cis -Arenediols as Versatile Chiral Synthons in the Synthesis of Prostaglandins, Cyclitols, Carbohydrates, and Alkaloids.
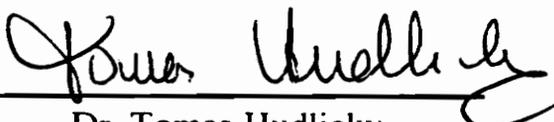
by

Hector Luna Contla

APPROVED:

_____
Dr. Tomas Hudlicky

_____
Dr. Joseph S. Merola

_____
Dr. John G. Mason

_____
Dr. Robert H. White

_____
Dr. David A. Becker

September, 1991
Blacksburg, Virginia

cis -Arenediols as Versatile Chiral Synthons in the Synthesis of Prostaglandins, Cyclitols, Carbohydrates, and Alkaloids
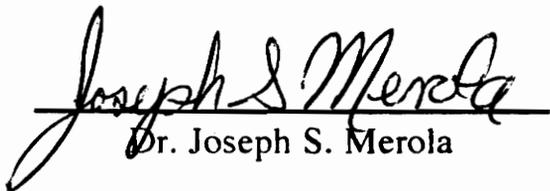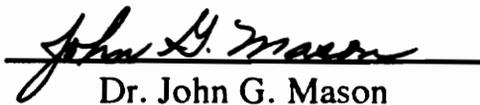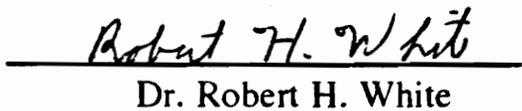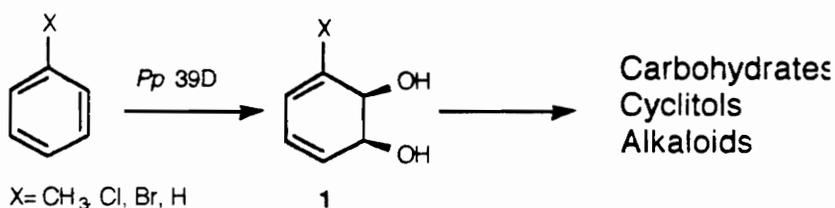
by

Hector Luna Contla

(Abstract)

The oxidation of simple benzene derivatives by a mutant of *Pseudomonas putida* , called 39-D, produces *cis*-arenediols (**1**). The diols are enantiomerically pure and can be used as synthons for the preparation of a variety of interesting compounds because of their stereochemistry and the special array of functional groups.

cis -Toluenediol (**2**) served as a chiral intermediate in an efficient synthesis of enone (**3**). which has been used to attain prostaglandin $F_2\alpha$ and Neplanocin A. The same diol (**2**) was transformed into both enantiomers of a terpene synthon (**4**).
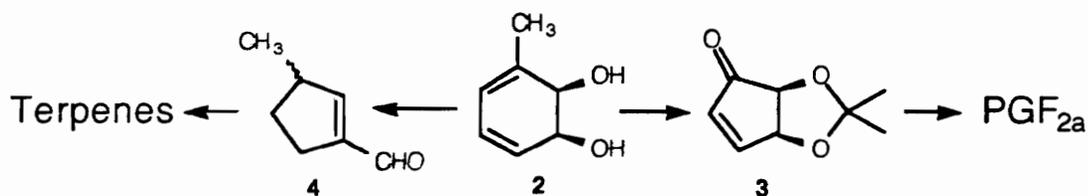
Oxidative functionalization of *cis*-chlorobenzenediol (**5**) afforded intermediates suitable for transformation into L-erythrose (**6**), conduritol C (**7**), dihydroconduritol C (**8**) and aminoconduritol F-4 (**9**).
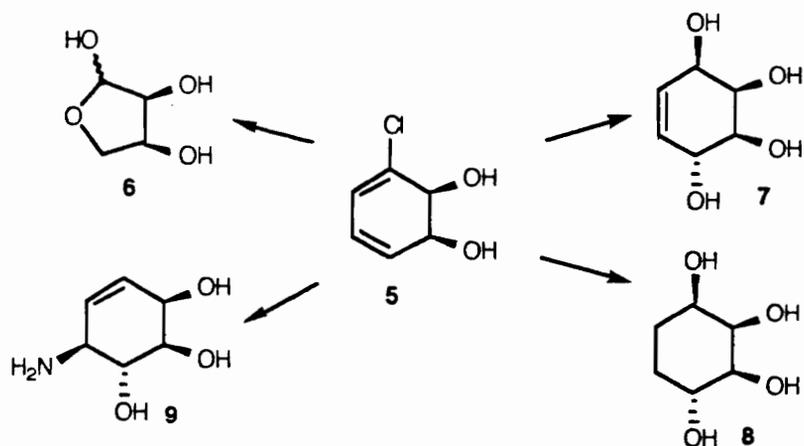
The application of this versatile synthetic protocol culminated in an approach to kifunensine (**10**), an important glycosidase inhibitor, which was approached according to the following retrosynthetic analysis:
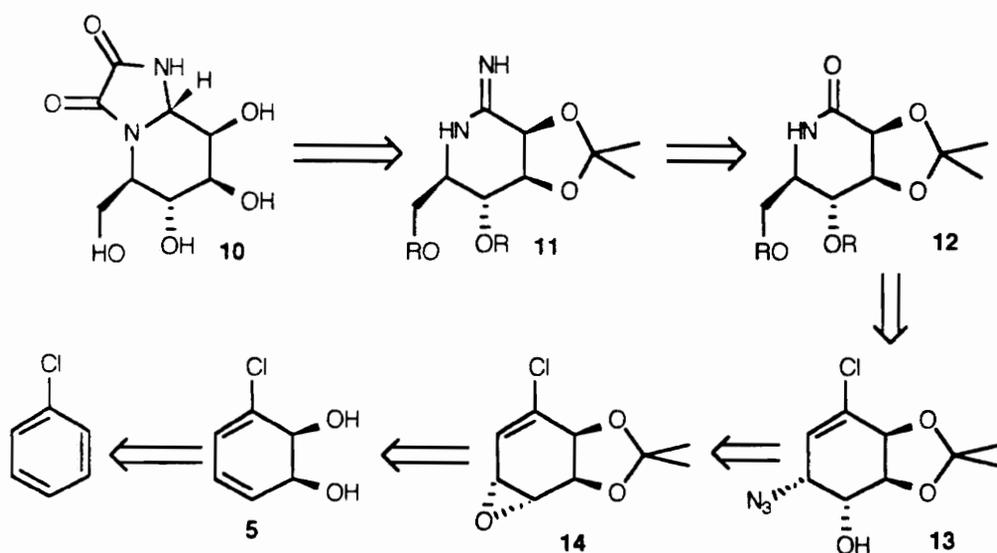
The advance in this approach reached the synthesis of amidine **12**.

A detailed study of the nucleophilic opening of epoxide **13** was carried out in order to better understand the parameters of the diastereoselective functionalization of arenediols. Details are provided for the oxidative functionalization of chlorobenzenediol (**5**), the key compound in all of the projects discussed.

ACKNOWLEDGMENTS

" Ph. D. program consists in a whole transformation of the person and it is not just the aquisition of technical knowledge and manual skills"

Hector Luna

To:

Maria Eugenia, my wife, for influencing me to study Chemistry and for all her support and sacrifices during all my career and life.

To:

Shizi, my son, for making me forget chemistry some of the time.

To:

Both, because without really knowing the situation they were going to live in, they got involved in my adventure called Ph. D.; which fortunately resulted in a valuable personal experience for me and both of them.

Table of contents

I. INTRODUCTION.

Synthetic organic chemistry has evolved over the years from emphasis on chemoselectivity to regioselectivity, to focus on diastereoselectivity and enantioselectivity, continuing into the area of enantiospecific and general design of synthesis. The state of the art of organic synthesis today, for both natural products or specifically designed molecules, is the incorporation of highly stereo- and regiocontrolled synthetic methods.

For the preparation of enantiomerically homogeneous molecules, the chemist has basically two options. First, the molecules can be synthesized in racemic form and later resolved into their optical antipodes, an option that is slowly becoming obsolete. The second choice is to perform the synthesis in an enantioselective (or preferably enantioespecific) fashion to produce chirally enriched products in <u>either</u> enantiomeric form.

The first option, namely the technique for resolving enantiomeric pairs of molecules, has been in practical use for well over a century.[1] One of the methods dictates that the compounds to be separated contain either an acid or an amine functionality in order to form a diastereoisomeric salt. Another method of resolution involves the formation of a covalent bond between the reaction substrate and a chirally pure molecule to form a pair of diastereomers, which in many instances may then be separated by chromatography. This method again requires that the substrates to be separated must possess some sort of a "handle" capable of reacting with the resolving agent. Molecules that are enantiomeric but relatively inert (biphenyl, alkanes, etc.) cannot be resolved by such means.

Advances have recently been made in resolution techniques by chromatography of racemates on chiral adsorbants.[2] The enantiomeric separation is achieved through a combination of π-acidity, hydrogen bonding, and steric interactions. The method is applicable to a wide range of compounds and can be performed on a preparative scale.

Even with the great advances in resolution methods previously mentioned, a number of problems continue. One of the major disadvantages of resolution is that half of the mixture is the undesired enantiomer, which is usually discarded, diminishing the chemical yield of the preparation.

The solution to the aforementioned problems relies on asymmetric procedures, which continue to form a current trend in organic synthesis. Strategically, this can be accomplished by two basic approaches; in one of them, asymmetry in the target molecule can be induced by means of an external chiral auxiliary reagent that, under ideal conditions, is recoverable and recyclable.[3] The asymmetry set in this fashion can then be used as a

1

further element of stereocontrol in subsequent transformations in the molecule, provided the operator takes an advantage of the actual knowledge of the reaction mechanism.

In the other approach, the synthesis of a target molecule can be designed so as to incorporate a chiral fragment whose absolute stereochemistry is already established. In this respect, nature's "chiral pool" offers a tantalizing array of such fragments that can be judiciously used to the chemist's advantage.

Among those natural products used as starting materials are amino acids[4] and alkaloids for nitrogen-containing compounds. For non-nitrogenous compounds, sugars, terpenes, and α-hydroxy acids are most frequently used.[5] An example of this strategy is the use of L-(+)-glutamic acid (**1**) in the synthesis of (-)-sulcatal (**2**)[6] and (+)-*trans*-bursevan (**3**).[7]
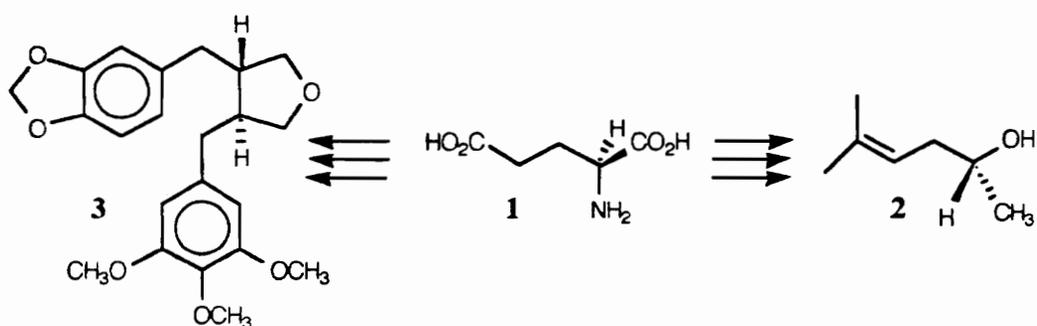
Sugars are among the most commonly used natural products to control stereochemistry in organic syntheses. For example, D-(+)glucose (**4**), a very common sugar. has been successfully transformed into $PGF_{2\alpha}$ (**5**)[8] and (+)-spectinomycin (**6**).[9]
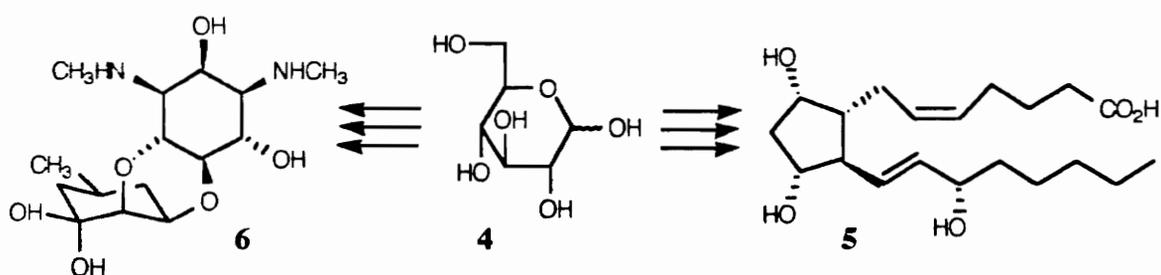
In the last 10 years a new approach has attracted the attention of researchers, namely the use of biocatalysis, biotransformations, or microbial transformations. These processes involve chemical transformations mediated by microorganisms or enzymes. The use of cell-free enzymes has attracted intensive attention as shown in several reviews.[10,11]

The practical importance of this synthetic strategy has been shown in the preparation of mevalonolactone,[12] the transformation of steroids and sterols, the synthesis of semisynthetic penicillins and cephalosporines, to name a few applications.[13] The main feature of biotransformations is that some functional group transformations can be performed stereospecifically and under mild conditions; such transformations by chemical means are sometimes impossible or require stringent conditions that produce low yields or present technical difficulties.

The objective of this work is to use one such biotransformation, dioxygenation of arenes developed by Gibson et.al.,[14] in the preparation of chiral synthons (in this case *cis*-benzenediols, **7**) from aromatic compounds. This transformation can be effected chemically in several steps in racemic fashion, but no equivalent asymmetric process is known to date.
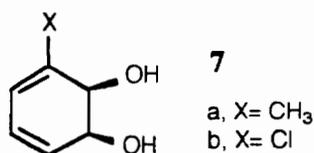
The versatility of two of these *cis*-cyclohexadienediols, those derived from toluene (**7a**) and chlorobenzene (**7b**), is demonstrated in this work. Both compounds are used for the synthesis of prostanglandins, derivatives of sugars, and an approach to the alkaloids kifunensine and azafuranose. The rich functional content of such diols is exploited in further transformations along with precisely defined symmetry considerations that lead to enantiodivergent synthetic protocols. This last feature conforms to the most recent trend in asymmetric synthesis, namely the requirement that any preparation of bioactive materials provide both antipodes for testing.

II. HISTORICAL.

II.1 Biotransformations.

During the past two decades, biochemical reactions performed by microorganisms or catalyzed by enzymes have been extensively evaluated from the viewpoint of synthetic organic chemists. The use of such reactions or methodology is referred to as *microbial transformations, microbial conversions, biotransformations, or bioconversions.*[15]

Microbial transformations are somewhat different from the so-called *fermentations.* A microbial transformation is the conversion of a chemical substance (substrate) to another substance (product) by a microorganism. The chemical reaction can be catalyzed by a particular cellular enzyme or organism or by an enzyme originally produced within the cells for a specific function that has evolved to catalyze reactions of compounds structurally related to the natural substrates.

The enzymes (or biocatalysts) used for microbial transformations increase the reaction rate by lowering the activation energy just as normal catalysts do. Enzymes, however, have more specificity, although this sometimes represents a limitation. Despite the high selectivity of enzymes, side reactions also occur in microbial transformations, and they can be avoided only by the use of single purified enzymes. High catalytic efficiency is another characteristic property of enzymes; for example, the rate of hydrolysis of an amide by chymotrypsin is about 4000 times faster than that for the corresponding base-catalyzed reaction.[16] Enzymes also exhibit their activity under milder conditions than normal chemical catalysts, as the usual conditions for enzymatic reactions are temperatures in the range of 20 to 40 °C with pH near neutrality. This topic has been widely reviewed by both biotechnologists[17-26] and chemists.[27-35] The International Union of Biochemistry lists more than 2000 enzymes[36] which are classified according to the reactions they catalyze. In addition, more than 800 kinds of purified enzymes are commercially available.[37]

The use of microorganisms to produce chemicals and fuels in the broadest sense dates back to the production of ethanol and acetic acid (as vinegar) by fermentation in prehistoric times. The latter process has been involved in the production of wine, which has been practiced since at least 10 000 BC.[38] The earliest recorded production of ethanol as a beverage was reported around 2000 BC in Assyria.[38] The preparation of distilled alcohol was first reported in the 12th century version of Mappae Clavicula (Key to

4

Painting),[39] with the first reported (non-beverage) use of spirits as incendiaries, solvents, and later in medicinal applications.

Apparently the earliest record which refers to vinegar are the Old and New Testaments.[40] The use of vinegar, an aqueous solution of acetic acid, as a condiment and in pickling has been known and practiced for as long as the art of wine making has existed. Another important fact in the history of fermentations is the preparation of acetone and butanol. The earliest work on this fermentation was performed by Pasteur in 1862.[41] The fact that acetone was also a fermentation product was not known until Schardinger made the discovery in 1905.[42]

In general, there are two ways of using microorganisms in the preparation of useful compounds: microbial transformations and fermentations. Living or growing cells are required for fermentation where the products are always those that result from the natural enzymatic pathways of that particular organism. In microbial transformations it is not important whether the cells are alive or not, as the life process is required only for the production of enzymes and the microorganism itself is simply used as a convenient source of that enzyme. Table 1 shows some of the fundamental differences between fermentations and microbial transformations.

Table 1. Differentiation Between Microbial Transformations and Fermentations.

	Microbial transformation	Fermentation
Microorganism	growing, resting, or treated cells	generally growing cell
Reaction	simple catalytic reaction (one or several steps)	life process (multistep reaction sequence)
Reaction time	short	long
Starting materials	sometimes uses expensive subtrates	inexpensive carbon and nitrogen sources
Product	natural or unnatural	natural
Product concentration	high	usualy low
Product isolation	easy	tedious

The use of biotransformations is an ancient art, the oldest and best known prototype of this kind of reaction being the aforementioned vinegar production, which has been

performed since the dawn of recorded history. In 1921, a microbial reaction for the stereospecific preparation of D-(-)-ephedrine was described.[43] It was only much later that microbial transformations gained great significance through their use in the modification of steroids via bioxidations.[44] Some of the reactions reported using this methodology cannot be achieved by chemical synthesis.
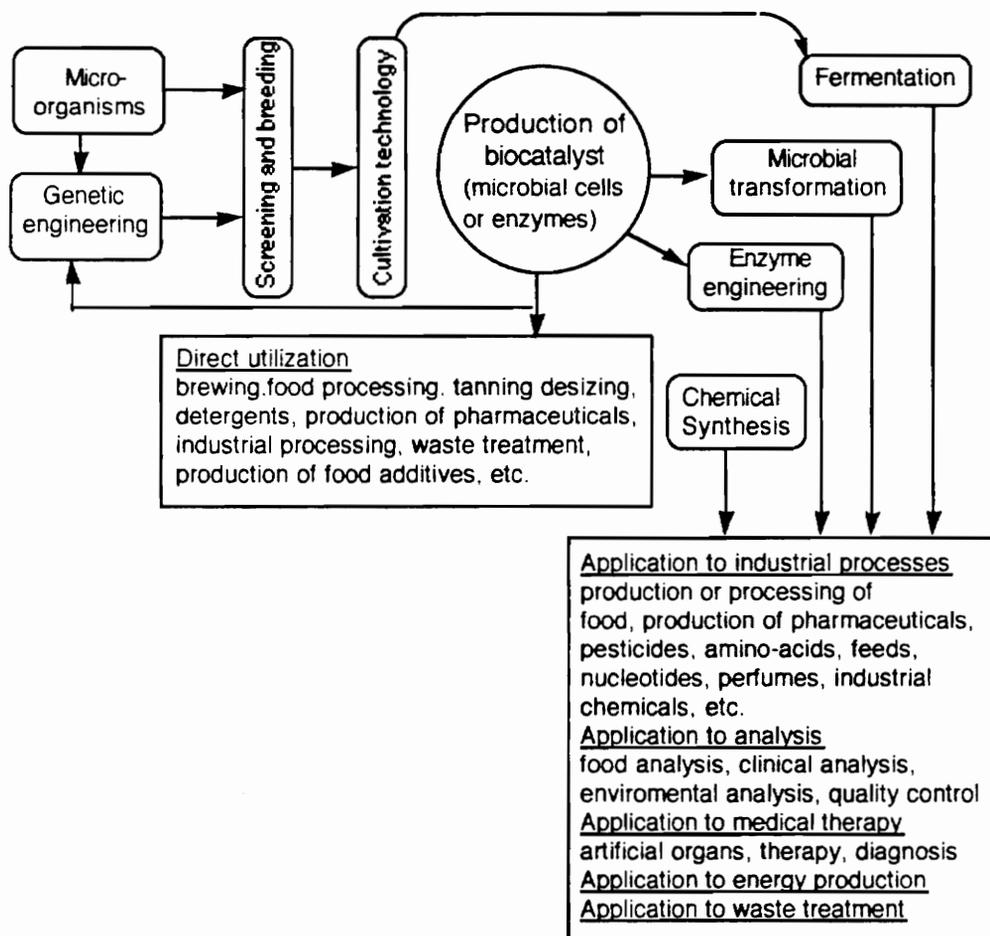
Figure 1. Overview of Biotransformations

Thousands of microbial transformations involving different types of reactions with organic compounds and natural products are now known.[45] The current technological importance of microbial transformations is well recognized in many fields of biotechnology as shown in Fig. 1.

In certain cases enzymes are considered the best practical catalysts for the preparation of specialty fine chemicals. There are many commercial processes for the production of such biological molecules as amino-acids, nucleic acid-related compounds, sugars, and lipids. For example L-alanine,[46] L-aspartic acid,[47] high-fructose corn syrup,[48] and 6-aminopenicillanic acid[49] are produced almost solely through enzymatic processes.[50]

The preparation of chiral synthons through enzyme catalysis is currently an area of intense activity in organic and pharmaceutical chemistry. The practical importance of this area has been shown in the preparation of mevalonolactone,[12] carbapene antibiotics,[51] nucleoside antibiotics,[52] insect hormones,[53] α-tocopherol,[54] muscone,[55] a prostaglandin synthon,[56] and others.

In designing a microbial transformation process, the following aspects require careful consideration:
1. Microbial physiology
2. Microbial metabolism and biosynthesis
    New microbial reactions
    New synthetic routes
3. Enzyme catalysis
    Properties of enzymes
    Mechanism of enzyme reaction
    Evaluation of potential as catalyst
4. Enzyme production
    Mechanism of enzyme production (inducers, regulators,etc.)
    Strain improvement (screening, mutation, gene engineering,etc)
    Optimization (environmental conditions)
5. Enzyme modification
    Chemical and/or biochemical modification, immobilization,etc.
6. Microbial or enzymatic synthesis and transformation
    Optimization
    Process designing

Even vegetative cell cultures are commonly used, but there are other alternatives and those are described as below:
1. Transformation with growing cells
2. Transformation with previously grown cells
3. Transformation with spores
4. Transformation with immobilized cells
5. Transformations with cell-free enzymes or purified enzymes.

The types useful for microbial transformations include whole or treated cells, organelles, cell-free multienzyme systems and simple cell-free enzymes. A new approach is emerging that has great potential for organic synthesis, the enzymatic catalysis in organic

solvents. It was believed for a long time that enzymes were only active in an aqueous medium.[57]

When a purified enzyme is used, the organic "substrate" to be transformed binds to an "active site" of the protein, which is complementary to its shape and chemical function; the chemical reactions are catalyzed by the functional groups in the vicinity of the substrate.[58] The initial binding of the substrate to the enzyme involves some selectivity, because the functional groups are arranged in particular orientation relative to the substrate. At this stage the stereochemistry of the product is determined. And consequently enzyme-catalyzed reactions are highly stereoselective and even stereospecific.

Enzyme reactions are performed in one of two ways: 1. Treatment of the solution (usually aqueous) of the compound with a purified enzyme. Recent technological advances in immobilized enzymes technology where an active form of an enzyme is attached to an inert support made enzyme-catalyzed reactions more attractive. 2. Addition of the compound to a suspension of a growing microorganism that produces the required enzyme. The use of the whole microorganism is usually simpler and cheaper than the use of isolated enzymes. Furthermore, any cofactors required in the transformation are regenerated automatically by the living microorganism. There are limitations to this method, however; the compound must be sufficiently soluble in the growing media and be taken up by the microorganism.

A new approach in this area, explored only recently, is the fascinating concept of using antibodies as catalysts. The basic idea involves raising antibodies to a stable mimic of the transition state of the reaction under investigation and then using these for the actual transformations.[59]

In conclusion, the future holds many fascinating rewards for workers in the area of biotransformations, and many more application of these techniques are expected as this area gains recognition as a "clean " alternative to chemical processes.

II.2. Microbial Oxidation of Arenes.

Pseudomonads have played an important role in our current understanding of the pathways and mechanisms used by bacteria in the degradation of a wide range of natural and man-made compounds. In 1955 Hayaishi and his colleagues showed that pyrocatechase (catechol:oxygen 1,2-oxidoreductase; EC 1.13.1) from a *Pseudomonas* species incorporated both atoms of molecular oxygen into catechol to form *cis,cis*-muconate.[60] Subsequent studies revealed that at least two hydroxyl substituents are required for oxidative fission of the benzenoid nucleus. Because of this phenomenon the enzymes involved in the incorporation of two oxygen atoms are called dioxygenases.[61]

Aromatic hydrocarbons, ranging in size from benzene to benzo[a]pyrene, are also known to be metabolized by this microorganisms.[62] Consequently pseudomonas oxygenases must be broad in specificity, as some of these enzymes may allow aromatic compounds with various ring substituents to be metabolized.

It is clear that living organisms have been in contact with aromatic hydrocarbons throughout evolutionary periods of time, leading therefore to the prediction that microorganisms would have evolved the enzymes necessary to convert these aromatic compounds to carbon dioxide and water. This assumption is supported by the fact that it is relatively easy to isolate bacteria that utilize simple aromatic hydrocarbons as sole sources of carbon and energy for growth. Attention was originally focused on the initial reactions utilized by a strain of *P. putida* to perform the incorporation of oxygen into benzene and toluene; the objective of these studies was to determine the mechanism of oxidation of aromatic nuclei by this microorganism.[63] These studies led to the discovery of a novel hydroxylation reaction and later to the use of chiral aromatic hydrocarbon metabolites in the synthesis of new polymers and pharmaceutical products.[64]

A fluorescent pseudomonad that utilizes ethylbenzene as the sole source of carbon and energy for growth was isolated.[65] This organism was identified as *P. putida* biotype B. Cells grown with ethylbenzene or toluene rapidly oxidized benzene, toluene, ethylbenzene, catechol, 3-methylcatechol, 4-methylcatechol, and, to a lesser extent, phenol, o-cresol, and m-cresol. Gibson in 1968 reported for the first time the role of *cis*-arenediols in the microbial metabolism of aromatic compounds, showing a marked difference with the equivalent transformation performed in mammalian metabolism.[66] The most surprising result was that both whole cells and cell extracts were able to rapidly oxidize *cis*-1,2-dihydroxy-1,2-dihydrobenzene (*cis*-benzene dihydrodiol). This indicated that *cis* benzene

dihydrodiol was an intermediate in benzene degradation, and this was confirmed by the isolation of a mutant, *P. putida* 39/D, that accumulated *cis*-benzene dihydrodiol in the culture medium after growth on glucose in the presence of benzene. Experiments with $^{18}O_2$ showed that both oxygen atoms in the dihydrodiol were derived from atmospheric oxygen.[67] The initial reactions in the oxidation of benzene by *P. putida* are shown in Fig. 2.

   P. putida 39/D also oxidized toluene to a dihydrodiol that was isolated in crystalline form. This product was unstable and, at pH values below 7.0, spontaneously eliminated
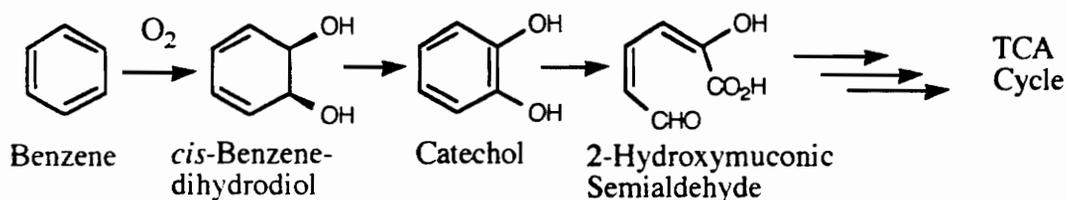
Figure 2. Oxidation of Benzene to 2-Hydroxymuconic Acid by *P. putida*

water to yield o-cresol. The position and relative stereochemistry of the hydroxyl groups in the dihydrodiol were determined by proton magnetic resonance spectrometry of a Diels-Alder adduct.[68] The latter was formed by reacting the diacetate of toluene dihydrodiol with maleic anhydride. The absolute stereochemistry of the metabolite was determined by X-ray crystallography of a Diels-Alder adduct formed from the diacetate by reaction with 4-(p-bromophenyl)-1,2,4-triazoline dione.[69] The absolute stereochemistry of the toluene dihydrodiol was also determined by converting it to methyladipic acid of known absolute stereochemistry and by application of the dibenzoate chirality rule of Nakanishi and Harada.[70,71] These experiments confirmed that the product formed from toluene is (+)-*cis*-(1S.2R)-dihydroxy-3-methylcyclohexa-3,5-diene (*cis*-toluene dihydrodiol).

   Crude cell extracts prepared from toluene-grown cells of *P. putida* oxidize toluene to *cis*-toluene dihydrodiol (Fig. 3). The enzyme was designated toluene dioxygenase and

10

its activity was shown to depend on the presence of NADH and oxygen. In addition, the
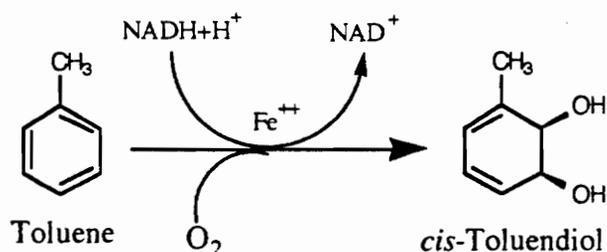
Figure 3. Oxidation of Toluene to *cis*-Toluenediol by *P. putida* F1.

rate of toluene oxidation is stimulated by the presence of Fe^{II}.[72] Subsequent studies led to the isolation of three protein components that are essential for toluene dioxygenase activity. These have been characterized as a flavoprotein (ferredoxin$_{TOL}$ reductase),[73] a [2Fe.2S] ferredoxin (ferredoxin$_{TOL}$),[74] and an iron sulfur protein (ISP$_{TOL}$), which is the terminal

151,000	15,400	46,000	Mol. Weight
52,500 20,800	15,400	46,000	Subunit Mol. Weight
todC1	todB	todA	Gene Designation

todC1   todC2
52,500 20,800

Figure 4. Organization and Gene Designation of the Components of Toluene Dioxygenase from *Pseudomonas putida*.

oxygenase in the sequence as shown in Fig. 3.[74] It was at this point that it was given to *P. putida* the designation F1 strain.[75] It has been recently reported the standardized methods for the isolation of toluene dioxygenase (from *P. p* F1),[77] as well as for other two related enzymes, benzene dioxygenase from *P. p* ML[NCIB 12190][78], and naphtalene dioxygenase from *P. p* NCIB 9816.[79]

The genes encoding the first four enzymes in toluene degradation are coordinately induced and form part of the Tod operon.[80] DNA from *P. putida* F1 was cleaved with EcoRl, and the resulting fragments were cloned into *Escherichia coli* HB101 by using the cosmid cloning vector pLAFR1.[81]. The recombinant organism *E coli* JM109(pDTG601), after induction with isopropyl-β-D-thiogalactopyranoside, oxidized toluene to *cis*-toluenediol.
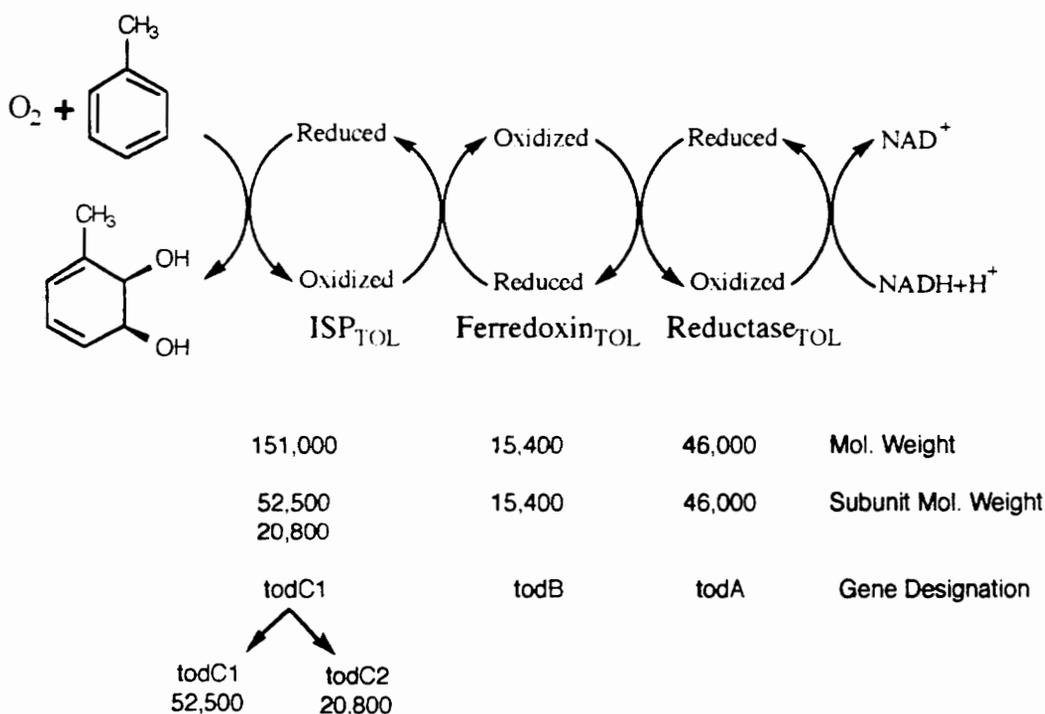
Toluene dioxygenase is a remarkable enzyme that has the ability to oxidize a wide range of substrates. The enzyme produces optically pure hydroxylated products as mentioned above. The same absolute stereochemistry is found in the dihydrodiols formed from ethylbenzene, chlorobenzene, *p*-fluorotoluene, and biphenyl.[70] In addition, toluene dioxygenase oxidizes (±)-3-methylcyclohexene to *cis*-diols that have the same absolute stereochemistry in the carbon atoms bearing the hydroxyl groups.[82] The relative importance of steric and electronic factors can be seen in *p*-halogenated toluene derivatives, in which the size of the halogen increases as its electronegativity decreases in the series of F. Cl. and Br. All three *p*-halogenated substrates are oxidized to the corresponding *cis*-dihydrodiols. However, only *p*-fluorotoluene dihydrodiol is optically active. Apparently, the enzyme can not distinguish between the sizes of methyl, chloro, or bromo in substituted benzenes. and the electronegativity of the substituents seems not influence the course of the oxidation.[70]

Phenol and certain substituted phenols are oxidized by toluene dioxygenase to the corresponding catechols.[83]. Catechol formation was observed with *P. putida* F39/D, which contains a defect in the structural gene for *cis*-toluene dihydrodiol dehydrogenase.[84] These observations suggest that toluene dioxygenase catalyzes dioxygenation of phenol to form the first intermediate shown in Fig 5, which is a hydrated ketone that rearomatizes to give catechol.[85] Substrates oxidized by *P. putida* F 39/D and *E. coli* JM109(pDTG601) are listed in Table 2.[64]
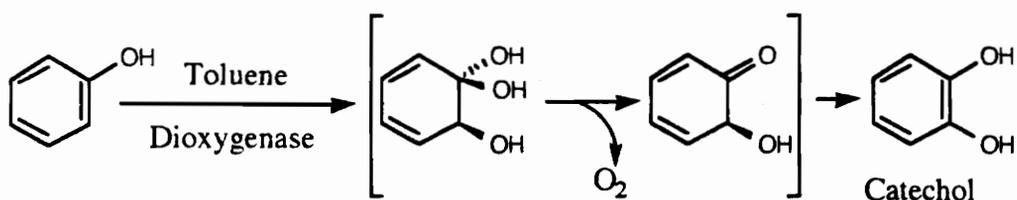
Figure 5. Proposed Mechanism of Phenol Oxidation by Toluene Dioxygenase from
Pseudomonas putida

It has been proposed that the presence of halogen, as well as nitro and sulfonate substituents, appears to reduce or prevent the complete biodegradation of many aromatic compounds; whereas carboxylic and phenolic hydroxyl groups favors degradation of substituted benzenes. The *meta* isomers were commonly found to be the most resistant.[86]

However, *Pseudomonas putida* F1 has shown utility not only in the preparation of *cis*-dihydrodiols but also as a potential antipollution agent. Toluene dioxygenase has been implicated as an enzyme of degrading trichloroethylene (TCE). This has been confirmed with *E. coli* JM109(pDTG601) that contains the structural genes of toluene dioxygenase. The latter microorganism was shown to degrade TCE.[87]

Because the utility of *cis*-dihydrodiols as synthetic intermediates has been already proved, the necessity to produce these kind of compounds in a multigram scale has arisen, generating studies related to the optimization and the scale-up of the process. An example is the recent report related to the preparation of cis-toluene diol.[88]

The utility of the microbial oxidation can be expanded by the use of a variety of substrates. For example it has been observed that other microorganism can perform these kind of hydroxylations in polycyclic arenes; for example, *cis*-1,2-dihydroxy-1,2-dihydronaphtalene was produce in appreciable quantities.[89] Later *P. putida* 119 was shown carry out the same transformation.[90] On the other hand, Gibson and Ziffer demonstrated that single double bonds can be transformed in *cis*-diols using *P. putida* 39/D, demonstrating that the oxidation was stereoselective, providing the same stereochemistry present in *cis*-toluenediol.[82] A recent review of compounds prepared by dioxygenation of arenes has been published.[91]

In summary the hydroxylation of olefins by means of microorganisms seems to have a promising future, especially when the stereochemistry of the outcoming diols is properly handled in further synthetic manipulations.

Table 2. Substrates oxidized by Toluene Dioxygenase

Substrate	Major Product(s)[a]	Organism	
		P.putida F39/D	E. coli JM109(pDTG601)
Benzene	cis-1,2-dihydrodiol	+	+
Toluene	cis-2,3-Dihydrodiol	+	+
Ethylbenzene	cis-2,3-Dihydrodiol	+	+
Propylbenzene	cis-2,3-Dihydrodiol	+	+
Cyanobenzene	cis-2,3-Dihydrodiol	+	NT[b]
Fluorobenzene	cis-2,3-Dihydrodiol	+	NT
Chlorobenzene	cis-2,3-Dihydrodiol	+	+
Bromobenzene	cis-2,3-Dihydrodiol	+	NT
Trifluorotoluene	cis-2,3-Dihydrodiol	+	+
Anisole	cis-2,3-Dihydrodiol	+	+
Styrene	cis-2,3-Dihydrodiol	+	NT
4-Fluorotoluene	cis-2,3-Dihydrodiol	+	+
4-Chlorotoluene	cis-2,3-Dihydrodiol	+	+
4-Bromotoluene	cis-2,3-Dihydrodiol	+	NT
1.2-Dimethylbenzene	2,3-Dimethylphenol	NT	+
1.3-Dimethylbenzene	2,4-Dimethylphenol	+	+
1.4-Dimethylbenzene	cis-2,3-Dihydrodiol	+	+
1.2-Dichlorobenzene	cis-3,4-Dihydrodiol	+	+
1.3-Dichlorobenzene	cis-4,5-Dihydrodiol	+	+
1,4-Dichlorobenzene	cis-2,3-dihydrodiol	+	+
Biphenyl	cis-2,3-Dihydrodiol	+	+
2-Chlorobiphenyl	cis-2,3-Dihydrodiol[c]	NT	+
3-Chlorobiphenyl	cis-2,3-Dihydrodiol[c]	NT	+
4-Chlorobiphenyl	cis-2,3-Dihydrodiol[c]	NT	+
Naphthalene	cis-1,2-Dihydrodiol	+	+
2-Chlorophenol	3-Chlorocatechol	NT	+
3-Chlorophenol	3-Chlorocatechol	NT	+
4-Chlorophenol	4-Chlorocatechol	NT	+
2-Methylphenol	3-Methylcatechol	NT	+
3-Methylphenol	3-Methylcatechol	NT	+
4-Methylphenol	4-Methylcatechol	NT	+
indan	1-Indanol	+	+
Indene	cis-1,2-		
	Dihydroxyindan	+	+
	1-Indanone	+	+
	1-Indenol	+	+
Indole	Indigo	+	+
Trichoroethylene	Unidentified	+	+

[a] Monosubstituent arbitrarily assigned position 1.

[b] NT, Not tested.

[c] Hydroxylation occurs on the unsubtituted ring.

II.3. Functionalization of Olefins and Dienes by Oxidation.

II.3.1. Ozonolysis.

Ozone has proved to be highly useful and powerful oxidizing agent not only on a laboratory scale, but also in the chemical industry. The use of ozone in organic chemistry dates back to 1903.[92] An important step in this area was the proposition of the reaction mechanism by Staudinger,[93] followed by the determination of the ozonide structure.[94] However, Criegge can be considered the father of modern organic chemistry of ozone, thanks to his contributions in the understanding of the reaction mechanism. In this discussion the term ozonolysis will be used as a synonym of ozonization, because the cleavage of a C-C double bond is involved throughout; the term ozonide will be given to the 1.2.4-trioxolane structure.[95]

The structure of the ozone molecule can be described as a resonance hybrid of the four canonical forms represented in Fig. 6; each oxygen atom is sp^2-hybridized, with two
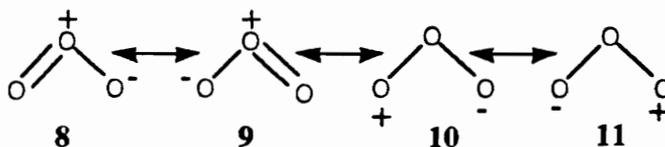

Figure 6. Resonance Structures of Ozone.

of the three sp^2 orbitals the apex atom forming localized sigma molecular orbitals by combining with bonding sp^2 orbitals of the terminal oxygen atoms. The ground state angle of the ozone molecule is 116°45".

Ozonolysis is the most important of all reactions involving ozone. The mechanism of this reaction, as proposed by Criegee, is outlined in Scheme 1.[96] The first intermediate (12), assumed to be very unstable, cleaves to the zwitterion 13. A carbonyl compound (15) and a carbonyl oxide (14) are then produced from the cleavage of 13. The direction or regiochemistry of this last fission depends on the structure of the reacting molecule. Stabilization of these two intermediates can occur by: (a) an intermolecular rearrangement to give the ozonide (16), accompanied by some polymeric material; (b) dimerization of the carbonyl oxide to give the tetraoxane , with polymeric peroxides; (c) reaction with the solvent, usually called a participating solvent; and, (d) rearrangement to give "abnormal"

ozonolysis products; this situation occurs when not only the C-C double bond is cleaved but also one of the C-C single bonds next to it is cleaved..
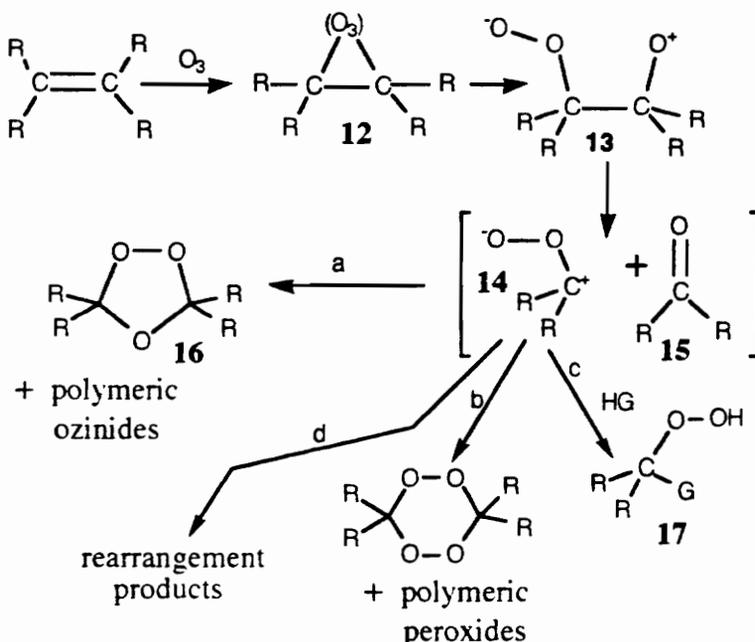

Scheme 1. Criegee Mechanism

Although the mechanism depicted in Scheme 1 is universally accepted, research continues on various details of ozonolysis. A complete understanding of the ozonolysis must include the following: (a) the nature of the initial attack of ozone on the olefin; (b) the structure of the initial ozone-olefin adduct; (c) the mechanistic route for the decomposition of the initial adduct; and, (d) the elements controlling the regiochemistry of formation of the carbonyl oxide.

Although ozonolysis has been widely used and studied, it is surprising that ozonolysis of conjugated dienes has not been seriously addressed. The few reports in the literature are mainly concerned with the description of non-peroxidic stable end products resulting from the cleavage of one olefin,[96-98] or both[99-104] in acyclic and of cyclic diene compounds. Thus, in some cases it was reported that diozonolysis of a diene system followed a conventional pattern and resulted in the cleavage of the double bonds only, whereas in other cases abnormal reactions have been observed, in which the carbon skeleton has been cleaved both at the double bonds and at the central single bond.

16

All of these previous reports did not provide a unified view of the reaction because of the existence of many rearranged unexpected products. For example Griesbaum
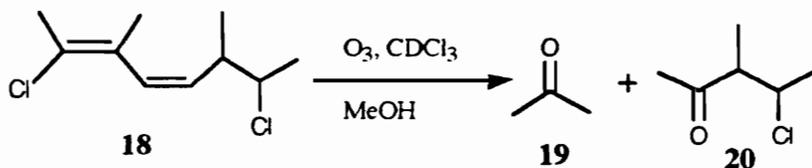
Scheme 2.

observed that ozonolysis of **18** led to product s**19** and **20** (Scheme 20), which resulted from the cleavage of both double bonds and the single bond in the conjugated diene.[104] The objective was frequently not the desire to understand of the reaction, but only as structure proof of an exocyclic diene.[99,100]

More insight into the mechanism came when it was shown that the ozonolysis of non-conjugated dienes or trienes could be performed selectively.[98,105] These results indicated that the attack of ozone on the double bond was sequential and not simultaneous. The eighties marked the initiation of some studies on the ozonolysis of dienes. Veysoglu demonstrated that selective mono-ozonolysis can be controlled.[106]

Mechanistic studies were initiated by Grisbaum, who demonstrated the striking influence of the solvent on the products of the reaction and therefore on the reaction pathway.[107] In further studies the cleavage of not only the double bond but also the single bond was corroborated by the monitoring of the stepwise ozonolysis of **7**.[97]

In the first step, 2,3-dimethyl-1,3-butadiene (Scheme 3) was cleaved by ozone to afford the fragments **22** and **23**. In a subsequent ozone cleavage step, **22** was postulated to react to give fragments **24** and **25**. Of these, **24** can undergo subsequent reactions with **22** and/or **21** to form **27** and by acid catalysis to form **19**, as had been previously demonstrated with authentic **24**.[108] Compound **25** is essentially completely cleaved at the central carbon single bond and at the peroxide bond to form **29**, **30**, and minor amounts of **31**. The predominance of **30** over **29** after 22 h of reaction time was probably due to partial esterification of **29** in the methanolic medium.

The latter results show that the anomalous cleavage of the central single bond in 2,3-dimethyl-1,3-butadiene does not occur in the ozonolysis step itself. This report also indicated the great influence of the solvent in controlling this reaction: the use of non-polar

solvents resulted in unstable products (a violent explosion occurred), but a participating solvent, like methanol, allowed the safe completion of the reactions.[97]
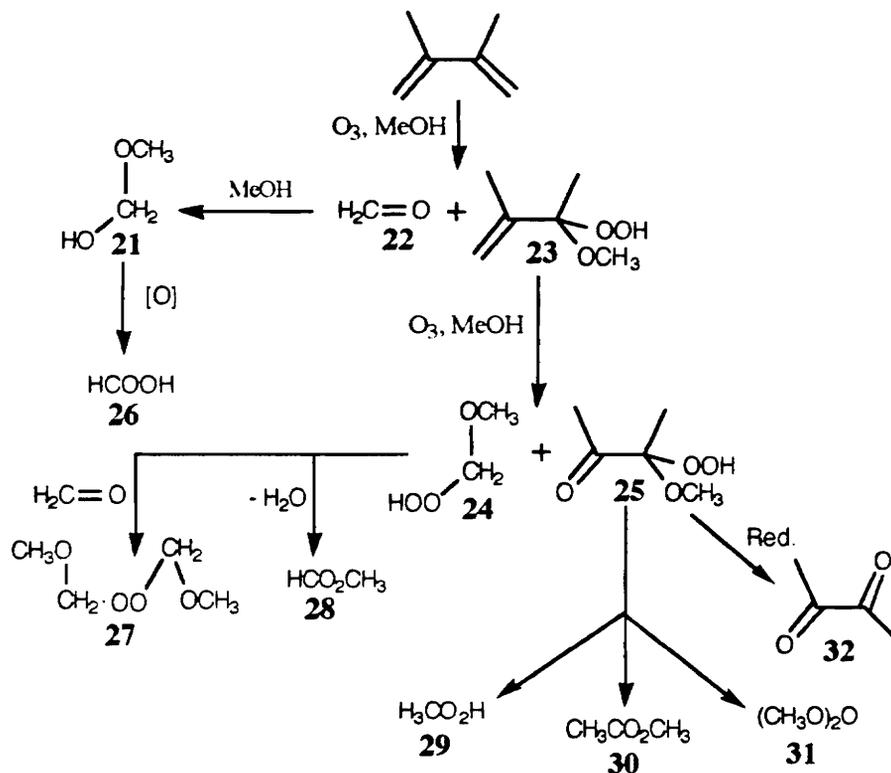
Scheme 3. Ozonolysis of 2,3-dimethyl-1,3-butadiene

A similar result using cyclic 1,3-dienes was reported by Hudlicky and coworkers. In a non-participating solvent it was shown that the reaction was a stepwise process, with
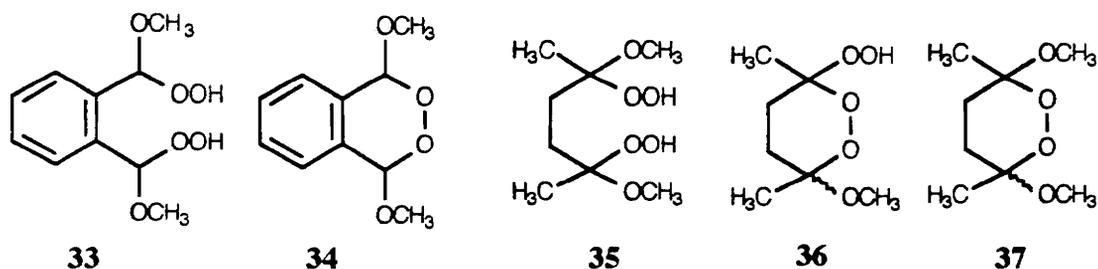
one of the double bonds (more electron-rich) attacked first. This was demonstrated by subjecting the products of mono-ozonolysis to further ozonolysis.[109] Details of this topic will be discussed later.

