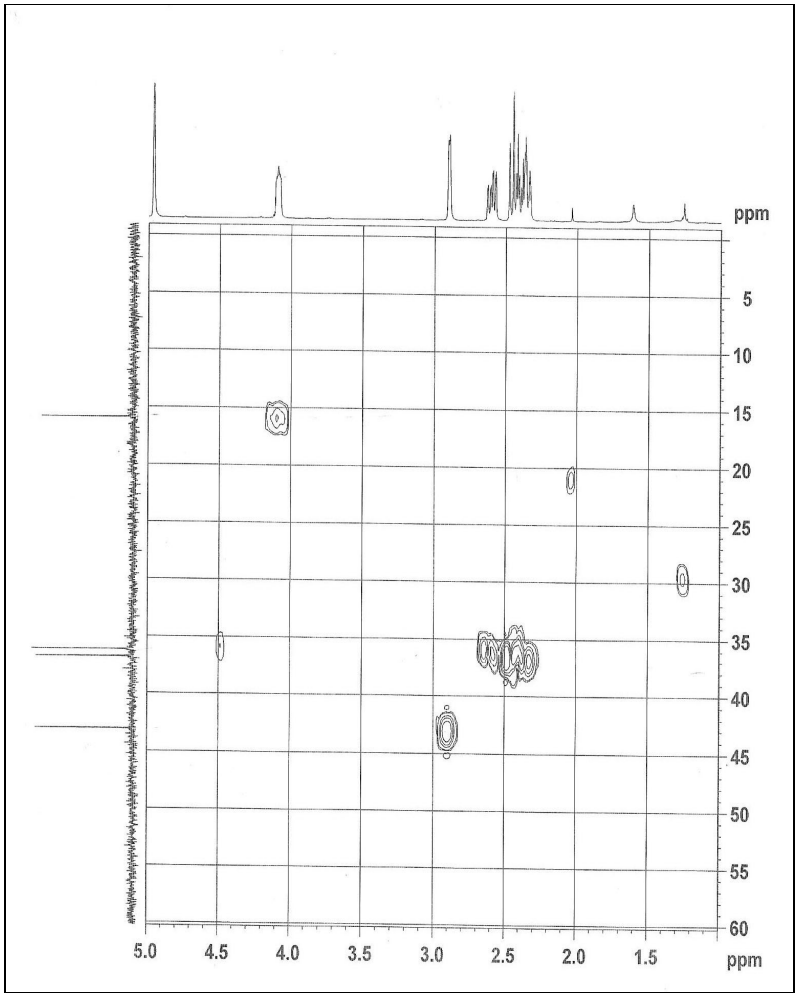


Figure **26**. HMQC of **32**

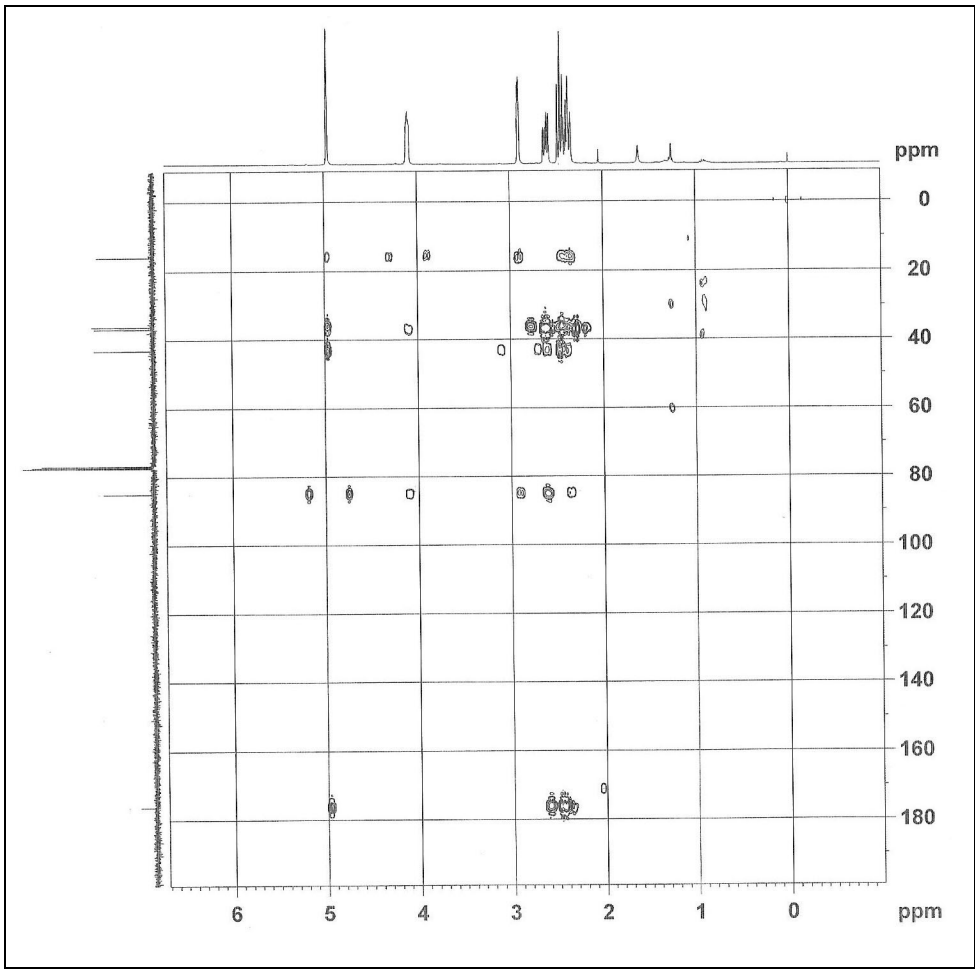


Figure **27**. HMBC of **32**

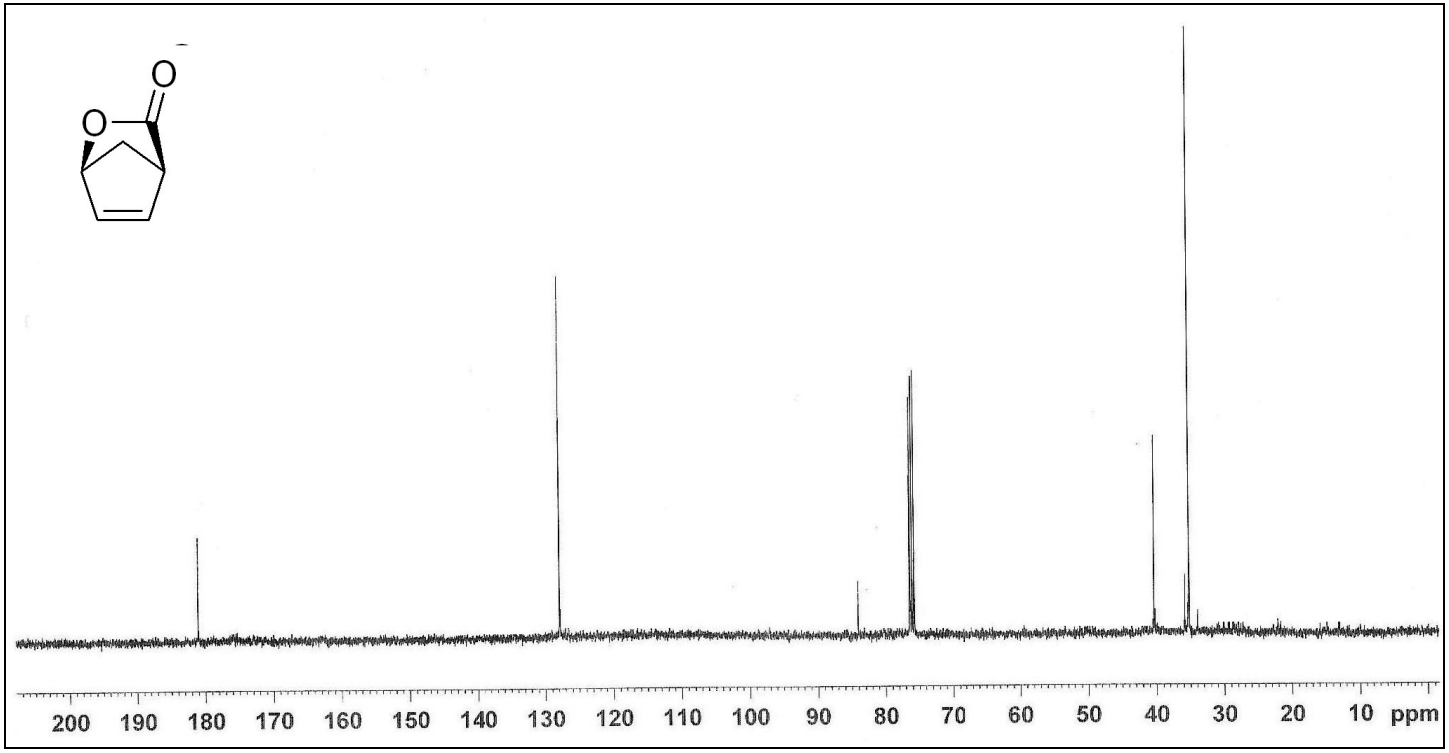


Figure 28. ^{13}C-NMR of **32**

REFERENCES

1. Alexandre Alexakis, Fixel Tomassini and Stephane Leconte, *Tetrahedron* **2004**, 60, 9479-9484

2. Kumar, P.; Upadhay, R.K. ; Pandey, R.K. , *Tetrahedron: Asymmetry*, **2004**, 15, 3955-3959

3. Gawley R. E.; Aube J.; *Tetrahedron Organic Chemistry Series,* Volume 14, Principles of Asymmetric Synthesis **1996,** Elsevier Science Ltd., pp xiii

4. Alan Fersht Imperial College of Science and Technology, *Enzyme Structure and Mechanism*, **1985,** 221

5. Blashke, G.; Kraft, H.P.; Fickenscher, K.; Kohler, F.; *Arziniem-Forsch/Drug Res.*, **1979,** 29,10

6. Blashke, G.; Kraft, H.P.; Fickenscher, K.; Kohler, F.; *Arziniem-Forsch/Drug Res.*, **1979,** 29,1640

7. Gary M. Coppola, Herbert F. Schuster, *Asymmetric Synthesis*, **1987**, 1

8. T. Scott and M. Brewer, *Concise Encyclopedia of Biochemistry*, **1983**, 14

9. H. Gutfreund , *An Introduction to the Study of Enzymes*, **1965**, 22