18

More recently the presence of α-methoxyhydroperoxides as intermediates in the ozonolysis of dienes in methanol, as well as the sequential nature of this reaction, have been demonstrated.[110] The work described in this report indicated that the course of the
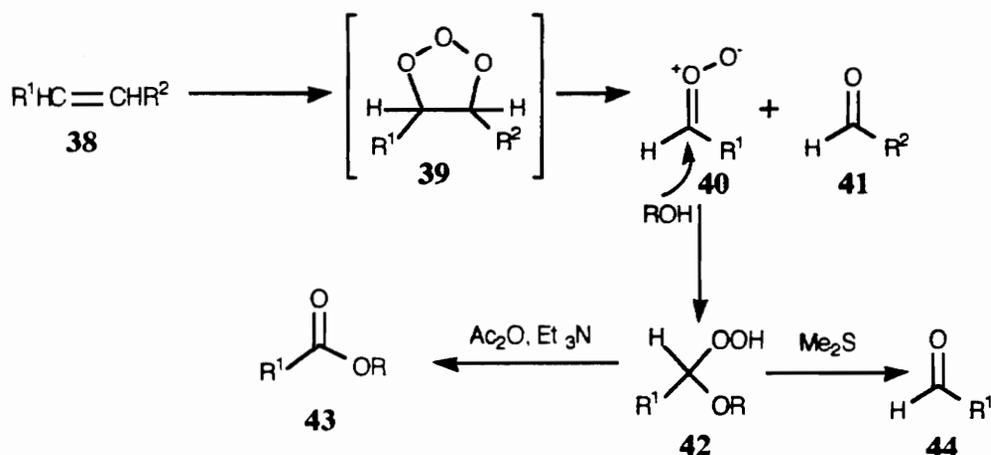

Scheme 4. Mechanism of Formation of α-alcohoxyhydroperoxide Intermediate

ozonolysis of conjugated dienes deviates considerably from that of similarly structured non-conjugated dienes both in the sequence of ozone attack and in the direction of cleavage of the two double bonds. Furthermore, the previously postulated[111,112] intermediacy of 1,4-bis-methoxyhydroperoxides like 24 was proven by the isolation of 27. It was shown that these species serve not only as precursors for compounds like 25 or 28, but also react also to give the hitherto unknown class of compounds 27, Scheme 3.

The formation of the α-alkoxyhydroperoxide 42 resulted from the nucleophilic attack of the solvent (ROH) on the carbonyl oxide 40 (Scheme 4).[113,114] Also as a consequence of the chemical manipulation of the α-methoxyhydroperoxides, different functional group arrays can be obtained, as shown by Wang (Scheme 5)[114] and others.[115-119] The stabilization of the carbonyl oxides could also lead to rearranged products, as shown by Griesbaum.[120,121] The author concluded that, especially for cyclodienes,[121] the fragmentation pattern of the primary ozonides may depend on the size of the ring.

An additional complication of the reactivity of conjugated cyclodienes is encountered when a halogen is attached to the conjugated system, because the reactivity of
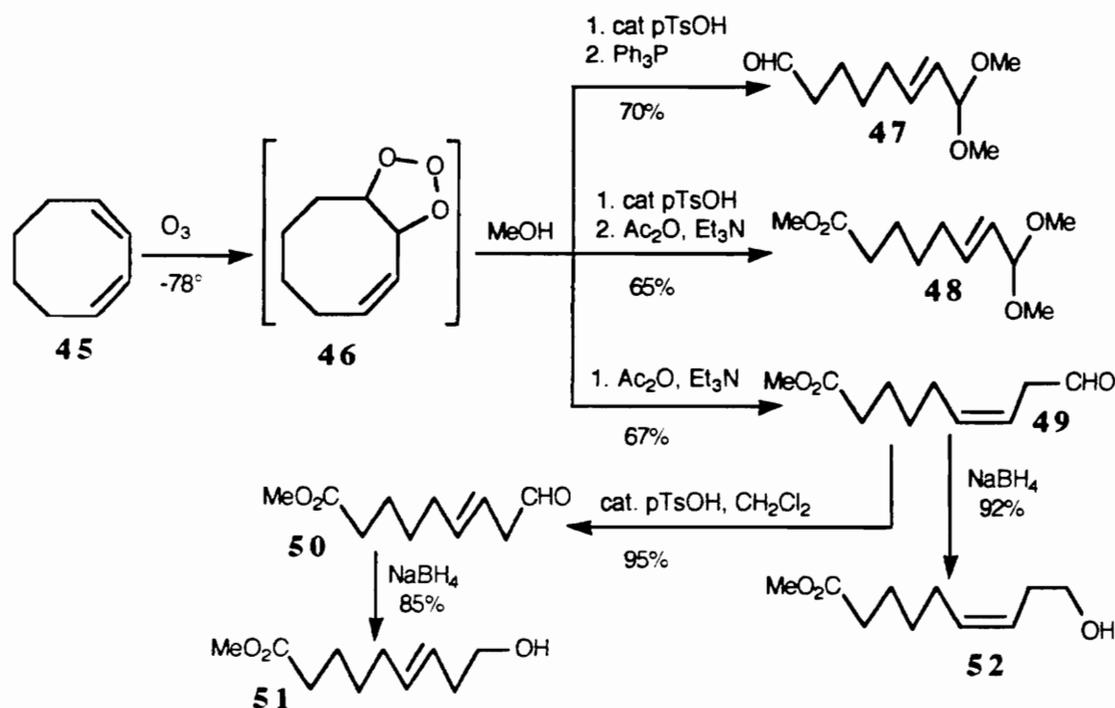
Scheme 5.

the diene is decreased, especially in the double bond directly attached to the halogen atom.[123-126] Also the cleavage of that double bond will produce a carboxylic acid derivative, whose type will be dependent on the solvent of the reaction; in a non-participating solvent that intermediate will be an acid chloride.[127-129] The complexity of the reaction is exemplified by the course of the ozonolysis of *trans*-2,3-dichloroethene as proposed by Griesbaum, Scheme 6.[128]

On the other hand the halogen confers regioselectivity to the reaction, allowing the ozonolysis to occur selectively at the double bond not bearing the halogen.[130] This brief survey of the ozonolysis of conjugated dienes shows its potential in the functionalization of dienes such as *cis*-benzenediols.
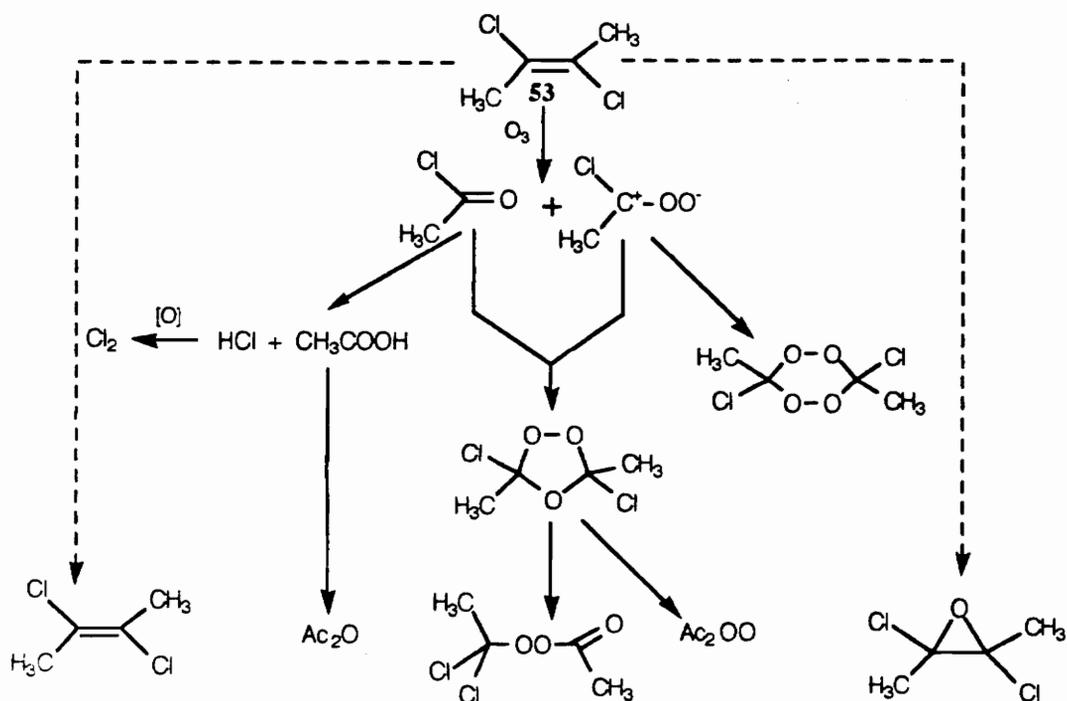
Scheme 6. Products from the Ozonolysis of *trans*-2,3-dichloro-2-butene in Non-participating Solvent and Probable Course of the Ozonolysis Reaction.

II.3.2. Singlet Oxygen Addition.[131,132]

Singlet oxygen was discovered by Kautsky in Germany in 1931,[132] but the theory behind his discovery was not accepted and his work remained unnoticed for about 25 years. The rediscovery of singlet oxygen was made in 1964 by Foote and Wexler[133] and Corey and Taylor[134] while working in the photoaddition of oxygen to double bonds. Since then the use of singlet oxygen has been widely applied in organic chemistry.

Although O_2 has a "double bond", its outermost pair of electrons are in different orbitals, and have their spins parallel making the ground state a triplet. The singlet state ($^1\Delta_g$) lies only 22.54 Kcal/mol above the ground state and has a lifetime of 1 h in the absence of collision with other molecules. This excited state of oxygen is reached by the absorption of ultraviolet (UV) or visible light.

Table 3. Lifetimes of Singlet Oxygen in Solution at Room Temperature (sec).[135-137]

Solvent	$10^6 \tau (O_2)$	Solvent	$10^6 \tau (O_2)$
H_2O	2	D_2O	20
H_2O-CH_3OH (1:1)	3.5	D_2O-CH_3OH (1:1)	11
C_2H_5OH	7	D_2O-CD_3OD (1:1)	35
Cyclohexane	17	tert-C_4H_9OH	34
Benzene	24	Pyridine	33
Acetone	26	CS_2	200
Acetone-d_6	26	CCl_4	700
Acetonitrile	30	C_6F_6	600
Chloroform	60	CF_3Cl (Freon-11)	1000

The shortest lifetime of singlet oxygen is found in solvents that contain hydroxyl groups, owing to the successful transfer of electronic energy to the vibrational energy levels of the O-H moiety.[135,137] Some characteristic data are shown in Table 2.

At present, a rather impressive variety of sources of singlet oxygen is available. These can be conveniently divided into the following categories, most of which also represent benchmarks in singlet oxygen history.

1. Photosensitization. The combined action of light, sensitizer, and oxygen is the basis for the oldest method employed for producing singlet oxygen. The basic mechanism is that singlet oxygen molecules are generated by transfer of energy from the excited state of the sensitized molecule to the oxygen molecule in the ground state, thereby raising the latter to its excited singlet state. This reaction suggests that both the singlet and triplet excited states of the sensitizer can transfer the energy to oxygen.

The chemical reactions of singlet oxygen generated by photosensitization in the liquid phase require an experimental set-up consisting of a light source and a reaction solution that contains the sensitizer, substrate, solvent, and oxygen. The specific conditions for photolysis differ according to the nature of the substrate, sensitizer, and solvent. Any light source whose emission spectrum matches the absorption band of the sensitizer can be employed.

In general, useful sensitizers for singlet oxygenations are the strongly colored thiazine and fluorescein-type dyes for protic and polar solvents, and porphyrins and aromatic compounds for aprotic, nonpolar solvents. The most commonly used sensitizers are methylene blue and rose bengal (for aqueous or alcoholic solutions) and zinc tetraphenylporphyrin (for benzene). A typical concentration of a dye sensitizer is 120 to 150 mg L^{-1}. The initial concentration of the substrate should not exceed 0.1 mol L^{-1}, in order to avoid undesirable side reactions.

The approach of using sensitizers bound to a solid support was developed in order to avoid the inherent drawbacks of homogeneous sensitization: difficulties in removing the dye from the reaction product, solubility restrictions of the dyes in various solvents, aggregation of dye molecules or complexation with certain substrates such as proteins, and bleaching of the dye over long reaction times.

The use of insoluble sensitizers is disarmingly simple. The sensitizer is suspended in a convenient solvent containing the substrate with mechanical stirring and bubbling oxygen, and then irradiated.

2. Gaseous Discharge.[132] The excitation of some molecules to the singlet excited level occurs in a stream of oxygen gas subjected to electrodeless discharge. By this procedure, concentrations of up to 10% O_2 ($^1\Delta g$) can be reached, and steady state concentrations of O_2 (^1Eg) of circa three orders of magnitude lower is produced as a result. In addition, the singlet oxygen is always mixed with a large excess of ground state oxygen, approximately 10% oxygen atoms in their ground ^3P state, and traces of ozone. The least desirable contaminants in this system are oxygen atoms and ozone. The formation of ozone is easily avoided by operating the discharge at a low oxygen pressure of a few torr.

The generation of singlet oxygen by microwave discharge is ideally suited for studying gas phase reactions. The equipment for production of singlet oxygen by discharge requires two basic components: a microwave generator and a vacuum system.

3. Decomposition of hydrogen peroxide.[132] Reactions using H_2O_2 are among the oldest chemical means of generating singlet oxygen. The singlet oxygen generated in the NaClO-H_2O_2 reaction was used in 1964 for the first time as a chemical reagent for preparative peroxidation of unsaturated compounds.[133] The reaction can be performed by adding either NaClO solution to a solution of H_2O_2 or chlorine gas to an alkaline solution of H_2O. This is an unambiguous way to generate singlet oxygen.[138] It should, however, be noted that the use of this method with unreactive substances can lead to side reactions of a free-radical nature.

The yield of oxidation products during decomposition of hydrogen peroxide is sensitive to the nature of the solvent. Based on hypochlorite, a yield of oxidation products higher than 60% is obtained in methanol, ethanol, and 1:1 methanol-t-butyl alcohol mixture; in isopropanol the yield drops to 40%.

A variation of this method uses bromine and alkaline H_2O_2.[139] This system provides a convenient peroxidation method for 9,10-disubstituted anthracene to the corresponding endoperoxides. For preparative purposes, 30% H_2O_2 and commercial NaClO are usually the reagents of choice; the amount of H_2O_2 in the solution must always be in excess of the amount of hypochlorite to be added, since in the absence of H_2O_2 the hypochlorite reacts with the acceptor.

The synthetic utility of this method is somewhat limited. Because of the hydrolytic solvent required and the resulting solubility restrictions, the method is not useful for all substrates. Obviously, it cannot be used with substrates that react with H_2O_2 or are sensitive to high alkalinity.

4. <u>Thermal decomposition of ozonides</u>.[132] Murray and Kaplen provided the experimental evidence that thermal decomposition of triphenyl phosphite ozonide released singlet oxygen.[133] The adduct between early phosphites and ozone was prepared at -78°C and subsequently decomposed to the corresponding phosphate by warming it above -35°C, where molecular oxygen was released. This molecular oxygen could oxidize typical singlet oxygen acceptors to give the same products as those formed by dye-photooxidation.

In contrast to the normal singlet oxygenation, this bimolecular reaction exhibits a different selectivity,[140] and is devoid of stereospecificity.[141] These facts are accommodated by a mechanism involving initial bond formation between the olefin and the center oxygen atom of the ozonide.

A large number of ozonide adducts have been suggested and tested as chemical sources of singlet oxygen; among them, two bicyclic phosphites have been reported to form ozonides of remarkable stability. Thus the ozone adduct of **54** produces singlet oxygen efficiently at temperatures above 0°C.[90] Similarly **55** yields an adduct that is 1.4 times more stable than the adduct of **54** at -5°C and can still decompose quantitatively to $^1O_2$.[142]
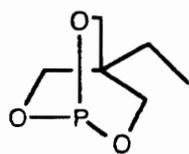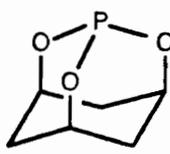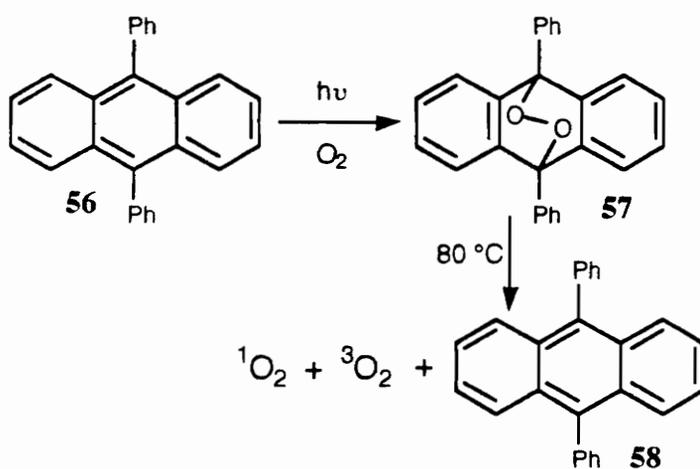
54   **55**

5. <u>Thermal decomposition of photoperoxides</u>. Many polycyclic aromatic transannular peroxides undergo loss of molecular oxygen upon thermolysis accompanied by regeneration of the parent hydrocarbon.[143] It has been proven that the oxygen released possesses singlet multiplicity (Scheme 7).

Scheme 7.

6. <u>Miscellaneous sources</u>. Many other physical and chemical processes have been reported to generate singlet oxygen,[132] but a complete description is out of the scope of this work. With regard to products of singlet oxygen reactions with hydrocarbons that contain one or more C-C double bonds, there appear to be only three types based on the primary products that are formed during the reaction:

    I. Endoperoxide synthesis. (Cycloaddition)
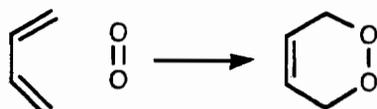
    II. Allyl hydroperoxide synthesis. (Ene Reaction)

25
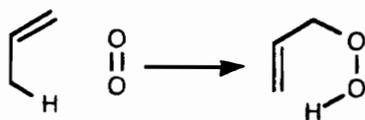
III. Dioxetane Synthesis. (Cycloaddition)

D = electron-donor group

This survey will includes only reaction where 1,3-dienes are involved, specifically cyclic dienes. These compounds react with singlet oxygen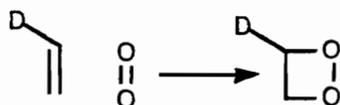 by cycloaddition reactions of type I, but the ene reaction cannot be disproved. In recent years several reviews have been published.[144]

The method used for the preparation of bicyclic endoperoxides consists of the interaction of a cyclic diene with the very reactive singlet oxygen. Minor differences arise in a particular technique depending on the methods used for the generation of singlet oxygen, as previously discussed. In the following paragraphs a few examples will be briefly discussed. Saito et al.[145] reported the reaction of singlet oxygen that involves a sequential ene reaction followed by a cycloaddition as shown below:
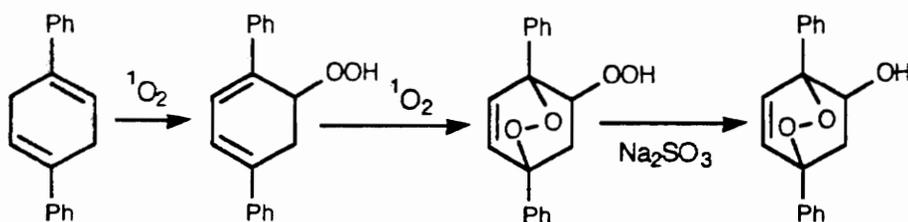
That the regiochemistry of the cycloaddition is governed by the principles of the Diels-Alder reaction is demonstrated in the preparation of **59**;[146] the reaction takes place at the more electron-rich diene system in the molecule.
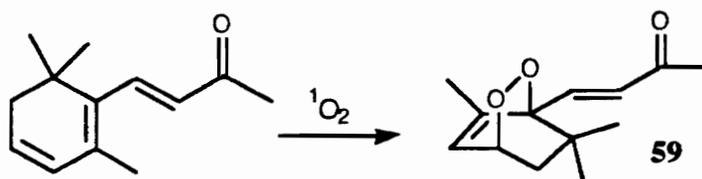
Uda reported the attack of singlet oxygen from the less hindered face, giving preferentially **60**. The same author observs no significant effect of solvent or temperature
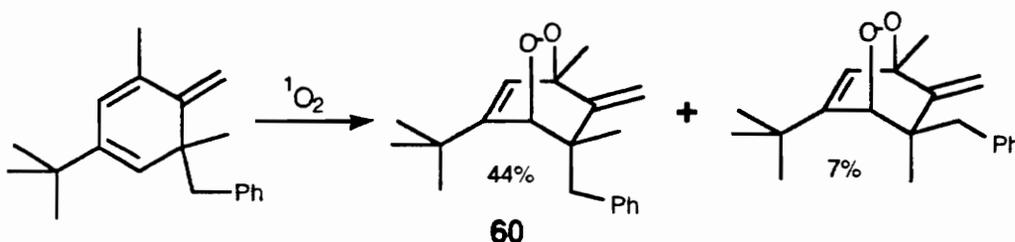
in the reaction.[147] The reaction producing **61** also gives some insight to the stereochemistry of the addition of singlet oxygen; the addition takes place from the side opposite to the methyl groups:[148]

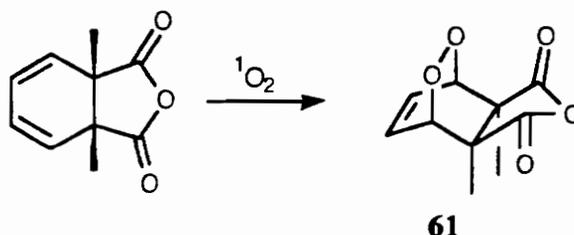On the other hand, some Lewis acids efficiently catalyze the insertion of triplet oxygen into certain conjugated dienes under mild conditions to yield cyclic peroxides identical with those obtained by the reaction with singlet oxygen.[149] Some examples are shown below: Endoperoxides can undergo a variety of reactions producing interesting functional group arrays; these reactions can be grouped into reductions, thermal decompositions, fragmentations, photolyses, and rearrangements.

The peroxide linkage is highly sucseptible to reductive cleavage by a variety of reducing agents. It is therefore not surprising that under catalytic hydrogenation of unsaturated endoperoxides, the peroxide bond as well as the double bond is reduced. Reduction of the peroxidic bond can be circumvented while reducing the C-C bond by the use of diimide. A variety of methods have been used for the generation of diimide.[152]

MoCl$_5$, O$_2$ Ref. 150
SO$_2$

C$_8$H$_{17}$

WCl$_3$, O$_2$
CH$_2$Cl$_2$, -80 °C Ref. 151

AcO
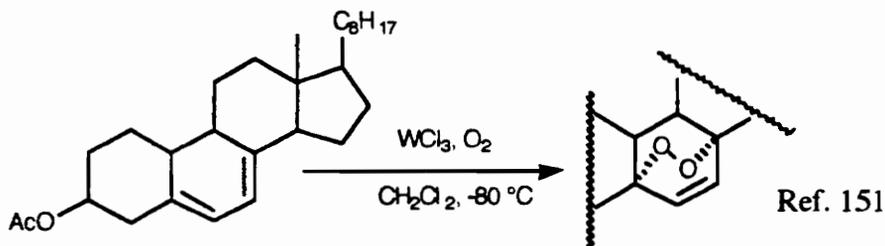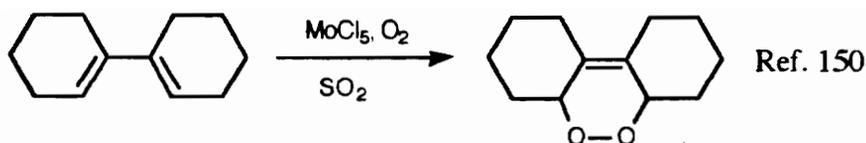
The generality of the diimide reduction under very mild conditions has been demonstrated by Adam. [153] Selective diimide reduction not only serves as an access to saturated bicyclic endoperoxides but also facilitates the trapping of the unstable singlet oxygen adducts of photooxygenation, especially at low temperature. The reactivity of diimide with olefins is influenced by the number and nature of the alkyl substituents present. Trans olefins are usually reduced somewhat more rapidly than cis olefins, and the ease of olefin reduction diminishes as the number of alkyl substituents is increased. These reactivity differences have been attributed to the differences in strain (torsion, bond angle, and eclipsing) with the more strained olefins reacting more rapidly.[154]

OH
LiAlH$_4$
OH

OH
LiAlH$_4$
OH

OH
Br
LiAlH$_4$
Br
OH
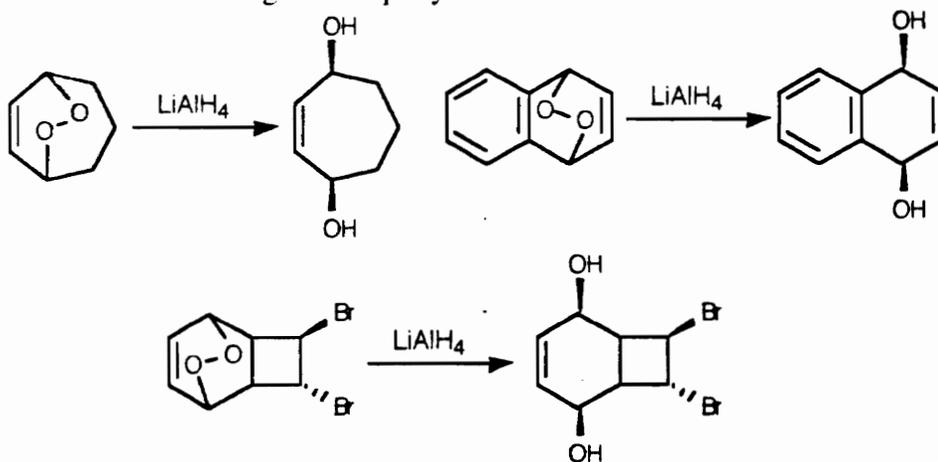
Scheme 8. Reduction of Peroxide Linkage.

The peroxide bond in bicyclic endoperoxides can be readily reduced by lithium aluminum hydride or thiourea to give 2-ene-1,4-diols. Catalytic hydrogenation normally leads to further reduction to 1,4-diols. Therefore the combination of singlet oxygenation of 1,3-dienes followed by reduction provides convenient and efficient access to the 2-ene-1,4-diols and 1,4-diols both with the syn configuration. A few interesting examples are listed

in Scheme 8. Thiourea reduction offers some advantages over both catalytic hydrogenation and lithium aluminum hydride reduction in that it reduces only the oxygen-oxygen bond and thus preserves most other functional groups in the molecule. For example, Schenck and Dunlap[155] synthesized endoperoxide **63** by 1,4-addition of singlet oxygen to cyclopentadiene (**62**) and after isolation reduced it with thiourea to the diol **64**, Scheme 9. Kaneko et al.[156] reported that the diol **64** can be obtained in one step by reaction of the
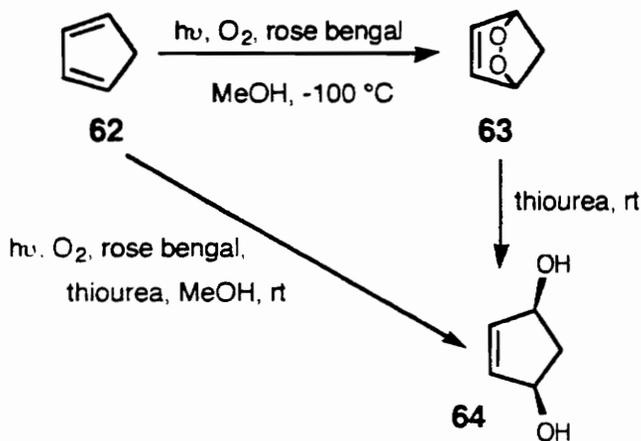
Scheme 9. Photooxygenation of cyclopentadiene

diene **62** with singlet oxygen in the presence of thiourea. This method therefore seems to be applicable to the synthesis of the diols by trapping of the corresponding labile endoperoxides.

1.4-Cycloaddition of singlet oxygen to 1,3-dienes followed by reductive extrusion of one oxygen atom with trivalent phosphorus compounds such as phosphines and phosphites provides a convenient entry to the unsaturated epoxides. The mechanism is analogous to that of the triphenylphosphine reduction of the dioxetanes, which leads to phosphoranes.[157] Phosphoranes are known to undergo thermal decomposition to give epoxides.[158]

It is interesting that bicyclic endoperoxides, in which backside displacement is precluded, can form the unsaturated epoxide by S_N2' displacement. When the suitable stereochemical array for a S_N2' displacement cannot be achieved, the side reactions would determine the final product, as exemplified by the production of **65**.[159]

65
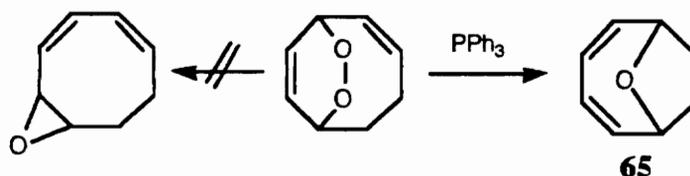
As benzene cannot be oxidized by peracids to form epoxides, the photooxygenation of oxepin or benzene oxide derivatives followed by triphenyl phosphite or triphenylphosphine reduction provides a unique entry into the biologically important arene oxides. The oxepin-benzene oxide system (**66-69**) readily undergoes a 1,4-Diels-Alder-type addition with singlet oxygen generated from hypochlorite-hydrogen peroxide[160] to form 1,4-epoxy endoperoxide **68**,[161,162] which has been deoxygenated[163] to the known *trans*-benzene dioxide[164].

66   **67**   **68**   **69**
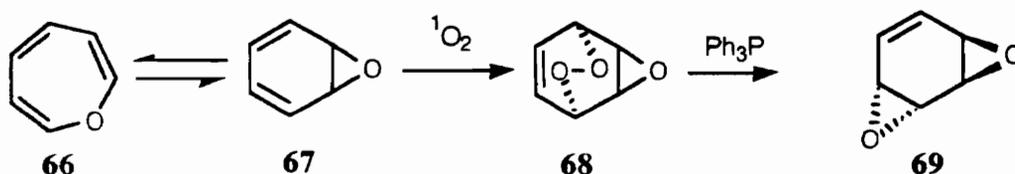
At least two different thermal decomposition modes must be considered in the thermochemical reaction of endoperoxides: (1) loss of molecular oxygen in a process that is similar to the reverse of the formation process and (2) cleavage of the O-O bond.[165,166] Thermal dissociation leading to the formation of diradicals (path b, Scheme 10) is favored
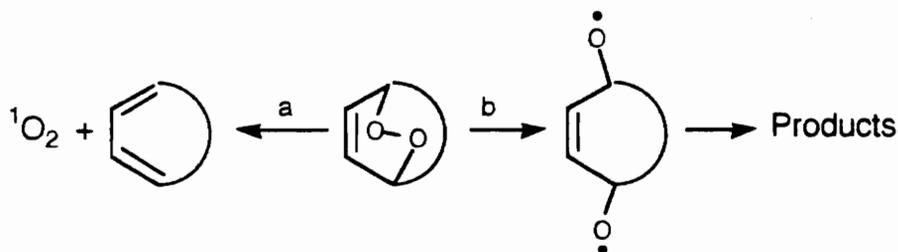
Scheme 10. Thermal Decomposition of Endoperoxides.

over thermal dissociation into the parent hydrocarbon and molecular oxygen (path a, Scheme 10) simply because the activation energy for the O-O cleavage appears to be lower than for the dissociation reaction (38 kcal).[167] One of the common reactions of unsaturated

30

[n.2.2] bicyclic endoperoxides is the cleavage of the weak oxygen-oxygen bond followed
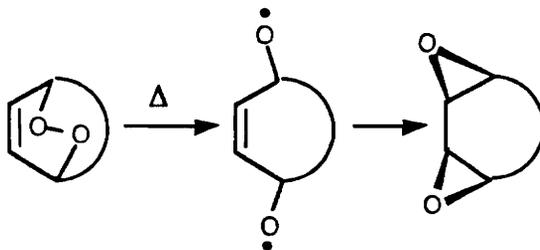
Scheme 11. Thermal Rearrangement of Endoperoxides.

by addition of the oxygen radicals to the adjacent double bond to give bis-epoxides with syn configuration (Scheme 11). A review was recently published that discusses the synthesis of cyclic polyepoxides.[168]

Rearrangement of an endoperoxide to a ketol and an acetyl aldehyde has been observed recently by Adam and Balci.[169] Thus, methanolysis of the [2+6] photoadduct of cycloheptatriene[169] (**70**) at room temperature gave an unusual product distribution (Scheme 12). The rate acceleration in polar solvents indicates that a polar mechanism is responsible for this transformation   In strained molecules like [2.2.1] systems thermolysis is always accompanied by side reactions.[170,171] One of these side reactions is the formation of epoxy ketones. Such side reactions have been observed recently also in cycloheptatriene systems.
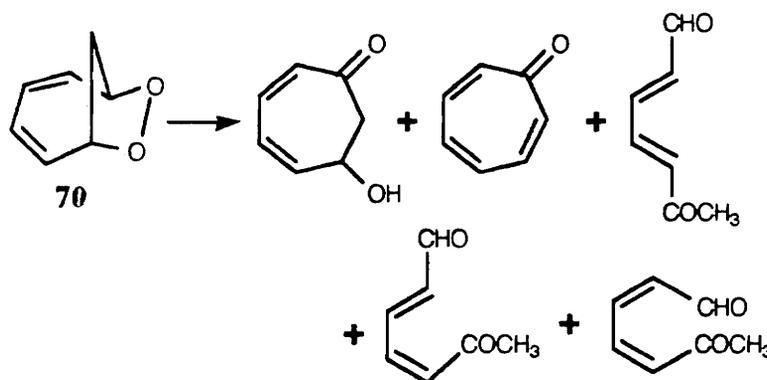
Scheme 12. Methanolysis of **70**.

The base-catalyzed decomposition of peroxides and hydroperoxides exemplifies the general type of elimination reaction that can be anticipated for compounds in which an anion or group X (capable of giving a relatively stable anion X⁻) is attached to oxygen.

31

The application of this mechanism to bicyclic endoperoxides and the oxidation of the resulting monocyclic hydroxy ketones should provide an efficient way to convert cyclic 1,2-dienes into the corresponding 1,4-diones. Le Roux and Goasdoue[172] reported the first isolation and characterization of dioxetanes formed by the acid-catalyzed rearrangement of 1,4-endoperoxides.

More recently a silica-gel-catalyzed rearrangement of a monocyclic endoperoxide to a 1,2-dioxetane has been demonstrated by Schaap et al.[173] These experiments demonstrate that 1,4-endoperoxides may be transformed by acid catalysis to cleavage products of the type found in aromatic dioxygenase enzymes and therefore suggest the possibility that such species are intermediates in these biological processes.[174]

Natsume et al.[175] showed that in the presence of $SnCl_2$ an endoperoxide[176] (obtained by photooxygenation of the corresponding dihydropyridine derivative) reacts with carbon nucleophiles, such as trimethylsilylated ketones, vinyl ethers, enamines, indole, N-methyl-pyrrole, and furan, to give products from the displacement of the peroxy linkage. This novel method leads to carbon-carbon bond formation without involving carbanions. The reaction is believed to proceed by a two-electron transfer from bivalent metal to the peroxide to form both a diol anion and a quadrivalent tin species. This may be in equilibrium with an allylic cation, or nucleophiles might attack directly by an S_N2 mechanism to afford trans products.

In conclusion, this brief survey indicates that a variety of functional group arrays can be derived by transformation of the products obtained from the reaction of singlet oxygen with dienes. In addition, the versatility of this reaction is broaded by the possibility of regioselective addition of singlet oxygen, via the ene reaction, and by the influence of the substituents directly attached to the diene.

II.3.3. Epoxidation[177,178]

Epoxidation of double bonds is also referred to as synthesis of oxiranes, the proper name for the functional group of the product. The most important method for the synthesis of oxiranes is the addition of oxygen to a C-C double bond. This epoxidation procedure was discovered by Prileshaeu in 1909.[179] Among other methods are the nucleophilic displacement of a vicinal leaving group by an oxygen, the preparation from 1,2-difunctional compounds, from carbonyl compound, and from other heterocycles. Olefins

Only the more general methods will be briefly presented in this discussion. The most common method for the preparation of epoxides is the conversion of an alkene by an electrophilic oxidant. The usual laboratory reagent is a peroxy acid (or peracid),[191-195] and the most widely used of these is 3-chloroperoxybenzoic acid (*m*-chloroperbenzoic acid or MCPBA). Solvents commonly used are dichloromethane, chloroform, and benzene. Oxidation with peracids involves an electrophilic addition in which the driving force is provided by the electron-donor nature of the double bond and the electron-acceptor nature of the $-CO_2OH$ group; the fine details of the mechanism have not been clarified in every respect.[178] The reaction is *syn*-stereospecific; and cases are influenced by steric effects, and other by polar effects or the geometry of the transition state. The stereochemistry is also affected by the substituents, especially polar groups, directing the epoxidation *syn* or *anti* to the substituent; this influence is dramatically observed in the case of allylic alcohols.

Some of the reagents used in addition to mCPBA, are performic,[196] peracetic[197] and perpropionic acids.[198,199] Imino-analogues of peracids are more reactive[200-203] but less selective.[203] Peroxycarbamic acids are very reactive epoxidizing agents that can be used under very mild conditions.[204] Others are ethers of peroxycarbonic acid,[205] benzene-peroxyselenic acids,[206,207] andperoxyphosphonic acid.[208]

71
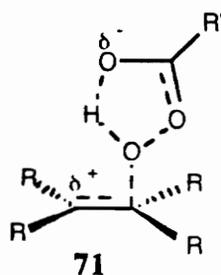
Figure 7. Transition State for Peracid Epoxidation

Peroxy acid epoxidation shows the characteristics of an attack on alkenes by a weakly electrophilic reagent, and the transition state has been represented as shown in Fig. 7, for the prototype reaction, although a more symmetrical transition state has been traditionally favored.[209] The stereospecificity shown by some (*tert*-butylhydroperoxide, TBHP) epoxidations of allylic alcohols may arise from intramolecular electrophilic attack by an oxygen in a metallocycle coordinated to the alkene, Scheme 14. As expected from

these mechanisms, these reactions are stereospecific and give oxiranes with the same configuration as the starting alkene.

On the other hand, electron deficient carbon-carbon double bonds (conjugated to electron-withdrawing groups) are resistant to attack by the electrophilc reagent and are usually converted to oxiranes by nucleophilic oxidants.[181] The most widely used of these is the hydroperoxy ion, Scheme 13. Since epoxidations by hydroperoxide ion proceed through an intermediate α-carbonyl anion (enolate anion), the reaction of acyclic alkenes is not necessarily stereospecific; because the intermediate enolate ion life-time is long enough for rotation to occur around the $C\alpha$-$C\beta$ bond. The stereochemistry aspects of this and other epoxidations are reviewed.[189]
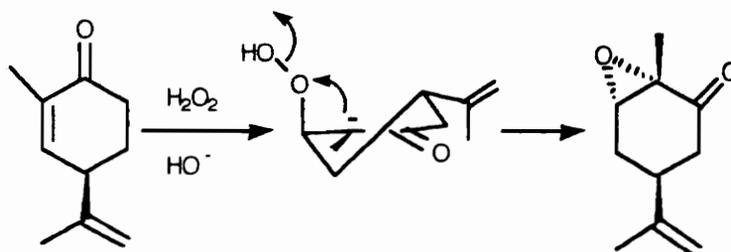
Scheme 13. Epoxidation of enones

In the preparation of some of the peracids, hydrogen peroxide plays an important role. Organic peroxides have provide a very useful method for epoxidation, especially associated with metal catalysts. Some of the advantages of organic peroxides are their preparation from inexpensive hydrocarbon sources, their solubility in hydrocarbons (the effects from polar solvents are avoided), their safe use, and the good selectivities achieved. The metals commonly used are molybdenum, tungsten, vanadium and titanium. The reaction is strongly affected by the structure of the olefin, the extent of alkyl substitution, and the number and presence of electron-donating groups.

More recently Sharpless and coworkers[210] have shown that *t*-butyl hydroperoxide (TBHP) epoxidation catalyzed by molybdenum or vanadium compounds offer advantages over peroxy acid with regard to safety, cost, and usually, selectivity. The remarkable

stereospecificity of TBHP-transition metal epoxidations of allylic alcohols has
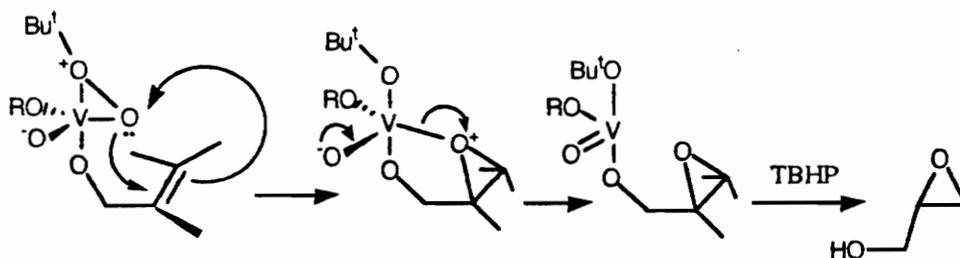
Scheme 14. Sharpless Epoxidation.

been exploited by Sharpless' group for the synthesis of chiral oxiranes from prochiral allylic alcohols and for diastereoselective oxirane synthesis from chiral allylic alcohols. It has been suggested that the latter reaction may enable the preparation of chiral compounds of complete enantiomeric purity.

Oxiranes can generally be prepared from difunctional compounds by 1,3-elimination.[181,211,212] The critical intermediate for this route (73) (Scheme 15) can be reached in several ways; the alkoxide produced in the basic medium participates in an internal nucleophilic attack, which promotes the departure of the substituent on the adjacent carbon, leading to ring closure. The most common procedure involves the reaction of an alkene with hypohalous acid, which proceeds with overall retention of configuration.

$X= Cl, Br, I, OSO_2R, OCOR, NR_3^{(+)}, N_2^{(+)}, OH.$
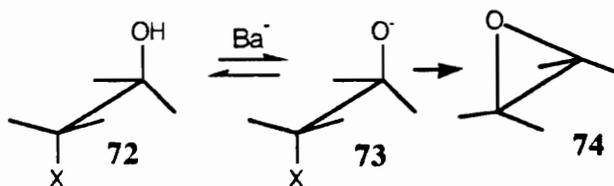
Scheme 15. 1,3-Elimination Route to Oxirane Ring.

Attack on the carbonyl group of a 2-halo ketone by a nucleophile or a reducing agent also generates oxiranes via 73, but this method has been used relatively little. Oxiranes can also prepared from a 1,2-diol; some examples are shown as follow:

35

Ref. 213

Ref. 214

Ref. 215

Ref. 216
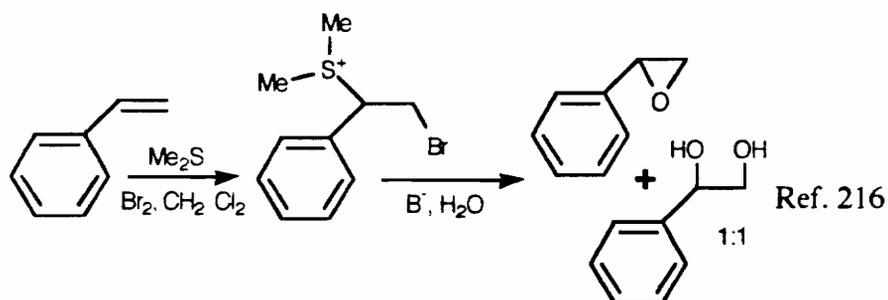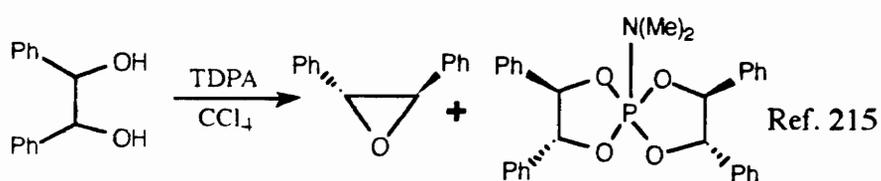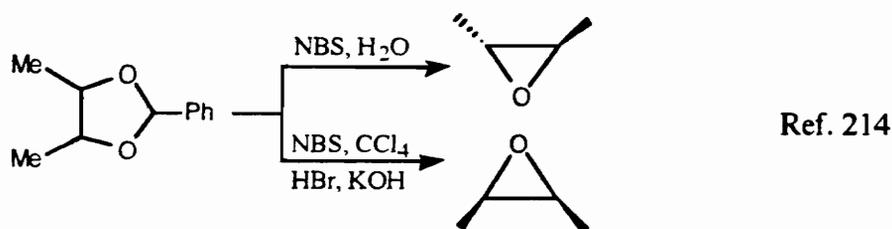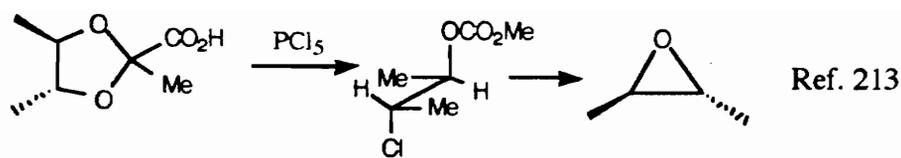
Regarding the synthesis of oxiranes from carbonyl compounds, where the oxygen atom is furnished by the carbonyl group, a very useful route to oxiranes is the Darzens reaction, which consists of the nucleophilic attack of an α-halo carbanion on an aldehyde
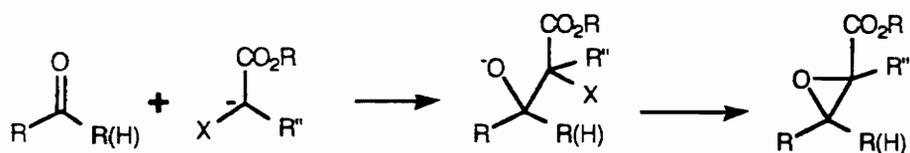
Scheme 16. Darzens Reaction.

or ketone carbonyl group (Scheme 16) . The "glycidic ester" is useful since it can be rearranged via elimination to an aldehyde or ketone homologous to the starting ketone. The α-halogen derivative can contain any electron-withdrawing group (i.e., carboxylate, carbonyl, amide, cyano, etc.).
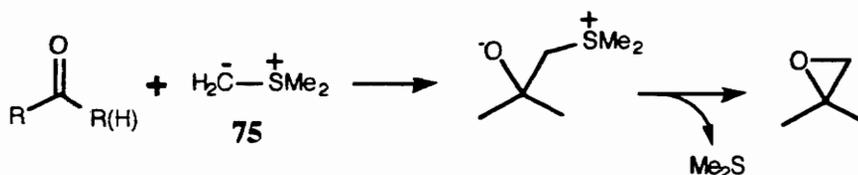
Scheme 17. Corey's oxirane Formation.

A versatile oxirane synthesis via a sulfur ylide approach, which in effect inserts a CR_2 (75) group into a carbonyl group, is shown in Scheme 17.[217] Other, less generally useful versions of this "insertion" utilize diazomethane or dibromomethane-butyllithium.

Other methods to prepare oxiranes are by the transformations of other heterocycles. Five-membered rings are transformed into analogous three-membered rings by extrusion. Oxiranes have been made by extrusion of nitrogen from oxadiazolenes (76), by flash thermolysis of dioxazolones (77) and (α-lactones), and by photolysis of 1,2-dioxole-3,5-diones (78). Some examples are shown below:

37

Ref. 218

Ref. 219

Ref. 220
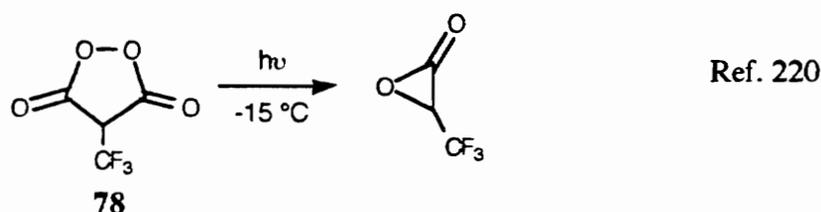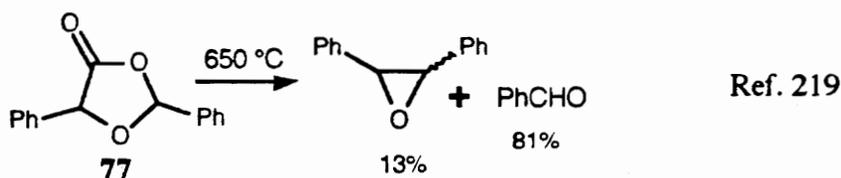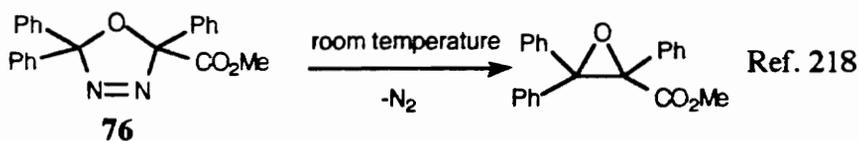
Oxiranes can be also prepared from six-membered heterocycles by rearrangement or extrusion. The thermal fragmentation of unsaturated bicyclic 1,4-peroxides, often readily made from the addition of singlet oxygen to 1,4-dienes, has become an important route to novel bis(oxiranes). Oxiranes have also been made by photolysis of cyclic oxalates.

Oxides of transition metals (tungsten, molybdenum, vanadium, titanium, etc) are very effective catalysts in the epoxidation of electron-poor olefins with hydrogen peroxide.[182,183,220-224] Oxides of tungsten and molybdenum are the most frequently used: in the presence of hydrogen peroxide these are oxidized to peracids. The mechanism of the reaction has been reasonably understood: a transition complex is formed with the double bond (Scheme 18).[225-226]
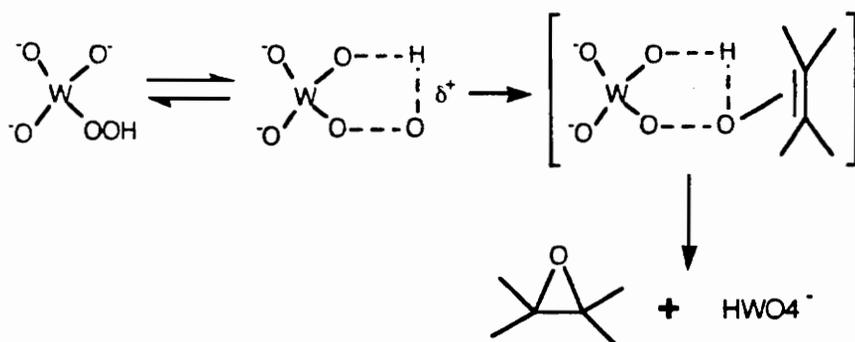
Scheme 18. Epoxidation with Tungstic Oxide and Hydrogen Peroxide.

Other methods use oxygen and a metallic complex, and there are some epoxidations effected with only oxygen, those having been reviewed in the literature.[178] Regioselectivity in the epoxidation of polyenes has not been widely studied, but there are some isolated results in this area. Some examples are shown in Scheme 19.

From the reactions described above, it can be inferred that the regioselectivity originates from the different electron density of the double bonds as well as the stereochemistry. There is one report of the epoxidation of conjugated dienes in the monoepoxidation of cyclic conjugated dienes;[227] there are several reports of selective epoxidation by means of biocatalysis.[228] The latter approach has also been applied to the asymmetric epoxidation of aliphatic alkenes.[229]

Scheme 19.

39

X= O, N-CHO

Ref. 232

Ref. 233

PdCl(NO₂)(CH₃CN)
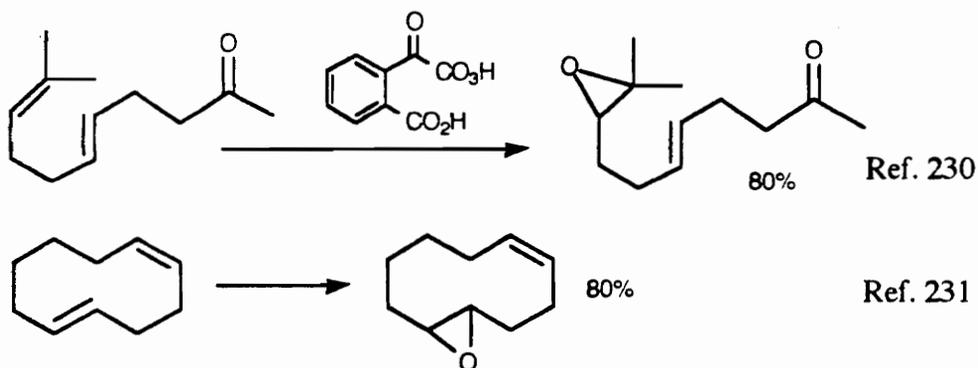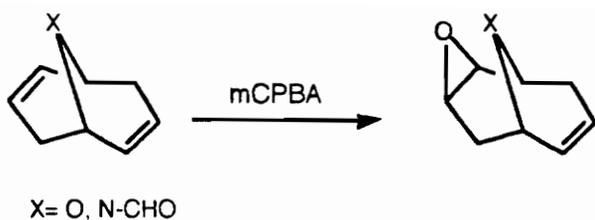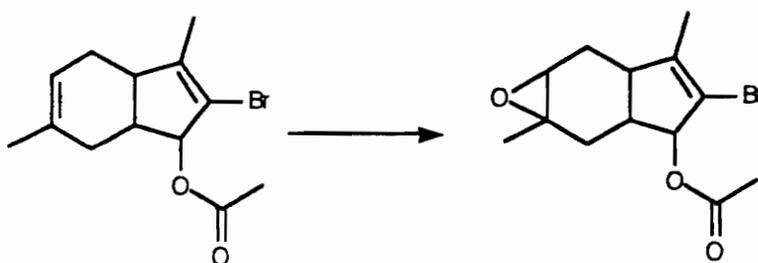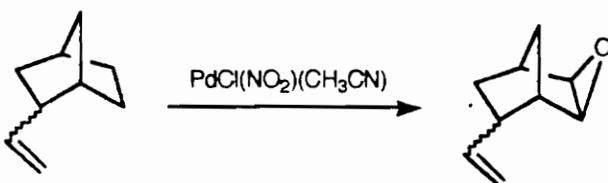
Ref. 225

Scheme 19. (contd.)

Chemical synthesis of **80** by epoxidation of the appropriate olefin (**79**) proceeds satisfactorily, but produces a racemic mixture of the antibiotic. However, epoxidation of **79** with *Penicillium spinulosum* gives the desired (-)-enantiomer of fosfomycin (**80**) in 90%
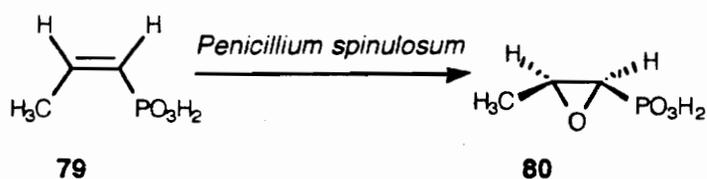
conversion.[234] Although brief, this survey indicates the wide repertoire of reaction conditions and reagents to perform epoxidation of olefins. It is important to mention the availability of enzymatic procedures, which have shown to be regio- and steroselective.

II.3.4. Hydroxylation.[235]

Reactions involving the stereoselective hydroxylation of alkenes[235,236] are prominent among the standard routes to 1,2-diols. The process can occur in a *syn* or *anti* manner with the actual mode depending on the reagents used. Of the two classic reagents for *syn* hydroxylation via the formation of cyclic esters, potassium permanganate ($KMnO_4$) and osmium tetroxide (OsO_4), the latter is more efficient and reliable but costly and hazardous. With care, alkaline $KMnO_4$ gives good results with alkenic acids, but yields
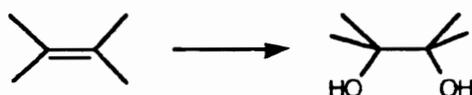
Figure 8. General Reaction of Hydroxylation.

are generally moderate with more lipophilic substrates. The problems associated with OsO_4 can be minimized by using it (or K_2OsO_4 neutralized with acetic acid[237]) catalytically along with a co-oxidant [a metal chlorate (Hofmann)[238] or H_2O_2 (Milas)[239]] though with some loss of efficiency compared with stoichiometric use of the OsO_4-pyridine reagent. The recently introduced co-oxidants *tert*-butyl hydroperoxide and N-methylmorpholine N-oxide appear to remove this disadvantage. With both the Mn^{VII} and Os^{VIII} reagents, hydroxylation of a double bond normally takes place from the less-hindered side.

Halohydrin esters are the key intermediates in a growing range of diol syntheses of which the Prévost reaction[240] and the Woodward reaction[240,241] are the prototypes. The latter leads to hydroxylation from the more hindered side (by inversion).

Alternative methods for the preparation of halohydrin esters and for their conversion to diol esters are now available. Suitably economical reagents are (1) iodine (I_2) with (a) a thallium (I) carboxylates,[242] (b) sodium acetate in anhydrous tetramethylene sulphone,[243] or (c) potassium periodate in acetic acid;[244] or (2) N-iodosuccinimide and a carboxylic acid in chloroform.[245] Similarly, N-bromoacetamide and silver (I) acetate have been used under Woodward conditions for the preparation of bromohydrin acetates.[246] For the conversion of iodohydrin acetates to the diol monoesters, adequate treatments[242,243] include heating under reflux with dimethyl sulfoxide, aqueous acetic acid, or potassium acetate in acetic acid. A comparable result has been obtained[247] with thallium (III) acetate in acetic acid, whereas thallium (III) sulfate in water has been recommended[248] for the anti hydroxylation of rigid cyclic alkenes. The diol esters formed by treating an

alkene with iodine (III) trifluoroacetate[249] correspond predominantly to net syn addition. In classical methods, peroxy acids are routinely used for the hydroxylation of alkenes via epoxidation (*syn* addition), acid-catalyzed ring-opening of the oxiranes (most commonly with inversion of configuration), and hydrolysis of the diol monoester formed. The reagent (usually prepared in *situ*) is conventionally chosen from performic acid, trifluoroperacetic acid, and peracetic acid-sulfuric acid, though the ability to obtain free diols directly from reactions employing o-sulfoperbenzoic acid[250] or disuccinoyl peroxide (a stable solid)[251] may be considered an advantage.

Pre-formed oxiranes can be used similarly to obtain 1,2-diols directly by hydrolysis (catalyzed by either an acid or a base) or indirectly *via* acid-catalyzed reaction with other nucleophiles.[252] Enol derivatives of various types have been subjected to hydroboration-oxidation. Applied to silyl ethers,[253] the sequence provides a useful route to *trans*-1,2-diols from certain cyclanones.

Recently, several groups have been actively involved in the development of chiral ligands for enantioselective dioxyosmylation of olefins,[254-259] including the improved ligands for dihydroxylations of terminal olefins reported recently by Sharpless.[260] Some examples are outlined below:

| R= Pr | CH$_2$Cl$_2$ | 54 | 43 | (R,R) |
| | Toluene | 65 | 54 | (S,S) |

Ref. 261

42

OsO₄(1eq.), CHCl₃   **90:10**

OsO₄ (0.001 eq), Me₃NO·2H₂O, Me₂CO-H₂O(10:1)   **65:35**

Ref. 262

Ref. 263
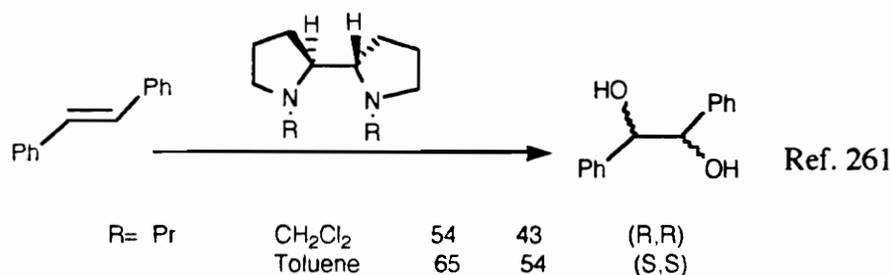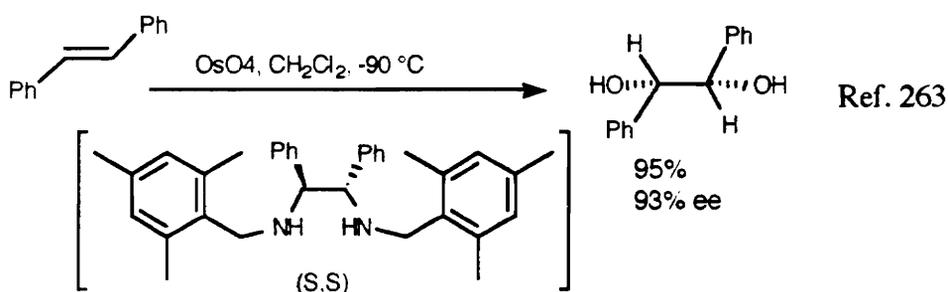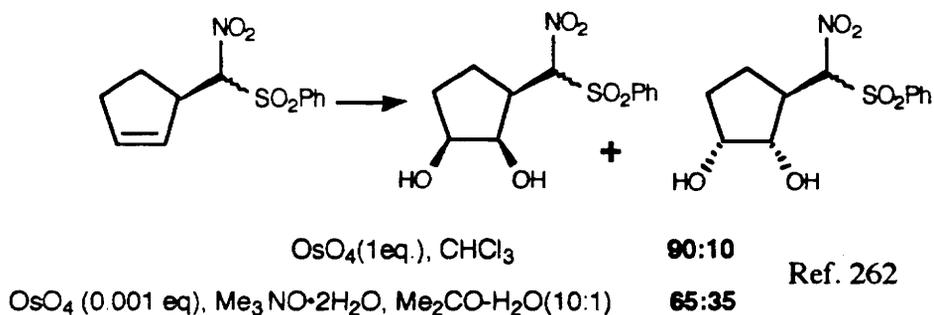
Cha reported a synthesis of lactone **82**, as the only product, from the hydroxylation of diene **81**. The regiochemistry in that reaction seemed to be directed by the electron density of the olefin.
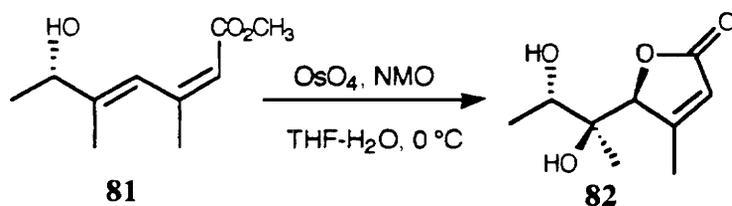
Scheme 20.

An area which has been attracting a lot of attention lately is the use of enzymes or microorganisms in chemical transformations, and hydroxylation of olefins is not an exception. Olefins may be hydrated or epoxidized by microorganisms, and the latter may be further hydrated. Thus, olefins can be converted to alcohols, to epoxides, or to diols. Although these reactions may be readily achieved chemically, there may be situations where the selectivity of the enzymatic method offers some advantages.

Synthetically, the most interesting reaction reported to date is the stereospecific mono epoxidation of 1,7-octadiene (**83**) to form (R)-(+)-7,8-epoxyl-1-octene (**84**) in about 25% yield.[264] This transformation opens great possibilities for the regioselective epoxidation of isolated dienes.
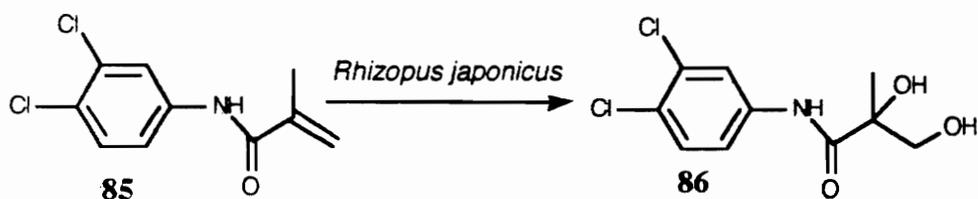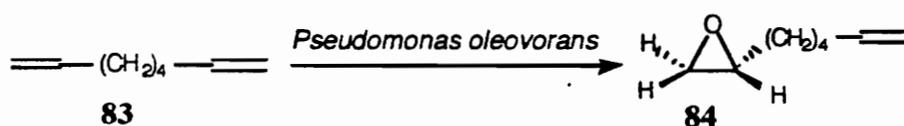
A mutant strain of *Pseudomonas putida* (*P. putida* 39/D) that has been used to effect the cis-1,2-dihydroxylation of various aromatic rings has been found to hydroxylate 3-methylcyclohexene in a similar fashion.[265] The herbicide N-(3,4-dichlorophenyl) methylacrylamine (**85**) is converted to a diol (**86**) by *Rhizopus japonicus* as a model for fungal metabolism in the soil.[266] Several monocyclic terpenes have been oxidized to diols with different species of the genus *Cladosporium* (Scheme 21).[267]
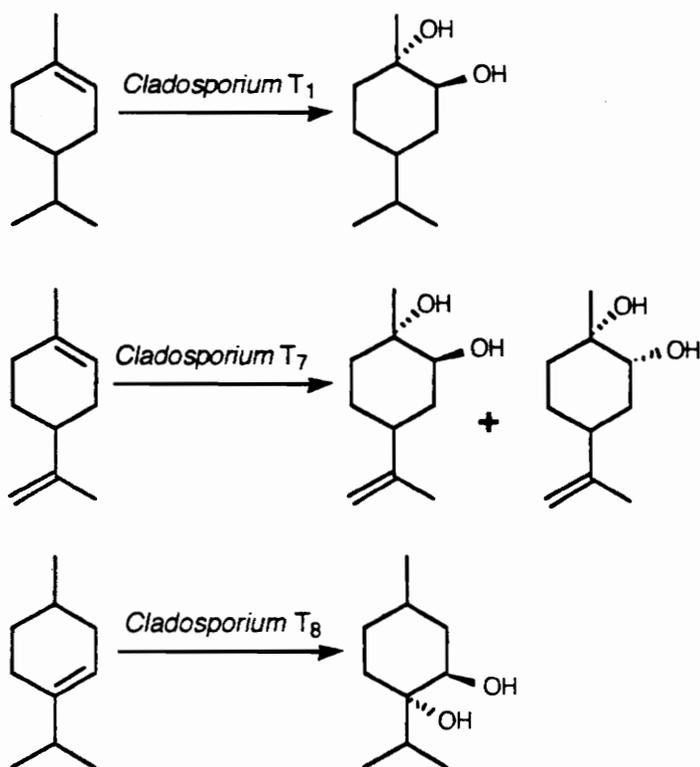
Scheme 21.

It can be seen that there exists a great repertoire of methods to perform the hydroxylation of olefins. in both regioselective and stereoselective fashion. This area has been developed in the synthesis of sugars and polyhydroxylate compounds.

II.3.5. Opening of Epoxides

Oxiranes are compounds with a three-membered ring composed of one oxygen atom and two formally $sp^{2.45}$-hybridized carbon atoms. The geometry of oxiranes (or epoxides) has been determined by x-ray diffraction studies of crystalline natural products. microwave spectroscopy, and electron diffraction studies;[267*] these studies showed that the strain in this system is mostly angle strain, although torsional interaction probably

contributes as well. The conventional ring strain energy of oxirane is comparable with that
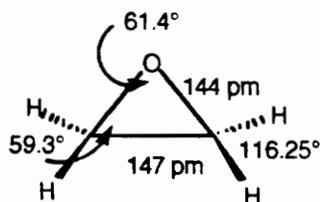
Figure 9. Geometry of Oxirane

of cyclopropane.[268] Ring strain is one of the most important properties of oxiranes, and is responsible for the high reactivity in ring-opening reactions and the low electron-donor ability of the oxygen. The geometry of the ring imparts a high rigidity to the system.

(1, 2) ring cleavage (probably homolytic), thermal and photochemical
(3) Electrophilic attack on ring oxygen
(4) Nucleophilic attach on ring carbon
"Nucleophilic attack on ring hydrogen (proton abstraction)
(6) Reaction with electrons and surface reaction
(7) Cycloadditions
(8) Reactions of a substituent
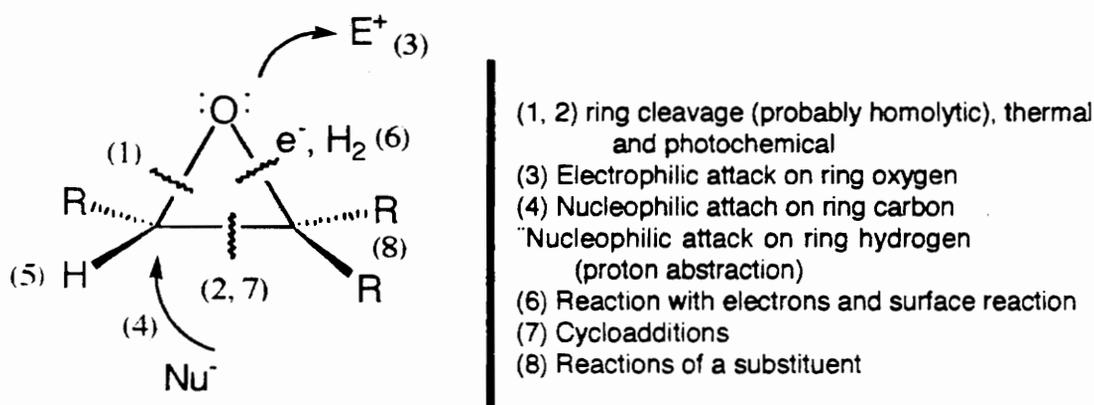
Figure 10. Reactivity Modes of Oxiranes.

In general the reactivity displayed by the oxirane ring is presented in Fig. 10[267] The chemical behavior of the oxirane ring is governed by two factors: the strain in the ring and the basicity of the oxygen of the ring. The extent to which these two factors contribute to the reactivity, either separately or jointly, can be assessed from the results of reaction under various conditions; the modes of reaction of opening of oxiranes are shown in Fig. 10.
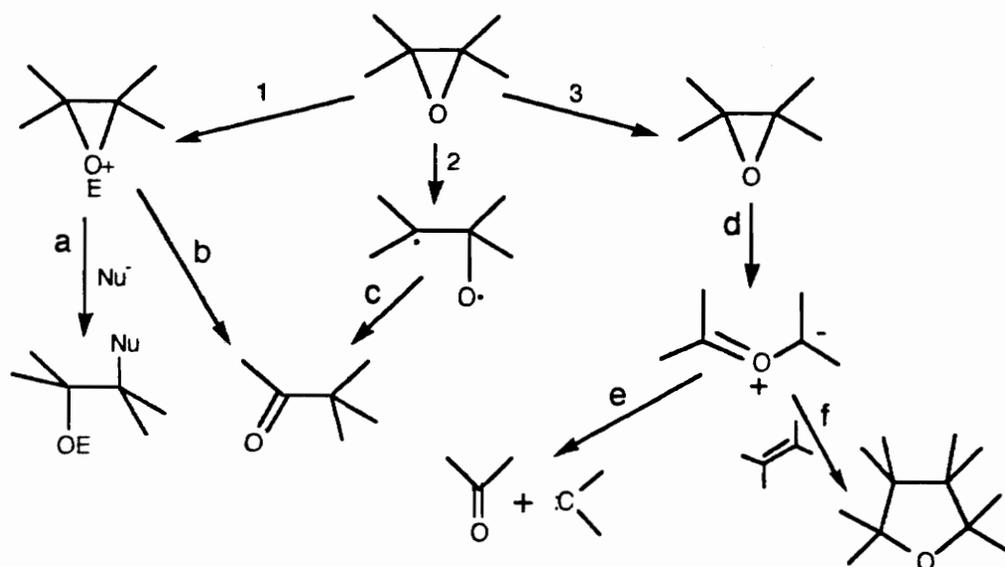
Scheme 22. Possible Pathways for Ring-opening of Oxirane.

The reactions generally involve heterolytic opening of the C-O bond (Scheme 22, 1); this opening, most often induced by an electrophile, is accompanied by the attack of a nucleophile (Scheme 22, a) or a 1,2-rearrangement (Scheme 22, b). Other comparatively rare reactions are the homolytic splitting of the C-O bond (Scheme 22, 2) or the C-C bond (Scheme 22, 3). The latter then suffers fragmentation (Scheme 1, e) or leads to a 1,3-dipolar cycloaddition products (Scheme 22, f). Ring-opening to an ylide can occur directly or via the homolytic mechanism by photochemical, thermal, or radical initiation. Another reaction pathway involves deoxygenation to olefins.
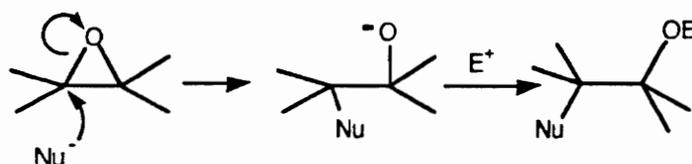
Scheme 23. Nucleophilic Ring-opening of Oxirane.

Because of the nature of the work described in the discussion section, only the reactions involving opening of the ring via C-O fission, mainly by a nucleophilic attack on a ring carbon, will be discussed. Nucleophilic attack on a ring carbon (Scheme 23) is the

most important reaction of these compounds. An electrophile may bond to the oxygen either before or after the nucleophilic attack. The mechanistic aspect of this topic has been thoroughly reviewed.[269] Regarding regiochemistry the main features of the effect of the structure on the site of attack are summarized in Table 3. These can be understood in terms of a "borderline S_N2" transition state, which somewhat resembles an S_N1 transition state in charge distribution because C-O bond breaking occurs before of Nu-C bond making.

The reaction is sensitive to steric effects, especially under non-acidic conditions, because the substituent sometimes deflects nucleophile from C_a; but this steric effect is to some extent offset by an electronic effect under acidic conditions. Under acidic conditions the protonated oxirane opens via transition states that are even more S_N1-like than the borderline S_N2 of the unprotonated oxirane. Thus electronic factors favor cleavage at the more substituted carbon or the carbon that can better support a partial positive charge.

Table 4. Favored position of a nucleophilic attack on substituted oxiranes.[269]

Substituent group (R)	Favored position of attack*	
	Non -acidic Conditions	Acidic Conditions
Alkyl	Cβ	Cα or Cβ
Electronegative, nonconjugative	Cβ	Cβ
P-Conjugative	Cα or Cβ	Cα or Cβ
n-Conjugative	Cα	Cα

* Cα is the carbon bearing the substituent.
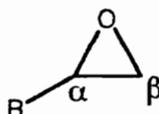
Electronegative, non-conjugating groups (groups able to stabilize carbocation via allylic interactions) prevent the attack at Cα, because of the interaction with an incipient carbocation by an inductive or field effect. This is due to destabilization of transition state by the juxtaposition of positive charge. π-Conjugative groups tend to favor attack at Cα; but the ratio of Cα:Cβ attack depends strongly on the balance of steric and electronic factors arising from both the substituent and the nucleophile. However, it can be rationalized that the presence of a good π-donor substituent will promote the stabilization of the incipient carbocation, thus offseting the steric hindrance. n-Conjugative groups consistently favor attack on Cα, as expected from their ability to stabilize carbocations.

Regarding stereochemistry, nucleophilic attack on an oxirane carbon usually proceeds with inversion of configuration (Scheme 24) as expected for S_N2 reactions, even
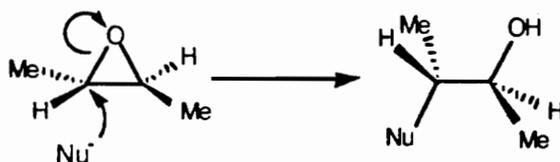
Scheme 24. Stereochemical pathway for ring-opening of oxirane

under acidic conditions. In the case of bicyclic oxiranes the opening takes place in a *trans*-diaxial manner. This is known as the Fürst-Plattner rule,[270] and there are only a few exceptions to it. A relatively few oxirane ring openings have been reported to give retention or a mixture of inversion and retention .[271]

The reactions of oxiranes involving nucleophilic opening of a C-O bond generally produce 1,2-difunctional compounds. Either the C-O bond is opened by direct nucleophilic attack on one of the carbons atoms, or the oxygen is first protonated (or a complex is formed with the electrophilic center of the reagent), then the oxonium ion suffers nucleophilic attack on the carbon (Scheme 25).
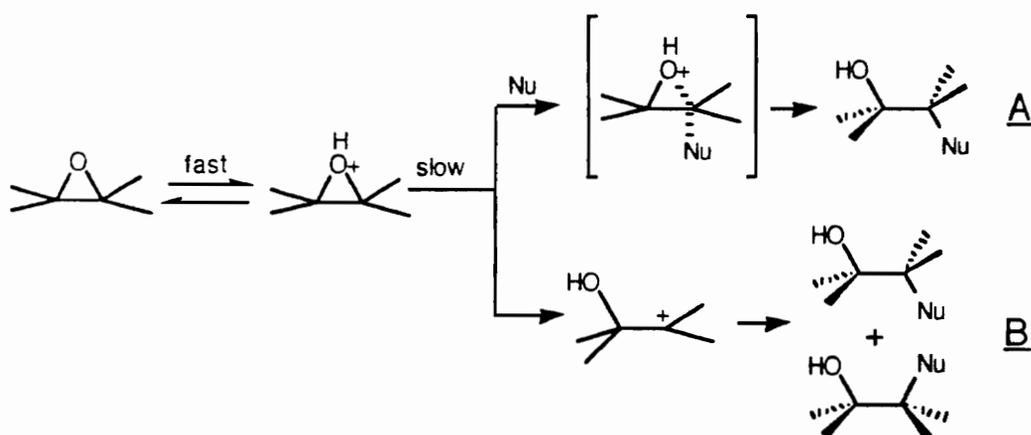
Scheme 25

Stereochemical consequences of this process depend on the pathway followed, as shown in Scheme 25. The route A leads to inversion of configuration at the carbon under attack, and route B produces a mixture of products with both retention and inversion. The mechanism and consequently the stereochemistry of a particular reaction depend on the structure of starting compound and on experimental conditions.

In general, reactions under basic and neutral conditions occur by an A2 mechanism (S_N2-like), and they involve stereospecifically *trans* stereochemistry; the attack of the nucleophile usually takes place on the less substituted carbon atom. In case of reactions under acid-catalyzed conditions the experimental results have been interpreted on the basis of an A2, an A1, or a borderline mechanism, Scheme 26.[272]
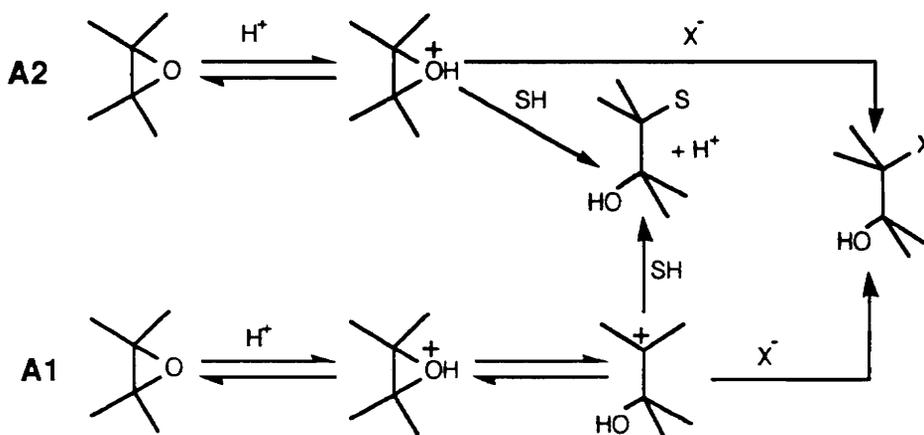
Scheme 26. A2 and A1 Mechanism of Opening of Epoxides.[273]

Comprehensive kinetic studies on acid-catalysis of ring opening of alkyl substituted oxiranes, in aqueous and non-aqueous media, point to a competition between the A2 and A1 mechanism, with the predominance of the former.[273] In order to resolve these contradictions, a different mechanistic concept was proposed in which the conjugated acid of the substrate forms a tight ion pair leading to products related to an A2 mechanism.

It has been observed, for acidic hydrolysis of *cis*- and *trans*-1-phenyl-4-*tert*-butyl cyclohexene oxides (**87**), that the rate and stereochemistry of the reaction depend to a large extent not only on the configuration of the substrate, but also on the type of solvent. In solvents with a low dielectric constant mainly *cis* opening occurs with retention of configuration. In water or alcohols the stereospecificity is lower (Scheme 27).[274]
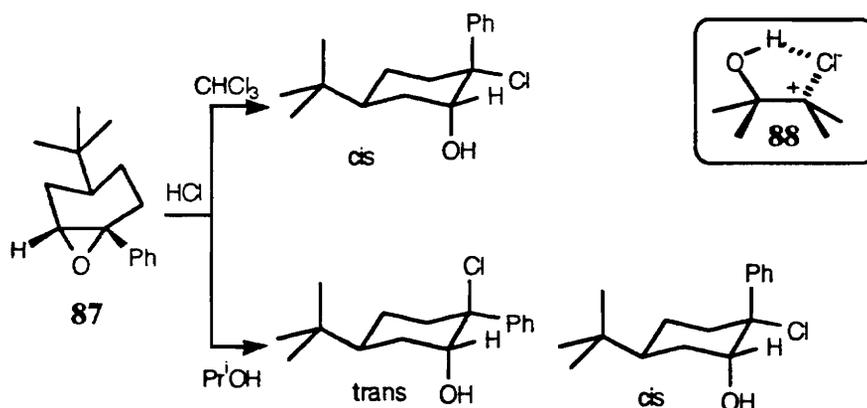
Scheme 27.

This observed retention can be ascribed in part to the formation of a solvent-protected ion pair (**88**) in which the attack by the anion proceeds internally on the electron defficient benzyl carbon atom.

Attempts have been made to separate inductive, conformational, and stereoelectronic effects.[275] It was concluded that the inductive effect plays a determinig role in the regioselectivity of the reaction, but the other factors, in some cases, are not negligible. In agreement with the regularities mentioned, neighboring-group participation is often involved in cis ring-opening.[276] More specific details will be futher discussed, with specific examples .

The discussion of the specific reactions of opening is been classified according to the identity of the atom in the nucleophile to be bonded to the ring carbon atom: i) oxygen, ii) nitrogen. iii) sulfur, iv) halides, v) carbanions vi) π-systems, vii) hydride ion and viii) phosphorous and silicon nucleophiles

i) Oxygen Nucleophiles. Most of the time during nucleophilic opening of oxiranes by an oxygen nucleophile produces 1,2-diols without isolation of the oxirane. This can be performed by oxiranation (epoxidation) of alkenes with unbuffered peroxiacetic acid or hydrogen peroxide in formic acid tends to give monoesters of 1,2-diols, which can be hydrolyzed to the corresponding diols (Scheme 28).

The opening of epoxides by the elements of water, (hydrolysis) results in the formation of a vic-diol, the derivative of which depends on the reaction conditions used. The stereochemistry of this addition is mostly trans, but in some cases neighboring group participation leads to cis-diols.[277] When alcohols serve as the nucleophile, 1,2-diol

51

monoethers are produced; these reactions can be performed under acidic or basic catalysis, and in both cases rearrangement products can be present.
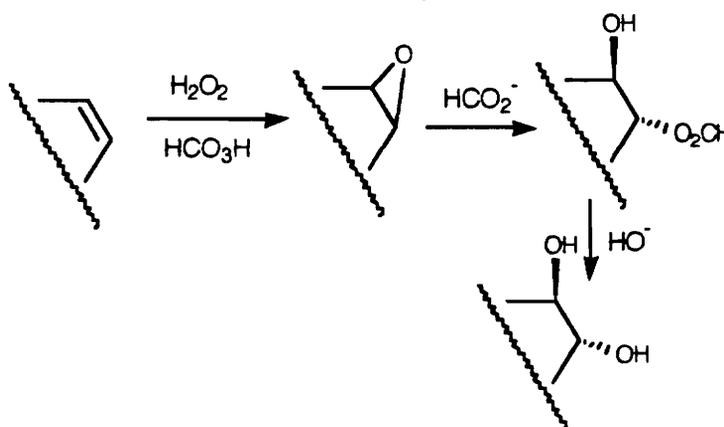
Scheme 28.

ii) Nitrogen-containing nucleophiles. The ring-opening of epoxides with ammonia or amines comprises a general method for the preparation of 1,2-amino alcohols. This procedure is widely used in the synthesis of amino sugars.[278-283] Base-catalyzed addition of oximes to oxiranes leads not only to O-alkyl but also N-alkyl derivatives (Scheme 29).[284] The nucleophilic opening of epoxides by the azide ion has been widely used, the resulting azidohydrins being valuable precursors of 1,2-amino-alcohols. Some examples are shown in Scheme 30, and, in some cases, suitably protected intermediates are produced.

Scheme 29.

52
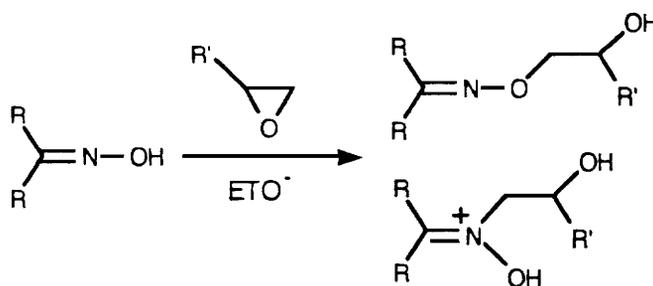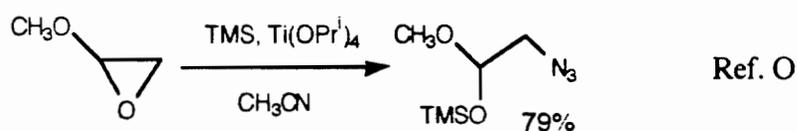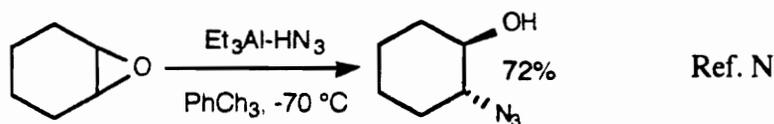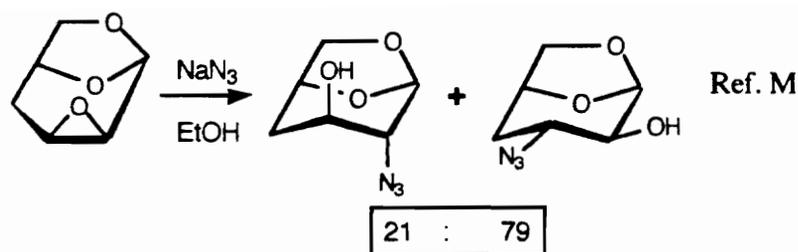
Scheme 30.

iii) <u>Sulfur-containing Nucleophiles</u>. The reaction with thiols is identical to that with alcohols: the only difference is the enhanced nucleophilicity of sulfur.[288]

iv) <u>Halides</u>. The reaction of oxiranes with certain halogen acids, HCl and HBr, can be performed easily with the production of the corresponding chloro- and bromohydrins. Simple aliphatic oxiranes are transformed to fluorohydrins with HF, but with great difficulty.[269] However, this transformation can be attained by using polyhydrogen fluoride[289] or amine-hydrogen fluoride complexes.[290] The regio- and stereochemistry of the reaction is influenced considerably by the nature and the stereochemistry of the substituents.

Other nucleophiles are discussed widely in general reviews[267*,269] and are out of the scope of this survey. Despite the great quantity of information about the reaction mechanism and the reagents available to perform the opening of epoxides, the reaction of each specific substrate must be tried, because of the particular structural factors present in each case.

II. 4. Synthetic Use of *cis*-Arenediols

After the "rediscovery" of *cis*-arenediols a few years ago, their use as chiral synthons has been growing, and the first review in this area will be soon published.[291] The array of functional groups present in these molecules makes them suitable for carrying out a variety of transformations. *cis*-Arenediols and their protected derivatives have been subjected to functional group transformations in practically every position of the molecule, producing phenols and catechols possessing substitution patterns difficult to achieve by other means.

The dienic functionality has been subjected to regioselective [2+2] cycloadditions and epoxidations, and of course to [4+2] cycloadditions. The diene has also been subjected to oxidative cleavage in order to transform these compounds to natural products.

The 1,2-diol group has been used as an auxiliary for the stereocontrol of further transformations and also to perform the cleavage of the cyclohexane ring. A survey of the reports on the use of cis-arenediols is presented below, with a special emphasis on the synthesis of natural products. The data presented is listed in chronological order. (Note: the information will be presented as schematic abstracts, with some comments; only the reports from Hudlicky's group not discussed elsewhere in this work will be presented in this section).

73%

14%

1.PhCOCl
2. MCPBA

1. MeOH,
2. OsO₄, NMO
3. hydrolysis

First report of Synthesis
based on *cis*-arenediol

(±)-Pinitol

Ref. 291

1. thiourea
2. MCPBA

hν

1 : 3 : 1

Et₃N

Intermediates to be used in the
synthesis of myo-inositol
triphosphate

Ref. 293

Other dienophiles:
Nitrosobenzene, Dimethylfumarate, Fumaronitrile,
Tetracyanoethylene, Methyl propionate, methyl acrylate
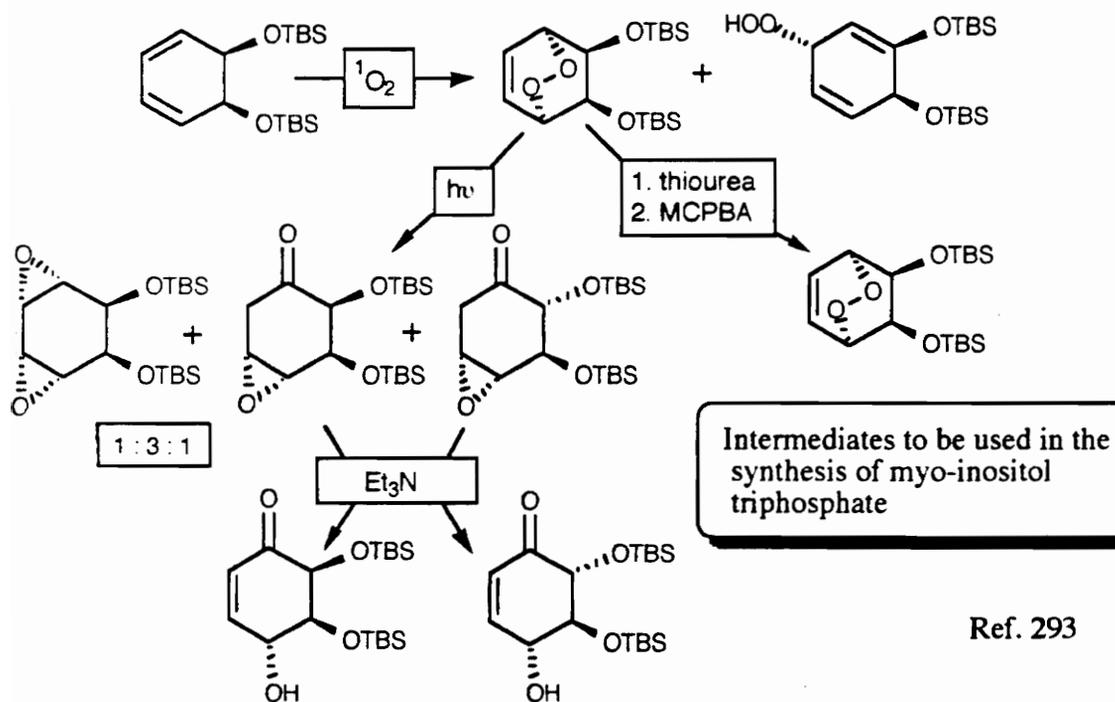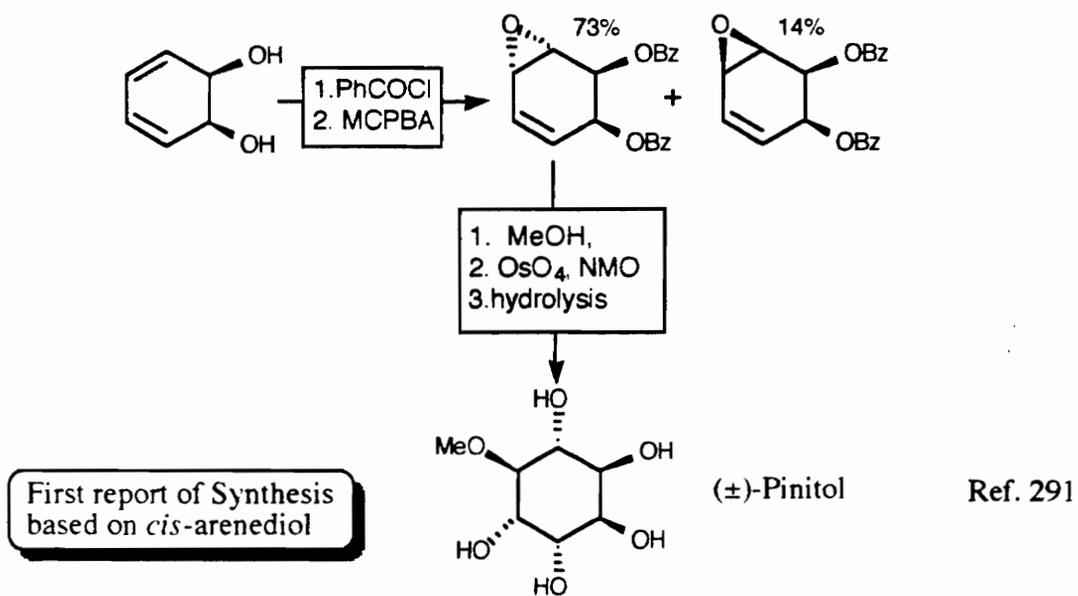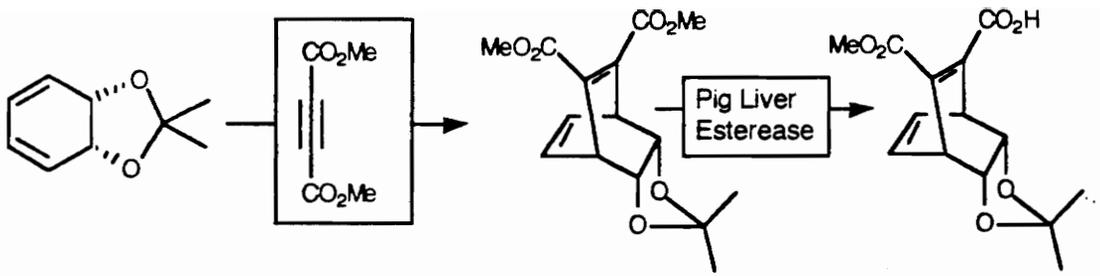
Enzymatic Resolution

Ref. 294

2.8 : 1 : traces

Coduritol A   Conduritol D   Ref. 295

5 steps. 49%

(±)-Pinitol

1. PhCOCl
2. MCPBA

1. Opening epoxide
   with chiral auxiliary
2. Resolution

(+)-Pinitol
and
(-)-Pinitol

17%

Enantioselective synthesis

Ref.296

DEAD

cis-trifluorobenzenediol behaves as diene and dienophile

MeO_2C N—N CO_2Me

F_3C

$\begin{bmatrix} \text{Other dienophiles:} \\ \text{Methyl propiolate, cyclopentadiene} \end{bmatrix}$

Ref. 297

Ph₂C=C=O

4 : 5

: CCl₂

Unexpected hetero Diels-Alder with diphenylketene

Ref. 298

---

1. (MeO)₂CO
2. MCPBA

1. BnOH, CSA
2. BnBr, AgO₂
3. Et₃N
4. MCPBA

1 : 9

Ref. 299

$OP(O)(OH)_2$

$(OH)_2(O)PO$

OH

HO

OH

$OP(O)(OH)_2$

Ref. 300
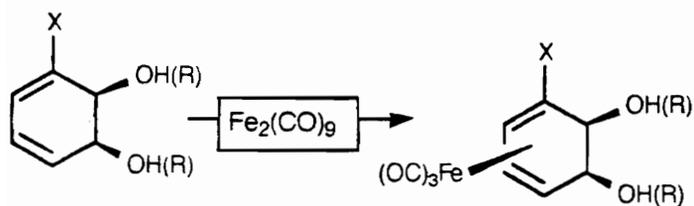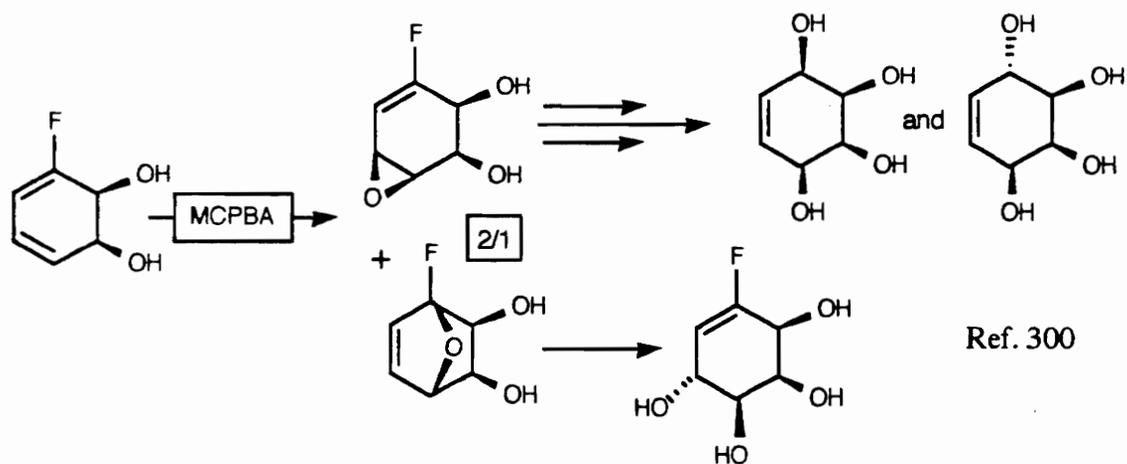
X= Me, Cl, OMe
R= Me, OAc

Method for determination of absolute configuration

Ref. 301

58

MCPBA ← → OsO₄, MNO

1. MeOH, Al₂O₃
2. LAH
3. OsO₄, MNO
4. HCl

(-)-Pinitol

(+)-Pinitol

1. LAH
2. MCPBA
3. MeOH, Al₂O₃
4. HCl
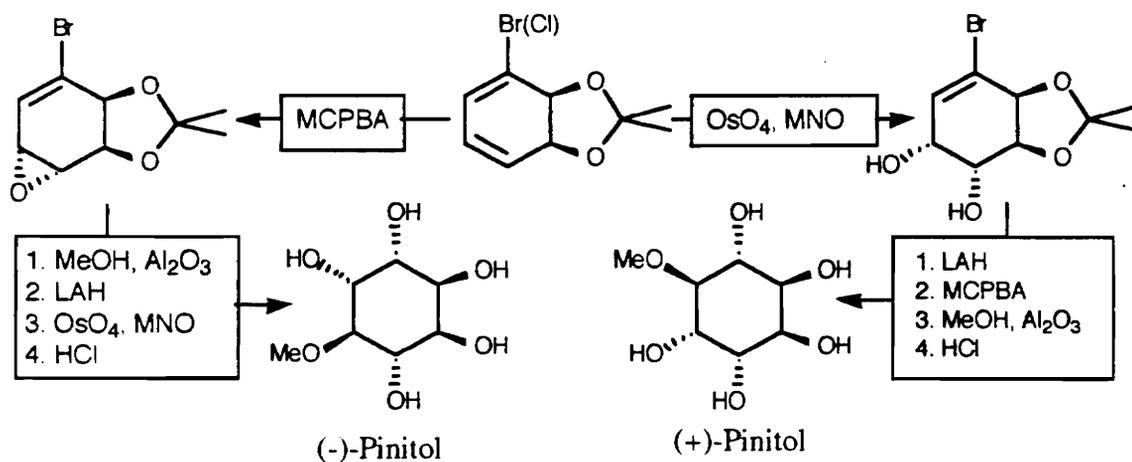
Enantiodivergent Synthesis

Ref. 301a

1. bis[(trimethylsilyl)ethyl]
   azodicarboxilate
2. THSCl
3. Ac₂O
4. TBAF

Enantioselective synthesis

1. cinnamic acid, DAED, Ph₃P
2. TBAF
3. Δ

1. O₃
2. NaBH₄
3. PhCO2H,
   Me₂CHCH₂OCOCl

Ref. 302

(-)-Zeylena

59

L-erythose

[D- erythrose was subjected to the same reaction sequence producing (-)-trihydroxyheliotredane]

1. Tf₂O
2. NaN₃
3. Δ

1. FVP, 250°
2. H2, Pd/C
3. LAH
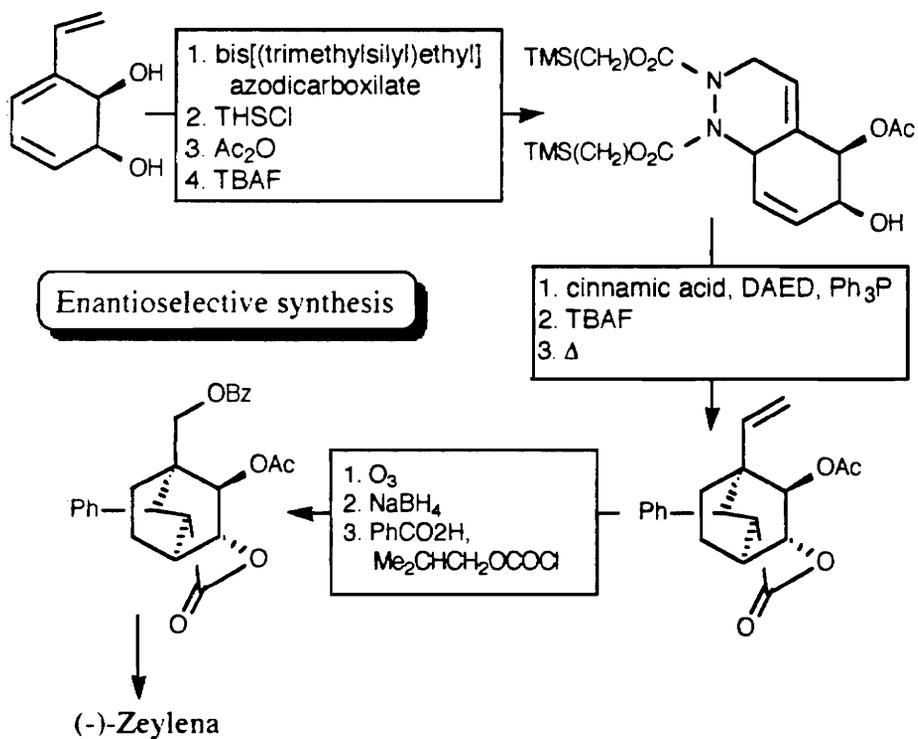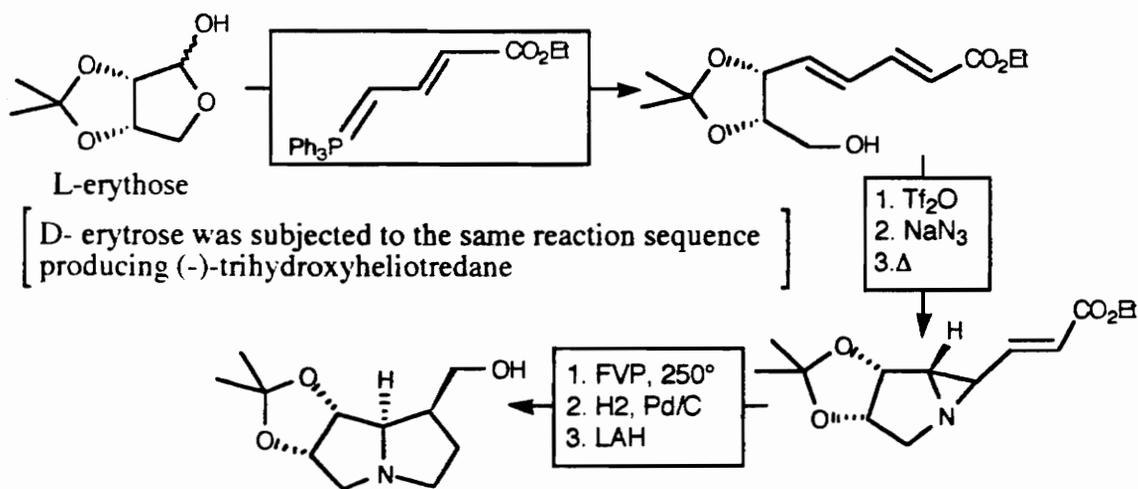
Enantiodivergent synthesis from *cis*-chlorobenzenediol   Ref. 303

1. O₃
2. Ph₃PCH₃Br

OsO₄
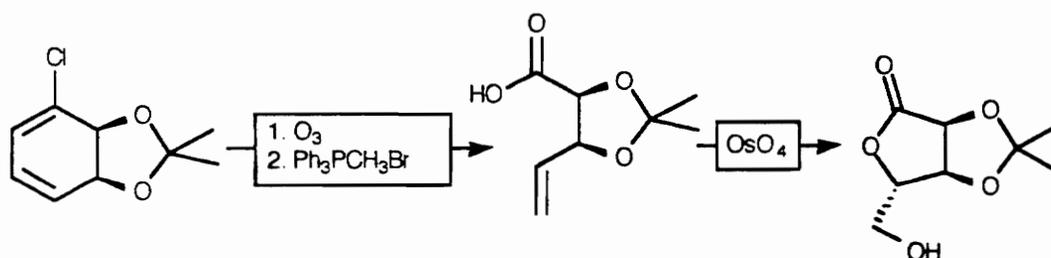
Enantioselective synthesis   Ref. 304

II.5.   Glycosidase inhibitors.