10. Ager D. J., East M. B. , *Asymmetric Synthetic Methodology*, **1996**, 17

11. Zhang D., Poulter C. D., *J. Am. Chem. Soc*., **1993**, 115,1270

12. G. M. Whitesides, C. H. Wong.; *Angew. Chem.,* **1985**, 97, 617

13. J. B. Jones, *Asymmetric Synthesis*, **1985**, 5, 309

14. O. Warburg, *Z. Physical Chem*., **1906**, 48, 205

15. J. B. Jones, *Tetrahedron*, **1986**, 42, 3351

16. W. Moore, *Physical Chemistry*, **1955**, 528-538

17. C. Toren, *J. Chem. Ed.* **1967**, 44,172

18. W. J. Blaedel and G. P. Hicks, *Advences in Analytical Chemistry and Instrumentation*, **1964**, 3, 105-140

19. H. Pardve, C. Frings and C. J. Pelarey, *Anal. Chem.* **1965**, 37, 34

20. H. Pardue, *Anal. Chem*., **1964**, 36, 633

21. H. Pardue and K. Simon, *Anal. Biochem*., **1964**, 9, 204

22. W. J. Bladel and G. P. Hicks, *Anal. Chem*. **1962**, 34, 388

23. Ibid., *Anal. Biochem*. **1962**, 4, 476

24. Robert L. Augustive, *Oxidation*, **1969**, 1, 1

25. Conolly, J. W. Urry. G. , *Inorg. Chem*. **1963**, 2, 645-646

26. G. J. Williams and N.R. Hunter, Can. *J. Chem.* **1976**, 4, 3830

27. Fristad W. E., Peterson J. R., *J. Org. Chem*. **1985**, 50, 1026-1029