In the hierarchy of enzymes that process carbohydrates, those taht break glycoside bonds to release mono or oligosaccharides are among the most important. Glycosidases are ubiquitous in nature and are essential for the normal growth and development of all organisms. They are involved in a variety of important cellular functions.

Because of its important role in carbohydrate technology, biochemistry, and organic chemistry, the hydrolytic cleavage of glycosides has been extensively studied with respect

to both acid[306-308] and enzyme catalysis.[309,310] Hen-egg lysozyme is the only enzyme for which detailed mechanistic information is available.[311] Some information is also available about the α-amylase from *Aspergillus wenttii*.[312] In general it is accepted that most of the processes follow the mechanism proposed by Phillips, described in Scheme 31.[313,314]
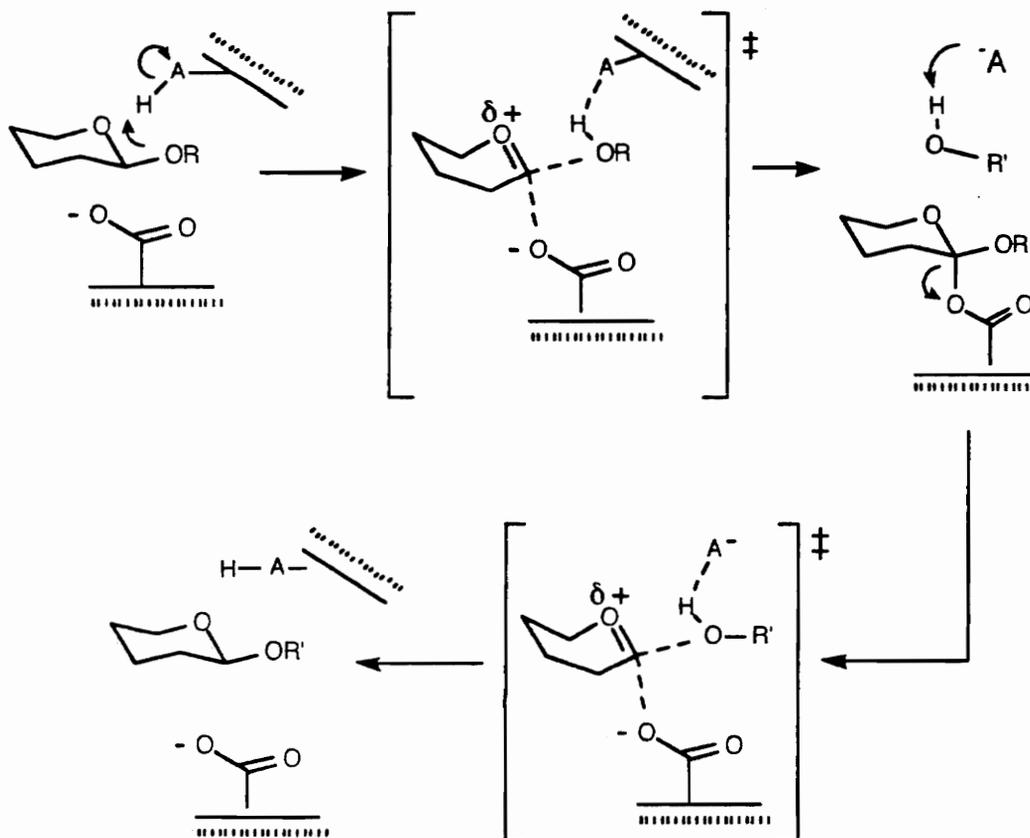
Scheme 31. Glycolysis Mechanism Proposed by Phillips.

Glycosidase inhibitors are classified as reversible and irreversible.[313] Among the first group are aldonolactone and 5-amino-5-deoxylactams; the inhibitory activity of aldonolactones was first mentioned in 1940, from studies on aspergillus and almonds.[315] Since then some authors have shown that aldonic acids themselves are non-inhibitory, and that 1,5-lactones are better inhibitors than their 1,4-isomers.[316] Leaback pointed out that the aldonolactones exert their powerful activity by virtue of their structural similarity to the glycosyl oxocarbonium ion intermediate or a related transition state.[317] Fleet reported that 1,4-dideoxy-1,4-iminomannitol was a potent competitive inhibitor of Jack Bean α-

61

mannosidase (*Canavalia ensiformis*), which was the first report of a specific inhibition of α glycosidase by a pyrrolidine analog of a furanose.[318]

Pyranosyl oxocarbenium ion   Aldono-1,5-lactone

Figure 11. Glycopranosyl Oxocarbenium *vs.* Aldono-1,5-lactone

Both the lactone and the oxocarbonium ion have a trigonal, planar configuration at C-1 and adopt a half-chair conformation. In addition to these geometrical factors, there have to be considerable electrostatic interactions arising from the large dipole moment of the
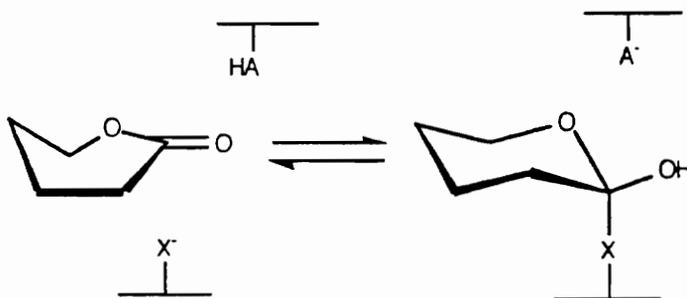
Figure 12. Possible Formation of an Enzyme-linked Acylal (-X- =Carboxylate) from an Aldono-1,5-lactone at the Active Site of a β-Glycosidase.

lactone that would enhance lactone binding if there is a negatively charged group in close proximity to C-1 of the bound inhibitor. A new, unorthodox concept to explain the strong inhibition by aldonolactones was advanced by Sinnott and Suchard,[319] who proposed the reversible addition of an active-site nucleophile X (possibly a carboxylate) to C-1.

The problems regarding ring size and stability that are encountered with aldonolactones disappear when the ring-oxygen atom is replaced by an NH group. The resulting 5-amino-5-deoxyglyconolactams constitute a new group of inhibitors, closely related to the lactones.[320,321] Like their oxygen analogs, they have a trigonal planar configuration at C-1; the dipole moment of the carbonyl group is expected to be even larger than that of the lactone carbonyl, because of the larger contribution of the dipolar resonance structure. The NH group may then cause steric repulsion, or a hydrogen-bond donor for the ring-oxygen atom may fail to interact properly with the NH group because of its amide resonance. The strong inhibition observed with the lactams tested so far shows that these effects are of minor importance. In cases where the inhibition constants for lactams and lactones can be compared, they have the same order of magnitude. In all cases, the inhibition is competitive; that is, the inhibitor competes with the substrate for the free enzyme. From structural considerations, it may be assumed that aldonolactones and lactams are bound by the glycon binding site of the enzyme, interacting with the same functional groups that are responsible for substrate binding and, possibly, catalysis.

The first report of sugar analogs bearing a basic group, which are strong inhibitors for glycosidases, was by Lai and Axelrod.[322] These researchers found that glycosylamines derived from D-glucose, D-galactose, and D-mannose were active at concentrations ranging from 0.002 to 0.22 mM; that is, up to 1000-fold better than the corresponding aldoses. Extension of these studies by Legler[323] and Legler and coworkers[324,325] showed that N-substituents increase the inhibitory potency of D-gluco- and D-galacto-sylamine, provided that they do not impair the basicity of the amino group.

In 1970 it was reported that nojirimycine, identified as 5-amino-5-deoxy-D-glucopyranose (89), is a powerful inhibitor of β-D-glucosidases.[326] It was discovered by virtue of its antibiotic activity over some streptomyces;[327] its 1-deoxy derivative (90) is a strong inhibitor of α-D-glucosidases.[328] Both compounds were shown to be fully competitive inhibitors of small intestinal sucrosose, demonstrating their high affinity for the enzyme.[329] Later, deoxynojirimycin, 2,5-dixydroxymethyl-3,4 dihydroxypyrrolidine and 1,5-dideoxy-1,5-iminomannitol were compared with respect to their glycosidase inhibitory activity. The chemistry of this class of sugar derivatives has been discussed by Paulsen and Todt.[330] Subsequent studies with 5-amino-5-deoxyhexopyranoses and 1,5-dideoxy-1,5-iminohexitols related to mannose,[331] D-galactose,[332] and 2-acetamido-2-deoxy-D-glucose[333] showed that strong inhibition of glycosidases by these compounds is a general

89   **90**   **91**
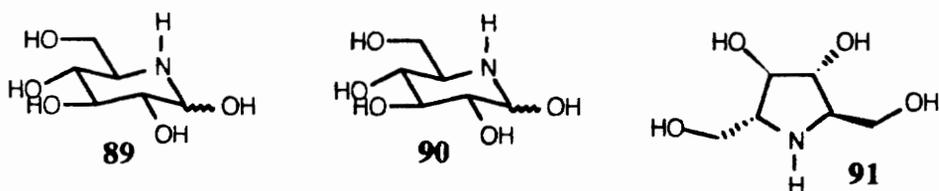
phenomenon probably based on common mechanistic features. (2R,5R)-Dihydrxymethyl-3R,4R-dihydroxypyrrolidine (**91**), isolated from *Derris eliptica*, proved to be more a effective inhibitor of α- and β-glucosidase than deoxynojirimycine. Deoxymannojirimycine is a α-mannosidase inhibitor and very potent α-fucosidase inhibitor.[334] Deoxymannojiimycine has been tested as an agent for the control of carbohydrate metabolism by comparison with swainsonine.[335]

The large inhibitory potential of these basic sugar derivatives, and their stability, have made them valuable tools for mechanistic and biological studies.[336] It has been demonstrated that 1,5-iminoalditols have an inhibitory potency similar to or even larger than that of glycosylamines and that 5-amino-5-deoxyaldopyranoses are even better inhibitors.[313]

92   **93**   **94**
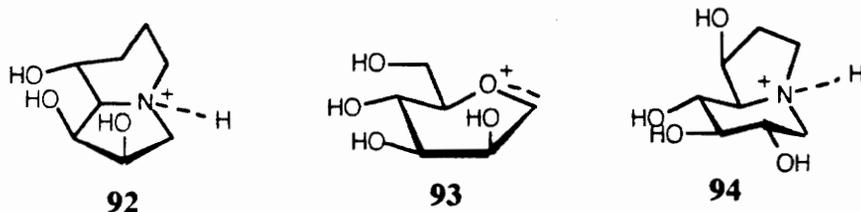
The isolation of valienamine, from *Flavobacterium charophilum*[337] and *Pseudomonas denitrificans*,[338] introduced a new class of glycosidase inhibitors having the basic structure of aminoconduritols.[339] In screening for new antibiotics, oligostatins were found to have amylase inhibitor activity; these oligosaccharides contain some aminosugar residues.[340]

Swainsonine (**92**, protonated form), first isolated from the Australian plant *Swainsonine canescens* by Colgate,[341] and latter from spotted locoweed (*Astralagus lentiginosus*) by Molyneux and James,[342] and others,[343,344] was characterized as a strong inhibitor of α-mannosidase by Dorling.[345] Its inhibitory potency can be rationalized by the spatial arrangement of its hydroxyl group and its nitrogen atom: protonated swainsonine (**92**) greatly resembles the mannosyl cation (**93**). Swainsonine (**92**) is possibly the agent

responsible for enzymatic and oligosaccharide changes in animals, producing a neurological condition similar to that observed in the hereditary mannosidosis, called *locoism* (dull-appearing eyes; dry, lusterless hair coats; depression; and lack of coordination).[346]

Castanospermine (**94**, protonated form) , isolated from *Castanospermun australe* by Hohenschutz[347] and characterized as strong inhibitor for β-D-glucosidase by Saul,[348] can be considered as a derivative of 1-deoxynojirimycine. The rigid, bicyclic structure of castanospermine and swainsonine may well be the cause of their strong inhibition of certain enzymes and weak inhibition of others.

The mechanism of glycolysis seems to be sufficiently understood and has been corroborated by the structure-activity relationship studies reported by Fleet and coworkers in the search for inhibitors of this process.

Inhibitors of glycosidases have the potential to produce a number of kinds of beneficial therapeutic effects. They have been used or suggested as antihyperglycemic compounds,[392] inhibitors of tumor metathesis,[393] antiobesity drugs,[394,395] fungistatic compounds,[396] insect antifeedants,[397-398] and antivirals.[399-402] Glycosidase inhibitors have been studied for anti-HIV activity.[403] Based on these studies, mannosidase inhibitors have attracted special attention.[404]

Because the envelope glycoprotein of HIV is heavily glycosated, it is plausible that inhibition of one of the enzymes required for processing glycoproteins may prevent envelop formation and affect virus infectivity. It has observed that Swainsonine restores the capacity of immunodeficient mice to produce antibodies against SRBC (Sheep Red Blood Cells). Furthermore, swainsonine inhibites completely the growth of sarcoma 180 ascites tumor in mice as well as reduced lung methatesis of B16 melanoma in mice. These results suggest that swainsonine has potential as an inmunomodulator for the treatment of inmunocompromised host.[389]

Table 13. Compounds with Glycosidase Inhibitory Activity.

Structure	Ref.	Structure	Ref.
1	318 349	**7**	358
2	350	**8**	351
3	352	**9**	353 354
4	352 355	**10**	356
5	353 354	**11**	352 357 349 358
6	358	**12**	359
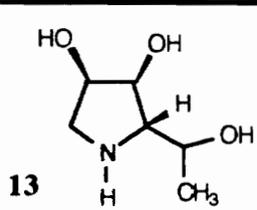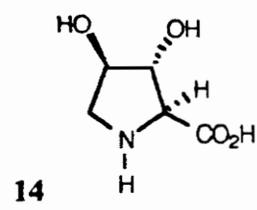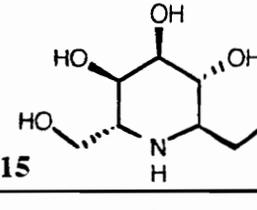
Structure	Ref.
13 (structure)	360
14 (structure)	364 365 366 367
15 (structure)	368
16 (structure)	331 373
17 (structure)	374
18 (structure)	376 367
19 (structure)	374, 379 331, 380 377, 361 381, 371 382, 362 383, 363
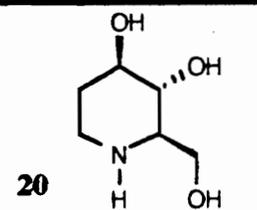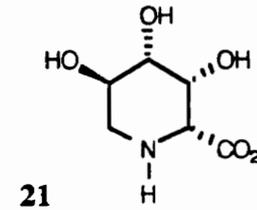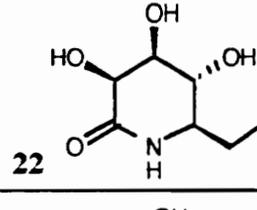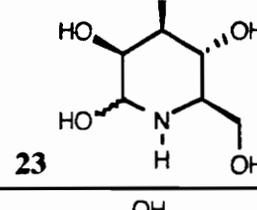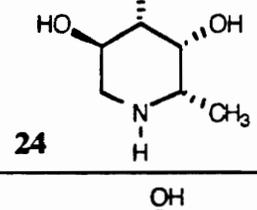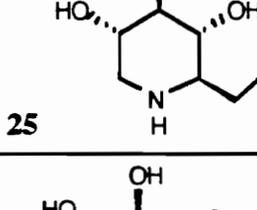
Structure	Ref.
20 (structure)	361 362 351 363
21 (structure)	367.
22 (structure)	369 370 371 372
23 (structure)	369
24 (structure)	375
25 (structure)	378 374 356 377 363
26 (structure)	381

67
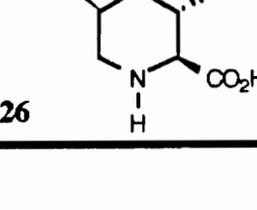
Structure	Ref.
27	384
28	386
29	386
30	386
31	377 363
32	388
33	349, 389 341, 390 344, 391

Structure	Ref.
34	365 385
35	385
36	358
37	387
38	358
39	353
40	353 352 388 349

The results of the investigation of biological activity of compounds like swainsonine and castanosermine, as well as norjirimycine and mannojirimycine, as well as kifunensine opens a great opportunity for searching inmunomodulating substances. Some of \ the compound which have been tested for glycosidase activity are shown in the Table 13.; note that the compounds listed do not represent an exhaustive search.

II.6. Kifunensine: Isolation, Structure, Biological Properties and Synthesis.

Kifunensine (**95**) was isolated from *Kitasatosporia kifunense* No. 9482 and was soon shown to be a new immunomodulator substance, initially designated as FR9000494 because its structure was not known.[406] This interesting compound also exhibited α-mannosidase inhibitory activity.[407] In 1989 its structure was reported to be a cyclic oxalamide derivative of 1-amino mannojirimycine (**95**).[408]
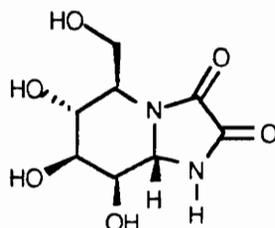
Kifunensine (**95**)

The structure was confirmed by a total synthesis from D-mannosamine (**96**), reported by Hashimoto, Scheme 32. The key step involved is the construction of the dioxoimidazolidine ring by a double cyclization of **97**.[409] The ring closure takes place in favor of the desired and energetically more stable diastereomer. Similar methodology has also been applied to the synthesis of 8-*epi*-kifunensine starting from D-glucosamine.[410]

The importance of this compound lies in its biological activity. Kifunensine (**95**) was initially reported to be a weak inhibitor of Jack bean α-mannosidase (IC$_{50}$ of 1.2 x 10^{-4} M).[408] Later it was found that kifunensine was a very potent inhibitor of the plant glycoprotein-processing enzyme, mannosidase I (IC50 of 2-5 x 10^{-8} M). However, kifunensine was inactive toward the plant mannosidase II.[407]

Studies with rat liver microsomes also indicated that kifunensine inhibited the Golgi mannosidase I. Consequently, kifunensine appears to be the most effective glycoprotein-processing inhibitor observed thus far. The most important activity
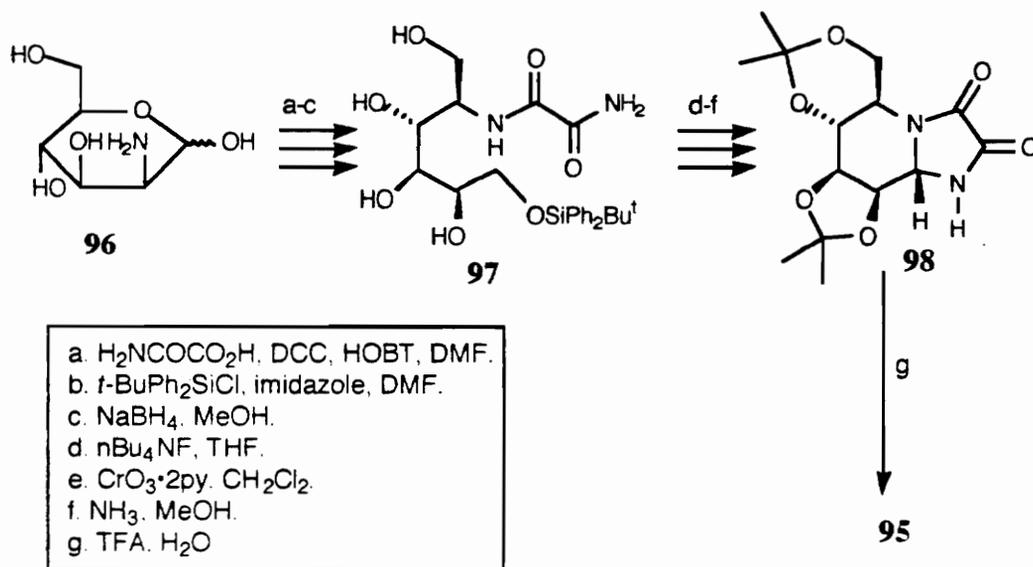
Scheme 32. Hashimoto's synthesis of Kifunensine.

presented by kifunensine is immunomodulation and the ability to restore the immune response in mice.[406] Its potential in the HIV screens has yet to be evaluated.

III. DISCUSSION.

III. 1. Microbial Oxidation and Isolation of cis-Arenediols.

As mentioned earlier (Section I) the main objective of this work is the utilization of cis-arenediols, produced by microbial oxidation of arenes, as chiral starting materials for synthesis of natural products. Consequently the first part of this work was the investigation of the microbial oxidation of arenes, specifically the oxidation of toluene and chlorobenzene. The bacteria used was a mutant of *Pseudomonas putida* 39D, which was reported by Gibson.[65]

The microbial oxidation consists of two stages. The first step, called induction, is the bacterial growth in the presence of limited amounts of an aromatic compound (which could be the substrate itself or a different compound) known to be an inducer for the operons of the enzyme. This phase is necessary in order for the cells to produce the enzymes needed for the oxidation. For this purpose the apparatus, as shown in Fig .14, contains a glass bulb in which the substrate was deposited for slow evaporation into a suspension of *P. Putida* 39D, which was incubated at 30 °C in a rotatory shaker for 6-8 h. It is noteworthy to mention that not all the arenes are able to induce the enzymatic system, only toluene, chloro- and bromobenzene have been shown to be inducers.
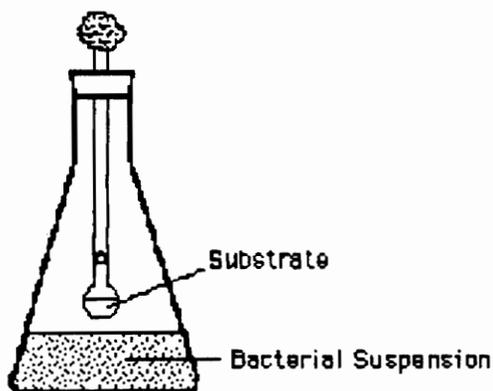
Figure 14. Induction flask

In the second step, production, the experimental set-up depends on the physical properties of the substrate. Liquid substrates were introduced into the suspension of cells by a stream of air as shown in Fig. 15, in an apparatus adapted from the one used by

71

Gibson.[67] A similar array can be used for solids with high vapor pressures; solids can be added to the cell suspension as powders or dissolved with the help of a co-solvent (DMSO, DMF, ethanol, glycerol) as this provides a steady release of low concentration of the substrate into the culture medium. High concentrations of substrates are usually detrimental to the microorganism. The temperature of the process is kept at 30 °C for about 24 h. At this stage the cells are grown in an excess of the substrate to be oxidized to provide the main carbon source for the development of the cells.
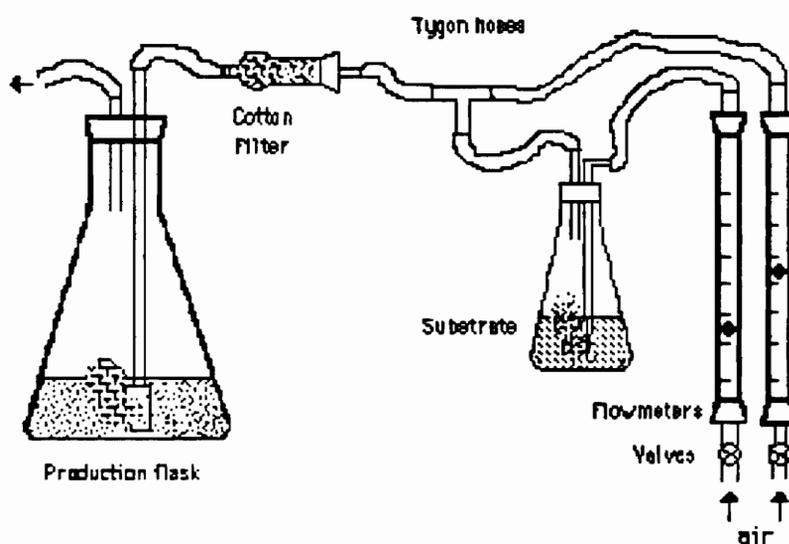
Figure 15. Initial Apparatus Used for Microbial Oxidation of Arenes.

The inoculum was prepared by streaking cells on a Petri plate containing an agar-MSB-arginine medium (Appendix I). The broth used for the fermentation was an MSB-arginine solution,[411] which contained all the necessary nutrients. This solution contained only arginine as the carbon source; this amino acid was selected because it is not metabolized efficiently by *Pp* 39D, thus allowing the aromatic substrate to act as an alternate carbon source

In practice cells from one agar plate, previously incubated at 30 °C, were transferred. in 10 mL of MSB-arginine solution to the induction flask (Fig. 9), which contained 50 mL of MSB-arginine. The flask was incubated at 30 °C on a rotatory shaker for 6-8 hrs. In this way the cells were subjected to low concentrations of toluene

72

(substrate) in order to effect the induction of the metabolic pathways in the cells so as to make them able to oxidize the aromatic substrate. This is possible because of the small solubility of the substrate in water. The cell suspension was then poured, under aseptic conditions, into the production flask for the second stage of the process. The stream of air and substrate served as a mixing device as well as a way to introduce the substrate. The apparatus (including the substrate container) was maintained at 30°C (±2°C) for 24 h. It is important to mention that the flow of substrate (toluene in this case) had to be carefully controlled because an excess inhibits the bacterial growth. The flow of air had to be controlled also because a strong flow could facilitate evaporation of the substrate whereas a slow stream might not furnish enough oxygen required by the bacteria to carry out the oxidation. During this process the diol accumulates in the solution as an exo-metabolite.

After the fermentation, the cells and solids were separated by centrifugation to give a clear solution containing the diol. This solution then was saturated with NaCl to allow for easy extraction. After extraction, purification of the crude product could be done by column chromatography or recrystallization, depending on the diol.

The diols are unstable to acidic conditions, which lead to generation of the corresponding phenols. Even the phenolic acidity of the decomposition product is enough to catalyze the process of decomposition of the arene diols. Consequently, precautions were taken in handling the arene diols by using acid-free solvents. The yields of this process under the described conditions were around 250 mg per 500 ml of MBS-arginine solution.

When initial optimization work was started, the first parameter to be evaluated was the optimal density of cells. The maximum amount of cells from the agar plate was transferred to the induction flask because the cells are the "limiting reagent" for the oxidation as they provide the necessary enzymes. Transferring all of the cell colonies from the plate to the medium resulted in an increase of yields up to 500 mg per 500 mL of MSB-Arginine.

In the second stage, the flow ratio of substrate and air was varied from 5:45 to 25:70 ml/min, resulting in improvement of yields to 700 mg per 500 mL of MSB-arginine. In another attempt to improve the yield and, at the same time, avoid contamination of the culture medium and substrate by the tygon tubing used for connections (see Fig. 16, A), an all-glass (bubbling) system was designed that had a 45° angle in the combining ducts (air and substrate) (see Fig 16, B) instead of the 90° angle in the old setting (Fig. 11, A). This feature avoided back pressure problems and produced a smooth merge of flows.
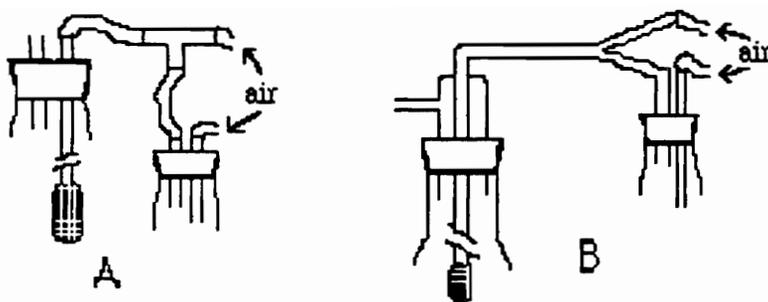
Figure 16. The two air/substrate introduction systems. A the original and initial setting; B the all-glass setting for bubbling.

These modifications in the initial process allowed us to get 1.0-1.2 g of *cis*-toluenediol per 500 ml of MSB-arginine solution. The new reaction conditions could be adapted to the preparation of other arenes, i.e. chlorobenzene, bromobenzene, and styrene. The all-glass system was especially useful for the oxidation of styrene because the tygon tubing had allowed the styrene to escape into the room producing an unpleasant environment for work.

A further attempt to improve the production of *cis*-diols involved the use of a fermentor,[413] with a working volume of 1.5 L. The process was carried out under similar conditions to those used with erlenmeyer flasks, but the yields were never improved. The parameters modified during the optimization were the ratio of air and substrate, their absolute flows and the stirring rate. The reason behind this apparent failure could be due to the different geometry of the reaction vessel, because it is known that the behavior of the fluid in the fermentation tank has a definite influence in the performance of the process.[412] On the other hand, the volume of solution displaced during the bubbling was greater, producing problems with the settings of the relative flows of substrate and air. This problem could be overcome by the use of different ports for the aeration and introduction of the substrate. However, the establishment of the optimum relative and absolute flows of substrate and air will require additional work.

Additional problems were encountered in the lack of monitoring of some important parameters of the process, i.e. pH, dissolved oxygen, and the growth curve. These

parameters did not seem very important in the erlenmeyer flask set-up where they were not controlled, but they became evident during the scale-up. Another important factor that was considered was the availability of the substrate for the bacteria, which has not been investigated: this availability could be dependent, not only on the partition coefficient of the substrate, but also on the rate of aeration.

Some of these scale-up problems were solved with the acquisition of a 15 L fermentor.[414] With this apparatus, the pH and dissolved oxygen were monitored and consequently adjusted during the process without disrupting the integrity of the fermentation vessel. With this facility a few experiments that led to the optimization of the diol production were performed; these are discussed below.

Once again, the optimization process began by using the fermentation conditions utilized in the erlenmeyer flask set-up and the fermentor. The objective of the first experiment was to determine the growth curve of *Pp* -39D in the fermentor. The first trial was performed with consideration of the need of larger initial amounts of cells. Consequently the cell content of one petri plate was divided into four 500-ml induction flasks, containing 250 ml of MSB-arginine solution. The cells were induced with chlorobenzene, and the flasks shaken at 150 rpm at 30°, for 24 h. The contents of all four induction flasks were poured into the fermentor containing 7 L of sterile MSB-Arginine solution. The initial fermentation conditions used were: temperature 30°C, stirring at 200 rpm, dissolved O_2 set at 100% , and pH=7.2. During the process, the change of pH, percentage of dissolved O_2 and cell growth (growth by optical density measurements) were monitored in order to have a better idea of the progress and profile of the fermentation. The data obtained in these experiments is summarized in the graph #1 (See Appendix V.2, Graph #1). Graphs #1 shows that the maximum growth was coincident with the maximum consumption of O_2, occurring 8 h after the initiation of the process. When the plateau of growth was reached, the consumption of oxygen was at a minimum, which meant that a stationary growth had been reach. The pH increased slowly to a maximum of 8.2, possibly from the presence of metabolites excreted by the cells. Regardless of the time required to reach the plateau in growth, the fermentation was continued for 24 h, after which time there were no significant changes other than the lysis of cells.

The next optimization involved adding substrate (chlorobenzene) to the culture at the stationary phase, which the same parameters monitored as before, in addition to the consumption of substrate and the concentration of *cis*-chlorobenzenediol produced. The

resulting data is shown in second part of graph #1 (after the first 24 h). (See Appendix V.2.) The consumption of oxygen did not increase, indicating no growth of cells (multiplication), and the pH also remained fairly constant. A maximum concentration of approximately 100 mg of diol in the whole solution was observed by UV quantitative analysis; extractive work-up was not performed for such a small amount.

These results suggested that only growing cells were able to oxidize the aromatic substrate and that the lack of diol production could be due to the fact that the cells were in a stationary phase. Another possibility was that most of the cells were the original wild strain, and the mutation, and therefore the ability of accumulating the diol, was lost during the process.

In order to answer the above questions, in the next experiment the substrate was added at the beginning of the fermentation, as was done in the erlenmeyer set-up. The induction was then continued for 9 h (using three erlenmeyer flasks with 50 mL of MBS-Arginine). The induced cultures were then transferred to the fermentor vessel containing 7 L of sterile MSB-arginine. The monitoring of the process is shown in graphs #2. (See Appendix V.2, Graph #2.) Once again the consumption of oxygen by the culture paralleled the growth, and the increase in the diol concentration followed the same trend as shown in the graphs. As observed before once the plateau of growth was reached the rate of the production of diol was at a minimum. Extraction of the broth produced 2.34 g of *cis*-chlorobenzenediol, an amount that was different from that expected from the UV analysis, which should have been 3.36 g. This difference could be due to incomplete extraction or decomposition during the storage of the crude fermentation solution.

In the next experiment the increase in air flow was studied because of the depletion of the concentration of the dissolved oxygen observed, oxygen being fundamental for the cell growth and the oxidation itself. The procedure used was similar to that used before, but this time the flow of air was doubled. The results are shown in the graphs #3 (see Appendix V.2, Graph #3). In this case the maximum depletion of dissolved oxygen took place 5 h after the initiation of the fermentation, coincidental with reaching of the plateau in the growth curve. The production of diol showed large increments during the first 10 h of fermentation, after which point the production of diol was slowed (5.6 g in the first 10 h and 1.1 g during the next 15 h).

Again there was a large discrepancy between the isolated yield, 7.11 g, and the concentration of 10.32 g estimated from the UV analysis. Because of these evaluations the

accuracy of the analytic determination of the diol concentration showed to be reconsidered and new and unambiguous procedures developed and optimized.

In summary, as of this writing the 15-L (12-L working volume) fermentation produces 20 g of diol which is a moderate improvement over the initial results. This is a preparatively useful amount, available for synthetic protocols on a regular basis.

III.2. Synthetic Design: General Considerations.

The monocyclic *cis*-arenediols of type **99** are interesting compounds from the point of view of their possible chemical synthesis, which would involve elaborate

99

X= H, CH_3, Cl, Br, F, I, F, $CHCH_2$,CCH, CF_3
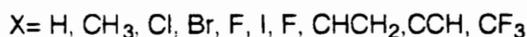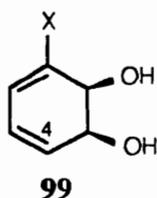
synthetic sequences and result in their production as racemates. The diols obtained by biocatalysis are of a known absolute configuration and possess an unusual array of functional groups that can be utilized in further functionalization of these molecules as shown in Fig. 17.[415,302]
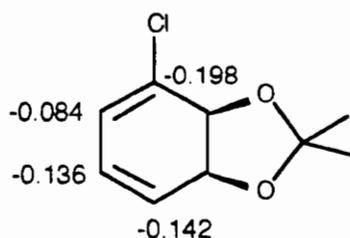
Figure 17. Electron Density of *Cis*-chlorobenzenediol Acetonide .[416]

One of the features of the structure of substituted *cis*-arenediols (**99**) is the differential reactivity that the substituent confers to the double bonds in the diene functionality, making it possible to functionalize the diene in a regiocontrolled manner with regard to the double bonds. That characteristic can be explained based on the different electron density content of each carbon atom in the diene system, Fig. 17.[416]
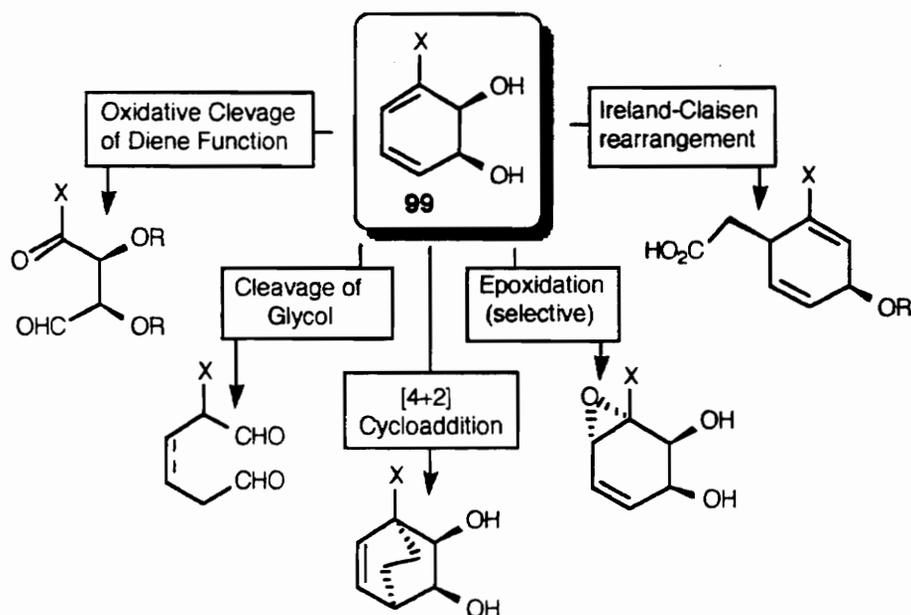
Figure 18. Synthetic Strategies

Another characteristic derived from this unique structure is the known absolute configuration of both hydroxyl groups, because that can be used as element of
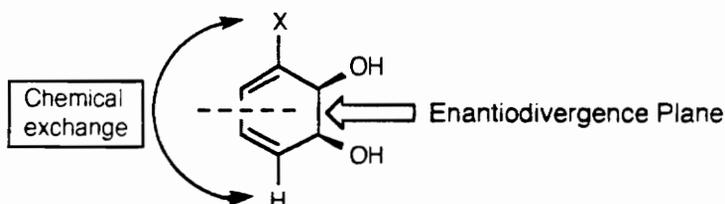
Figure 19. The Concept of Enantiodivergence .

stereocontrol in further functionalizations of the molecule. The diol function can either be protected or used unchanged providing an excellent opportunity for the face selectivity in subsequent steps. This would allow the functionalization of the diene or one of the double bonds in a selectively controlled fashion. Some of the strategies that can be explored in the functionalization these molecules are shown in Fig. 20.

78

Nu: Cl, Br, F, NH$_2$, N$_3$, SH, SR, OH, OR
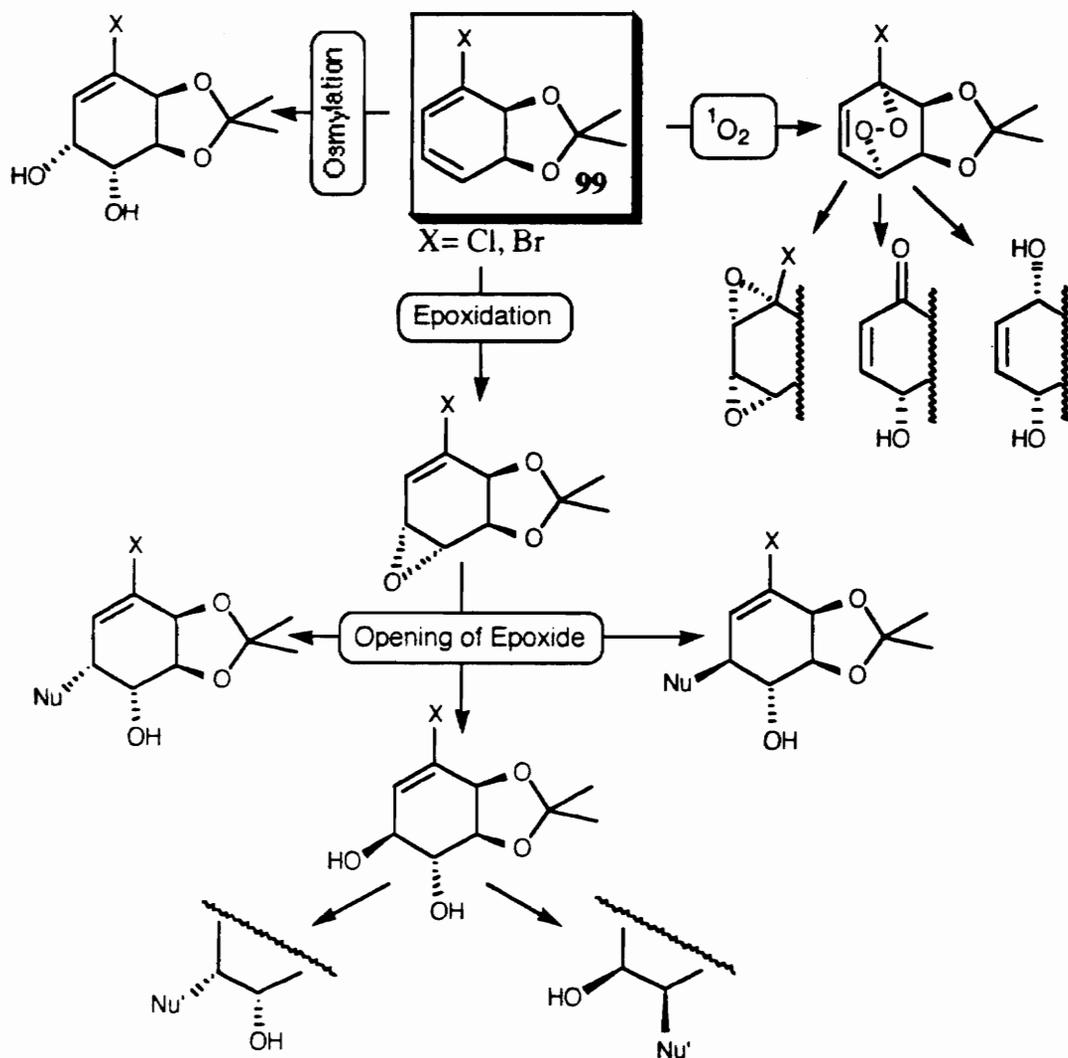
Figure 20. Strategies for regio- and stereoselective functionalization of halo *cis*--arenediols.

Possibly the most important feature is that, in some cases, there exists the possibility of chemical exchange of the substituent **X** with the hydrogen in C-4 which could lead to enantiodivergence (Fig. 19).[303] This latter fact greatly enhances the synthetic utility of cis-arenediols, as will be shown further in the discussion of their application to synthesis.

All the characteristics previously described for regio- and stereocontrolled functionalization of halo *cis*--arenediols (**99**, X= Cl, Br) lead to considerations of these compounds as chiral synthons. Structural features of these compounds allow their sequential oxygenation, in a stereo- and regiocontrolled fashion, at positions 1,4,5 and 6, providing the possibility of synthesis of any isomer of any cyclitol, Fig. 15. Furthermore, the oxygenated groups are ideal precursors of cyclitols (hexaoxygenated cyclohexanes) substituted with other groups resulting in the preparation of cyclitol derivatives of the amino-, halo-, and thio-cyclitols class of compounds. Some of the transformation that would be attempted are shown in Fig. 15.[417,416]

An additional potential utility of halo *cis*--arenediols derived from the regio- and stereocontrolled sequential oxygenation is their involvement in the synthesis of sugars. This topic will be discussed in section II.4.

III.3. Prostaglandin and Terpene Synthons.

Prostaglandins exhibit diverse pharmacological properties and are now recognized as local hormones that control a multitude of important physiological processes.[418] Since the elucidation of the structure of the prostaglandins in the early 1960's, their chemical synthesis has occupied a central and exciting place in organic chemistry. The total synthesis of these compounds is the only means to have sufficient quantities of these physiological bewildering but rare, naturally occurring substances for testing.
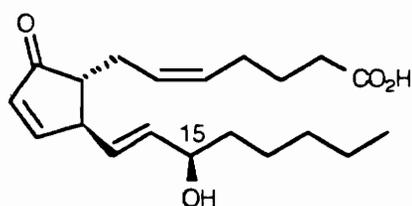
PGA$_2$-15R

The only possible source of prostaglandins from natural sources is the gorgonia coral *Plexaura homomalla*.[419] However, PGA$_2$, the major component (1.5% of the wet weight) of these corals, is contaminated in some species with 3 to 15% of the 5-trans isomer, which is difficult to remove. From the Mexican Caribbean area a different species of *Plexaura homomalla* contains up to 5% (dry weight) of PCA$_2$-15R.[420] Alternatively, Corey developed an efficient method to transform PGA into the primary prostaglandins

(PGE$_2$ and PGF$_2$).[421]   On the other hand, this methodology was adapted for the transformation of PGA$_2$-15R in PGF$_2$ and PGE$_2$, both having the R configuration in C-15.[420] The use of such natural resources for obtaining prostaglandins is prevented by little knowledge of artificial growth as well as the extremely low rate of growth of this organism.

100   **101**   **102**
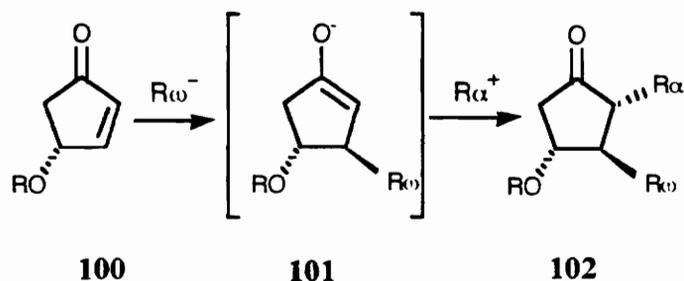
Scheme 33. Three Component Synthesis of Prostaglandins

The main problems associated with the synthesis of prostaglandins are: 1) selective generation of the four or five asymmetric centers ; 2) stereoselective insertion of the C-C double bonds; and 3) overcoming the stability of their β-hydroxycyclopentanone structure in Some strategies of synthesis involve rigid bicyclic intermediates which exert perfect stereocontrol around the five-membered ring, as shown in the elegant syntheses reported by the Upjohn[422] and Corey's groups.[423]

Possibly the most efficient methodology for the synthesis of prostaglandins is the so-called *three-component coupling* process, namely , a single-pot combination of the five-membered-ring unit and the two side chains (Scheme 33).[424] This strategy combines convergence, stereocontrol, and efficiency. It is also suitable for the preparation of non-natural analogs,containing modifications in one or both side chains. This method requires a properly functionalized chiral cyclopentenone. An approach to the preparation of enone **103** was designed by Johnson in the synthesis of PGE$_{2\alpha}$,[425] which has been prepared by different routes.[426] The possibility of using in *cis*-toluene diol as a precursor of the isopropylidenedioxy group of the enone **103** was recognized as shown in the retrosynthetic analysis (Scheme 34). The synthetic plan called for protection of the *cis*-toluene diol as the acetonide during the synthetic work and later as directing group needed

81

in **103**. The next step required an oxidative cleavage of the diene function
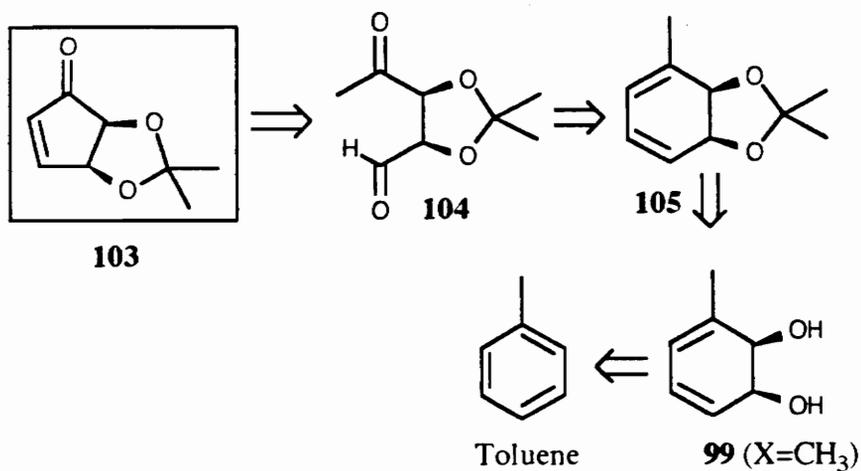
Scheme 34. Retrosynthetic Analysis.

Table 5. Total Ozonolysis of Cyclic Dienes.[415]

Diene	Reaction conditions	Dicarbonyl	Yield, %
106	CH$_2$Cl$_2$, -78 °C, excess O$_3$	**110**	70
107	CH$_2$Cl$_2$, -78 °C, excess O$_3$	**111**	89
108	CH$_2$Cl$_2$, -78 °C, excess O$_3$	**112**	52
109	CH$_2$Cl$_2$, -78 °C, excess O$_3$	**113**	50
105	EtOAc, -60 °C, excess O$_3$	R^1 = R^2 = H **114**	60-70
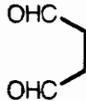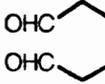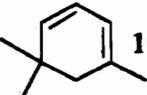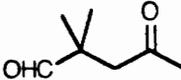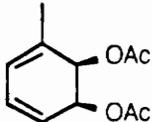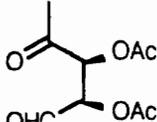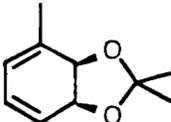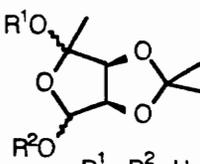
to provide the keto aldehyde **104**. For this purpose the logical option was found in ozonolysis. Aldol cyclization of **104** could lead to enone **103**. The whole process appeared to be short, efficient, and enantiospecific.

In practice *cis*-toluene diol (**99**, X=CH$_3$), obtained from microbial oxidation of toluene (**106**), was reacted with dimethoxypropane (DMP) under acid catalysis to produce the isopropylidene dioxy derivative **105**.[427] The yield ranged from 80-90% because of variable amounts of m-cresol formed during the acidic conditions of the reaction.

Next the oxidative cleavage of the conjugated diene, for which the literature showed no precedent, was to be performed. Most reports of direct ozonolysis of conjugated dienes indicated the production of undesired rearrangement products stemming from allylic ozonides.[96,98,105] Initial work on the ozonolysis of acetonide **105** produced an unexpected product (**114**) as a mixture of all possible stereoisomers, making its purification and identification difficult and challenging.

Table 6. Selective Ozonolysis of Cyclic Dienes.[415]

Diene	Reaction conditions	Dicarbonyl

In order to gain better insight into the ozonolysis of conjugated cyclic dienes, a study was performed on some simple dienes to determine the feasibility of such oxidation and its potential application in our synthetic effort. The results are shown in Table 5 (with isolated yields).[428] The product of ozonolysis was the corresponding acyclic dicarbonyl compound in all the cases, except in that of acetonide **105**. The dialdehydes derived from

1,3-cyclohexadiene (**106**) and 1,3-cyclooctadiene (**107**) (**110** and **111** respectively) were unstable and difficult to isolate; consequently the products of ozonolysis were transformed to the corresponding diols for complete characterization. The yields of these reactions were reasonably good. Even the diacetate of *cis*-toluenediol (**109**) gave the corresponding dicarbonyl product (**113**). The presence of the acetonide as protecting group of the diol seemed to interfere in the mechanism of the ozonolysis process.

In further experiments designed to obtain a better understanding of the irregular ozonolysis reaction of **109**, the group of conjugated dienes shown in Table 5 were subjected to a controlled mono-ozonolysis reaction. The results in Table 6 indicate that the cleavage of only one double bond followed the expected pathway, and also the regiochemistry of the reaction was directed toward the more electron-rich double bond.[95] The products of the mono ozonolysis were again subjected to the action of ozone. Again the keto aldehyde **104** was not detected from the reaction of **118**. The keto aldehyde **118** could not be isolated but it was used directly for further ozonolysis to give the bis-hemiacetal (**114**). In contrast the diacetate **109** was ozonized to give a stable keto unsaturated aldehyde.

In all the ozonolysis experiments we could not isolate or detect any products of rearrangement; the rest of the mass balance in each reaction were polar materials that
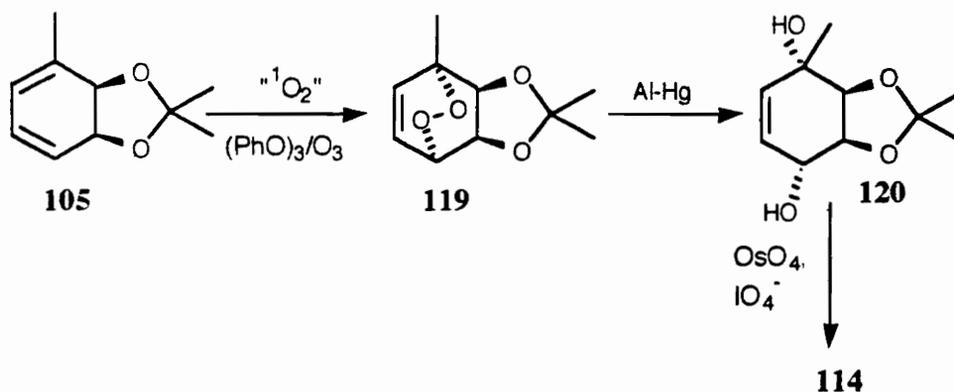
Scheme 35. Singlet Oxygen Approach to the bis-Hemiacetal **114**.

remained at the origin on TLC. After this work was reported,[56] a futher serious studies of the ozonolysis of dienes were published,[429] corroborating our early observations.

In order to prepare the desired keto aldehyde **104**, and at the same time prove or disprove that the bis-hemiacetal **114** was a product of rearrangement in the ozonolysis, another approach was tried (Scheme 35). The addition of singlet oxygen to introduce the two oxygenated groups on the diene by irradiation in presence of rose bengal[156] was not successful. Chemically generated "$^1O_2$" produced the corresponding endoperoxide (**119**) in good yield. This latter compound proved to be a stable crystalline solid whose stereochemistry was assumed to be as shown based on the face selectivity governed by the bulkiness of the acetonide. Reduction of the endoperoxide with aluminum amalgam[430] gave the diol **120** which upon in situ hydroxylation and glycol cleavage produced the bis-hemiacetal (**114**) in an overall yield comparable with that of the ozonolysis approach.

A last attempt at ozonolysis in methanol, resulted in the production of the methoxy derivatives of the bis-hemiacetal **114**. The resulting mixture of diastereoisomers once again complicated its complete characterization.

Other attempts to oxidatively cleave of the ring are shown in Scheme 36. Epoxidation of **109** produced a mixture of both possible epoxides (**121**), resulting from the reaction of the more electron rich double bond; all the attempts to epoxidize the second double bond were unsuccessful. The idea behind this approach was to generate the diepoxide **122** which would lead to the corresponding tetrol by hydrolytic opening. Glycol cleavage of the tetrol would lead to the keto aldehyde (**113**).
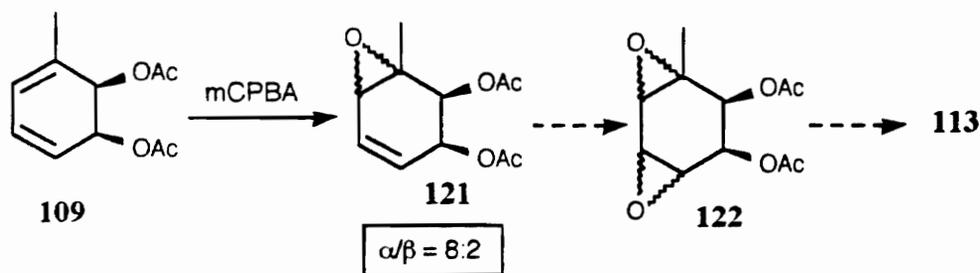
Scheme 36. Epoxidation Approach to Ketoaldehyde **113**.

At this point our synthetic strategy had to be modified to pursue the preparation of enone **103** from **114**. In addition, the availability of the keto aldehyde **113** led to attempts at the aldol cyclization of this compound. The reaction conditions used for the aldol cyclization of **113** are shown in the following table:

1. KOH, MeOH, rt, 2 h [431]
2. -LDA (1 eq.); -78 °C→15 °C
3. -Basic alumina (column) 11 h, rt followed by elution
4. -K$_2$CO$_3$, MeOH, rt, 3 h.[431]
5. -NaOH, MeOH

From all these reactions cyclopentenone **103** could not be isolated. The only recognizable product was the compound resulting from hydrolysis of both acetyl groups.

After a clean and homogeneous sample of hemiacetal **114** was prepared, it was adsorbed on neutral alumina,[432] and the mixture was heated at 100 °C followed by vacuum distillation (0.25 Torr.). The result was the recovery of the starting material. Later the same procedure was repeated at 150 °C with the same result. Finally, by refluxing the hemiacetal **114** with basic alumina in benzene, the enone **103** was isolated in a 3.6% yield. With these promising results, the reaction was monitored by ^{1}H-NMR spectroscopy, showing that after 20 min the reaction mixture was composed of keto aldehyde **104** and enone **103** in a 2:1 ratio. After 50 min of reaction time, the ratio changed to 1:2 and, after 1.5 h, the reaction mixture showed only the enone **103**. It was found that a ratio of alumina to 114 greater than 5:1 was necessary to promote the reaction to completion.

Later, in a series of experiments using different solvents, it was found that the best results were obtained in refluxing DME. These reaction conditions, reported in the experimental part gave reproducible results,[415] even when the opposite has been manifested[433]; this could be due to the critical role that the catalyst has in this transformation. Since then, other syntheses of enone **103** have been reported.[434] As noted before, enone **103** had been used in the synthesis of PGE$_{2\alpha}$.[425] The successful synthesis of enone **103** therefore allows us to claim the formal synthesis of PGE$_{2\alpha}$ from toluene. This was the first report of the use of *cis*-arenediols as intermediates for the enantiocontrolled synthesis of natural products.
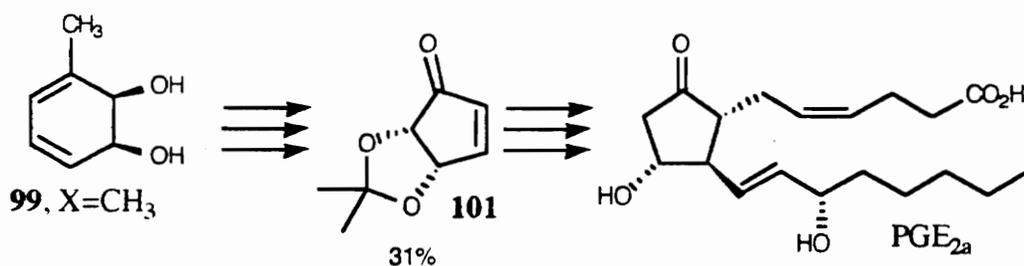
For the synthesis of enone **103,** of the diene functionality the six-membered ring was cleaved keeping the diol unchanged. In another approach, the transference of the stereochemistry of the diol was undertaken, it will be discussed latter in this section.

The preparation of the terpene synthons **126** and **127** started with the catalytic hydrogenation of *cis*--toluene diol as described by Ziffer.[70] chromatographic separation
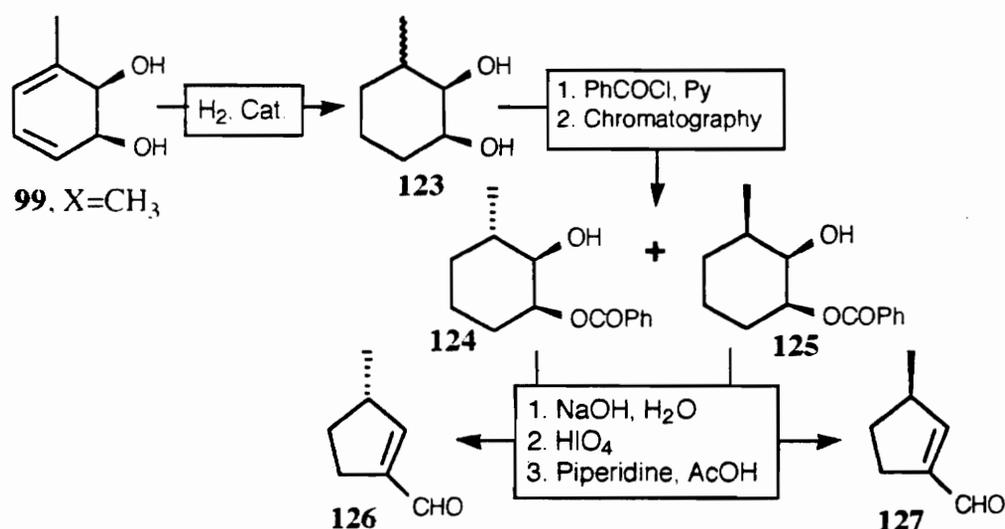
Scheme 37. Terpene Synthesis.

of the 3-monobenzoates of the epimeric methylcyclohexanediols **123** 3-monobenzoates provided both epimers in good purity.

In order to complete the syntheses of **126** and **127** (Scheme 36), **124** and **125** were subjected separately to the following reaction sequence: a) hydrolysis of benzoate (NaOH, MeOH-H_2O); b) cleavage of glycol functionality (HIO$_4$, THF); and, c) aldol cyclization (piperidine, AcOH). Both compounds showed the same physical and

spectroscopic properties, but differed in their optical rotations, the value of which demonstrated the enantiomeric relation of **126** and **127**.[415]

During the completion of the preparation of enone **103** and terpene synthons **126** and **127**, two other arene diols were prepared in our laboratories, namely *cis*-styrene diol and *cis*-chlorobenzene diol. The former was transformed to zeylena,[302] and the latter was planned to be used in the synthesis of monosaccharides as described in the next section.

III.4. Ozonolysis of Chlorobenzene diol; Synthesis of L-Erythrose.

Sugars have been widely used as chiral templates for the synthesis of natural products and their non-naturally occurring analogs.[5] Sugars by themselves are an important group of natural products with medical and research relevance. Consequently a synthetic methodology to prepare sugars, especially those which are present in small amounts in nature, could be important. Through biocatalysis a plan was implemented for a general methodology for the synthesis of sugars, tetroses, pentoses, and hexoses, based on the microbial oxidation of monocyclic arenes.

The general plan envisioned for the synthesis of sugars is shown in Fig 21. In general, the use of selected oxidative methods, combined with the stereocontrolled functionalization of the olefins by the assistance of the glycol group could lead to completion of this approach.
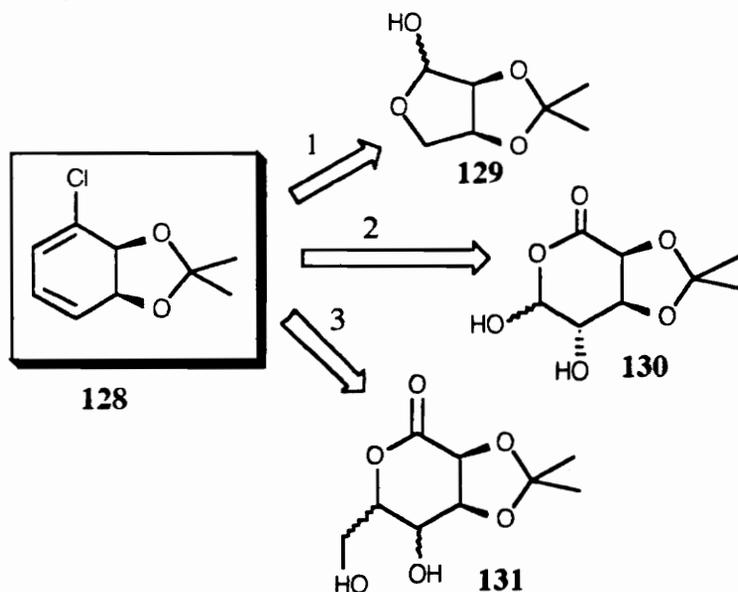
Figure 21. General Synthetic Plan for Sugars.

In a few words, the exhaustive oxidation of the diene functionality by ozonolysis could lead to the loss of two carbon atoms and consequently to the tetrose skeleton (route 1, Fig. 21). For the pentose skeleton (route 2, Fig. 21), the addition of singlet oxygen to the diene function followed by oxidative cleavage of the enone group, with loss of one carbon atom, was the strategy to follow. Finally hexoses can be prepared via different hydroxylation methods followed by the cleavage of the ring at a predeterminated C-C bond. The selection and order of those hydroxylations and the place for the cleavage of the ring will determine the stereochemistry of the product and consequently the type of sugar produced (route 3, Fig. 21).

The first target in this project was L-erythrose The starting material for this synthesis, cis-chlorobenzene diol (99, X=Cl), was prepared in similar fashion to the preparation of cis-toluene diol. This compound was previously mentioned in the Gibson work.[64] but never was characterized until reported as a result of this work.[415]
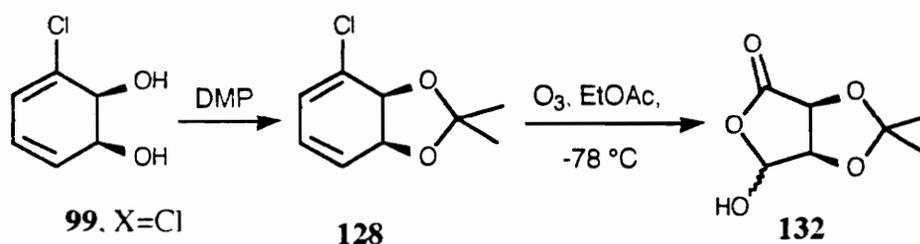
Scheme 38. L-erythruronolactone Synthesis.

The reaction conditions already used in the ozonolysis of cis-toluene diol acetonide were adapted for ozonolysis of 128. The ozonolysis of 128 followed by reductive work-up (DMS) produced a mixture of compounds that were identified as the epimers in C-4 of L-erythruronolactone (132, Scheme 38).[435] The ratio of these epimers varied from 7:3 to 8:2, as determined by ^{1}H NMR spectroscopy.

The structural characteristics of 132 make this compound extremely versatile because it can be considered as a chiral equivalent of meso-tartaric acid (Fig. 22), where the carbonyl group in C-1 in 133 corresponds to the carbonyl C-1 of the lactone and the carbonyl in C-4 of 133 is correspondent to the hemiacetal function at C-4 in 132. Both carboxyl groups of 133 are differentiated in 132 by a different oxidation state, and an

additional advantage found in **132** is that both terminal group can be considered as

L-Erythruronolactone, **132**   meso-Tartaric ac., **133**
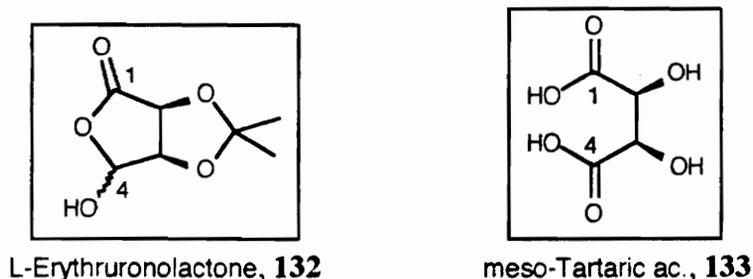
Figure 22.

mutually protected. This differentiation at the terminus of **132** makes it possible to use this compound in enantiodivergent syntheses just by selecting the order of subsequent synthetic operations.[436]

For the ozonolysis of **132**, the reaction conditions reported by Griesbaum for ozonolysis of vinyl chloride were tried.[437] It was performed in methanolic solution at -78 °C followed by stirring at room temperature for 48 h. This allowed the thermal decomposition of the peroxidic compound produced during the ozonolysis. After a troublesome isolation, two compounds were separated. ^{1}H NMR spectroscopic data
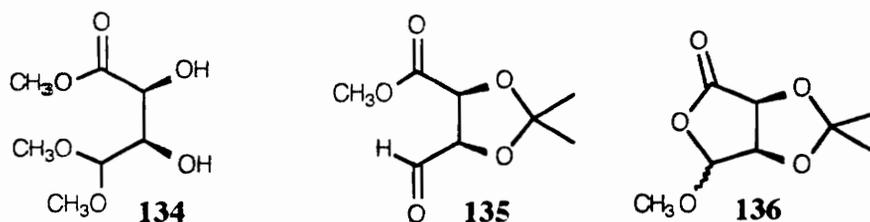
corresponded to structure **134** [R_f= 0.42 (silica gel; Et$_2$O-hexane-MeOH, 41:8:1)].[438] and **135** (R_f=0.76, same system).[439] It can be seen that these two compound present characteristics similar to those of **132** with respect to their potential synthetic utility. The acetonide protection was lost in **134** possibly because of HCl generated during the ozonolysis reaction. Further optimization of the preparation of both compounds was not pursued.

The change in the reductive work up of the ozonolysis reaction (CH$_2$Cl$_2$-MeOH-78 °C) to dimethylsulfide instead of NaBH$_4$[440] led directly to L-erythrose acetonide (**129**). L-erythrose derivative (**129**) could be later produced by the reduction of the aldehydic site

90

first followed by in situ reduction of the resulting lactone. Traces of the methoxylated L-erythrose were observed. The same result was obtained by Price, following a sequential reduction of **132** with NaBH$_4$ and DIBAL.[435] In the same report the successful synthesis of D-erythrose from **132** was achieved, demonstrating the enantiodivergent characteristics of L-erythruronolactone (**132**).[435]

To avoid the hydrolysis of the acetonide during ozonolysis, NaHCO$_3$ was added and methanol was the only solvent used. As expected no hydrolysis of the acetonide was observed, and the product of the reaction consisted in a mixture of two compounds, **135** and **136,** in an approximate ratio of 7:3.[441] No further optimization or repetition of this experiment was performed.

For the synthesis of the pentose skeleton the synthetic plan involved the preparation of the γ-hydroxyenone **137** from the acetonide of *cis*-chlorobenzene diol. The oxidative cleavage of the double bond followed by the loss of C-6 could lead to the pentose derivative **129**. The first transformation (**127→137**) was addressed by the
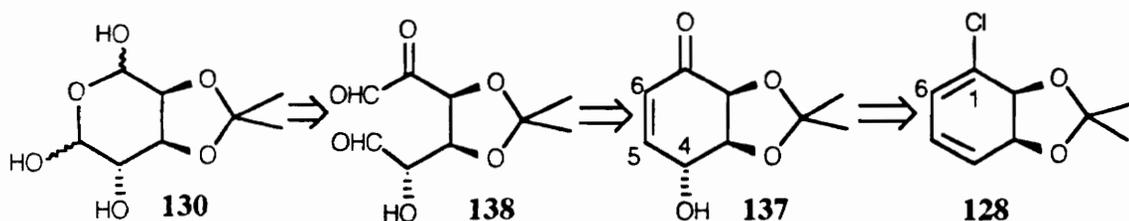
Scheme 39. Retrosynthetic Analysis of Pentose Skeleton.

addition of singlet oxygen to the dienic function. This reaction was first attempted with chemically generated $^1O_2$,[442,415] leading to the recovery of the unreacted **127**. The same result was observed even when the reaction was performed at higher temperatures. The
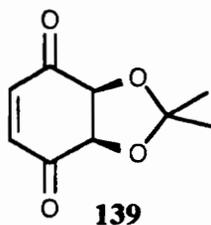
addition was successfully attained when the generation of $^1O_2$ was performed photochemically (tetraphenylporphine used as sensitizer)[443] with **137** as practically the only product. Traces of several by-products were obtained, among which **139** could be

identified (^{1}H NMR spectra showed agreement with proposed structure). The endoperoxide **140** was reduced with thiourea[156,444] to provide the γ-hydroxyenone **137**. The structure of the product indicated that an unstable *gem*-chlorohydrin could be an intermediate during this transformation. This compound has been previously prepared in a racemic form .[295] In order to continue with the synthetic plan, the hydroxyl group of the enone **137** was protected as the TBS-ether.[427] This protecting group was selected because it was planned to be used as a stereocontrol element in the next reaction.
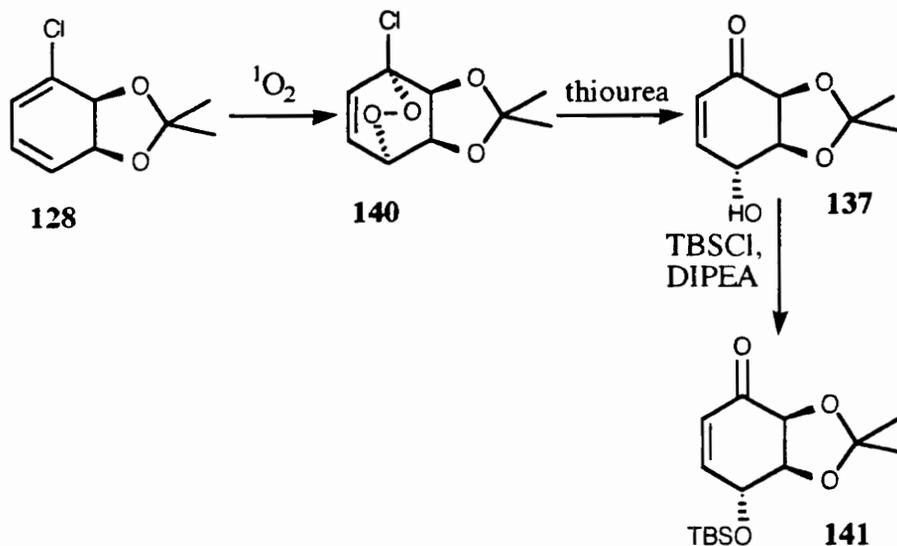
Initial work on the oxidative ring-cleavage was attempted. As a result of the ozonolysis of **141**, a mixture of at least two compounds was formed, and the mixture could not be separated by usual chromatographic methods; therefore, identification of the product(s) of the reaction was not possible. The preparation of **137** centered on the ability of the acetonide group to controlling the stereochemistry of the functionalization adjacent to it. That fact could be exploited in synthesis of cyclitols, specifically conduritols, which will be described in the next section.
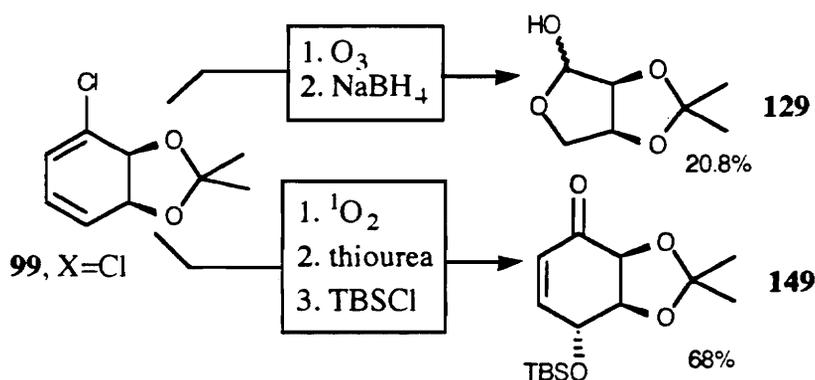
Figure 23. Overall Results in Sugars Synthesis

III. 5.   Synthesis of cyclitols.

Cyclitols are a group of naturally occurring polyhydroxylated cyclic compounds derived from cyclohexane.[446] This class of compound is widely spread in nature and has some interesting biological activities. Conduritols which belong to a subclass of cyclitols,
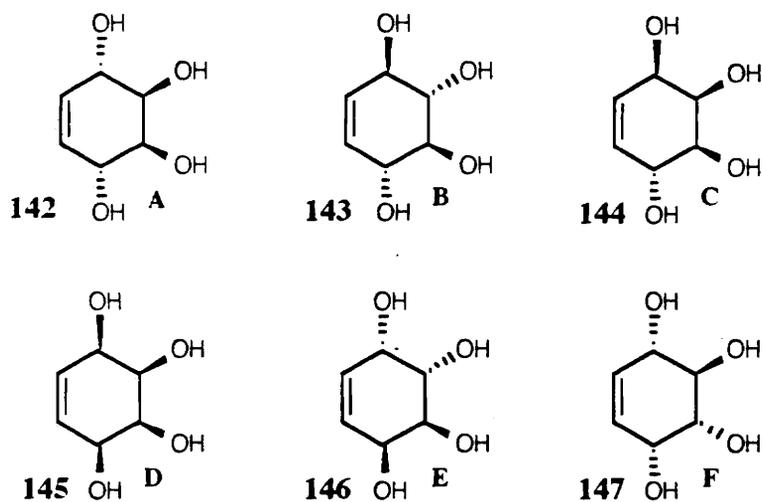
Figure 24. Structures of Some Representative Conduritols.

are 1,2,3,4-cyclohexenetetrol isomers with interesting potential glycosidase inhibitor activity.[447] Some representative structures are shown in Fig. 24. This group of compounds is important because its members are intermediates in the synthesis of inositols

and inositol phosphates. Conduritol A[448] and conduritol F[449] are found in nature; others are of synthetic origin.

According to the synthetic scheme described in Section III.2, *cis*-chlorobenzenediol (**99**, X=Cl) would provide the intermediates for the stereocontrolled synthesis of conduritols and some of their derivatives. It was reasoned that cyclitols could be prepared from the intermediates used in sugar synthesis. An example of stereocontrolled manipulation of *cis*-arenediols to a hexa-substituted cyclohexane can be found in the report of the enantiodivergent synthesis of both enantiomers of pinitol.[450] The γ-hydroxy enone was therefore thought to be a suitable intermediate for the stereocontrolled synthesis of conduritols.

Previously, the endoperoxide **140** was reduced with thiourea in order to prepare the γ-hydroxy enone **137**. Reduction of **140** with Al-Hg, the reagent that had been used for the reduction of the endoperoxide of *cis*-toluene diol, yield **148**, in which the extrusion of the chlorine atom occurred before the reductive cleavage of the peroxidic linkage as shown in scheme 40. The structure of product of this reaction (**148**) corresponds to that of the acetonide of conduritol A.[451] This successful result constitutes the shortest synthesis of this meso compound.[416]
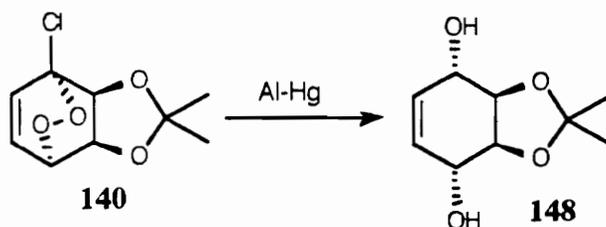
Scheme 40.

It was observed that reduction of the carbonyl group in the enone **141** could lead to conduritols. Consequently the enone **141** was subject to the action of $NaBH_4$,[417] which

produced the non-regio- and non-stereospecific reduction of the enone system. The
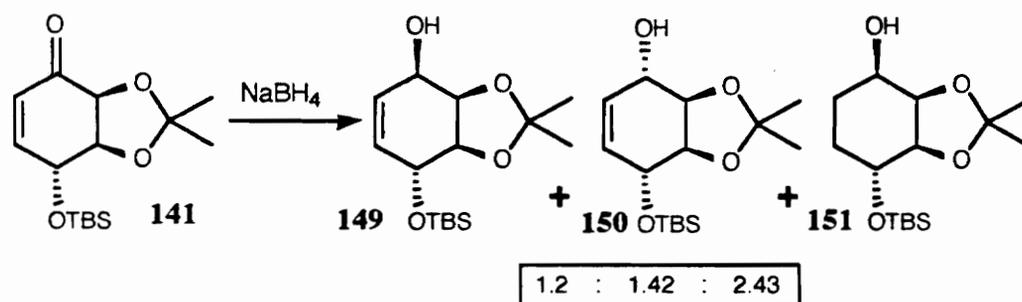
Scheme 41.

alcohols **149** and **150** resulting from the 1,2-hydride addition, and the greater proportion of **151** indicates that some stereocontrol has been exerted by the acetonide group. On the other hand, the major product came from the initial 1,4-addition of hydride followed by a 1,2-reduction from the less hindered face, demonstrating once more the stereocontrol ability of the acetonide.

These results encouraged the exploration of different 1,2-reducing agents of different steric parameters in order to achieve control in the stereochemistry of the products. The results are shown in Table 7.

Table 7[453,417]

Reducing Agent	**149:150** ratio
NaBH$_4$, CeCl$_3$	1:1
9-BBN	1:1
L-Selectride	>99:traces

From Table 7 can be observed that the highest stereoselective reduction was accomplished with L-Selectride,[453] producing **149** in 52% yield (unoptimized). Hydrolysis of the protecting group in **149**, led to conduritol C (**144**), whose properties matched those reported in the literature.[454] Similar hydrolytic treatment of **150** and **151** could lead to conduritol A and dihydroconduritol C,[455] respectively.

Amino conduritols are another important class of compounds[456] accessible through intermediates derived from *cis*-chlorobenzenediol. Hence, the 1,4-addition or Michael

addition of a nitrogen-nucleophile to the protected enone **141** could lead to a compound
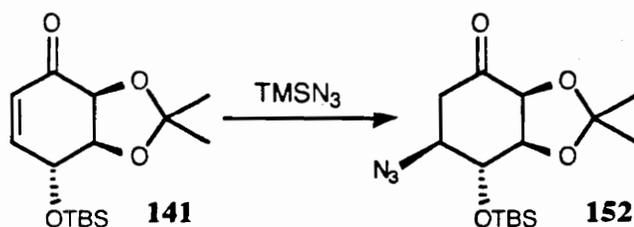
Scheme 42.

that would serve in the synthesis of amino conduritols and possibly other amino cyclitols. Enone **141** reacted with TMSN$_3$ producing the azido ketone **152** as the only isomer, as demonstrated by the analysis of its ^{1}H-NMR spectra,. The details of the preparation of this compound will be discussed in a later section. The reductive transformation of the
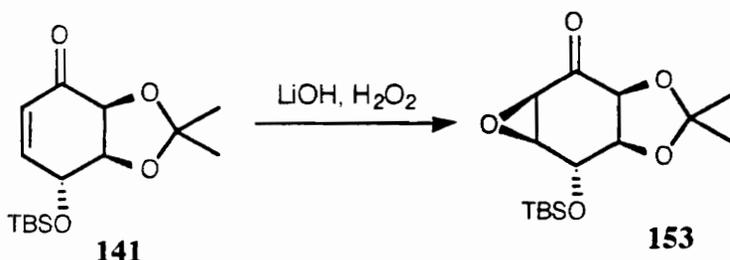
carbonyl group to a methylene group would produce the basic structure of dihydroconduramine F-4.[457] Also, transformation of the carbonyl to a C-C double bond would lead to a conduramine F-4 derivative.

Among conduritol derivatives showing biological activities are the conduritol-epoxides. Epoxidation of enone **141** produced a single compound whose structure was in agreement with that of **153**. This epoxide **153** could also serve as an intermediate for the preparation of cyclitols.

A different approach to this type of compound came from the opening of the epoxide (**154**) with a nitrogen nucleophile. In the first approach the azide ion was used as nucleophile, resulting in the regioisomer **155**, Scheme 42. This result was rather surprising. because in the opening of epoxide (**154**) with other nucleophiles (MeOH or MeO⁻) performed previously in the context of the synthesis of pinitol,[450] the nucleophilic opening took place at the allylic position. The reaction conditions were varied initially in

order to achieve regiocontrol in the azide ring-opening of the epoxide **154**. The results are presented in Table 8.

Assuming that the mechanism of the reaction with the azide ion could range from a complete S_N1 type to a complete S_N2, the reaction conditions were selected in order to
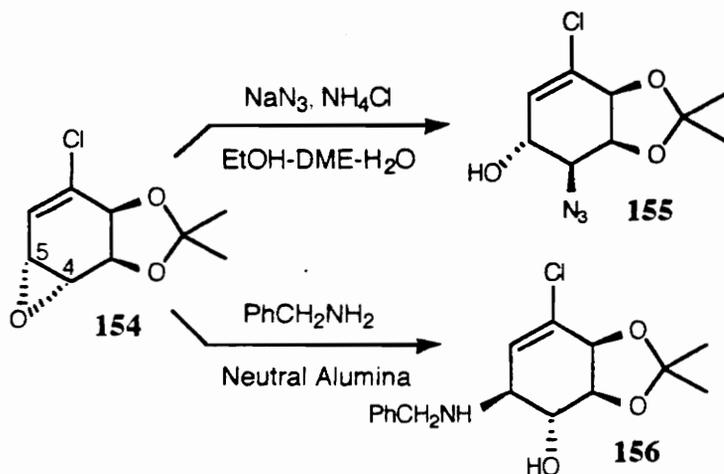
Scheme 43.

resemble more closely the S_N1 type, thereby maximizing the possibility of the formation of an allylic cation or its predecessor in the transition state. This would lead to the desired regiochemistry at C-5, regardless of the stereochemical consequences (Scheme 43).
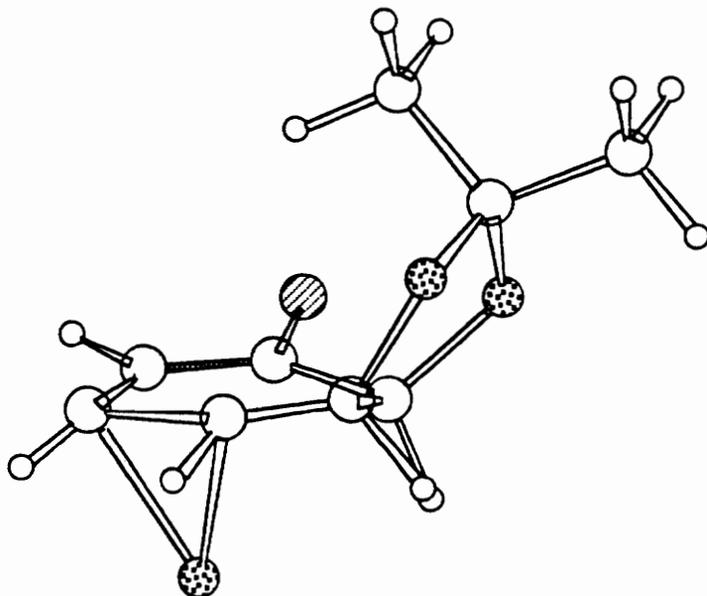
Conformation <u>B</u>.
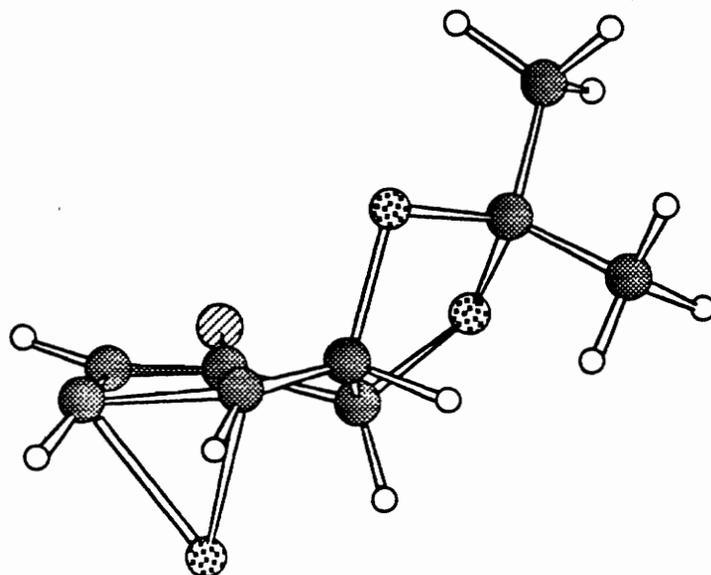
Conformation <u>A</u>.
Figure 25.

Entry 1 in Table 8 shows the conditions initially attempted for the expected synthesis of **154**, which led to the regiochemistry found in **153**, in complete agreement with the Fürst-Plattner rule,[270] and also reinforced the conformation <u>A</u> (Fig 25) as the likely entity in the context of recent work concerning the control of the regiochemistry of the oxirane opening by chelating processes.[458]

Table 8. Opening of Epoxide **154** with azide.[416]
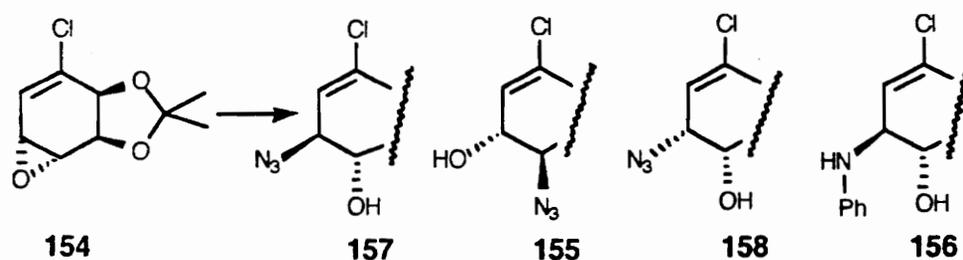
Entry	Reaction conditions	Product (ratio)	Reference
1	NaN$_3$, NH$_4$Cl, EtOH-DME-H$_2$O	**155**	
2	TMSN$_3$, DMF, rt, 9 h	**154**	459
3	TMSN$_3$, DMF, 90 °, 20 h	**155**	459
4	NaN$_3$, 18-C-6, rt, 12 h	**154**	460
5	NaN$_3$, 18-C-6, , H$_2$O, rt, 12 h	**155**	460
6	TMSN$_3$, MeOH, DMF, rt	**154**	459
7	TMSN$_3$, MeOH, DMF,	**154/155** (70/30)	459
8	NaN$_3$, dioxane-H$_2$O, 100 °, 18 h	**154/155** (33/77)	461
9	TMSN$_3$, Ti(OPr^i)$_4$(cat.), THF,rt, 20 h	**154/155** (80/20)	462
10	TMSN$_3$, Ti(OPr^i)$_4$, THF, rt, 20 h	2 unseparable products	462
11	TMSN$_3$, neutral alumina, CHCl$_3$,, rt, 18 h	**155**	450
12	HN$_3$, neutral alumina, CHCl$_3$, rt, 18 h	**155**	463
13	HN$_3$, silicic acid (pH=4), rt, 22 h	**155**+ aromatics	463
14	a) HN$_3$, silicic acid b) **33**, rt, 26 h		464
15	NaN$_3$, CuI, DMSO, rt, 24 h	mixture (**155**)	465
16	a) FeCl$_3$, Et$_2$O, rt, 2 h b) NaN$_3$, DMF, 70 °, 4 h	**158**	466
17	PhCH$_2$NH$_2$, neutral alumina, CHCl$_3$, 6 h	**156**	450

The use of Lewis acids as catalysts for the regiocontrolled opening of oxiranes, especially those derived from allylic alcohols, is well documented,[467] sometimes even leading to the protected alcohols.[462] The use of $Ti(OPr^i)_4$ was selected in combination with $TMSN_3$ in an attempt to produce **155** as its silyl ether. But when a catalytic amount of $Ti(OPr^i)_4$ was used (entry 9), azide **155** was again the main product. When 1.5 eq. of $Ti(OPr^i)_4$ was used (entry 10), the products could not be separated and identified. We also thought that acid conditions could lead to a stable allylic carbocation and attempted the use of NH_3 generated in situ by the reaction of $TMSN_3$ and methanol (entries 6 and 7); this again produced **155**.

During the pursuit of the synthesis of pinitols, the epoxide **153** was opened with methanol with neutral alumina as a catalyst. Following a report in the literature[463] the reaction of HN_3/alumina (entry 12) was attempted but the product was again **155**. Even when the nucleophile was $TMSN_3$ (entry 11) in $CHCl_3$, again closely resembling the conditions used in the pinitol synthesis, the result was the same. With more acidic surface catalysts such as silicic acid (pH=4) in conjunction with HN_3, the product was the *trans-*isomer of diol **159**, which was identified by comparison with a known sample.[464] Regardless of the condition used, the regioisomer **155** was obtained; the failure in the $Ti(OPr^i)_4$ reaction could be due to the rigidity of the ring system,which did not allow the complexation of the titanium atom with the "allylic" alcohol and the oxygen of the epoxide to take place.

Table 8 shows that the only nucleophile that does not react at C-5 is the azide ion. A rational explanation of this observation could be the possible complexation of the azide ion with the acetonide oxygens, resulting in the delivery of the azido ion to C-4 from the endo surface of the molecule. Azide is also the only nucleophile examined that possesses a formal charge. A reasonable explanation of the mechanism of the epoxide opening therefore is that it involves an S_N1-like process. This is reinforced by observation of traces of the *cis*-diol detected in the opening of the epoxide **153** under acidic conditions, although the *trans* -diol was formed almost exclusively.

MM2 calculations (PC model for Macintosh) show a small difference in charge at both carbons of the oxirane ring, lending evidence to the idea of a non-symmetrical oxirane ring, and therefore a highly polarized transition state resembling an allylic cation or possibly a tight ion pair, Fig 26. The small differences shown in Fig. 26 may be increased by polar interactions with the solvent. This possibility is reinforced by observing the opening of the epoxide by an attack at the allylic position.

Charge difference   C-O Bond length
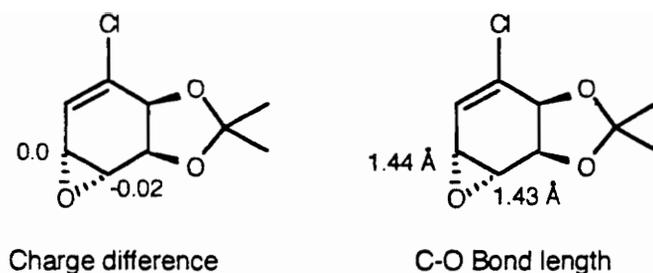
Figure 26. Monoepoxide of *cis*-Chlorobenzendiol

Finally the regio- and stereochemistry of **158** were achieved in two reactions, Scheme 43. When epoxide **152** was reacted with FeCl$_3$ [468] and the intermediate chlorohydrin further reacted with NaN$_3$[381] (entry 16), **158** was produced in 43% overall yield. On the other hand, the reaction with the primary amine, benzylamine with alumina catalysis, produced **154**, possessing the regiochemistry observed in case of methanol or hydroxide under similar reaction conditions.[450]
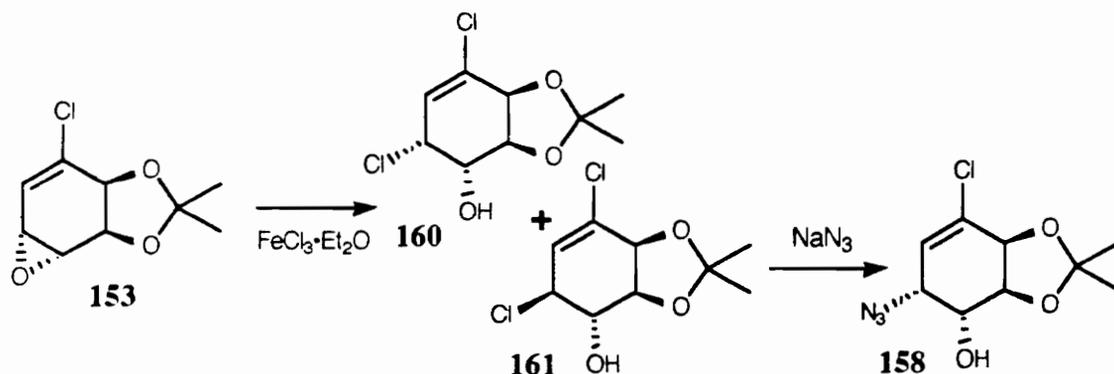
Scheme 44.

The chlorohydrins **160** and **161** can serve as precursors of haloconduritols or other conduritols (i.e., E and F), just by selecting the appropriate transformations. In all the cases leading to amino conduritols, the reductive extrusion of the chlorine atom at the vinyl position has to be included. The reduction of vinylic chlorides is easy, consequently the use of the bromo equivalent of epoxide **153**, prepared from *cis*--bromobenzenediol would be more appropriate as the reductive extrusion of the bromine atom would be easier.

As envisioned in Section III.2, *cis*--chlorobenzenediol could be synthetically manipulated to produce intermediates that could be transformed into conduritols in an

enantiocontrolled fashion. Some of the derivatives prepared (azido-, amino- and halosubtituted) would serve for the synthesis of amino- and haloconduritols and hence amino- and halocyclitols. One of the main consequences of this work was a better understanding of the parameters involved in the opening of oxiranes of type **153**. In addition, a better insight into the methodologies of generation of functional groups in *cis*--arene diols was attained, which can be applied for further synthetic purposes. An example of the application of this knowledge will be presented in the next section, where the synthetic approach to kifunensine is addressed.
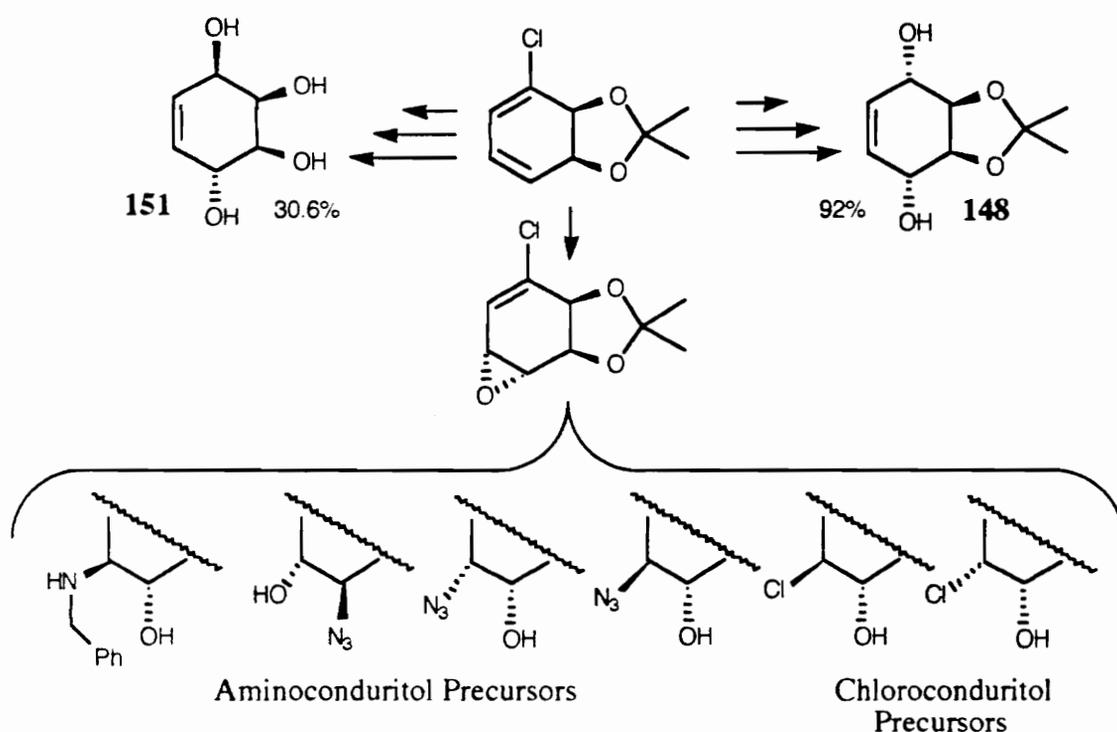
Figure 27. Overall Results on Conduritols Synthesis.