28. Fristad W. E., Herbersberger S. S., *J. Org. Chem*. **1985**, 50, 1026-1029

29. Christian Döbler, Gerald M. Mehltretter, Uta Sundermeier, Matthias Beller, *J. of Organometallic Chemistry*, **2001**, 621, 70-76

30. *J. of Org. Chem*, **1998**, 63, 7322-7327

31. (a) Schronder, M. *Chem. Rev*. , **1980**, 80, 187-213

(b) Kolb, H.C.; Van Nieuwenhze, M. S.; Sharpless, K. B. *Chem. Rev*. , **1994**, 94, 2483-2547

32. Hammond C. R., *In CRC Handbook of Chemistry and Physics*, **2001**, 4-21

33. Kolb, H. C.; Van Nieuwenzhe, M. S.; Sharpless, K. B. *Chem. Rev*., **1994**, 94, 2483-2547

34. V. VanRheeren, D. Y. Cha, W. M. Hartley, *Org. Synth. Coll*., **1988**, 6, 342

35. V. VanRheeren, R. C. Kelly, D. Y. Cha., *Tetrahedron Lett*., **1976**, 1973

36. S. A. Hermitage, A. Murphy, P. Nielsen, S. M. Roberts, *Tetrahedron,* **1998**, 54, 13185

37. K. Narkunan, M. Nagarajan, *J. Chem. Soc*., *Chem. Commun*., **1994**, 1705

38. A. Tubul, M. Bertrand, *Tetrahedron Letter*, **1979**, 2381

39. Minato M. , Yamamato K., Tsuji J.*, J. Org. Chem*., **1990,** 55, 766

40. (a) Billington, D. C. *The Inositol Phosphates – Chemical Synthesis and Biological Significance*; VCH: Winheim, **1993**