III. 6. Approach to the Total Synthesis of Kifunensine.

The total synthesis of kifunensine[406-408] was approached in two stages: first a model study leading to 5-epi-kifunensine was explored, then the synthesis of kifunensine was addressed. This work was based on the experience obtained during the development

of the results described in section III.5. From the synthesis of cyclitols, it was proven that
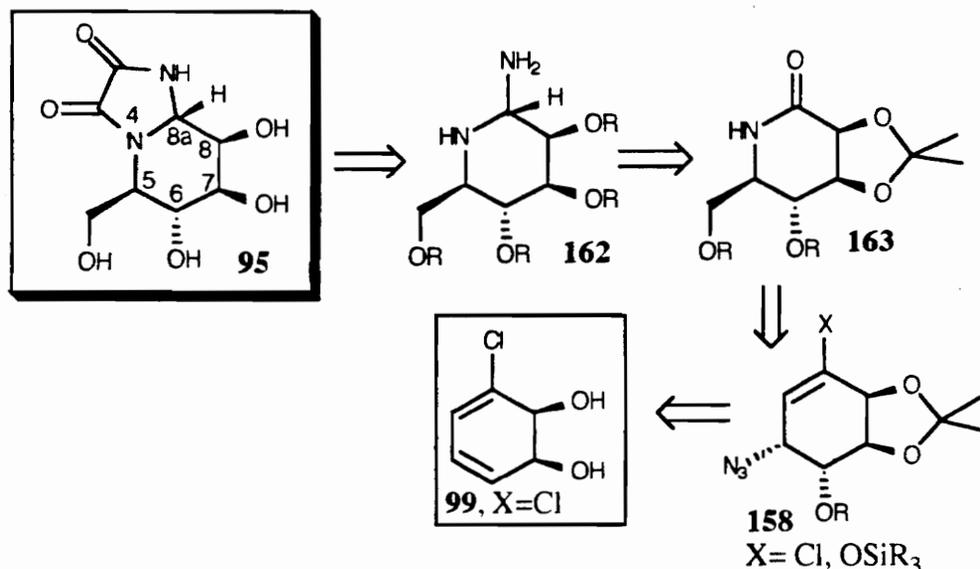
Figure 28. Retrosynthetic Analysis for Kifunensine.

it was feasible to introduce sequentially a variety of functional groups around the cyclohexane ring in an stereocontrolled way. That that methodology could be applied to the preparation of the lactam **163**. The synthetic strategy was based on the retrosynthetic analysis shown in Fig 28.

It was proposed that kifunensine **95** could be accessed from t aminal **162** by a cyclization reaction with some oxalic acid derivative. The aminal **162** could be prepared from the lactam **163** (mannonolactam protected), itself a natural product.[469] The mannojirimycine derivative (**163**) was thought to arise from the cis-azido hydrin **158**, to be prepared from cis-chlorobenzenediol (**99**, x=Cl). From this retrosynthetic analysis it is obvious that cis-chlorobenzene diol (**99**, =Cl) was selected because it possesses the cis-- diol functionality with the same absolute stereochemistry of the cis-diol function present in kifunensine (**95**). The vinyl substituent (X) in **158** should be a group such that, during the cleavage of the double bond, could provide a carboxylic acid derivative as the precursor of the lactam carbonyl.
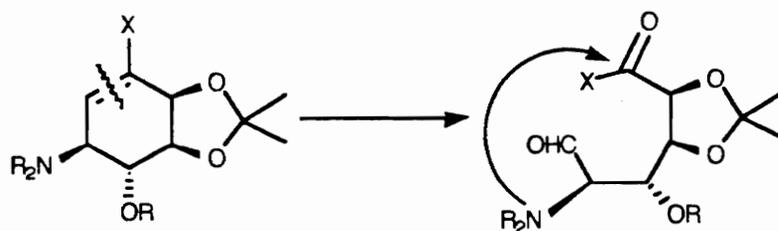
Scheme 45. General Strategy for Lactam (163) Preparation.

At the onset of the synthesis of kifunensine, the thought of using one of the intermediates already available from the other projects, namely **141**, was addressed. The introduction of the nitrogen functionality should be the first transformation to be considered. The introduction of a nitrogen functionality to enone **141** could provide a good model to study the behavior of this kind of molecule toward the synthetic operations planned for the synthesis of **95**, even though this would lead to an epimer of **157** (5-epi-kifunensine,**164**). The utility of **165** in design of amino conduritols or amino hexoses was also considered.

The logical means of introduction of nitrogen functionality to the proper position in **141** was the Michael reaction. Azide ion was selected as the nucleophile because it would present less interference in the further reactions (ozonolysis) in the synthetic plan. It was reported in the literature that under a variety of reaction conditions, the Michael addition of TMSN$_3$ to acyclic α,β-unsaturated carbonyl compounds leads to β-azido enol ethers.[470]
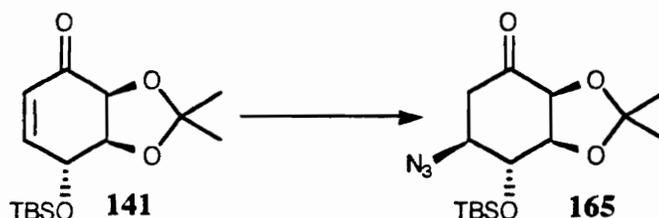
Scheme 46.

Table 9. Reaction Conditions Used for the Transformation of **141** to **165**.

TMSN$_3$, TMSOTf, Et$_2$O, rt, 43 h	Ref. 472
TMSN$_3$, Et$_2$O, rt, 50 h	Ref. 473
TMSN$_3$, NaN$_3$, 18-C-6, Et$_2$O-THF	Ref. 474

The reaction was performed according to those reports,[471] resulting in the isolation of **165** as the only product, regardless of the reaction conditions (catalyst, exclusion of water, temperature) and reagents used (Scheme 46).[470]

One explanation of this results was the possibility of internal quenching of the resulting enolate ion by contaminating moisture. In order to evaluate this possibility, a ^{1}H-NMR monitoring experiment was performed; this showed that the reaction proceeded by an equilibrium process, because it took 288 h for the reaction to go to completion. On the other hand, by TLC analysis, the reaction mixture always showed starting material left (**141**) even though this could not be correlated with GC and ^{1}H NMR analysis. When 2-cyclohexenone was subjected to the same reaction conditions, in order to clarify the results in this transformation, only starting material was recovered

Cleavage of the ring could also be accomplished by a Baeyer-Villiger oxidation, which could lead to **166**. The structural features of this compound were
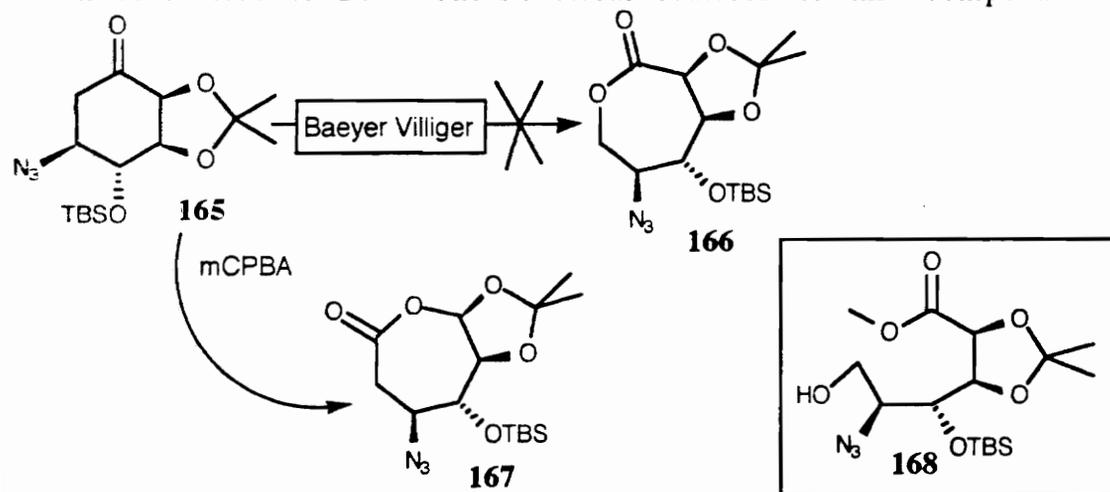
Scheme 47.

equivalent to the product that we expected from the ozonolysis and further reduction of **58**, namely **168** (Scheme 46). The reaction of **165** with MCPBA[475] produced the product

(**167**, Scheme 48) of oxygen insertion with the regiochemistry opposite to that expected according to Köll,[475] compound **167**, Scheme 46. The assignment of the structure was based on observation of a doublet at 5.8 ppm in the ^{1}H NMR spectrum, corresponding to a hemiacetal proton. Compound **167** could, however, be exploited in the synthesis of amino sugars.
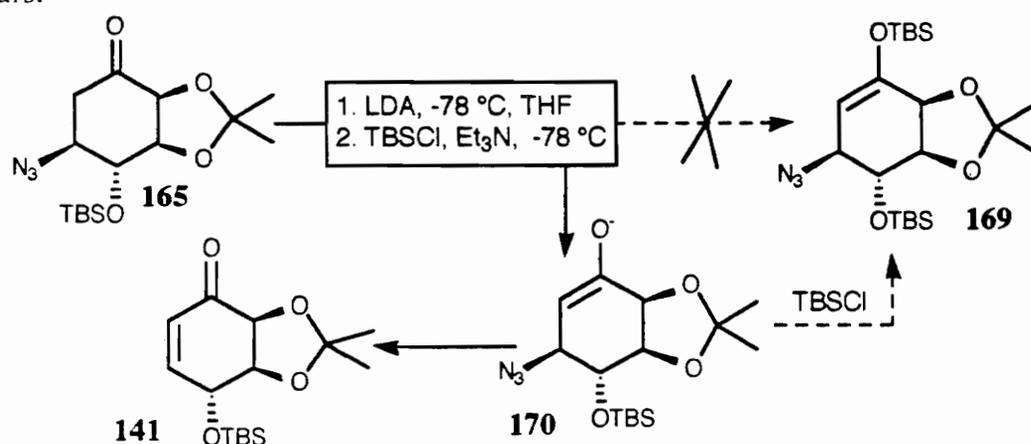
Scheme 48.

To prepare the silyl enol ether **169**, Scheme 48, the enolate ion **170** was prepared by the reaction of **165** with LDA at low temperature. Trapping the enolate with TBSCl was attempted, but the reaction resulted in the extrusion of the azide ion giving the starting enone **141**[476] as the only isolated product.

The previous results forced a change in strategy and dictated the use of intermediate **160** (Scheme 44) for the ring cleavage. The vinyl chloride **171**
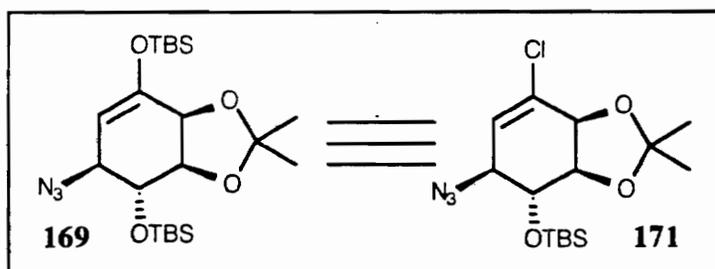
Figure 29. Equivalence of Synthons.

could also serve as a synthetic equivalent to the silyl enol ether **169**. The reaction sequence used for its preparation is shown in Scheme 49. The retrosynthetic analysis was based on
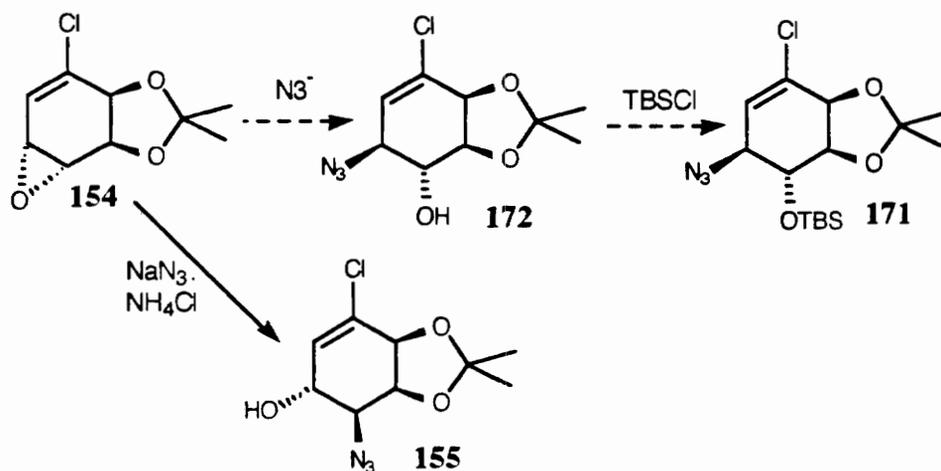
Scheme 49.

our previous experience in the opening of epoxide **154**. Unfortunately the regiochemistry of the nucleophilic opening of **154** took a different course, as already described previously (section lll.5). This regiochemistry would lead to azafuranose.

Because compound **155** could be prepared in an appreciable amount, this was used as a model to test the series of reactions planned for the transformation of **171** into 5-epi - kifunensine **164**. After protection of the hydroxyl group in **155** as TBS ether,[427] **155**
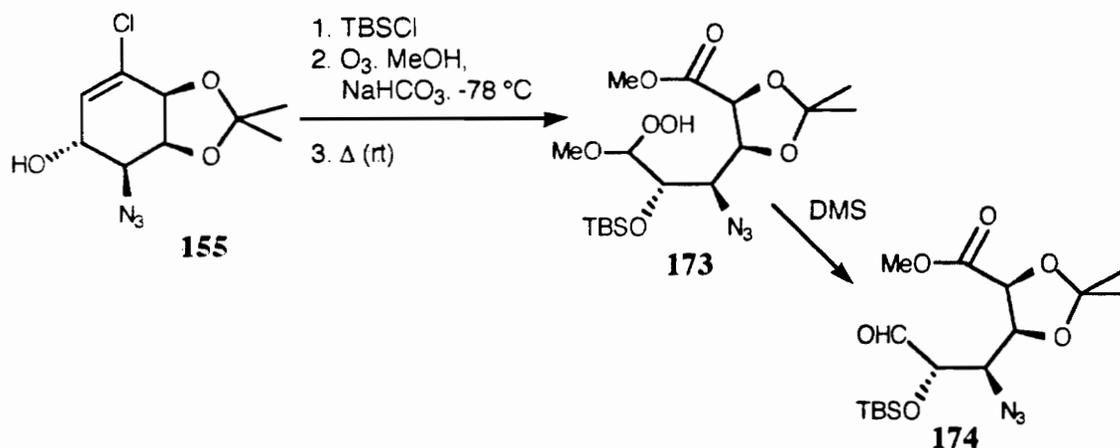
Scheme 50.

was subjected to ozonolysis under Griesbaum conditions[477] producing the α-metoxyhydroperoxide **173** in almost quantitative yield. Treatment of **173** with DMS led to the aldehyde ester **174**, which could be detected spectroscopically but could not be isolated due to its instability. The assignment of the structure as **173** was confirmed by the study of ozonolysis of 1-chlorocyclohexene[478] under the same reaction conditions followed by its transformation to known compounds. This study gave us information about the chemical behavior of the α-methoxy hydroperoxyl group. Application of those chemical reactions to **173** led to the confirmation of the functional group array in **173**.

The reduction of **173** with excess NaBH$_4$ (THF-MeOH)[479] (Scheme 50) produced a mixture, of the alcohol ester **175** and the diol **176** in a 23% and 40% yield respectively; it was obvious that overreduction took place. It was surprising that the azide group remained untouched because the reaction conditions used were reported for the selective reduction of azides. In a more careful experiment, where methanol was the limiting reagent, the alcohol ester **175** was produced in good yield (Scheme 51). The same transformation could also be performed by a sequential treatment of **173** with DMS and NaBH$_4$, but the yields were not reproducible.
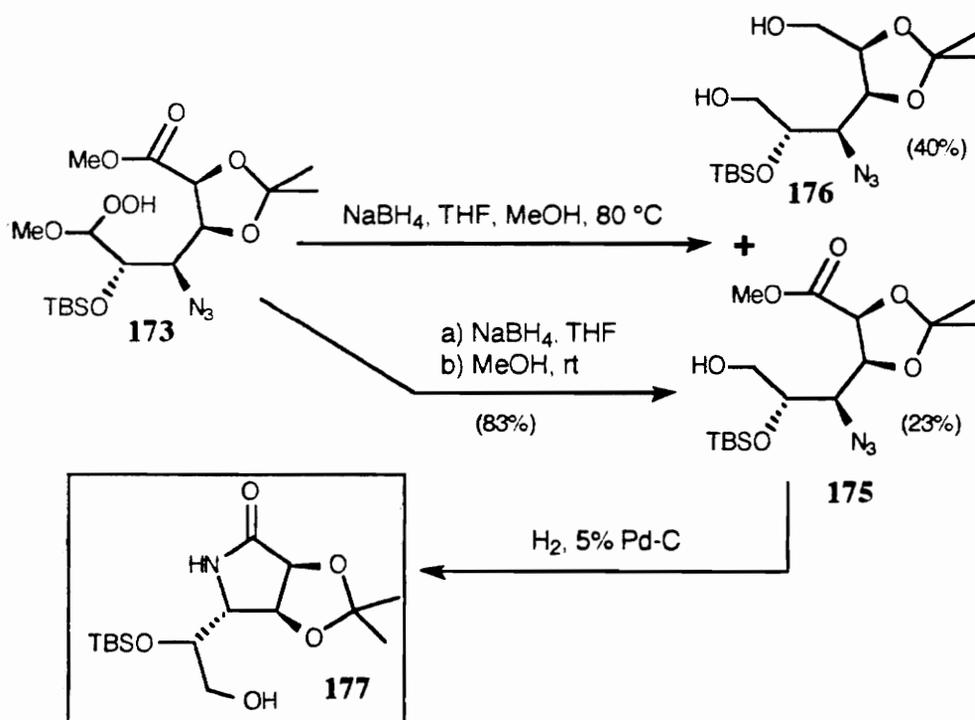
Scheme 51.

The catalytic hydrogenation of the alcohol ester **175** produced the five-membered lactam **177**, as reported by Fleet in similar systems.[476] Prior protection of the free hydroxyl group in **175** did not interfere with the cyclization that followed the catalytic hydrogenation.

The above discussion demonstrates the feasibility of the reactions involved in the synthetic plan. Synthetic work was then focused on the preparation of the intermediates required in the synthesis of kifunensine, which is shown in Scheme 52.

A cis relationship is required in the azido hydrin derivative **158**, so this compound became the first target. For the preparation of **158**, two routes were selected (Scheme 53). In route A the plan was to transform chlorobenzene diol acetonide (**128**) into the *cis*-diol **179**, then monoprotect selectively the hydroxyl group at C-4 and substitute the free alcohol in C-5 in such way as to lead to retention of the configuration to give the relative stereochemistry shown in **158**. In route B, the *trans*-diol (**180**), prepared from the opening of the epoxide **153**, would be again selectively protected, leaving the hydroxyl group at C-5 free for its substitution with inversion of configuration.
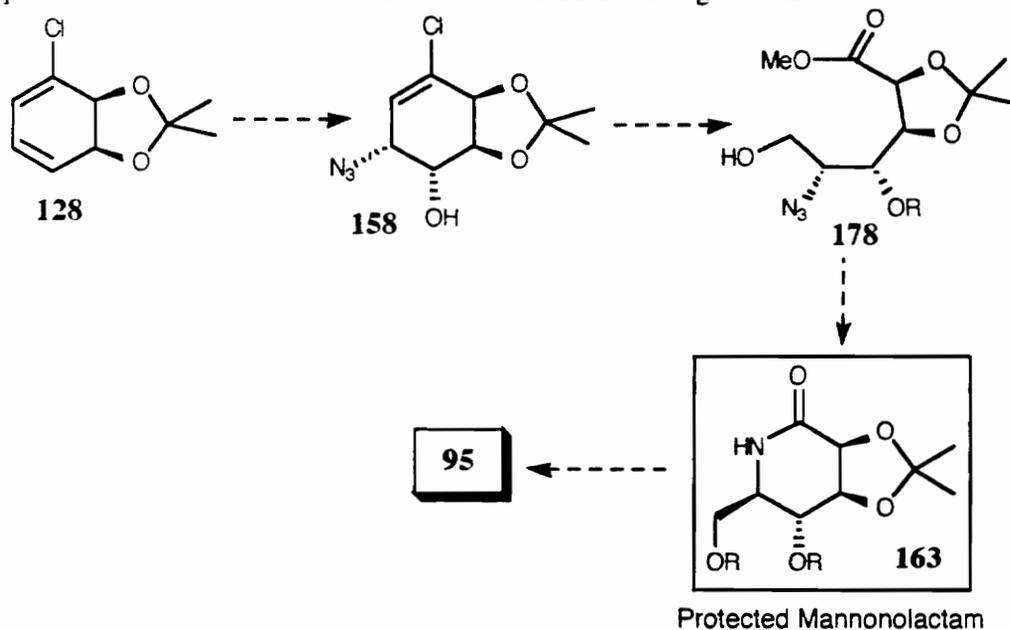
Scheme 52.

The *cis*-diol **179** was produced from the reaction of the acetonide of *cis*-chlorobenzene diol with OsO_4, in 85% overall yield from *cis*-chlorobenzene diol (**99**,

x=Cl). The first protecting group tried was the TBS ether, but was changed to the benzyl
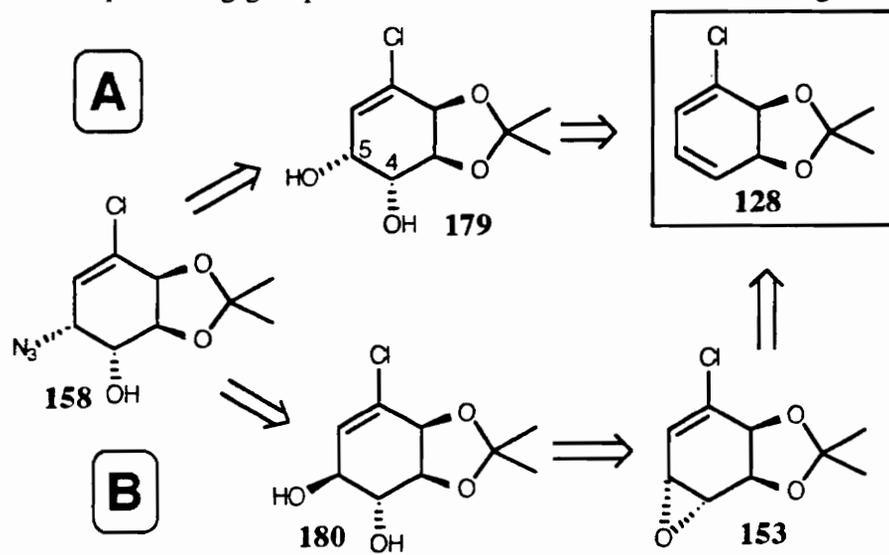
Scheme 53.
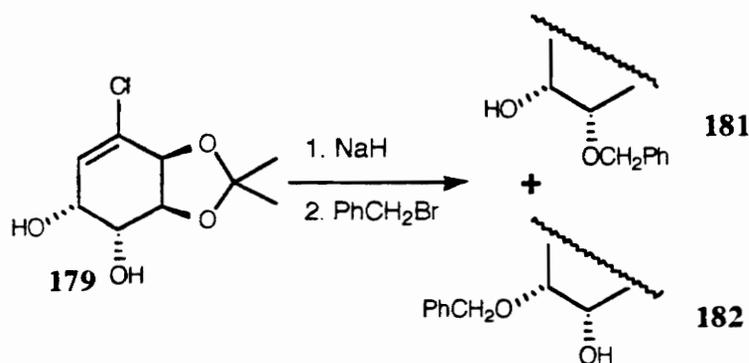
Scheme 54.

ether because of difficulties in separation of the regioisomers. This transformation (. NaH,then PhCH$_2$Br) produced both isomeric monoprotected *cis* diols **181** and **182** in a 2.3:1.0 ratio, (Scheme 53). Recently the transformation of an alcohol to an azide with retention of configuration via a double inversion process was reported.[480] The authors proposed a stabilized planar complex with cuprous ion as the agent responsible for the stereochemistry of the reaction. This kind of intermediate may not be possible in a rigid structure such as **181**, consequently the route was aborted.

The effort was then concentrated on route B (Scheme 53). The *trans*-diol (**180**) was prepared by opening epoxide **153**, based on the methodology that has been used in

Hudlicky's laboratory.[417] The monoprotection in this case was performed by a
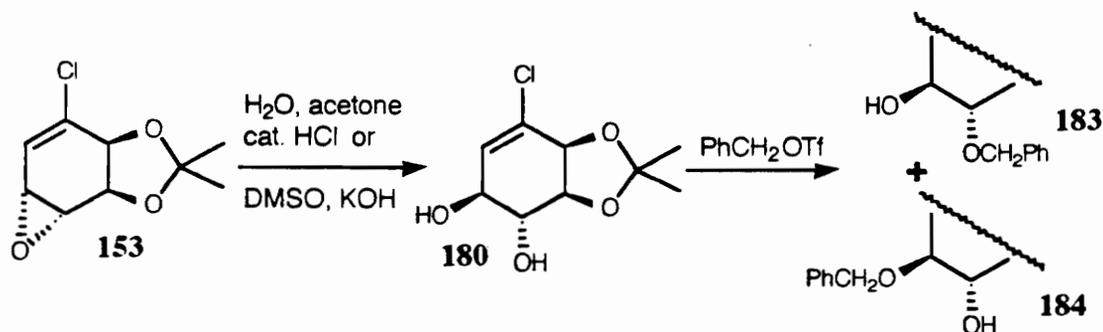
Scheme 55.

different method,[481] producing **183** and **184**, in 49% and 37% respectively, with some of the diprotected product. In order to transform the free alcohol to the azide group an straightforward method was selected, which involved the transformation of the free alcohol in **183** to the corresponding tosylate followed by the nucleophilic displacement by the azide ion.
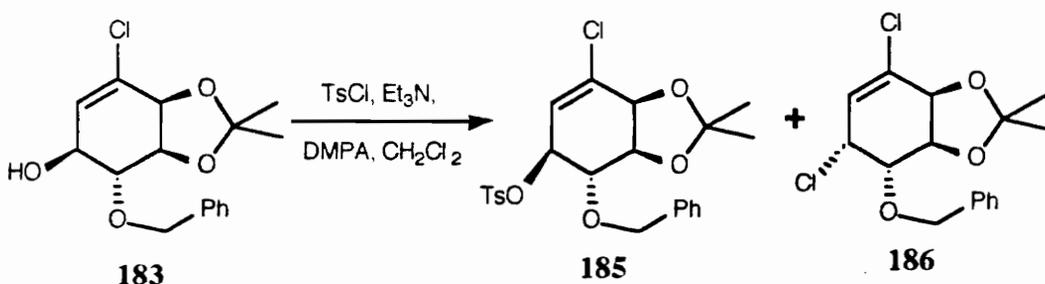
Scheme 56.

The reaction resulted in the formation of a mixture of the expected tosylate and the *cis*-chlorohydrin derivative **186** as the main compound in the mixture, as shown in Scheme 56. The latter resulted from the nucleophilic displacement of the tosylate by the chloride ion.[482] This result, even though undesired, opened a new alternative to the preparation of *cis*-azidohydrin (**158**); this new approach is shown in Scheme 57.
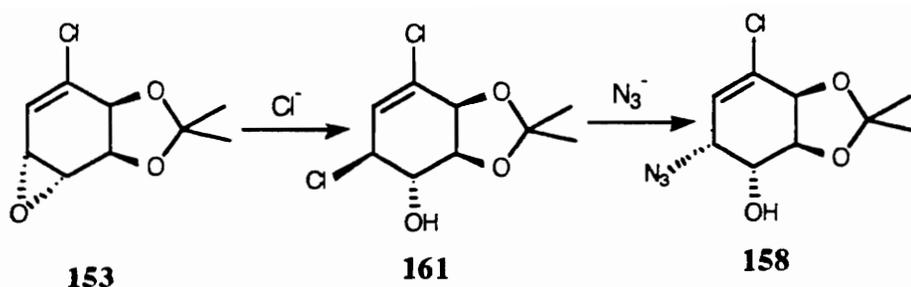
Scheme 57.

The *trans*-chlorohydrin **187** was prepared according to two different procedures: one with an ethereal solution of ferric chloride and the other with TBSCl as the chlorinating

Table 10. Reaction Conditions Used in the Preparation of the Chlorohydrin **158**.

Reaction Conditions	cis/trans ratio	Ref.
TBSCl, Ti(Opr^i)$_4$, THF, rt, 48 h	1:7.8	484
TBSCl, Ph$_3$P, CHCl$_3$, 85 °C, 17 h	>1:99	485
FeCl$_3$, Et$_2$O, rt, 20 min	8:2	486

agent. Table 10. As can be seen from the Table 10, the second method for preparation of *trans*-chlorohydrin (**161**) was successful, and at the same time small amounts of the *cis*-chlorohydrin were available giving the possibility of access to isomeric targets.

Scheme 58.

112
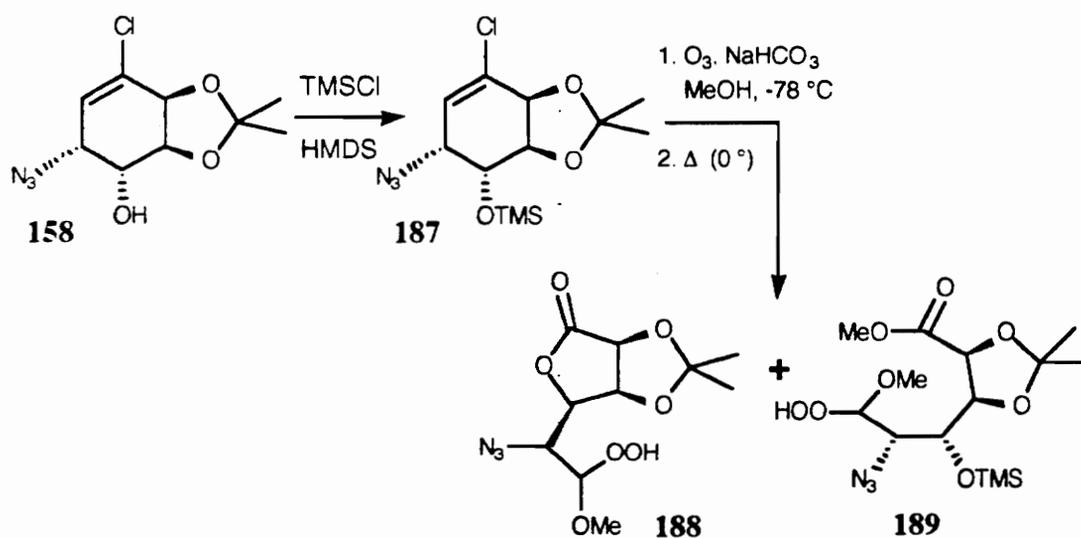
The displacement of the chloride took place under usual conditions (NaN₃, DMF)[476] producing the *cis*-azidohydrin **158**, in 60% yield; the low yield could be due to manipulations during the isolation of the product. The next step was the protection of the hydroxyl group. Attempts to form a TBS ether were unsuccessful, but the TMS ether was achieved successfully by the reaction with TMSCl/HMDS, Scheme 58.[486]

Ozonolysis of **187** (NaHCO₃, MeOH, -78 °C) followed by thermal decomposition of the peroxidic ozonolysis products produced two compounds: the ester hydroperoxide, **189**, which was the expected product according our previous experience; and the lactone, **188**, which resulted from the hydrolysis of the TMS ether group group by intramolecular transesterification. The yield of the reaction was very good (90%) but during separation by chromatography decomposition of both was observed, especially of the lactone **188**. The ratio of **188** to **189** varied, but it was observed that under conditions that assured complete exclusion of moisture, the lactone **188** was the only product in the crude mixture. However, when the ozonolysis reaction was performed with the addition of 2% water to the solvent of the reaction, the ratio of **188** to **189** changed to 70:30 in the crude mixture. This indicated the necessity of having a homogeneous base during the reaction to provide more effective neutralization of the HCl generated during the reaction.
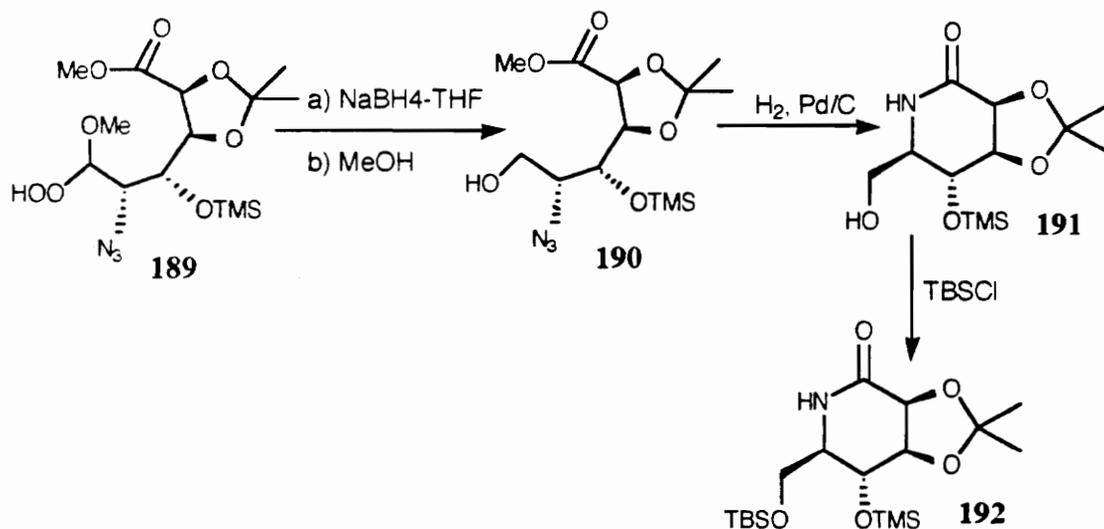
Scheme 59.

The peroxide **189** was reduced under the reaction conditions used for **173** to produce the ester alcohol **190** in low yield, possibly due to overreduction. As before,

hydrogenation of **190** produced the expected lactam **191**, which was reacted with TBSCl to form the derivative **192.** This completely protected lactam **192** was identical with a sample prepared from L-guconolactone following Fleet's procedure.[476] In this way the structure and consequently the stereochemistry proposed for all the intermediates in this synthetic sequence were assigned correctly.

On the other hand, the selective transformation of the peroxidic functionality in **188** should lead to intermediate **194**, which by catalytic hydrogenation could lead to intermediate **195** whose structure is equivalent to **191** (Scheme 60).
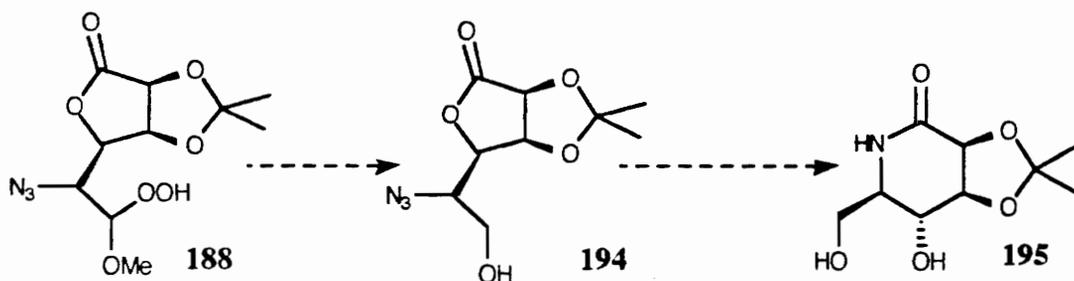
Scheme 60.

Table 11. Results in the Reduction of Lactone **189**.

Reaction conditions	Product	Ref.
NaBH$_4$. THF. rt (as work up of ozonolysis)	**196**	487
Mg°. MeOH	decomp.	488
NaBH$_4$. MeOH (as work up of ozonolysis)	**196**	487
NaBH$_3$CN, MeOH. 0 °C	**196**	489
NaBH$_4$. 2-propanol. 0 °C	**196**	
a) DMS, CH$_2$Cl$_2$, rt		
b) NaBH$_3$CN, 2-propanol	decomp.	
H$_2$. Pd/ CaCO$_3$. EtOAc	**195 (188)**	
NaBH$_4$. CH$_2$Cl$_2$-MeOH, -78 °C	**196, 197**	
SnCl$_2$•2H$_2$O, MeOH, rt	**195 (188)**	490
(t-amO)$_3$LiAlH, -78→0 °C	**196, 197**	491
1,3-propanedithiol, Et$_3$N, MeOH, rt	complex mixture	492

Several reducing conditions were used and most of them led to either a reduction that stopped at the stage of an aldehyde, which was to be too unstable for isolation or the reduction of peroxide that accompanied the reduction of the lactone to lactol. These results are shown in Table 11.
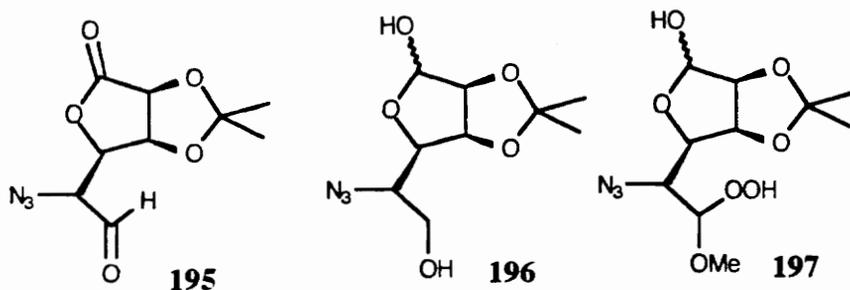
The production of **198** in the reactions at low temperature [NaBH₄, (t-AmO)₃LiH] indicates that the reduction of the lactone was faster than the reduction of the peroxidic group.

It was reported in the literature that the transformation of **198**[493] by catalytic hydrogenation leads to **199**, which is a natural product, through the intermediacy of a compound similar to **196**. Hence **196** might be used for synthesis of **199**.
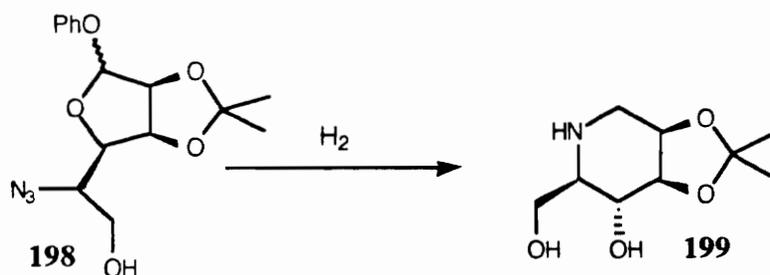
After the successful synthesis of **191**, we continued the synthesis of kifunensine, using **201** derived from L-gulonolactone in ample quantities. The reaction of **201** with chlorosulfonyl isocyanate (CSI) cleanly produced the amidine **202**.[494] Without further purification, **202** was reacted with diethyl oxalate in the presence of a catalytic amount of potassium t-butoxide in DMF,[495] resulting in a complex mixture where the lactam **201** was one of the components.[496] Then, the oxalamide formation was tried with oxalyl chloride.[497] This time spectroscopic analysis (especially ¹³C NMR) of the crude product showed signals that may correspond to the oxalamide **203**.

Scheme 61.

In summary, at this point the synthesis of kifunensine cannot be claimed.

III. 7 Conclusion.

When this work was initiated there were no reports of the use of *cis*-arene diols, either in synthesis of natural products or general organic synthesis. The number of reports recently published in this area demonstrate that the use and hence utility of *cis*-arenediol are increasing. Furthermore, this work is another contribution to the symbiotic biocatalysis-organic synthesis area which has led to excellent results.

Analysis of the functionalities of cis-arenediols and the stereocontrol provided by this singular structure allowed the successful enantiospecific preparation of the synthons for prostaglandins (**103**) and terpenes (**126** and **127**). This constituted the first formal synthesis of prostaglandins from an aromatic compound, namely toluene.

The ozonolysis of the acetonide of *cis*-chlorobenzenediol (**128**) produced L-erythruronolactone **132** in an enantiospecific fashion. Compound **132** can be considered a chiral equivalent of meso-tartaric acid, whose carboxyl groups are mutually protected and differentiated from each other by a different oxidation state. Also by the proper selection of

116

reaction conditions for the ozonolysis of *cis*-chlorobenzenediol different acyclic compounds structurally closely related to L-erythruronolactone **132** could be prepared, as well as L-erythrose **129**.
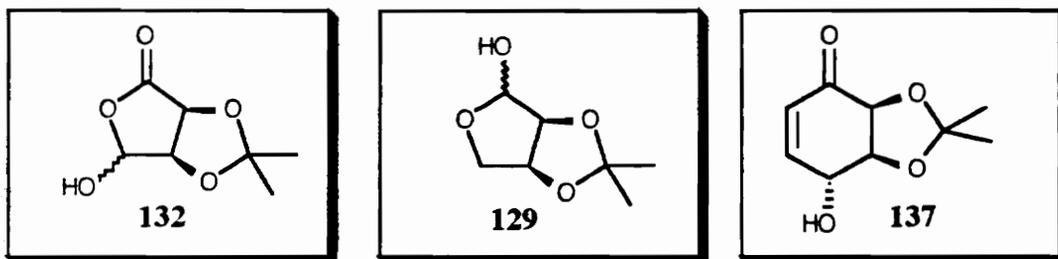
The structural characteristics of compound **132** make it suitable for its use in enantiodivergent synthesis, as demonstrated by the synthesis of both enantiomers of trihydroxyheliotradene[303] and the enantioespecific syntheses of L- (**129**) and D-erythrose acetonides.

In the same vein, working towards the synthesis of sugars from *cis*-chlorobenzene diol (**131**) led to the synthesis of γ-hydroxy enone **137** was synthesized. This enone contains a functional group array suitable for its use in the synthesis of cyclitols, as well as monosaccharides (hexoses and pentoses).

Derivatization of γ-hydroxy enone **137** led to the enantioselective synthesis of conduritols A (**148**) and C (**149**) and dihydroconduritol C (**150**). Further refining of the methodology led to the enantiospecific synthesis of conduritol C (**149**). In addition a second stereocontrolled synthesis of Conduritol A (**148**) was achieved, which can be considered one of the shortest approaches (three steps).
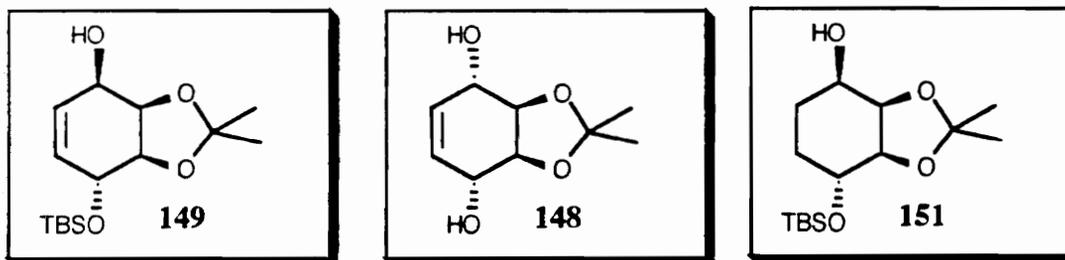
The studies into the introduction of a nitrogenous functionality to the *cis*-chlorobenzene diol molecule led to the establishment of the methods to prepare several

intermediates (**151-155**) regio- and stereoselectively. The significance of this achievement
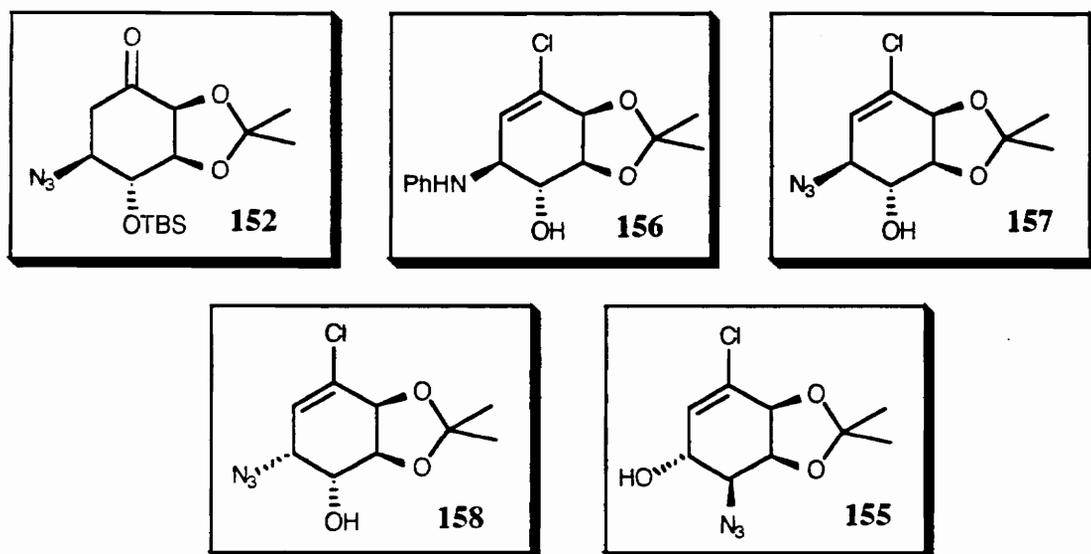
bodes well for a great synthetic potential of these intermediates in the synthesis of amino conduritols, amino inositols, amino sugars, imino alditols, and alkaloids.

In the same context it was shown that halogen atoms could be added to the molecule (**157, 158**). These intermediates would either be transformed to haloconduritols or, because of the reactivity of the halogen atom, serve as synthons for other types of cyclitols and sugar derivatives.
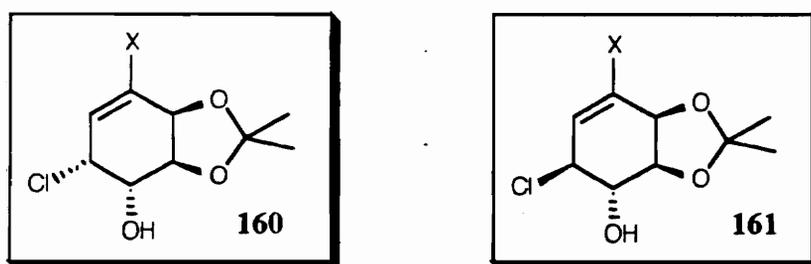
As a result of the synthetic approach to kifunensine, an enantioespecific synthesis of an L-mannonolactam derivative (**201**) was achieved, which rivaled that reported with L-gulonolactone as starting material. This fact allowed the claim of the formal enantioespecific synthesis of mannojirimycine (**204**) from chlorobenzene; as well as a formal synthesis of deoxymannojirimycine (**205**). An approach to kifunensine (**95**) was based on the use of this compound as well.
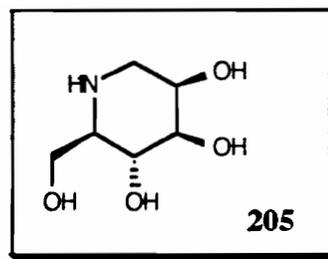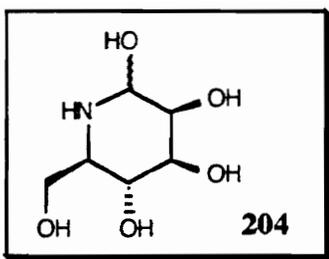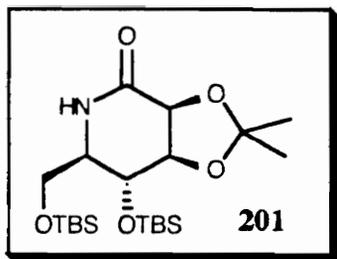
In a general sense, it can be concluded that a valuable insight into the stereocontrolled functionalization and reactivity of the cis-arenediols was gained. More specifically, this work produced some pioneering contributions in the area of ozonolysis of cyclic conjugated dienes; and also a valuable experience regarding the opening of epoxides, which led to the possibility of preparation of any combination (regio- and stereochemical) of 1.2-difunctionalities in the cyclohexane ring.

The potential biological activity of several of the compounds synthesized adds a significant importance to the biocatalysis methodology described in this work. In addition, all the syntheses resulted in shorter routes than those that originated from sugars. In view of the fact that in the syntheses based on sugars, all or most of the chiral centers are set from the beginning, the methodology described here is more efficient because all of the chiral centers were sequentially built, in most of the cases in stereocontrolled fashion. The cis diol produced during the microbial oxidation is the ultimate element of stereocontrol.

119

IV. Experimental.

All nonhydrolytic reactions were carried out in an argon atmosphere with standard techniques for the exclusion of air and moisture. Glassware used for moisture sensitive reactions was flame-dried under vacuum. THF, ether, DME, and benzene were distilled from benzophenone ketyl; dichloromethane, diisopropylamine, diisopropylethylamine, triethylamine, and toluene from calcium hydride.

Analytical TLC was performed on silica gel 60F-254 plates. Flash chromatography[103] was performed on Kieselgel 60 (EM Reagents, 230-400 mesh). Mass spectra were recorded on a Varian MAT-112 instrument (low resolution) or on a double focusing VG 7070 E-HF instrument (exact mass). Infrared spectra were recorded on Perkin-Elmer 283B or 710B instruments. Proton NMR spectra were obtained on a Bruker WP-270 instrument. Proton chemical shifts are reported in parts per million (ppm) relative to TMS, with $CHCl_3$ as standard (7.24 ppm). Carbon NMR spectra were recorded on Bruker WP-270 or NR-80 instruments. Carbon chemical shifts are reported in ppm relative TMS, with the center line of the $CHCl_3$ triplet (77.0 ppm) as standard and the multiplicity is indicated by CH_3, CH_2 CH, C (INEPT experiments). Rotations were recorded on a Perkin Elmer 241 digital polarimeter.

(2R,3S)-2,3-Dihydroxy-1-methylcyclohexa-4,6-diene (99, X=CH$_3$). *P putida* 39D was grown in MBS-arginine medium (80 mL) and incubated in a 250-mL Erlenmeyer flask at 28-30 °C. Toluene was supplied by an scale bulb attached to the flask by a neoprene stopper, and the culture was aereated on a reciprocal shaker. After 6 h, the culture was poured into a 1-L Erlenmeyer flask and medium was added until the total volume was 400 mL. A stream of air/toluene (5:1by volume) was bubbled for 24 h (the total volume of toluene consumed was 20 mL). The cell suspension was centrifuged at 5000 rpm for 10 min, and then the pH of the solution was brought to 8.4 with 50% NaOH solution. The solution was saturated with NaCl, centrifuged again, and extracted with ethyl acetate (acid-free, 5x100 mL). The organic extracts were dried (Na_2SO_4), and the solvent was evaporated. The crude diol was chromatographed (10% deactivated silica gel; methylene chloride/acetone, 2:1) to give 1.5 g of pure **104** (yield 3g/L of culture medium); R_f= 0.2 (hexane/ethyl acetate, 2:1);$[\alpha]^{25}_D$= +26.4° (c 0.38, MeOH); **IR** ($CHCl_3$) υ 3575, 3400-3200, 1400, 1390 cm^{-1}; **^{1}H NMR** ($CDCl_3$) δ 5.95 (ddd, J= 14, 7, 2 Hz, 1H), 5.8 (dd, J= 14, 4 Hz, 1H), 5.75 (br d, J= 7 Hz, 1H), 4.22 (m, 1H), 3.96 (m, 1H), 1.90 (m, 3H).