(b) Potter, B. V. L.; Lamp, D. Angew. Chem., *Int. Ed. Engl*. **1995**, 34, 1933 - 1972

41. (a) Kwon, Y. K.; Lee, C.; Chung, S. K. , *J. Org. Chem*. , **2002,** 67, 3327-3338

(b) Riley, A. M.; Jenkins, D. J. ; Potter, B. V. L. *Carbohydrate Res*. **1998**, 314, 277-281

(c) Husson, C.; Odier, L.; Vottero, P. J. A. *Carbohydr. Res*. **1998**, 307, 163-165

(d) Trost, B. M.; Patterson, D. E.; Hembre, E. J. *J. Am. Chem. Soc*. , **1999**, 121, 10834-10835

42. (a) Hudlicky, T.; Gonzalez, D.; Gibson, D. T. *Aldrichim. Acta*, **1999**, 32, 35-62

(b) Brammer, L. E. , Jr.; Hudlicky, T. , *Tetrahedron: Asymmetry*, **1998**, 9, 2011-1014

43. Takahashi, H.; Kittaka, H.; Ikegami, S. *J. Org. Chem.*, **2001**, 66, 2705-2716

44. Yadav, J.S.; Maiti, A,; Sankar, A.R.; Kunwar, A. C. *J. Org. Chem.*, **2001**, 66, 8370-8378

45. Angelaud, R.; Babot, O.; Charvat, T.; Landais, Y., *J. Org. Chem.* **1999**, 64, 9613-9624

46. Stöckigt, J. *In Indole and Biogenetically Related Alkoloids*, **1980**, 6

47. Le Men, J. and Taylor, W. I. Experientia, **1965**, 21, 508-510

48. Leonard, J. Quali, D., and Rahman, S. K., *Tetrahedron Lett.* , **1990**, 31, 739-742

49. Izawa, H., Shirai, R., Kawasaki, H., Kim, H., and Koga, K., *Tetrahedron Lett.* **1989**, 30, 7221-7223

50. a) Wolfrom M.L., Thompson A., Evans E. F. *J. Am. Chem. Soc.* **1945**, 67, 1793

b) Fenselau C. *Steroid Reactions*, ed. Djerassi C, Holden-Day, San Francisco, **1963**, pp. 537-591

51. a) Green T. W., Wuts P.G. M. Protective Groups In *Organic Synthesis*, Wiley, New York, **1991**, pp. 87-104

b) Kocienski P. J. *Protecting Groups*, Thieme, Stuttgart, **1994**, pp. 22-29

52. Rawlinson, D. J.; Sosnovsky, G. *Synthesis* **1973**, 567-603

53. (a) Criegee, R. *Angew. Chem.* **1958**, 70, 173.

(b) Criegee, R. In *Oxidation in Organic Chemistry, Part A*, Wiberg, K. B., Ed.; Academic: New York, **1965**; Chapter 5

54. Murai, A.; Ono, M.; Abiko, A.; Masamune, T. *J. Am. Chem. Soc.* **1978**, 100, 7751-7753

55. Mimoun, H.; Seree de Roche , L.; Sajus, L. *Bull. Soc. Chim. Fr.* **1969**, 1481- 1492

56. Iwata, C.; Takemoto, Y.; Nakamura, Y.; Imanishi, T. *Tetrahedron Lett.* **1985**, 26, 3227-3230

57. Iwata, C.; Takemoto, Y.; Kubota, H.; Kuroda, T.; Imanishi, T. *Tetrahedron Lett.* **1985**, 26, 3231-3234

58. Rubottom, G.; Gruber, J. M. *J. Org. Chem.* **1978**, 43, 1599-1602

59. Musser, A. K.; Fuchs, P. L. *J. Org. Chem.* **1982**, 47, 3121-3131

60. Tanyeli, C.; Turkut, E.; Akhmedov, İ. M. *Tetrahedron: Asymmetry* **2004**, 15, 1729-1733.

61. Tanyeli, C.; Tosun, A.; Turkut, E., Sezen, B. *Tetrahedron* **2003**, 59, 1055.

62. Sugimura, T.; Iguchi, H.; Tsuchida, R.; Tai, A.; Nishiyama, N.; Hakushi, T. *Tetrahedron: Asymmetry* **1998**, 9, 1007

63. Jonsson, S. Y.; Farnegardh, K.; Backvall, J.-E. *J. Am. Chem. Soc*. **2001**, 123, 1365-1371

64. Mishima, M.; Ide, J.; Muramatsu, S.; Ono, M. J. Antibiot. **1983**, 36,980

65. (a) Varki, A. Glycobiology **1993**, 3, 97-130; (b) Dwek, R. A. *Chem. Rev*. **1996**, 96, 683-720

66. van Boeckel, C. A. A.; Petitou, M. *Angew. Chem*., Int. Ed. Engl. **1993**, 32, 1671-1690

67. Ogawa, S.; Hirai, K.; Odagari, T.; Matsunaga, N.; Yamajaki, T.; Nakajima, A. Eur. *J. Org. Chem*. **1998**, 1099-1109

68. Saumi, T.; Ogawa, S. *Adv. Carbohydr. Chem. Biochem*. **1990**, 154, 257-283

69. Ogawa, S.; Ara, M.; Kondoh, T.; Saitoh, M.; masuda, R.; Toyokuni, T.; Suami, T. Bull. *Chem. Soc. Jpn*.**1980**, 53, 1121-1126

70. Gómez, A. M.; Danelón, G. O.; Valverde, S.; López, J. C. *J. Org. Chem.* **1998**, *63,* 9626-9627.

71. Gómez, A. M.; Danelón, G. O.; Moreno, E.; Valverde, S.; López, J. C. *Chem. Commun.*, **1999**, 175-176.

72. Gómez, A. M.; Company, M. D.; Valverde, S.; López, J. C. *Organic Letters*, **2002**, *4***,** 383-386.

73. Hobbs, K. B. *In Comprehensive Medicinal Chemistry*; Hansch, C.; Ed.; Pergamon: New York, **1990**, 2, 306

74. (a) De Clercq, E. Biochem. Pharmacol. **1987**, 36, 2567. (b) Cools, M.; De Clercq, E. *Biochem. Pharmacol*. **1989**, 38, 1061

75. Bennett, L. L.; Jr.; Brockman, R. W.; Rose, L. M.; Allan, P. W.; Shaddix, S. C.; Shealy, Y. F.; Clayton, J.D. Mol. Pharmacol. **1985**, 27,666

76. Patil, S. D.; Schneller, S. W.; Hosoya, M.; Snoeck, R.; Andrei, G.; Balzarini, J.; De Clercq, E*. J. Med. Chem*. **1992**, 35, 3372

77. S.M; Chen, X.; Schneller, S. W.; Ikeda, S.; Snoeck, R.; Andrei, G.; Balzarini, J.; De Clercq, E. *J. Med. Chem*. **1994**, 37, 551

78. (a) Hamill, R. L.; Nagarajan, R. U.S. Patent 4 087603.

(b) Florent, J.; Lunel, J.; Mancy, D. U.S. Patent 4 189 349

79. Trager, W.; Tershakovac, M.; Chiang, P. K.; Cantoni, G. L. *Exp. Parasitol.* **1980**, 50, 83

80. Zweygarth, E.; Schillinger, D.; Kaufmann, W.; Rottcher, D. *Trop. Med. Parasitol.* **1986**, 37, 255

81. (a) Wahnon, D.C.; Shier, V.K; Benkovic, S. J . *J. Am. Chem. Soc.* **2001**,123, 976.

(b) Van Lanen, S. G.; Iwata-Reuyl, D. Biochemistry **2003**, 42, 5312

82. V.M. Kolb, *Prog. Drug Res*., **1997**, 48, 195

83. C. Perigaud, G. Grosslin, J.L. Imbach, *Nucleosides Nucleotides,* **1993**, 11, 903

84. Wu, Z.; Minhas, G. S.; Wen, D.; Jiang, H.; Chen, K.; Zimniak, P.;Zheng, J. *J. Med. Chem*. **2004**, *47*, 3282

85. Dowle, M. D.; Davies, D. I. *Chem. Soc. Rev.* **1979**, *8*, 171-197

86. Fuji, K.; Node, M.; Naniwa, Y.; Kawabata, T. *Tetrahedron Lett.* **1990,** *31*, 3175-3178

87. Fang, F. G.; Kishi, Y.; Matclich, M. C.; Scola, P. M. *Tetrahedron Lett*. **1992**, *33*, 1557-1560.