(2R,3S)-2,3-Dihydroxy-1-chlorocyclohexa-4,6-diene (**99**, X=Cl). *P putida* 39D was grown in MBS-arginine medium (80 mL) and incubated in a 250-mL Erlenmeyer flask.at 28°C. Chlorobenzene was supplied by an scale bulb attached to the flask by a neoprene stopper, and the culture was aerated on a reciprocal shaker. After 6 h, the culture was poured into a 1-L Erlenmeyer flask and medium was added until the total volume was 500 mL. A stream of air/chlorobenzene (5:1,by volume) was bubbled for 24 h at 28-30°C. The cell suspension was centrifuged at 5000 rpm during 10 min, and then the pH of the solution was brought to 8.4 with 50% NaOH solution. The solution was saturated with NaCl, centrifuged again, and extracted with ethyl acetate (acid-free, 5x100 mL). The organic extracts were dried (Na_2SO_4), and the solvent was evaporated. and filtered through a small plug of silica gel (10% deactivated, hexane/ ethyl acetate, 1:1) to give 0.4 g of pure chlorobenzendiol (**131**) as a white solid. R_f= 0.32 (hexane/ethyl acetate, 1:1); **mp**= 82-84 °C; **^{1}H NMR** ($CDCl_3$) δ 6.12 (m, 1H), 5.87 (m, 2H), 4.48 (m, 1H). 4.19 (t, J= 7.3 Hz, 1H), 2.74 (d, J= 7.3 Hz, 1H), 2.63 (d, J= 8.4 Hz, 1H); **^{13}C NMR** ($CDCl_3$) δ 134.9 (C). 128.0 (CH), 123.4 (CH), 122.7 (CH), 71.4 (CH), 69.1 (CH).

(2R,3S)-1-Methyl-2,3-O-isopropylidenecyclohexa-4,6-diene (**105**). To a solution of (2R,3S)-2.3-dihydroxy-1-methylcyclohexa-4,6-diene (**99**, X=CH_3) (116 mg. 0.92 mmol) in 2,2-dimethoxypropane (DMP, 15 mL). a crystal of *p*-toluenesulfonic acid was added then it was stirred at room temperature for 10 min. The methylene chloride (20 mL) was added, and the solution was washed with 10% NaOH (1x30 mL), and brine (1x30 mL). The organic layer was dried (Na_2SO_4), and the solvent was evaporated to yield 129.8 mg (0.782 mmol. 85%) of acetonide (**105**). R_f= 0.8 (hexane/ethyl acetate, 2:1); $[\alpha]^{25}_D$= +93.74° (c 2.98, MeOH); **IR** (neat) υ 2980, 1380, 1050 cm^{-1}; **^{1}H NMR** ($CDCl_3$) δ 5.95 (dd, J= 9.5, 5.5 Hz, 1H), 5.80 (dd, J= 9.5, 4.0 Hz, 1H), 5.72 (br d, J= 5.5 Hz. 1H). 4.64 (dd, J= 8.7, 4.0 Hz, 1H), 4.45 (br d, J= 9.0 Hz, 1H), 1.9 (s, 3H), 1.42 (s, 3H). 1.38 (s, 3H);

(2R,3S)-2,3-Diacetoxy-4-oxopentanal (**113**). To a solution of (2R,3S)-1-me-thyl-2,3-diacetoxycyclohexa-4,6-diene (**109**) (ll9 mg, 0.566 mmol) in ethyl acetate (2 mL) was treated with a stream of O_2/O_3, at -78 °C, until a light blue color persisted, at which time nitrogen was bubbled through for 15 min. Then dimethyl sulfide (2 mL) was

added dropwise at -78°C, the temperature was immediatly raised to 0°C, and the reaction was stirred overnight. The solvent was removed at reduced pressure, and the residue (160 mg) was purified by Kugelrohr distillation (85-90°C/0.005 Torr) to give 62mg (0.283 mmol, 50%) of pure **113**. R_f= 0.33 (hexane/ethyl acetate, 1:1); **IR** (neat) υ 1742, 1365, 1225 cm^{-1}; **^{1}H NMR** (CDCl$_3$) δ 9.51 (s, 1H), 5.62 (d, J= 2.9 Hz, 1H), 5.48 (d, J= 2.9 Hz, 1H), 2.19 (s, 3H), 2.18 (s, 6H); **^{13}C NMR** (CDCl$_3$) d 194.5, 169.8, 169.5, 77.0, 76.5, 26.8, 20.5, 20.4; **MS** (CI) m/e (relative intensity) 217 (M$^+$+1, 5), 156 (100), 114 (20), 96 (15); **HRMS Calcd. for** C$_7$H$_9$O$_4$: (M-59) 157.0501; **Found:** 157.0476.

(3S,4R)-2,5-Dihydroxy-3,4-O-isopropylidene-2-methyltetrahydro furan (114). Method A. by Ozonolysis of 105.

A solution of (2R,3S)-1-methyl-2,3-O-isopropylidenecyclohexa-4,6-diene (**105**) (0.106 g, 0.638 mmol) in ethyl acetate (7 mL) was cooled to -78°C and then a stream of O$_2$/O$_3$ was passed throuhg until the blue color persisted. Nitrogen was bubbled for 10 min, and then dimethyl sulfide (1 mL) was added, and the temperature was allowed to rise overnight until 0°C, whereupon the solvent was removed at reduced pressure. The resulting residue was purified by Flash chromatography (10% deactivated silica gel, hexane/ethyl acetate, 9:1 to 7:3) to yield 67 mg (0.059 mmol, 56%) of a mixture of isomers of **114**: R_f= 0.39 (hexane/ethyl acetate, 1:1); **IR** (neat) υ 3450, 2990, 1750, 1390 cm^{-1}; **^{1}H NMR** (CDCl$_3$) δ 5.34 (d, J= 7.1 Hz, 1H), 4.74 (d, J= 5.6 Hz, 1H), 4.55 (d, J= 5.6 Hz, 1H), 3.76 (d, J= 7.6 Hz, 1H), 3.60 (s, 1H), 1.55 (s, 3H), 1.48 (s, 3H), 1.32 (s, 3H); **^{13}C NMR** (CDCl$_3$) δ 112.8 (C), 108.9 (C), 84.0 (CH), 25.0 (CH), 24.8 (CH$_3$), 21.8 (CH$_3$); **MS** (CI), m/e (relative intensity) 173 (100), 155 (56), 143 (29), 129 (33), 113 (42), 87 (18); **HRMS Calcd. for** C$_7$H$_9$O$_4$: (M-33) 157.0500; **Found:** 157.0519.

1,4,4-Trimethyl-3,5,8,9-tetraoxa-tricyclo-[2.2.2.3^{2,6}]undec-10-ene (119).

A solution of triphenylphosphite (186 mg, 0.6 mmol) in dichloromethane (15 mL) was ozonized at -78°C until a blue color persisted. It was then purged with nitrogen for 20 min, and a solution of (2R,3S)-1-methyl-2,3-O-isopropylidene-4,6-cyclo-hexadiene (**105**) (100 mg, 0.6 mmol) in dichloromethane (2 mL) was added. It was then slowly warmed up to 0°C and stirred for 20 min. The solvent was removed under reduced pressure, and the residue was purified by Kugelrohr destillation (80°C/0.075 mmHg) to yield 99.8 mg (0.504 mmol, 84%) of a white crystalline solid; R_f=0.79 (hexane/ethyl acetate, 8:2); **mp**= 79-75 °C; **IR** (CHCl$_3$) υ 2970, 2930, 1450, 1375 cm-1; **^{1}H NMR**

(CHCl3) δ 6.5 (dd, J= 8.3, 6.1 Hz, 1H), 6.3 (d, J= 8.3 Hz, 1H), 4.8 (dt, J= 6.1, 4.5, 1.2 Hz, 1H), 4.6 (dd, J= 6.9, 4.5 Hz, 1H), 4.2 (d, J= 6.9 Hz, 1H),1.31 (s, 6H);^{13}C NMR (CDCl3) δ 134.6, 130.1, 110.1, 72.6, 72.0, 25.7, 25.6, 19.1.

2,8,8-Trimethyl-2,5-dihydroxy-7,9-dioxabicyclo[4.3.0]non-3-ene

(120) To a solution of 1,4,4-trimethyl-3,5,8,9-tetraoxatricyclo[2.2.2.3^{2,6}]undec-10-ene (119) (23 mg, 0.13 mmol) in dry ether (5 mL) and MeOH-H$_2$O (9:1, 0.5 mL), was added aluminum amalgam (freshly prepared from 114 mg of aluminum foil). After the addition was complete, the reaction mixture was stirred for 25 min, then filtered through Celite and rinsed with ether. The ethereal solution was evaporated under reduced presure to yield 25 mg (0.125 mmol, 96%) of practically pure diol (120). An analytical sample was obtained by Flash chromatography (10% deactivated silica gel; hexane/ethyl acetate, 3:1); mp= 85 °C; R$_f$= 0.25 (hexane/ethyl acetate, 1:1), IR (CHCl3) υ 3650, 3410, 3015, 2965, 1470, 1400 cm_1; ^{1}H NMR (CHCl3) δ 6.10 (dd, J= 9.7, 5 Hz, 1H) , 5.98 (dd, J= 9.7, 0.8 Hz, 1H), 4.48 (dd, J=7.2, 2.0 Hz, 1H), 4.29 (d, J= 7.2 Hz, 1H), 4.21 (m, 1H), 3.60 (m, 1H), 3.40 (s, 1H), 1.38 (s, 3H), 1.33 (s, 3H); ^{13}C NMR (CHCl3) δ 137.9 (CH), 131.3 (CH), 108.4 (C), 81.3 (CH), 78.6 (CH), 69.3 (C), 66.1 (CH), 26.3 (CH$_3$), 25.1 (CH$_3$), 24.3 (CH$_3$); MS (EI, 70eV) m/z (rel. intensity) 185 (10), 167 (6), 125 (30), 113 (57), 100 (100), 95 (030), 85 (32), 71 (35); MS (CI) m/z (rel. intensity) 201 (4), 183 (13), 165 (13), 125 (100), 109 (32), 97 (33); HRMS Calcd. for C$_{10}$H$_{17}$O$_4$: (M+1) 201.1127; Found: 201.1125.

(3S,4R)-2,5-Dihydroxy-3,4-O-isopropylidene-2-methyltetrahydro furan (114).

To solution of 2,8,8-trimethyl-2,5-dihydroxy-7,9-dioxabicyclo[4.3.0]non-3-ene (120) (15 mg, 0.075 mmol) in dioxane (0.7 mL) and phosphate buffer (pH 8, 0.5 mL) was added a small crystal of osmium tetraoxide at 0-5°C. After 10 min of stirring, sodium periodate (96 mg, 0.448 mmol) was added in four portions, each one after the previous one was dissolved. After 1 h of stirring, the reaction temperature was raised to room temperature, and the stirring was continued for 20 h. Then 10% sodium sulfite solution (3 mL) was added and the mixture was extracted with ether (3x3 mL). The organic layer was washed with saturated sodium bicarbonate solution (2 mL), brine (2 mL) and dried (Na$_2$SO$_4$), and the solvent was removed to yield 11 mg (0.06 mmol, 81%) of a homogeneous material whose spectral data matched that of 114 prepared from ozomolysis of 105.

(4S,8S)-6,6-Dimethyl-5,7-dioxabicyclo[3.3.0]oct-2-en-1-one (103).
To a stirred suspension of neutral alumina (Fluka, activity V) in 70 mL of freshly distilled
1,2-dimethoxyethane (DME) was added a solution of **113** (86.9 mg, 0.457 mmol) in DME
(15 mL). After 15 min of reflux under argon, (2R,3S)-2,3-O-isopropylidene-4-oxo-
pentanal (**104**) was identified by ^{1}H NMR analysis as the sole product. ^{1}**H NMR**
(CDCl$_3$) δ 9.83 (s, 1H), 4.62 (d, J= 5.5 Hz, 1H), 4.55 (d, J= 5.5 Hz, 1H), 2.33 (s, 3H),
1.48 (s, 3H), 1.45 (s, 3H). After 30 min of reflux under argon atmosphere, the reaction
was filtered and the solvent was removed at reduced pressure. The crude enone (**101**)
(45.5 mg, 64.6 %), 95% pure by GC (capillary column SE-30 chromosorb; 80°C [0.5
min] to 120°C [0.5 min] at 10°C/min), was distilled (Kugelrohr, 40°C/0.005 torr); mp= 42
°C (lit. mp 36-38°C); R$_f$= 0.37 (ethyl acetate/hexane, 1:1); [α]^{25}$_D$= +62.8° (c 0.7,
CHCl$_3$) (lit. [α]^{25}$_D$= +71.8° [c 0.91, CHCl$_3$]); **IR** (CHCl$_3$) υ 3000, 2940, 1730, 1380,
cm^{-1}; ^{1}**H NMR** (CDCl$_3$) δ 7.65 (dd, J= 5.9, 2.2 Hz, 1H), 6.22 (d, J= 5.9 Hz, 1H),
5.27 (dd, J= 5.5, 2.2 Hz, 1H), 4.46 (d, J= 5.5 Hz, 1H), 1.42 (s, 3H).

(4S,5S)-Diacetoxy-6-oxo-2-heptenal (117). To a solution of (2R,3S)-2,3-
diacetoxy-1-methylcyclohexa-4,6-diene (**109**) (243.5 mg, 1.16 mmol) in dichloromethane
(5 mL), at -55 °C a saturated solution of ozone in dichloromethane (cooled at -78°C) (65
mL) was added. After the addition was completed, the reaction was stirred at -55°C for 10
min, whereupon dimethyl sulfide (3 mL) was added. The mixture was stirred overnight at
0 °C, and then the solvent was evaporated under reduced pressure to give 267 mg (95%) of
crude product **117**. Flash chromatography (10% deactivated silica gel, hexane/ethyl
acetate, 1:1) yielded 138.7 mg (0.754 mmol, 65%) of pure **117**; R$_f$= 0.33 (hexane/ethyl
acetate, 3:1); **IR** (neat) υ 1750, 1685, 1115 cm^{-1}, ^{1}**H NMR** (CDCl$_3$) δ 9.59 (d, J= 8 Hz,
1H), 6.73 (dd, J= 16, 5 Hz, 1H), 6.27 (ddd, J= 16, 8, 1.5 Hz, 1H), 5.95 (m, 1H), 5.38
(d, J= 3.5 Hz, 1H), 2.21 (s, 3H), 2.20 (s, 3H), 2.16 (s, 3H); ^{13}**C NMR** (CDCl$_3$) δ
202.0, 192.1, 169.1, 146.9, 133.7, 77.8, 70.8, 27.3, 20.5, 20.4; **MS** (CI) m/e (relative
intensity) 243 (M$^+$+1, 100), 183 (32), 141 (53), 123 (100), 85 (20); **HRMS Calcd for**
C$_9$H$_{11}$O$_4$ (M-59): 183.0657; **Found:** 183.05750.

(4S,5S)-4,5-O-isopropylidene-6-oxo-2-heptenal (118). (2R,3S)-1-
methyl-2,3-O-isopropylidenecyclohexa-4,6-diene (**105**) was reacted with ozone, by an
analogous procedure used for **109** (above) to give **117** as the main reaction product: R$_f$=
0.48 (hexane/ethyl acetate, 1:1); ^{1}**H NMR** (CDCl$_3$) δ 9.56 (d, J= 8.0 Hz, 1H), 6.61
(ddd, J= 4, 13 Hz, 1H), 5.1 (ddd, J= 13.0, 8.0, 2.0 Hz, 1 H), 4.66 (d, J= 8.0 Hz, 1H),
2.24 (s, 3H), 1.40 (s, 3H).

(1S,2R,3R)-Dihydroxy-3-methylcyclohexane (124) and (1S,2R,3S)-dihydroxy-3S-methylcyclohexane (123) (a) A solution of (2S,3R)-2,3-dihydroxy-1-methylcyclohexa-4,6-diene (**99**, X=CH₃) (1.1 g, 8.8 mmol) in ethyl acetate/benzene (1:1, 25 mL) was hydrogenated, with 5% Pd/C as catalyst, at 50 psi for 14 h. The reaction mixture was filtered, and the solvent was removed at reduced pressure. The liquid residue was purified by Flash chromatography (10% deactivated silica gel, hexane/ethyl acetate, 9:1) to produce 915.5 mg (7.08 mmol, 80%) of mixture of *cis* and *trans* methyl diols and (in a 7:3 ratio). R_f= 0.16 (hexane/ethyl acetate, 1:1). This mixture was dissolved in dry pyridine (10 mL) cooled to 0-5°C, and then cold benzoyl chloride (0.91 mL, 7.74 mmol), was added. After the reaction mixture was stirred overnight at rt, it was diluted with ether (15 mL) and washed with water (2x10 mL), saturated cooper sulfate solution (6x10 mL), and brine (2x10 mL). The organic layer was dried (Na₂SO₄), and the solvent was removed under reduced pressure. The crude mixture of monobenzoates was separated by Flash chromatography (10% deactivated silica gel; hexane/ethyl acetate, 9:1), yielding 1.4 g (4.81 mmol, 68%) of (1S,2R,3R)-benzoyloxy-2-hydroxy-3-methylcyclohexane and 0.514 g (1.77 mmol, 25%) of (1S,2R,3S)-benzoyloxy-2-hydroxy-3-methylcyclohexane.

For "R" epimer : R_f= 0.7 (ethyl acetate/hexane, 1:1); **mp**= 84-85 °C; **IR** (CHCl₃) υ 3620, 2930, 2870, 1710 cm⁻¹; **¹H NMR** (CDCl₃) δ 8.4 (d, J= 7.5 Hz, 2H), 7.56 (t, J= 7.5 Hz, 1H), 7.44 (t, J= 7.5 Hz, 2H), 5.0 (m, 1H), 3.97 (br s, 1H), 1.58-1.98 (m, 5H), 1.34-1.48 (m, 3H), 1.07 (d, J=6.8 Hz, 3H). For "S" epimer : R_f= 0.58 (ethyl acetate/hexane, 1:1); **IR** (neat) u 3525, 2980, 2890, 1768 cm⁻¹; **¹H NMR** (CDCl₃) δ 8.05 (d, J= 7.5 Hz, 2H), 7.56 (t, J= 7.5 Hz, 1H), 7.46 (t, J= 7.5 Hz, 3H), 4.80 (dd, J= 13.0, 2.6 Hz, 1H), 4.17 (br s, 1H), 2.2 (m, 1H), 1.43-1.95 (m, 7H), 0.95 (d, J= 6.6 Hz, 3H).

(b) Hydrolysis of monobenzoates (general method) To a solution the corresponding monobenzoate (536 mg, 2.292 mmol) in methanol (5 mL) was added 10N sodium hydroxide solution.(0.5 mL). The solution was refluxed for 1 h, and the solvent was removed at reduced pressure. The solid residue was taken up in a water (15 mL) and saturated with sodium chloride, this solution was extracted with ethyl acetate (5x5 mL). The combined organic extracts were dried (Na₂SO₄), and the solvent was evaporated, yielding 240.5 mg (1.856 mmol, 81%) of the corresponding diol.

For (1S,2R,3R)-dihydroxy-3-methylcyclohexane (**124**); R_f=0.32 (methanol/chloroform, 5:95); **IR** (CHCl₃) υ 3620, 3480, 2940, 1460, 1040, 980 cm⁻¹; **¹H NMR** (CDCl₃) δ 3.75 (t, J= 3 Hz, 1H), 3.54 (ddd, J= 11.0, 4.2, 3.0 Hz, 1H), 2.26

(br s, 2H), 1.59-1.74 (m, 2H), 1.43-1.59 (m, 2H), 1.17-1.36 (m, 3H), 1.04 (d, J= 7 Hz, 3H).

For (1S,2R,3S)-dihydroxy-3-methylcyclohexane (123): R_f= 0.32 (methanol/chloro-form, 5:95); IR (CHCl₃) υ 3650, 3600, 2950, 1470, 1040 cm⁻¹ ¹H NMR (CDCl₃) δ 3.96 (dt, J= 5.0, 3.0 Hz, 1H), 3.22 (br s, 2H), 3.18 (dd, J= 9.0, 3.0 Hz, 1H), 1.83-1.94(m, 1H), 1.36-1.83 (m, 6H), 1.02 (d, J= 7 Hz, 3H).

2(R)-Methylhexanedial. To a solution of (1S,2R,3R)-dihydroxy-3-methyl-cyclohexane (227.5 mg, 1.75 mmol) in anhydrous THF (10 mL), cooled to 0°C, was added a solution of periodic acid (442 mg, 1.93 mmol) in THF (6 mL). After 10 min, the reaction mixture was warmed to room temperature, and the stirring was continued for 1h. The reaction was then diluted with ether (20 mL) and washed with saturated NaHCO₃ solution (10 mL) and brine (2x10 mL), and the organic layer was dried (Na₂SO₄). The solvent was evaporated under reduced pressure to yield 207 mg (1.59 mmol, 91%) of 2(R)-methylhexanedial as a light yellow oil, which was used without purification: R_f= 0.55 (hexane/ethyl acetate, 1:1); IR (neat) υ 2930, 2850, 1715 cm⁻¹; ¹H NMR (CDCl₃) δ 9.83 (t, J= 1.8 Hz, 1H), 9.63 (d, J= 2.0 Hz, 1H), 2.44-2.54 (td, J= 7.0, 1.8 Hz, 2H), 1.59-1.81 (m, 3H), 2.31-2.42 (m, 1H), 1.35-1.46 (m, 1H), 1.16 (d, J= 7.8 Hz 3H).

2(S)-Methylhexanedial. This compound was prepared via the same procedure used above, in 86% yield; R_f= 0.55 (hexane/ ethyl acetate, 1:1); IR (neat) υ 2945, 2870, 1720 cm⁻¹; ¹H NMR (CDCl₃) δ 9.83 (t, J= 1.8 Hz, 1H), 9.63 (d, J= 2 Hz, 1H), 2.44-2.54 (td, J= 7.0, 1.8 Hz, 2H), 2.31-2.42 (m,1H), 1.59-1.81 (m, 3H), 1.35-1.40 (m, 1H), 1.16 (d, J= 7.8 Hz, 3H)

3(R)-Methylcyclopentene-1-carboxaldehyde (126). To a solution of 2(R)-methylhexanedial (155 mg, 1.21 mmol) in anhydrous ether (15 mL), at 0°C, was added dropwise freshly distilled piperidine (114.7 mg, 1.35 mmol). After 2.5 h at 0°C, glacial acetic acid was added dropwise (324 mg, 5.40 mmol). The reaction mixture was warmed to room temperature, and the stirring continued for 3 h. The reaction was quenched with saturated NaHCO₃ solution (10 mL), after the layers were separated, the organic layer was washed with brine (2x5 mL) and dried over Na₂SO₄. The solvent was distilled off at atmospheric pressure, and the liquid residue was distilled (Kugelrorh 30°C/2 mmHg) to yield 80 mg (0.73 mmol, 60%) of pure 126: R_f= 0.58 (silica gel, hexane/ethyl acetate, 8:2); $[\alpha]^{25}_D$= +193.3° (c 1.058, CHCl₃); IR (neat) υ 2960, 2870, 1680, 1615 cm⁻¹; ¹H NMR (CDCl₃) δ 9.8 (s, 1H), 6.77 (dd, J= 4..0, 2.0 Hz, 1H) 2.93-3.08 (m, 1H), 2.52-

2.66 (m, 1H), 2.38-2.52 (m, 1H), 2.19-2.31 (m, 1H), 1.46-1.6 (m, 1.17 (d, J= 8 Hz, 3H).

3(S)-Methylcyclopentene-1-carboxaldehyde (127). The "S" enantiomer was prepared from 3(S)-methylhexanedial via the same procedure used for **126** in a 44.8% yield ; R_f= 0.58 (hexane/ethyl acetate, 8:2); $[\alpha]^{25}_D$= -214.9° (c 1.1, CHCl$_3$); **IR** (CHCl$_3$) υ 2980, 2890, 1675, 1605 cm^{-1}; **^{1}H NMR** (CDCl$_3$) δ 9.75 (s, 1H), 6.73 (dd, J= 4.0, 2.0 Hz, 1H), 2.87-3.04 (m, 1H), 2.50-2.63 (m, 1H), 2.13-2.27 (m, 1H), 1.42-1.56 (m, 1H), 1.13 (d, J= 8 Hz, 3H).

L-erythruronolactone (132). A solution of (2S,3S) 2,3-O-isopropylidene-1-chlorocyclohexa-4,6-diene (**128**) (94 mg, 0.5 mmol) in ethyl acetate (7 mL), was cooled to -78 °C and a stream of O$_2$/O$_3$ was passed through until a blue color persisted. Nitrogen was bubbled through the solution to remove the excess of ozone. Then dimethylsulfide (DMS) (1.4 mL) was added and the temperature was immediately raised to 0 °C. The reaction was stirred for 12 h, then diluted with ether (30 mL). The ethereal solution was washed with water (1x5 mL), and brine (1x5 mL). The solution was dried (Na$_2$SO$_4$), filtered and the solvent evaporated, yielding 70.7 mg (0.4 mmol, 80 %) of crude product. After purification by flash chromatography (10% H$_2$O silica gel; dichloromethane/acetone,7:3) clean L-erythruronolactone (**132**) was obtained (30.4 mg, 34.4%). R_f= 0.2 (chloroform/methanol, 8:2); **IR** (KBr) υ 3400, 1723 cm^{-1}; **^{1}H NMR** (CDCl$_3$) δ 5.81 (s, 1H); 4.91 (m, 1H); 4.60 (d, 1H, J= 5.4 Hz); 1.47 (s, 3H); 1.40 (s, 3H); **^{13}C NMR** (CDCl$_3$) δ 174.5, 114.5, 99.4, 95.9, 80.0,, 74.7, 26.5, 25.9 .
L-erythruronolactone = (2S,3R)-O-isopropylidene-4-hydroxybutyrolactone.

2,3-O-Isopropylidene-L-erythrose (129). A solution of (2S,3S)-O-isopropylidene-1-chlorocyclohexa-4,6-diene (**128**) (78.4 mg, 0.5 mmol) in methanol-dichloromethane (8:2, 7 mL) was cooled to -78 °C and a stream of O$_3$/O$_2$ was passed through until the persistance of a blue color. Nitrogen was bubbled through the solution to remove excess of ozone. To the stirred reaction, at - 78 °C under nitrogen atmosphere, was added NaBH$_4$ (36 mg, 0.5 mmol), stirring continued for one hour; then the temperature was raised to 0 °C and the solution was stirred for an aditional hour. After that, 10 drops of a saturated aqueous solution of NH$_4$Cl was added, and the solvent was removed under reduced pressure, at room temperature. The semisolid residue was taken up in ethyl acetate (5 mL), and filtered; this operation was repeated twice. The combined

organic extracts were evaporated to produce 74 mg of a colorless viscous liquid. Separation by preparative TLC (Silica gel; hexane/ethyl acetate, 6:4) produced methoxylactone (11.6 mg,0.06 mmol, 12%) and 2,3-O-isopropylidene-L-erythrose (**129**) (41.6 mg, 0.26 mmol, 52%). **Methoxylactone: R$_f$**= 0.55 (hexane/ethyl acetate,1:1); **mp**= 76-78 °C; **IR** (neat) υ 2985, 2920, 1710 cm^{-1}; **^{1}H NMR** (CDCl$_3$) δ 5.34 (s, 1H); 4.8 (d, J= 5.5 Hz, 1H), 4.55 (d, J= 5.5 Hz, 1H), 3.53 (s, 3H), 1.46 (s, 3H), 1.38 (s, 3H); **^{13}C NMR** (CDCl$_3$) δ 114 (C), 105 (C), 79 (CH), 74 (CH), 60 (CH), 26 (CH), 25 (CH); **HRMS calcd. for** C$_8$H$_{12}$O$_5$: 188.07629; **Found**: 188.07574. **2,3-O-isopropylidene-L-erythrose (129); R$_f$**=0.34 (hexane/ethyl acetate,1:1);[α]$_D^{25}$= -66.35° (c 4.75, MeOH); **^{1}H NMR** (CDCl$_3$) δ 5.4 (br s, 1H), 4.85 (m, 1H), 4.59 (d, 5.9 Hz, 1H), 4.05 (m, 2H), 1.48 (s, 3H), 1.32 (s, 3H).

(1S,2S,3S,4R)-1-chloro-2,3-O-isopropylidene-5,6-dioxabicyclo [2.2.2]octa-7-ene (140) Three milligrams of tetraphenylporphine were dissolved in chloroform (5 mL) 10 min before starting the reaction; this solution was transferred to the reaction vessel (a water jacketed flask, with 2 necks and a glass frit bubbler attached) using of carbon tetrachloride (CCl$_4$, 50 mL). Then (2R,3S)-2,3-O-isopropylidene-1-chlorocyclohexa-4,6-diene (**128**) (736 mg, 4.646 mmol) was added to the reaction vessel in 50 mL of CCl$_4$. Oxygen was bubbled through the solution as it was irradiated with a 500-W quartz lamp. The temperature of the reaction mixture was kept ~20°. The reaction was monitored by TLC (hexane-ethyl acetate,9:1), and it was completed in 2.5 h. The solvent was removed in rotatory evaporator without heating and the resulting dark green residue was triturated with hexane (80 mL) and shaken for 5 min with 0.5 g of charcoal; the mixture was filtered through celite. Evaporation of the solvent produced 950.9 mg (93%) of clean endoperoxide (**140**), which could be used for the next reaction without further purification (mp= 69-71 °C). An analytical sample was obtained by flash chromatography followed by sublimation (<30 °/0.005 Hg mm) **mp**= 74-76 °C; **R$_f$**= 0.5 (chloroform); [α]^{25}$_D$= +57.42 (c 0.5, MeOH); **IR**. (KBr) υ 2994, 2936, 1381, 1263,1214, 1054 cm^{-1} ; **^{1}H NMR** (CDCl$_3$) δ 6.6 (ddd, J= 8.7, 5.0, 0.9 Hz, 1H), 6.5 (dt, J= 8,7, 1.4, 1.2 Hz, 1H), 4.95 (ddd, J= 7.0, 4.4, 0.9 Hz,1H), 4.73 (ddd, J= 5.0, 4.4, 1.4 Hz, 1H), 4.5 (dd, J= 7.0, 1.2 Hz, 1H), 1.37 (s,3H), 1.34 (s, 3H); **^{13}C NMR** (CDCl$_3$) δ 133.7, 131.7, 111.3, 94.9, 78.8, 74.1, 73.1, 25.6, 25.4.

(2S,3S,4R)-2,3-O-Isopropyledene-4-hydroxycyclohexa-5-enone

(137). In a flask protected from moisture the endoperoxide (950.9 mg, 4.36 mmol) **(140)** was dissolved in methanol (6 mL) and the solution was stirred in a cold water bath (~ 10°), then a solution of 364 mg (4.79, 4.79 mmol) of thiourea in 5 mL of methanol was added, dropwise. After the addition was complete the reaction mixture was stirred at room temperatue for 0.5 h; the solvent was evaporated under reduced presure at room temperature; the residue was quickly diluted with 50 mL of ether, the precipitate produced was filtered through celite. The filtrate was evaporated to produce a viscous yellow liquid. The crude product was purified by flash chromatography (silica gel; dichloromethane-acetone, 8:2) to produce 680 mg (3.73 mmol, 85%) of a light yellow liquid, which slowly solidified. R_f= 0.21 (ethyl acetate-hexane,1:1); **IR** (neat) υ 3444, 2989, 2937, 1682, 1378, 1079, 1047 cm^{-1}; **^{1}H NMR** (CDCl$_3$) δ 6.87 (dd, J= 10.3, 3.2 Hz, 1H); 6.11 (dd, J= 10.3, 1.6 Hz, 1H), 458 (br s, 1H), 4.46 (m, 2H), 2.53 (d, J= 4.7 Hz, 1H), 1.4 (s, 6H); **^{13}C NMR** (CDCl$_3$) δ 195.0, 148.3, 127.9, 110.0, 79.0, 74.0, 65.9, 27.1, 25.6.

(2S,3S,4R)-4-t-butyldimethylsilyloxy-2,3-O-isopropylidene-5-cyclohexenone (141).

To a solution of *t*-butyldimethylchlorosilane (1.0 g, 6.67 mmol) in dry DMF (4 mL) diisopropylethylamine (1.5 mL, 8.58 mmol) was added under Argon at room temperature. After the hydrochloric acid was removed by the stream of Argon, the enone **(137)** (415.4 mg, 2.258 mmol) in dry DMF (4 mL) was added dropwise. The reaction mixture was stirred at room temperature for 8.5 h.; then brine (10 mL) and ethyl acetate (20 mL) were added, the stirring was continued for 10 min. The two layers were separated and the aqueous layer was extracted with ethyl acetate (2x4 mL). The combined organic extracts were washed with brine (3x5 mL), dried (Na$_2$SO$_4$), and the solvent was removed in rotatory evaporator yielding a dark liquid which was immediately purified by flash chromatography (silica gel, ethyl acetate/hexane, 3:7) producing 579.5 mg (1.942 mmol, 86%) of a viscous liquid which produced a waxy, light yellow solid on refrigeration (mp= 50-54 °C). this product was suitable for the next reaction. An analytical sample was obtained by distillation of the solid (Kugelrohr, 100-110 °C/0.1 torr). R_f= 0.35 (hexanes/ethyl acetate, 9:1); **mp**= 56.5-57 °C; **IR** (neat) υ 2955, 1694, 1383, 1251, 1220, 839 cm^{-1}; **^{1}H NMR** (CDCl$_3$) δ 6.88 (ddd,J= 10.2, 1.1, 1.0 Hz; 1H), 6.09 (dd, J= 10.2, 1.0 Hz; 1H); 4.56 (m,1H), 4.44 (m, 2H), 1.41 (s,3H), 1.40 (s, 3H), 0.90 (s, 9H), 0.17 (s, 3H), 0.15 (s, 3H);**^{13}C NMR** (CDCl$_3$) δ 194.3, 148.4, 127.9, 110.1, 79.7,

74.4, 67.2, 27.4, 25.7, 18.0, -4.7; **Anal. Calcd.** for $C_{15}H_{26}SiO_4$: C, 60.36; H,8.78. **Found**: C, 60.23; H, 8.81.

Conduritol A acetonide (148). Chlorobenzene diol (**99**, X=Cl) (340 mg, 2.14 mmol) was dissolved in DMP (5 mL) and acetone (2 mL), with stirring at room temperature. A small crystal of p-TsOH was added; and the stirring continued for 1h. Then 10% NaOH solution (2 mL), brine (2 mL), and Et_2O (10 mL) were added. After 10 min of stirring the layers were separated, the aqueous layer was extracted with Et_2O (5 mL); the combined ethereal extracts were washed with brine (3x3 mL). The organic solution was dried (Na_2SO_4) and evaporated to produce 450 mg (112%) of crude acetonide (#). The crude acetonide was dissolved in CCl_4 (50 mL) and mixed with tetraphenylporphine (6.1 mg); the solution was irradiated, as oxygen was bubbled through; the process continued, at ~20°, for 4 h. Evaporation of 90% of the solvent followed by trituration of the dark green solution, with hexane and treatment with activated charcoal (400 mg) produced a light yellow solution which upon evaporation gave 510 mg (109%) of crude crystalline endoperoxide (**139**). The crude endoperoxide was dissolved in Et_2O (50 mL) and two drops of water were added. The solution was treated with aluminum amalgam (freshly prepared from 0.5 g of aluminum foil). The reaction mixture was stirred for 25 min, filtration through celite and evaporation of the solvent gave 310 mg (1.648 mmol, 77%) of essentially pure Conduritol A acetonide(**148**). It was recrystalized from CH_2Cl_2-hexane. **Mp** =100.5-101° C (Lit. 101-102° C); **¹H NMR** (CDCl₃) δ 5.88 (s, 2H), 4.20 (s, 4H), 2.49 (br s, 2H), 1.43 (s, 3H), 1.34 (s,3H). Physical data were in agreement with those in literature.[451]

(1R,2R,3S,4S)-4-t-butyldimethylsilyloxy-5,6-O-isopropylidene-1-hydroxycyclo hexa-2-ene (149); (1S,2R,3S,4S)-4-t-butyldimethylsilyloxy-5,6-O-isopropylidene-1-hydroxycyclohexa-2-en (150); (1R,2R,3S,4S)-4-t-butyldimethylsilyloxy-5,6-O-isopropylidene cyclohexanol (151). (2S,3S,4R)-4-t-Butyldimetylsilyloxy-5-cyclohexenone (**141**) (83.6 mg, 0.28 mmol) was dissolved in dry methanol (2 mL), protected from moisture; the solution was cooled in an ice bath and a solution of $NaBH_4$ in MeOH (10.6 mg in 5 mL) was added. Stirring was continued for 35 min (the reaction was monitored by TLC). Then 10 drops of saturated solution of NH_4Cl were added and the mixture stirred for 10 min at room temperature. The reaction was diluted with ethyl acetate (5 mL) and washed with brine (5 mL); after the separation of , the aqueous layerwas extracted with ethyl acetate (2x3 mL). The combined

organic extracts were dried and evaporated to produce 83.7 mg of a mixture of three compounds, which were separated by preparative TLC (silica gel; hexane/ethyl acetate, 7:3). **149**: R_f= 0.44 (hexane/ethyl acetate, 7:3); $[\alpha]_D^{25}$= -120.4° (c 0.0116, MeOH); **IR** (neat) υ 3476, 2955, 2930, 2857, 1472 cm^{-1}; **^{1}H NMR** (CDCl$_3$) δ 5.91 (s, 1H), 5.90 (s, 1H), 4.48 (dd, J= 6.8, 4.6 Hz, 1H), 4.37 (dd, J= 7.2, 4.2 Hz, 1H), 4.32-4.24 (m, 2H), 2.55 (d, J= 7.7 Hz, 1H), 1.38 (s, 3H), 1.35 (s, 3H), 0.84 (s, 9H), 0.06 (s, 3H), 0.03 (s, 3H); ^{13}**C NMR** (CDCl$_3$) δ 132.9, 132.1, 109.1, 79.0, 75.8, 67.3, 65.0, 26.3, 25.7, 24.6, 18.0, -4.2, -4.5; **MS** (EI, 70 eV) m/z (rel. intensity) 184 (M+, 11), 169 (26), 155 (20), 100 (66), 97 (100); **Anal. calcd.** for C$_{15}$H$_{28}$O$_4$Si: C, 59.96; H, 939. **Found** : C, 59.85; H, 9.40. **150**:R_f= 0.-- (hexane/ethyl acetate, 7:3); **IR** (neat) υ 3448, 3052, 2888, 2857, 1472, 662 cm-1; **^{1}H NMR** (CDCl$_3$) δ 6.06 (dd, J=9.8, 4.2 Hz, 1H), 5.92 (dd, J=9.8, 4.2 Hz, 1H), 4.28 (m, 2H), 4.19 (t, J= 3.8 Hz, 1H), 4.11 (m, 1H), 2.83 (d, J= 8.4 Hz, 1H), 1.37 (s, 3H), 1.31 (s, 3H), 0.87 (s, 9H), 0.11 (s, 3H), 0.09 (s, 3H). **151**: R_f= 0.-- (hexane/ethyl acetate, 7:3); $[\alpha]_D^{25}$= -40.2° (c 0.395, MeOH); **IR** (neat) υ 3472, 2986, 2892, 1472, 665 cm^{-1}; **^{1}H NMR** (CDCl$_3$) δ 4.30 (dd, J= 5.8, 3.9 Hz, 1H), 3.94 (t, J= 5.8 Hz, 2H), 3.75 (m, 1H), 2.01 (d, J= 8.0 Hz, 1H), 1.85-1.60 (m, 4H), 1.48 (s, 3H), 1.34 (s, 3H), 0.85 (s, 9H), 0.05 (s, 3H), 0.04 (s, 3H); ^{13}**C NMR** (CDCl$_3$) δ 109.0, 80.4, 71.0, 67.6, 27.4, 27.1, 25.8, 25.5, 18.0; **Anal. calcd.** for C$_{15}$H$_{30}$O$_4$Si: C. 59.56; H, 9.99. **Found** : C, 59.47; H, 10.05.

(1R,2R,3S,4S)-1-Hydroxy-2,3-O-isopropylidene-4-t-butyldimethyl silyloxycyclohex-5-ene (149). To the enone **141** (107 mg, 0.359 mmol) in THF (0.15 mL) L-Selectride (1M solution, 0.35 mL, 0.35 mmol) at 0 °C. The solution was stirred for 50 min, then 10% NaOH solution (1.0 mL) was added followed by 30% hydrogen peroxide (1.0 mL); stiring was continued for 20 min at 0 °C. The mixture was saturated with K$_2$CO$_3$ and extracted with ethyl acetate (4x3 mL); the combined organic fractions were dried (Na$_2$SO$_4$), filtered and concentrated to leave a yellow oil. The crude product was purified by Flash chromatography (silica gel; hexane/ethyl acetate, 8:2) affording 55 mg (0.183 mmol, 51%) of protected Conduritol C **(149)**. R_f= 0.44 (hexane/ethyl acetate, 7:3); $[\alpha]_D^{25}$= -120.4° (c 0.0116, MeOH); **IR** (neat) υ 3476, 2955, 2930, 2857, 1472 cm-1; **^{1}H NMR** (CDCl$_3$) δ 5.91 (s, 1H), 5.90 (s, 1H), 4.48 (dd, J= 6.8, 4.6 Hz, 1H), 4.37 (dd, J= 7.2, 4.2 Hz, 1H), 4.32-4.24 (m, 2H), 2.55 (d, J= 7.7 Hz, 1H), 1.38 (s, 3H), 1.35 (s, 3H), 0.84 (s, 9H), 0.06 (s, 3H), 0.03 (s, 3H); ^{13}C **NMR** (CDCl$_3$) δ 132.9, 132.1, 109.1, 79.0, 75.8, 67.3, 65.0, 26.3, 25.7, 24.6, 18.0, -

4.2, -4.5; **MS** (EI, 70 eV) *m/z* (rel. intensity) 184 (M+, 11), 169 (26), 155 (20), 100 (66), 97 (100); **Anal. calcd.** for $C_{15}H_{28}O_4Si$: C, 59.96; H, 939. **Found** : C, 59.85; H, 9.40.

Epoxidation of 141 (153). (2S,3S,4R)-4-*t*-butyldimethylsilyloxy-2,3-O-isopropylidene-5-cyclohexenone **(141)** (65 mg, 0.215 mmol) was dissolved in isopropanol-methanol (6:4, 5 mL), the solution was cooled to -45 °C (temperature of the cooling bath) and a mixture of 30% H_2O_2 (0.7 mL) and 1.7M LiOH (0.1 mL) was added dropwise with an efficient stirring. When the addition was completed the temperature of the bath was raised to -35 ° and the stirring continued for 23 min. (the reaction progress was monitored by TLC). Then brine (5 mL) was added at -35° and the temperature allowed to go up to room temperature, sodium chloride was added to saturate the solution, which was then extracted with Et_2O (1x15, 2x5 mL). The combined ethereal extracts were dried and evaporated producing a light yellow liquid which was filtered through a small column of flash silica gel (hexane-ethyl acetate, 8:2) to produced 66 mg (0.208 mmol, 97%) of a colorless liquid. An analytical sample was obtained by distillation (Kugelrohr, 100 °C/0.05 Torr). R_f= 0.5 (hexane/ethyl acetate,8:2); $[\alpha]^{20}_D$= -55.8° (c 6.4, MeOH); **IR** (neat) υ 2955, 2858, 1742, 1255, 1212, 1117, 899, 838 cm^{-1}; **^1H NMR** (CDCl3) δ 4.73 (d, J= 9.1 Hz, 1H), 4.45 (dd, J= 9.1, 6.1 Hz, 1H), 4.00 (d, J= 6.1 Hz, 1H), 3.50 (m, 2H), 1.45 (s, 3H), 1.33 (s, 3H), 0.93 (s, 9H), 0.14 (s, 3H), 0.13 (s, 3H); **^{13}C NMR** (CDCl3) δ 201.4, 109.2, 78.5, 76.0, 72.5, 59.7, 54.9, 26.3, 25.6, 23.9, 18.0, -4.56, -5.0; **Anal.Calcd** for $C_{19}H_{26}SiO_5$: C, 57.29; H, 8.33. **Found**: C, 57.21; H, 8.37.

(1S,2S,3R,4S)-6-Chloro-4-(N-benzylamino)-1,2-O-isopropylidene-3-hydroxycy-clohexa-5-en (156). To a stirred solution of the epoxide **(154)** (140 mg. 0.691 mmol) in CHCl3 (15 ml),at room temperature, neutral alumina (Fluka, 4.0 g) was added followed by Benzylamine (0.2 mL, 1.83 mmol). The reaction continued for 4 h. and MeOH (5 mL) was added, the was stirring continued for 15 min, and then the mixture was filtered through celite and the solid residue was washed thoroughly with 10% MeOH in CHCl3 . The solvent was evaporated under reduced pressure and the resulting residue was purified by flash chromatography (Silica gel; hexane/ethyl acetate, 7:3) to give 145 mg of the aminoalcohol **(156)** (0.469 mmol, 68%). Analytical sample was obtained by recrystalization from dichloromethane-hexane. R_f= 0.5 (hexane/ethyl acetate, 1:1); **mp**= 106-106.5 °C; $[\alpha]_D^{23}$= +38° (c 0.25, MeOH); **IR** (film) υ 3307, 2921, 1217, 1073 cm^{-1}; **^1H NMR** (CHCl3) δ 7.32-7.30 (m, 5H), 6.06 (d, J= 1.6 Hz, 1H), 4.60 (d, J= 6.5

Hz, 1H), 4.14 (dd, J= 8.5, 6.5 Hz, 1H), 3.93 (d, J= 12.9 Hz, 1H), 3.74 (d, J= 12.9 Hz,
1H), 3.51 (t, J= 8.5 Hz, 1H), 3.16 (br d, J= 8.5, 1H), 1.51 (s, 3H), 1.39 (s, 3H); ^{13}C
NMR (CHCl$_3$) δ 139.5 (C), 129.2 (CH), 128.6 (CH), 128.2 (CH), 127.4 (C, CH),
110.9 (CH), 78.4 (CH), 75.7 (CH), 71.9 (CH), 58.3 (CH), 50.7 (CH$_2$), 28.1 (CH$_3$),
25.9 (CH$_3$); MS (CI) m/z (rel. intensity) 310 (M+1, 100), 252 (15), 209 (2), 106 (15),
91 (18); Anal. calcd for C$_{16}$H$_{20}$O$_3$ClN: C, 62.03; H, 6.50; Found: C, 61.46; H,
6.37.

**(1S,2S,3R,4S)-6-Bromo-4-(N-benzylamino)-1,2-O-isopropylidene-
3-hydroxycyclo hexa-5-ene (156a).** To a solution of the epoxide (154) (140 mg,
0.691 mmol) in CHCl$_3$ (15 ml), was added neutral alumina (Fluka, 4.0 g) and then under
efficient stirring benzylamine (0.2 mL, 1.83 mmol) was added. The reaction was
continued for 4 h. and then MeOH (5 mL) was added, the stirring was continued for 15
min. The mixture was filtered through celite and the solid residue was throroughly washed
with 10% MeOH in CHCl$_3$. The solvent was evaporated under reduced pressure and the
resulting residue was purified by flash chromatography (Silica gel; Hexane/ethyl acetate,
7:3) to give 145 mg of the aminoalcohol (156a) (0.469 mmol, 68%). An analytical sample
was obtained by recrystallization from dichloromethane-hexane. R$_f$= 0.5 (hexane/ethyl
acetate, 1:1); mp= 117-118 °C; [α]$_D^{23}$= +40.88° (c 0.38, MeOH); IR (film) υ 3500-
2800, 1638, 1227, 1073 cm^{-1}; ^{1}H NMR (CDCl$_3$) δ 7.32 (m, 5H), 6.30 (d, J= 1.9 Hz,
1H), 4.66 (d, J= 6.5 Hz, 1H), 4.13 (dd, J= 8.5, 6.5 Hz, 1H), 3.94 (d, J= 12.9 Hz, 1H),
3.74 (d, J= 12.9 Hz, 1H), 3.53 (t, J= 8.6 Hz, 1H), 3.11 (dt, J= 8.6, 1.6 Hz, 1H), 3.0-
2.0 (br s, 1H), 1.75 (br s, 1H), 1.53 (s, 3H), 1.41 (s, 3H); ^{13}C NMR (CDCl$_3$) δ 139.3
(C), 133.4 (C), 128.1 (CH, C), 128.1 (CH, C), 127.3 (CH), 119.0 (C), 110.0 (C), 78.3
(CH), 77.0 (CH), 71.5 (CH), 59.2 (CH), 50.6 (CH$_2$), 28.1 (CH$_3$), 25.8 (CH$_3$); MS (CI)
m/z (rel. intensity) 354 (M+1, 100),276 (20), 106 (3), 91 (25) ; Anal. calcd for
C$_{16}$H$_{20}$O$_3$BrN: C, 54.42; H, 5.69; N, 3.95; Found: C, 54.19; H, 5.69; N, 3.97.

**(2S,3S,4R,5S)-5-azido-2,3-O-isopropylidene-t-butyldimethylsilyloxi
cyclohexanone (165).** To the dry enone (dried by melting it under vaccum) (141) (259
mg, 0.87 mmol), trimethylsilylazide (TMSN$_3$, 5 eq., 0.57 mL) was added under argon
atmosphere. The reaction mixture was stirred at room temperature for 45 h. After
evaporation of the reaction mixture in high vaccum, 307 mg of a liquid were obtained
(103%). GC showed only one compound, but TLC showed the azido ketone (165) with

some starting material left. An analytical sample was obtained by HPLC (reverse phase). R_f= 0.31 (CH$_2$Cl$_2$); $[\alpha]_D^{25}$= +15 (c 0.6, MeOH); **IR** (neat) υ 2954, 2858, 2102, 1736, 1254, 1064 cm^{-1}; **^{1}H NMR** (CDCl$_3$) δ 4.32 (m, 2H), 4.13 (dd, J= 2.9, 2.1 Hz, 1H), 3.95 (ddd, J= 8.9, 6.8, 2.1 Hz, 1H), 2.72 (d, J= 4.1, 1H), 2.69 (d, J= 1.88 Hz, 1H), 1.41 (s, 3H), 1.32 (s, 3H), 0.89 (s, 9H), 0.14 (s, 3H), 0.12 (s, 3H); **^{13}C NMR** (CDCl$_3$) δ 203.5 (C), 111.1 (C), 78.5 (CH), 77.1 (CH), 70.8 (CH), 57.4 (CH), 38.5 (CH$_2$), 26.7 (CH$_3$), 25.6 (CH$_3$), 24.9 (CH$_3$), 18.(C), -4.9 (CH$_3$), -5.1 (CH$_3$); **Anal.Calcd.** for C$_{15}$H$_{27}$N$_3$SiO$_4$: C, 52.76; H, 7.97; N, 12.30. **Found**: C, 52.87; H, 8.01; N, 12.22.

(3S,4R,5R,6R)-3-azido-6,5-O-isopropylidene-4-t-butyldimethylsilyl oxy-1,6-lactone (167). The ketone **165** (47.8 mg, 0.14 mmol) was dissolved in CHCl$_3$ (1 mL) and a solution of MCPBA (36.3 mg, 0.21 mmol) in CHCl$_3$ (2 mL). The reaction mixtute was stirred at room temperature for 7 h; then saturated solution of NaHCO$_3$ was added and the stirring continued for 15 min. After separation of layers the organic layer was washed with satd. NaHCO$_3$ solution (2x3 mL), brine (2x3 mL), dried and the solvent evaporated to produce 54.7 mg. The crude product was purified by flash chromatography (silica gel; ethyl acetate/hexane, 2:8) to give 35.5 mg (0.099 mmol, 71%) of lactam **167**. R_f= 0.3 (ethyl acetate/hexane, 2:8); **IR** (film) υ 2930, 2900, 2130, 1720, 1250 cm^{-1}; **^{1}H NMR** (CDCl$_3$) δ 5.78 (d, J= 3.0 Hz, 1H), 4.36 (dd, J= 7.8, 3.0 Hz, 1H), 3.97 (m, 2H), 3.03 (dd, J= 14.8, 9.9 Hz, 1H), 2.30 (dd, J= 14.8, 7.0 Hz, 1H), 1.56 (s, 3H), 1.39 (s, 3H), 0.89 (s, 9H), 0.12 (s, 3H), 0.10 (s, 3H).

(3S,4R,5S,6S)-5-azido-1-chloro-4-hydroxy-5,6-O-isopropylidene cyclohexane (155). The epoxide (**154**) (133 mg, 0.657 mmol), sodium azide (2.628 mmol, 171 mg) and dry ammonium chloride (2.628 mmol, 141 mg) were dissolved in a mixture of DME-EtOH-H$_2$0 (1.5:1:1). The solution was heated at 80° for 1 h. After cooling, brine (15 mL) and ethyl acetate (5 mL) were added, stirring continued for 10 min and separated; the aqueous layer was extracted with ethyl acetate (3x5 mL). The combined organic extracts were dried (Na$_2$SO$_4$) and evaporated to produce 192 mg of a slightly yellow solid, which was purified by flash chromatography (Silica gel; hexanes/ethyl acetate,7;3) producing 141 mg (0.574 mmol, 87.8%) of pure azidohydrin (**155**). An analytical sample was obtained by recrystallization from CH$_2$Cl$_2$-hexane. R_f= 0.09 (Silica gel; hexanes/ethyl acetate, 8:2); **Mp**=94-94.5° ;$[\alpha]_D^{23}$= -10.2 (c 0.96, MeOH); **IR** (film)

υ 3454, 2113, 1250, 1085, 1074, 869 cm^{-1}; **^{1}H NMR** (CDCl$_3$) δ 5.87 (d, J= 2.1 Hz, 1H), 4.6 (d, J= 6.4 Hz, 1H), 4.16 (dd, J= 8.7, 6.4 Hz,1H), 3.96 (dd, J= 8.7, 1.4 Hz, 1H), 3.69 (td, J= 8.6, 3.0 Hz, 1H), 2.88 (d, J= 3.0 Hz, 1H), 1.53 (s, 3H), 1.40 (s, 3H); **^{13}C NMR** (CDCl$_3$) δ 131.0 (C), 126.6 (CH), 111.5 (C), 77.9 (CH), 75.6 (CH), 73.1 (CH), 61.3 (CH), 28.1 (CH$_3$), 25.9 (CH$_3$); **Anal. calcd for** C$_9$H$_{12}$N$_3$O$_3$Cl: C, 44.0; H, 4.92; N, 17.10; **Found:** C, 44.11; H, 4.92; N, 17.07.

(3S,4R,5S,6S)-4-azido-1-chloro-3-t-butyldimetylsilyloxi-5,6-O-iso propylieden cyclohexane (155). TBSCl (96.5 mg, 0.64 mmol,) was dissolved in dry DMF (2 mL) under argon, then diisopropylethylamine (0.81 mmol, 0.14 mL) was added; the HCl fumes produced were removed by the flow of Argon. Then a solution of the azidoalcohol (**155**), in dry DMF (0.5 mL), was added , at room temperature. The reaction mixture was stirred at room temperature for 26 h, then H$_2$0 (3 mL) and Et$_2$O were added and stirred for 15 min followed by separation of both layers. The aqueous layer was extracted with Et$_2$O (2x3 mL); the combined ethereal extracts were washed with brine (2x5 mL), then dried and evaporated. The product was purified by flash chromatography (Silica gel; hexanes/ethyl acetate, 9:1) to give 39.6 mg (51%) of protected alcohol (**165**). An analytical sample was obtained by preparative TLC. **R$_f$**= 0.69 (hexanes/ethyl acetate, 8:2); [α]$_D^{23}$= 32.85 (c 0.56, MeOH); **^{1}H NMR** (CDCl$_3$) δ 5.88 (d, J= 2.73 Hz, 1H), 4.55 (d, J= 6.2 Hz, 1H), 4.14 (td, J= 6.2, 0.8 Hz, 1H), 3.75 (m, 2H), 1.49 (s, 3H), 1.38 (s, 3H), 0.88 (s, 9H), 0.13 (s, 3H), 0.11 (s, 3H); **^{13}C NMR** (CDCl$_3$) δ 133.0 (C), 124.6 (CH), 110.9 (C), 77.8 (CH), 75.2 (CH), 72.3 (CH), 27.8 (CH$_3$), 25.7 (CH$_3$), 18.1 (C), -4.4 (CH$_3$), -4.9 (CH$_3$); **Anal Calcd.** for C$_{15}$H$_{26}$N$_3$O$_3$ClSi: C,50.05; H, 7.28; N, 11.67; **Found:** C, 50.18; H, 7.30; N, 11.60.

(1S,2S,3S,4R)-4,6-Dichloro-1,2-O-isopropyledene-3-hydroxycyclohexa-5-ene (160), (1S,2S,3S,4S)-4,6-Dichloro-1,2-O-isopropyledene-3-hydroxycyclohexa-5-ene (161). Method A. A solution of epoxide **152** (270 mg, 1.33 mmol) in anhydrous Et$_2$O (4 mL) was titrated with a solution of ferric chloride in anhydrous Et$_2$O and the reaction was monitored by TLC (silica gel; hexane/ethyl acetate, 8:2) for the disappearence of epoxide. The reaction mixture was cooled in an ice bath and quenched with saturated NaHCO$_3$ (2 mL) and brine (3 mL) and diluted with Et$_2$O (10 mL). After separation the aqueous layer was extracted with Et$_2$O (3x3 mL); the combined ethereal extracts were washed with brine (1x3 mL), dried (Na$_2$SO$_4$) and the solvent

135

evaporated to give 302.6 mg of crude material, which was separated by flash chromatogarphy (silica gel, hexane/ethyl acetate, 7:3) producing 10.6 mg of *cis*-chlorohydrin **160** (0.044 mmol, 3.3%), and 192.4 mg of *trans*-chlorohydrin **161** (0.805 mmol, 60.5%).

Method B. To a solution of epoxide **154** (350 mg, 1.78 mmol) in anhydrous THF (10 mL) was added a solution of TBSCl (518 mg, 3.456 mmol) and Et3N (3 drops) in THF (3 mL), at room temperature. After stirring 5 min under argon, titanium isopropoxide (0.17 mL, 0.518 mmol) was slowly added and the stirring was continued for 24 h at room temperature. Then more TBSCl (280 mg, 1.86 mmol) and titanium isopropoxide (0.15 mL) were added and the reaction was stirred for another 24 h. The reaction was quenched with saturated NaHCO3 (6 mL), brine (4 mL) and diluted with Et2O (30 mL). After separation the aqueous layer was extracted with Et2O (1x10 mL) and ethyl acetate (1x5 mL). The organic extracts were washed with brine (1x15 mL), dried with Na2SO4, and the solvent evaporated. The crude product was purified by flash chromatography (silica gel, hexane-ethyl acetate, 7:3) producing 277 mg of *trans*-chlorohydrin **161** (1.162 mmol, 67.2%) and 6.3 mg of epoxide **154** (0.03 mmol). *cis*-Chlorohydrin (**160**) : R_f= 0.4 (hexane-ethyl acetate, 7:3); **mp**= 73-74 °C ; $[\alpha]_D^{26}$= -120° (c 0.12, MeOH); **IR** (KBr) υ 3436, 2990, 1649, 1081 cm^{-1}; **^{1}H NMR** (CDCl3) δ 6.00 (d, J= 3.9 Hz, 1H), 4.76 (dd, J= 3.9, 3.6 Hz, 1H), 4.65 (d, J= 5.6 Hz, 1H), 4.49 (t, J= 6.0 Hz, 1H), 4.17 (dd, J= 6.0, 3.6 Hz, 1H), 2.48 (br s, 1H), 1.48 (s, 3H), 1.43 (s, 3H); **^{13}C NMR** (CDCl3) δ 130.6 (C), 128.6 (CH), 111.5 (C), 77.6 (CH), 75.6 (CH), 74.2 (CH), 58.1 (CH), 28.1 (CH3), 25.9 (CH3); **HRMS Calcd for** C9H12Cl2O3: 239.024174; **Found**: 239.023682. *trans*-chlorohydrin (**161**): R_f= 0.32 (hexane-ethyl acetate, 7:3); $[\alpha]_D^{26}$= -7.3° (c 2.08, CHCl3); **IR** (neat) υ 3436, 2990, 1649, 1081 cm^{-1}; **^{1}H NMR** (CDCl3) δ 6.04 (dd, J= 2.0, 1.0 Hz, 1H), 4.63 (d, J= 6.3 Hz, 1H), 4.38 (ddd, J= 8.4, 2.0, 1.0 Hz, 1H), 4.18 (dd, J= 8.4, 8.4 Hz, 1H), 3.81 (t, J= 8.4 Hz, 1H), 3.11 (br s, 1H), 1.56 (s, 3H), 1.43 (s, 3H); **^{13}C NMR** (CDCl3) δ 130.5 (C), 128.7 (C), 111.6 (C), 77.5 (CH), 75.7 (CH), 74.3 (CH), 58.2 (CH), 28.0 (CH3), 25.9 (CH3); **MS** (CI) *m/z* (rel. intensity) 239 (M+1, 100), 223 (20), 145 (20), 89 (18); **HRMS Calcd for** C9H13Cl2O3: 239.024175; **Found**: 239.021317.

(1R,2R,3R,6R)-6-azido-4-chloro-2,3-O-isopropyledene-1-hydroxy cyclohex-4-ene (158). To a solution of chlorohydrin (**161**) (277.7 mg, 1.59 mmol) in dry DMF (10 mL) sodium azide (151 mg, 2.324 mmol) was added under argon

atmosphere. The reaction mixture was stirred at room temperature for 15 h, and then at 80 °C for 4.5 h. The reaction mixture was diluted with ether (20 mL) and extracted with 10% $Na_2S_2O_3$ (1x3 mL), brine (2x5 mL); the organic layer was dried (Na_2SO_4) and the solvent evaporated. The oil resulted was purified by flash chromatography (silica gel; hexane/ethyl acetate, 7:3) producing 257.9 mg of the azidohydrin (**158**) (1.431 mmol, 66%). R_f= 0.55 (hexane/ethyl acetate, 7:3); **mp**= 93.5-94 °C (from CH_2Cl_2-hexane) ; $[\alpha]_D^{27}$= - 99 (c 0.68, MeOH); **IR** (KBr) υ 3884, 2115, 1651, 1383 cm^{-1}; **^{1}H NMR** ($CDCl_3$) δ 5.9 (dd, J= 3.6, 0.5 Hz, 1H), 4.58 (dd, J= 5.6, 1.1 Hz, 1H), 4.39 (t, J= 5.6 Hz, 1H), 4.23 (m, 1H), 4.19 (m, 1H), 2.49 (br s, 1H), 1.42 (s, 3H), 1.38 (s, 3H); **^{13}C NMR** ($CDCl_3$) δ 134.7 (C), 122.2 (CH), 110.9 (C), 75.9 (CH), 75.0 (CH), 69.4 (CH), 27.6 (CH_3), 26.0 (CH_3); **MS** (CI) m/z (rel. intensity) 246 (M$^+$, 100), 160 (35), 145 (60), 96 (100); **Anal. calcd for** $C_9H_{12}ClN_3O_3$: C, 44.00; H, 4.92; N, 17.10; **Found**: C, 44.05; H, 4.95; N, 17.03.

Methyl (2S,3S,4S,5R)-4-azido-6-hydroperoxy-6-methoxy-5-*t*-butyl dimethylsilyloxy-2,3-O-isopropylidene hexanoate (173). To a solution. of (3S,4R,5S,6S)-3-azido-4-*t*-butyldimethylsilyloxy-5,6-O-isopropylidenecyclohexene (**155**) (6.31.4 mg, 1.85 mmol) in methanol (25 mL) sodium bicarbonate (933 mg, 11.11 mmol) was added. The mixture was cooled to -78° C and a stream of O_3/O_2 was passed until a blue color persisted for 5 min. After the excess of O_3 was removed at -78°, the temperature was rised to room temperature and stirring continued for 20.5 h. The solvent was evaporated, without heating (CAUTION: The solution was peroxide active), in a rotatory evaporator. The reside was taken in ethyl acetate and filtered through celite, the flask and solid were thoroughly washed with ethyl acetate. The filtrated was evaporated to give a viscous liquid (~100%). The crude material was homogeneous by TLC and could be used for the next reaction without purification. An analytical sample was obtained by flash chromatography (Silica Gel[dry packing]; hexane/ethyl acetate, 7:3) and subsequent recrystalization from CH_2Cl_2-hexane. R_f= 0.5 (hexane/ethyl acetate,7:3); **mp**= 95.5-96 °C; $[\alpha]^{25}_D$= +38.31 (c 0.355; MeOH); **IR** (film) υ 3346, 2094, 1712, 1116, 837 cm^{-1}; **^{1}H NMR** ($CDCl_3$) δ 8.6 (br s, 1H), 4.9 (d, J=8.1 Hz, 1H), 4.5 (t, J= 5.8 Hz, 1H), 4.4 (t, J= 8.8 Hz, 1H), 4.0 (d, J= 8.8 Hz, 1H), 3.7 (s, 3H), 3.6 (s, 3H), 1.56 (s, 3H), 1.37 (s, 3H), 0.87 (s, 3H), 0.10 (s, 3H), 0.07 (s, 3H); **^{13}C NMR** ($CDCl_3$) δ 171.5, 110.9, 105.2, 79.2, 75.3, 61.3, 56.7, 26.6, 25.5, 18.5, -4.0, -4.8; **Anal Calcd.** for $C_{17}H_{33}N_3O_8Si$: C,46.88; H, 7.63; **Found:** C, 46.99; H, 7.84.

Methyl (2S,3S,4S,5R)-4-azido-6-hydroxy-5-*t*-butyldimethylsilyloxy-2,3-O-isopropylidene hexanoate (175). A solution of hydroperoxide (**173**) (617.7 mg, 1.42 mmol) in anhydrous THF (5 mL) was cooled in an ice bath under argon. Then NaBH$_4$ (67 mg, 1.42 mmol) was added, with strong stirring MeOH (0.21 mL) was then slowly added. The reaction was continued for 35 min and quenched with saturated ammonium chloride solution.(ten drops) and H$_2$O (1 mL). The mixture was saturated with sodium chloride, and extracted with ethyl acetate (3x5 mL). Evaporation of the solvent gave a liquid, which crystalized on storing in the refrigerator. Purification by Flash chromatography (Silica gel [dry packing]; hexane/ethyl acetate,6:4), produced 288.2 mg (0.74 mmol, 52%) of the ester alcohol (**175**). Washing the column with ethyl acetate provided 67.4 mg (0.18 mmol; 12.7%) of the corresponding 1,6 diol (**176**). An analytical sample of the ester alcohol (**175**) was obtained by recrystalization from ethyl acetate-hexane. **R$_f$**= 0.5 (hexane/ethyl acetate, 1:1); **mp**= 95.5-96.5° C; [α]^{25}$_D$= +56.73° (c 0.49, MeOH); **IR** (film) υ 3484, 2116, 1755, 1209 cm^{-1}; **^{1}H MNR** (CDCl$_3$) δ 4.50 (t, J= 6.0 Hz, 1H), 4.45 (t, J= 6.0 Hz, 1H), 3.93 (m, 2H), 3.81 (dd, J= 10.9, 6.7 Hz, 1H), 3.71 (m, 1H), 3.30 (td, J= 6.7, 1.5 Hz, 1H), 1.92 (br s, 1H), 1.56 (s, 3H), 1.37 (s, 1H), 0.87 (s, 3H), 0.09 (s, 3H), 0.06 (s, 3H); **^{13}C-NMR** (CDCl$_3$) δ 170 0 (C), 110.9 (C), 78.9 (CH), 75.5 (CH), 71.4 (CH), 61.3 (CH$_2$), 52.0 (CH), 26.5 (CH$_3$), 25.9 (3 CH$_3$), 25.6 (CH$_3$), 18.4 (C), -4.1 (CH$_3$), -4.8 (CH$_3$); **Anal Calcd for:** C$_{16}$H$_{31}$N$_3$O$_6$Si : C, 49.34; H, 8.02: **Found:** C, 49.58; H 8.17.

(2S,3S)-2,3-O-isopropylidene-4-[1(R)-1-*t*-butyldimethylsilyloxy ethyl-2-hydroxy]-γ-butyrolactam (177). The azido-ester (**175**) (189.6 mg, 0.487 mmol) was dissolved in MeOH (15 mL) and 10% Pd/C (41 mg) was added. The mixture was hydrogenated at 45 psi for 13 h. The mixture was filtered through celita and the solvent evaporated to produce 157.7 mg ; this crude material was purified by flash chromatography (silica gel; ethyl acetate) to give 99.3 mg (0.297 mmol, 60.9%).The material was used for the next reaction.

(2S,3S)-2,3-O-isopropylidene-4-[1(R)-1,2-di-*t*-butyldimethylsilyl oxyethyl]-γ-butyrolactam. To a solution of lactam **177** (99 mg, 0.295 mmol) in dry DMF (1.5 mL) TBSCl (133.5 mg, 0.886 mmol) and DIPEA (0.19 mL, 1.123 mmol) were added at room temperature, under argon atmosphere. The mixture was stirred at room

138

temperature for 19 h; then diluted with ether (10 mL) and brine (3 mL), stirring continued for 15 min and the layers separated. The organic layer was washed with brine (2x3 mL), dried (Na2SO4) and the solvent evaporated to produce a yellow oil. The crude product was purified by flash chromatography (silica gel; ethyl acetate/hexane, 7:3) producing 104.7 mg (0.232 mmol, 78.8%) of colorless oil which crystallized on storage. R_f= 0.31 (hexane/ethyl acetate, 7:3); **mp**= 147-148 °C; $[\alpha]_D^{24}$= 16.9° (c 0.88, MeOH); **^{1}H NMR** (CDCl3) δ 6.54 (br s, 1H), 4.46 (d, J= 6.7 Hz, 1H), 4.29 (dd, J= 6.7, 4.8 Hz, 1H), 3.95 (m, 1H), 3.73 (dd, J= 10.6, 8.1, 1H), 3.59 (m, 2H), 1.43 (s, 3H), 1.34 (s, 3H), 0.85 (s, 9H), 0.83 (s, 9H), 0.08 (s, 3H), 0.03 (s, .9H); **^{13}C NMR** (CDCl3) δ 169.1, 110.4, 78.9, 76.5, 73.6, 68.3, 62.4, 54.3, 26.9, 25.8, 25.6, 24.9, 18.2, 17.9, –4.5; **Anal.** calcd. for $C_{21}H_{43}NO_5Si_2$: C, 56.58; H,9.72; N,3.14; **Found**: C, 56.30; H, 9.67; N, 3.10.

(1S,2R,3S,4S)-5-chloro-3,4-O-isopropylidene-2-benzyloxi-1-hydroxycyclohex-5-en (183) and (1S,2R,3S,4S)-5-chloro-3,4-O-isopropylidene-1-benzyloxy-2-hydroxycyclohex-2-en (184).

To a solution of triflic anhydride (0.1 mL, 0.565 mmol) in dry CH2Cl2 (5 mL), at -78 °C in an argon atmosphere, a solution of benzylic alcohol (0.06 mL, 0.565 mmol) and 2,6-*t*-butyl-4-methyl pyridine (116 mg, 0.565 mmol) in CH2Cl2 (5 mL) was added dropwise. The mixture was stirred at -78 ° for 15 min, then a solution of trans-diol (179) (96 mg, 0.435 mmol) and 2,6-*t*-butyl-4-methyl pyridine (134 mg, 0.652 mmol) in CH2Cl2 (5 mL) was added dropwise; the stirring was continued at -78 ° for an additional 15 min. the reaction was slowly warmed to room temperature and continued for 1.5 h. Brine (10 mL) was added and the layers were separated. The organic layer was washed with brine (2x5 mL), dried (Na2SO4) and concentrated to give a light brown solid. The mixture was separated by flash chromatography (silica gel; hexane/EtOAc, 8:2) to afford 66 mg of **183** (0.214 mmol, 49%) and 50.6 mg of **184** (0.163 mmol, 37%). Analytical samples were obtained by recrystallization from petroleum ether for **183** and from CH2Cl2-petroleum ether for **184**. **183: mp**= 77.0-77.5 °C; R_f= 0.55 (silica gel; hexane/EtOAc, 8:2); $[\alpha]_D^{25}$= -28.53 ° (c 0.34, MeOH); **FTIR** (neat) 3416, 2989, 2935, 1383, 1217, 1091, 1069, 910, 732 cm^{-1}; **^{1}H NMR** (CDCl3) δ 7.35 (m, 5H), 6.02 (d, J= 4.0 Hz,1H), 4.78 (d, J= 11.7 Hz, 1H), 4.66 (d, J= 11.7 Hz, 1H), 4.58 (d, J= 5.7 Hz, 1H), 4.42 (t, J= 5.7 Hz, 1H), 4.18 (br t, J= 4.2 Hz, 1H), 3.79 (t, J= 5.3 Hz, 1H), 2.66 (br s, 1H), 1.51 (s, 3H), 1.39 (s, 3H); **^{13}C NMR** (CDCl3) δ 138.2 (C), 132.6 (C), 128.9 (2CH), 128.5 (CH), 128.3

(2CH), 111.5 (C), 78.3 (CH), 77.4 (CH), 75.9 (CH), 73.5 (CH$_2$), 68.3 (CH), 28.2 (CH3), 26.6 (CH3); **mass spectrum** (El, 70 eV) m/e (rel intensity) 295 (M-15, 0.03), 191 (0.5), 91 (1.0), 59 (0.8); **Anal. calcd for** C$_{16}$H$_{19}$O$_4$Cl : C,61.83; H, 6.16; **Found**: C, 61.99; H, 6.13. **184: mp**= 91.5-92.5 °C; **R$_f$**= 0.35 (hexane/EtOAc, 8:2); **[α]$_D^{25}$**= +16.67 ° (c 0.51, MeOH); **FTIR** (neat) 3444, 2988, 2933, 1381, 1072 cm^{-1}; **^{1}H NMR** (CDCl$_3$) δ 7.31 (m, 5H), 6.02 (d, J= 1.4 Hz, 1H), 4.70 (d, J= 11.6 Hz, 1H), 4.63 (d, J= 11.6 Hz, 1H), 4.59 (br d, J= 6.6 Hz, 1H), 4.14 (dd, J= 8.7, 6.6 Hz, 1H), 3.91 (dd, J= 8.7, 0.9 Hz, 1H), 3.72 (t, J= 8.7 Hz, 1H), 2.67 (br s, 1H), 1.53 (s, 3H), 1.40 (s, 3H); **^{13}C NMR** (CDCl$_3$) δ 138.0 (C), 129.6 (C), 129.2 (CH), 128.9 (2CH), 128.4 (2CH), 128.2 (CH), 111.7 (C), 78.3 (CH), 78.0 (CH), 76.3 (CH), 73.6 (CH), 72.7 (CH$_2$), 28.5 (CH$_3$), 26.3 (CH$_3$); **MS** (CI) m/z (rel intensity) 311 (M+1, 8), 293 (13), 235 (80), 145 (60), 91 (100); **Anal. Calcd. for** C$_{16}$H$_{19}$O$_4$Cl: C,61.83; H, 6.16; **Found**: C, 61.65; H, 6.14.

(1R,2R,3R,6R)-6-azido-4-chloro-2,3-O-isopropyledene-1-trimethyl silyoxicyclohex-4-ene (187). The azidohydrin **(158)** (460 mg, 1.87 mmol) was dissolved in dry pyridine (5 mL) under anhydrous conditions, then hexamethyldisilasane (HMDS) (0.78 mL, 3.74 mmol) was added followed by trimethylchlorosilane (TMSCl) (0.23 mL, 1.87 mmol) at room temperature. The reaction mixture was stirred at room temperature for 12 h. The mixture was diluted with Et$_2$O (20 mL) and quenched with water (5 mL); the organic layer was washed with saturated CuSO$_4$ solution (5x3 mL), water (1x5 mL) and brine (2x5 mL). The ethereal solution was dried (Na$_2$SO$_4$) and the solvent evaporated to produce 571 mg of homogeneous material (by TLC). Purification by flash chromatography (silica gel; hexane/ethyl acetate, 9:1) gave 538 mg (1.28 mmol, 68.7%) of **187. R$_f$**= 0.45 (hexane/ethyl acetate, 9:1); [α]$_D^{27}$= - 83.4 (c 1.64, MeOH); IR (film) ʋ 2940, 2095, 1380, 1230, 845 cm^{-1}; **^{1}H NMR** (CDCl$_3$) δ 5.87 (d, J= 3.2 Hz, 1H), 4.53 (m, 1H), 4.23 (m,1H), 3.85 (m, 1H), 1.40 (s, 3H), 1.38 (s, 3H), 0.22 (s, 9H); **^{13}C NMR** (CDCl$_3$) δ 134.4, 122.9, 110.4, 76.7, 75.2, 71.0, 58.1, 27.6, 26.6, - 0.05.

(2S,3R)-2,3-O-Isopropylidene-4(1(R)-azido-2-hydroperoxy-2-methoxyethyl)-γ-butyrolactone (188) and Methyl (2S,3R,4R,5R,6X)-5-azido-6-hydroperoxyl-2,3-O-isopropylidene-4-trimethylsilyloxy-6-methoxylhexanoate (189). Method A. To a solution of (2S,3R,4R,5S)-5-azido-1-

chloro-2,3-O-isopropylidene-4-trimethylsilyloxycyclohex-6-ene (**187**) (874 mg, 2.093 mmol) in dry methanol (20 mL) sodium bicarbonate (1.054 mg, 12.55 mmol) was added; the mixture was cooled to -78 ° and ozonized, until the blue color persisted. The excess of ozone was removed using Argon, at -78 °. Then the cooling bath was substituted by an ice-water bath and stirring continued for 5.5 h. The solvent was evaporated under reduced pressure and without heeating (CAUTION: the solution gave positive test for peroxides). The residue was taken in ethyl acetate and filtered through celite; evaporation of the solven gave the peroxide (**188**) practically pure in quantitaive yield. An analytical sample was obtained by susesive recrystalizations from ethyl acetate-hexane. R_f= .0.36 (hexane/ethyl acetate, 1:1); **mp**= 101-102 °C (decomp., gas evolution) ; $[\alpha]_D^{24}$= + 28.32 (c 0.925, MeOH); **IR** (neat) υ 3250, 2900, 2045, 1755, 1160 cm^{-1}; ^{1}**H NMR** (CDCl$_3$) δ 5.0 (d, J= 2.2 Hz, 1H), 4.84 (m, 2H), 4.39 (dd, J= 9.8, 3.2 Hz, 1H), 4.10 (ddd, J= 9.8, 3.2, 1.5 Hz, 1H), 3.64 (s, 3H), 1.46 (s, 3H), 1.40 (s, 3H); ^{13}**C NMR** (CDCl$_3$) δ 172.9, 114.6, 106.4, 76.1, 75.8, 75.6, 60.5, 58.4, 26.7, 25.9; **MS** (CI) m/z (rel. intensity) 272 (M-17, 30), 244 (77), 184 (100), 142 (65), 128 (80), 85 (75); **HRMS calcd for** C$_{10}$H$_{15}$O$_7$N$_3$: 272.0882604; **Found**: 272.088516.

Method B. The procedure described above was repeated using 2% water MeOH, after work-up the crude product showed to be a mixture of **188/189** in a 7:3 ratio.

Method C. The procedure described in Method A was repeated but using 10% water MeOH. (NOTE: the reaction mixture did not turn blue). After work-up 92% of crude product was isolated consisting in the peroxide **189**, contaminated with traces of **188** and the starting material (**187**). Peroxide **189**: R_f= .0.63 (hexane/ethyl acetate, 1:1); $[\alpha]_D^{22}$= + 37.39 (c 0.845. MeOH); **IR** (neat) υ 3360, 2960, 2095, 1740, 1090 cm^{-1}; ^{1}**H NMR** (CDCl$_3$) δ 8.81 (br s, 1H), 4.9 (d, J= 7.2 Hz, 1H), 4.64 (d, J= 6.0 Hz, 1H), 4.48 (dd, J= 9.0, 6.0 Hz, 1H), 3.82 (m, 2H), 3.74 (s, 3H), 1.56 (s, 3H), 1.36 (s, 3H); ^{13}**C NMR** (CDCl$_3$) δ 170.8, 110.5, 106.5, 78.6, 75.8, 75.7, 73.2, 62.5, 56.9, 51.8, 26.5, 25.7.

Methyl (2S,3R,4R,5R,6X)-5-azido-6-hydroxy-2,3-O-isopropylidene-4-trimethylsilyloxyhexanoate (190). To a cooled (0 °C) solution of the peroxide (**189**) (113.8 mg, 0286 mmol) in dry THF (10 mL) sodium borohydride (10.8 mg, 0.287 mmol) was added; then MeOH (0.035 mL, 0.858 mmol) was slowly added, with efficient stirring. The reaction progress was monitored by TLC 9silica gel, hexane/ethyl acetate, 1:1), and it was cmpleted in 50 min. The reaction was quencehed with saturated amonium chloride solution (15 drops), brine (2 mL) and Ethyl acetate (5 mL). Afte the separation of

layers, the organic layer was extracted with ethyl acetate (2x3 mL); the combiend organic extracts werre dried (Na_2SO_4) and the solvent evaporated. The crude product was purified by chromatography (silica gel; hexane/ethyl aceteta, 1:1) to produce 44.7 mg (0.125 mmol. 44%) of the alcohol **190**. R_f= 0.27 (hexane-ethyl acetate, 1:1); $[\alpha]_D^{24}$= +45.7° (c 0.0155, MeOH); **IR** (neat) υ 3480, 2940, 2080, 1740, 1375 cm^{-1}; **^{1}H NMR** (CDCl$_3$) δ 4.45 (d, J= 6.3 Hz, 1H), 4.30 (dd, J= 7.9, 6.3 Hz, 1H), 3.96-3.92 (m, 2H), 3.82 (m, 1H), 3.75 (s, 3H), 3.54 (m, 1H), 1.56 (s, 3H), 1.35 (s, 3H), 0.11 (s, 3H); **^{13}C NMR** (CDCl$_3$) δ 170, 110.8, 78.8, 75.8, 72.9, 64.2, 61.6, 26.4, 25.6, 0.3; **HRMS** calcd. for $C_{13}H_{26}O_6NSi$: 320.1529413; **Found**: 320.152995.

2,3-O-isopropylidene-4-trimethylsilyloxy-6-t-butyldimethylsilyloxy-1,5-mannonolactam (192).

(2S,3R,4S,5R)-2,3-O-isopropylidene-4-hydroxy-6-*t*-butyldimethylsilyloxy-1,5-mannonolactam (90.2 mg, 0.269 mmol) was dissolved in dry pyridine (1.5 mL) under argon, then hexamethyldisilazane (HMDS) (0.04 mL, 0.29 mmol) and trimethylchlorosilane (TMSCl) 0.12 mL, 0.591 mmol) at romm temperature. The reaction mixture was stirred for 20 h, then the mixture was diluted with ethyl acetate (10 mL) and water (3 mL). Pyridine was removed by washing with $CuSO_4$ solution; the organic layer was then washed with water (1x3 mL), brine (2x3 mL); then the organic solution was dried and evaporated to give 100 mg. The crude product was purified by flash chromatography (silica gel; ethyl acetate/hexane, 1:1) producing 83.3 mg of **192** (0.242 mmol. 90%). R_f= 0.42 (hexane/ethyl acetate, 1:1); $[\alpha]_D^{27}$= 16.4° (c 1.43, MeOH); **IR** (neat) υ 3300, 2910, 1660, 810 cm^{-1}; **^{1}H NMR** (CDCl$_3$) δ 6.17 (br s, 1H), 4.6 (d, J= 7.8 Hz, 1H), 4.27 (t, J= 7.8 Hz, 1H), 3.96 (dd, J= 8.7, 4.0 Hz, 1H), 3.57 (m, 2H), 3.4 (td, J= 8.7, 4.0 Hz, 1H), 1.48 (s, 3H), 1.37 (s, 3H), 0.86 (s, 9H), 0.05 (s, 9H), 0.03 (s, .6H); **^{13}C NMR** (CDCl$_3$) δ 168.0, 110.9, 78.9, 73.0, 71.4, 63.4, 54.7, 27.2, 25.8, 25.0, 18.2, 1.9, −4.2; **MS** (CI) *m/z* (rel. intensity) 446 (M+1, 0.8), 332 (100), 274 (60), 89 (45).

2,3-O-isopropylidene-4,6-dit-butyldimethylsilyloxy-1,5-mannono lactam (201).

2,3-O-isopropylidene-4-hydroxy-6-t-butyldimethylsilyloxy-1,5-mannonolactam (87.1 mg, 0.263 mmol) was dissolved in dry CH_2Cl2 (3 mL) and DBU was added at room temperature under argon. Then a solution of TBSCl (86.8 mg, 0.578 mmol) in CH2Cl2 was added. The reaction mixture was stirred for 24 h at room temperature and the diluted with ether (20 mL) and brine (5 mL). After separation of layers,

the aqueous layer was extracted with ether (2x5 mL). The ethereal extracts were washed with water (1x3 mL), brine (1x5 mL) and dried. The solvent was evaporated to give a yellow oil which cristallized on storing at low temperature. The crude product was purified by flash chromatography (silica gel, ethyl acetate/hexane, 1:1) producing 80.4 mg (0.184, 70%). R_f= 0.37 (ethyl acetate/hexane, 1:1); **IR** (neat) υ 2940, 2850, 1690, 1250, 830 cm⁻¹; **¹H NMR** (CDCl₃) δ 6.0 (br s, 1H); 4.57 (d, J= 7.2 Hz, 1H), 4.25 (dd, J= 8.6, 7.2 Hz, 1H), 3.95 (dd, J= 10.0, 3.6 Hz, 1H), 3.55 (dd, J= 9.0,6.5 Hz, 1H), 3.45 (t, J= 10.0 Hz, 1H), 3.32 (m, 1H), 1.5 (s, 3H), 1.37 (s, 3H), 0.90 (s, 9H), 0.14 (s, 6H), 0.09 (s, 3H), 0.08 (s, 3H).

Preparation of amidine 202. Lactam **201** (229.4 mg, 0.52 mmol) was dissolved in dry chloroform (20 mL) under argon and then chlorosulfonyl isocyanate (CSI) (0.068 mL, 0.52 mmol) was added at room temperature. The reaction mixture was stirred for 3 h 40 min. Then 30% NaOH (3 mL) was added and the stirring continued for 1 h 10 min. After separation of layers the aqueous layer was extracted with CHCl3 (2x5 mL). The combined organic extracts were dried (Na2SO4) and evaporated to give 293.3 mg of a white solid. The crude product was used without purification. **¹H NMR** is showed in Appendix V.3, #10.

V. APPENDIX

V. 1. Culture Medium Composition

MSB-Arginine Solution

Solution A......................................40 mL
Solution B......................................20 mL
Solution C.......................................5 mL
Arginine•HCl....................................2 g
                 H_2O to make one liter, then adjust pH to 6.0-6.2.

Solution A composition:

$NaHPO_4•7H_2O$	1 M
KH_2PO_4	1 M

Solution B composition:

Nitrilo triacetic acid	10 00 g
$MgSO_4$	14.45 g
$CaCl_2•H_2O$	3.33 g
$(NH_4)_6Mo_7O_{24}•4H_2O$	9.25 mg
$FeSO_4•7H_2O$	99.0 mg

              H_2O to make one liter.

Solution C composition:

$(NH_4)_2SO_4$	60 g/300 mL

V. 2. Graphs from the initial optimization of diol production.(Section III.1)
Graph # 1
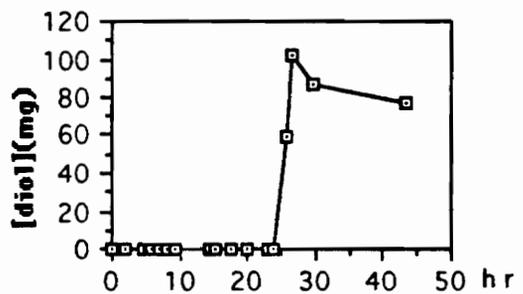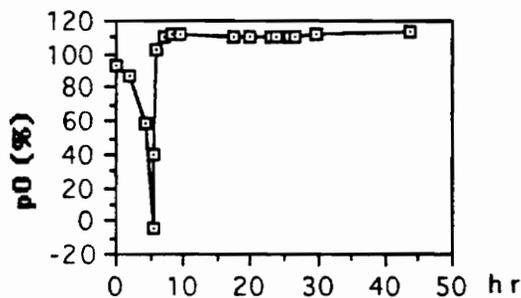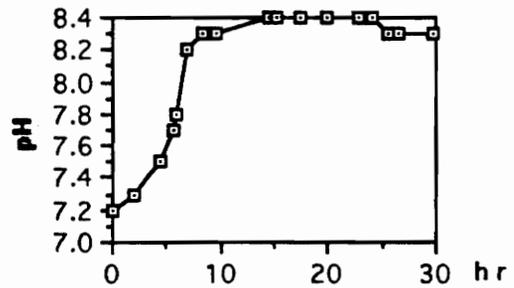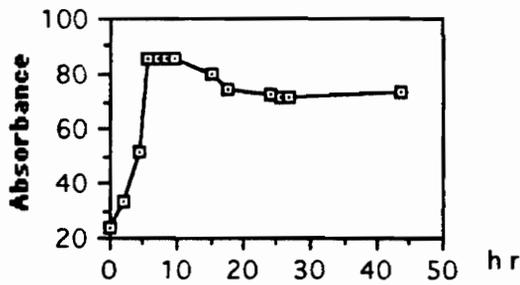
Appendix. Data #1

Data	time (hr)	Absorbance	pH	pO (%)	[Diol](mg)
1	0	23.5	7.2	94	-
2	2	33.4	7.3	87.5	-
3	4.5	51.6	7.5	58	-
4	5.6	85.7	7.7	-4.2	-
5	5.7	-	-	40	-
6	5.9	-	7.8	103	-
7	7	85.7	8.2	111.3	-
8	8.3	85.5	8.3	111.6	-
9	9.5	85.4	8.3	111.8	-
10	14.5	-	8.4	110.2	-
11	15.	80.2	8.4	-	-
12	17.5	73.9	8.4	110.7	-
13	19.9	-	8.4	110.6	-
14	22.9	-	8.4	110.3	-
15	24	72.6	8.4	110.4	-
16	25.6	71.9	8.3	110.6	59.5
17	26.6	71.6	8.3	111	101.5
18	29.7	-	8.3	111.8	87.5
19	43.5	73.5	-	113.2	77

Graph # 2
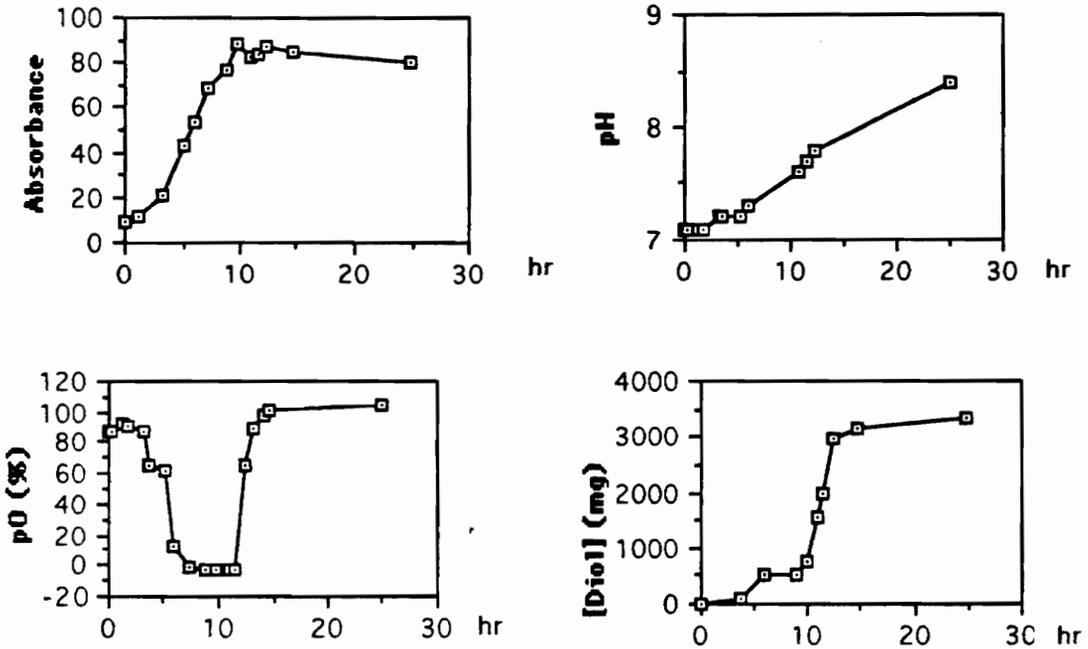
Data Graph # 2

Data	time (hr)	Absorbance	pH	pO (%)	[Diol] (mg)
1	0	9.3	7.1	87	0
2	0.3	-	7.1	87	-
3	1.2	11.3	7.1	92.7	-
4	1.7	-	7.1	91.2	-
5	3.2	21	7.2	87.7	-
6	3.6	-	7.2	65.4	91
7	5.2	42.8	7.2	61.1	-
8	5.9	53.7	7.3	12	525
9	7.2	68.4	-	-1.3	-
10	8.9	77	-	-2.6	525
11	9.8	88.9	-	-2.6	770
12	10.8	82.5	7.6	-2.6	1575
13	11.5	83.9	7.7	-2.7	1995
14	12.6	86.8	7.8	65	1975
15	13.6	-	-	89.8	-
16	14.1	-	-	97.3	-
17	14.6	84.7	-	101.1	3150
18	24.9	79.9	8.4	103.8	3360

146
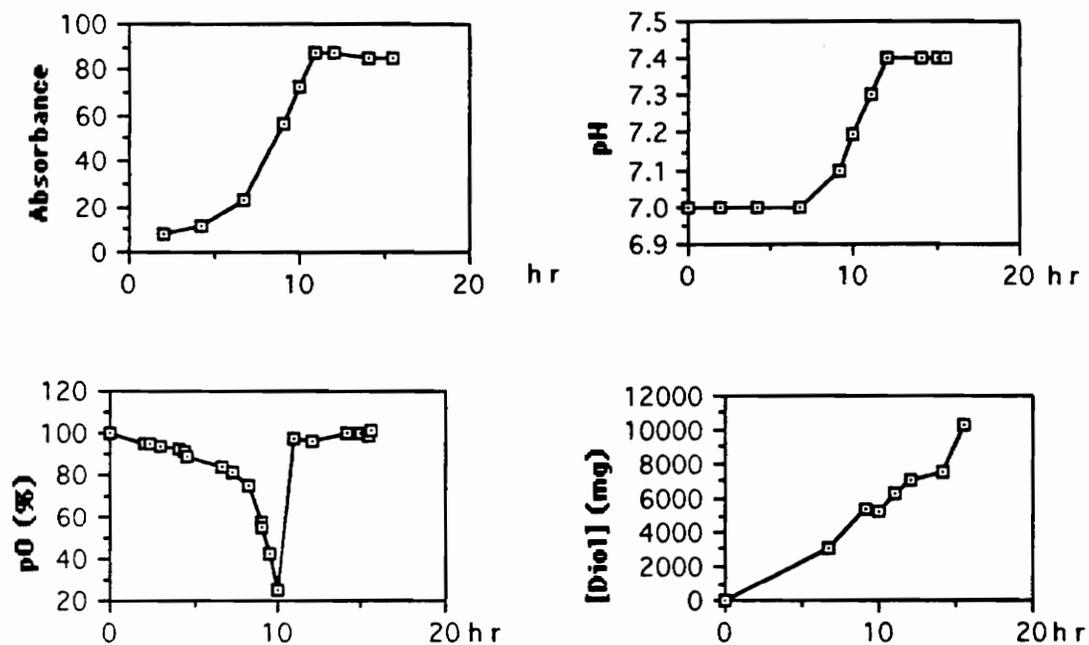
Graph #3

Data Graph # 3

Data	time (hr)	Absorbance	pH	pO (%)	[Diol](mg)	v. PhCl
1	0	-	7.0	100	o	73
2	2	8	7.0	94.6	-	-
3	2.4	-	-	94.5	-	-
4	3	-	-	93.9	-	-
5	4.2	11.2	-7.0	92.1	-	-
6	4.5	-	-	91.3	-	-
7	4.6	-	-	88.8	-	-
8	6.7	22.5	-7.0	83.3	3045	63
9	7.3	-	-	80.7	-	-
10	8.2	-	-	74.7	-	-
11	9	-	-	58.0	-	-
12	9.1	56.6	7.1	54.7	5320	60
13	9.6	-	-	43	-	-
14	10	72.3	7.2	25.4	5250	59
15	11	86.6	7.3	97.2	6300	57
16	12	87.8	7.4	96.8	7000	55
17	14.1	85.1	7.4	100.4	7560	-
18	15	-	7.4	100.2	-	-
19	15.4	-	-	98.4	-	-
20	15.5	84.4	7.4	100.7	10325	50

Graph # 4

Data Graph # 4

Data	time (hr)	Absorbance	pH	pO (%)	[Diol](mg)	v. PhCl
1	0	11.7	7.1	99.9	-	80
2	0.96	-	7.1	102.0	-	79
3	1.49	-	7.0	99.8	-	-
4	2.20	20.6	7.1	96.5	1050	78.5
5	2.48	-	7.1	93.9	-	-
6	3.00	-	7.3	88.9	-	-
7	3.66	42.2	6.8	69.1	1750	76
8	4.26	-	-	39.5	-	-
9	4.49	-	-	28.3	-	-
10	4.65	-	-	20.6	-	-
11	4.83	-	-	10.0	-	-
12	5.14	-	-	2.0	-	-
13	5.37	-	-	24.9	-	-
14	5.38	85	7.2	76.1	3500	74
15	5.98	-	-	99.9	-	-
16	7.03	88.5	7.3	101.9	4550	72
17	7.6	-	-	102.0	-	-
18	9.1	85.8	-	102.3	5600	69
19	25	81.9	7.5	106.7	6650	50

148

V. 3. Selected Spectra.

1. (4S,8S)-6,6-Dimethyl-5,7-dioxabicyclo[3.3.0]octa-2-en-1-ona (**103**).

2. 3(R)-Methylcyclopentene-1-carboxaldehyde (**126**).

3. L-Erythruronolactone (**132**).

4. (3S,4R,5S,6S)-5-Azido-1-chloro-4-hydroxy-5,6-isopropylidenecyclohexane (**155**).

5. Methyl (2S,3S,4S,5R)-4-Azido-6-hydroxyperoxy-6-methoxy-5-t-butyldimetylsilyloxy-2,3-O-isopropylidenehexanoate (**173**).

6. (1S,2S,3R,4S)-6-Chloro-4-(N-benzylamino)-1,2-O-isopropylidene-3-hydroxycyclohexa-5-ene (**156**).

7. (1S,2S,3S,4S)-6,4-Dichloro-1,2-O-isopropylidene-3-hydroxycyclohexa-5-ene (**161**).

8. (1R,2R,3R,6R)-6-Azido-4-chloro-2,3-O-isopropylidene-1-hydroxycyclohexa-4-ene (**158**).

9. Methyl (2S,3R,4R,5R,6X)-5-Azido-6-hydroperoxy-6-methoxy-2,3-O-isopropylidene-4-trimethylsilyloxyhexanoate (**189**).

154

173

MeO, OMe, HOO, TBSO, N₃

P591C#18

BpM=0   I=10⁶   Hm=0   TIC=65

HL, II-237

Sys DICI

Cal FFKJL19A

Acid TH   FT= 0°

100
50
0
133   159   189   MASS
80   100   120   140   160   180   200   220

#18   1.0
65534000

100
50
0
245   252   274   303   316   348   360   374   MASS
380   400   420   440   460   480   500   520

#18   1.0
65534000

332

50
25
0
390   408   432   444   474   490   MASS

#18   2.0
65534000

156

S137A04   ×1   Bgd=1   21-MAR-91 15:34+0:01:07   70E-HF   C1+
BpM=0   I=804mV   Hm=0   TIC=23464000   Acnt:TH   Sys:DICI
HL III-025   PT= 0°   Cal:PFKMR21A

100   #4   1.0
                                                                    5274000
50

0   85   91   106   209   MASS
      80   100   1.0   140   160   180   200   220

100   310   #4   1.0
                                                                    5274000
50

0   234   252   MASS
     240   260   280   300   320   340   360   380   400

161

161

161

158

189

MeO—C(=O) OMe O HOO N₃ OTMS

HLIV024
AU PROG:
POWGATE.AU
DATE 18-7-91

SF   67.925
SY 67.9300000
O1   3300.000
SI   8192
TD   8192
SW 17241.379
HZ/PT   4.209

PW   0.0
RD   0.0
AQ   .238
RG   800
NS   6000
TE   297

FW   21600
D2   4416.000
DP   5H DO

LB   1.000
GB   0.0
CX   25.00
CY   0.0
F1   180.003P
F2   -9.998P
HZ/CM 516.231
PPM/CM 7.600
SR   -4986.77
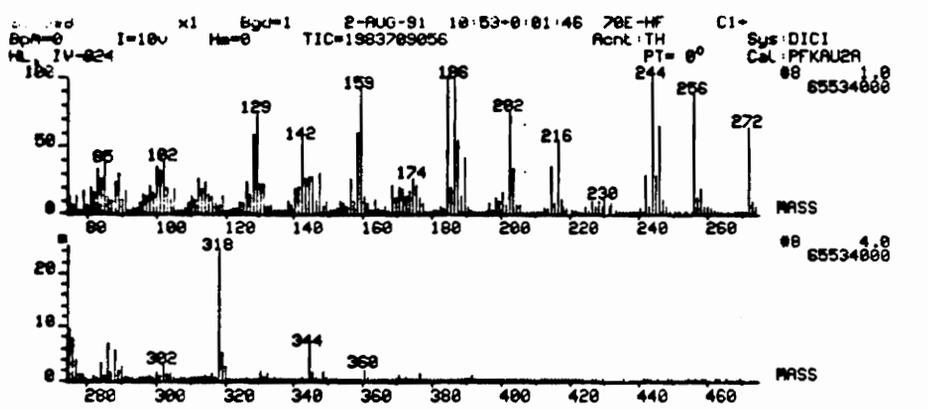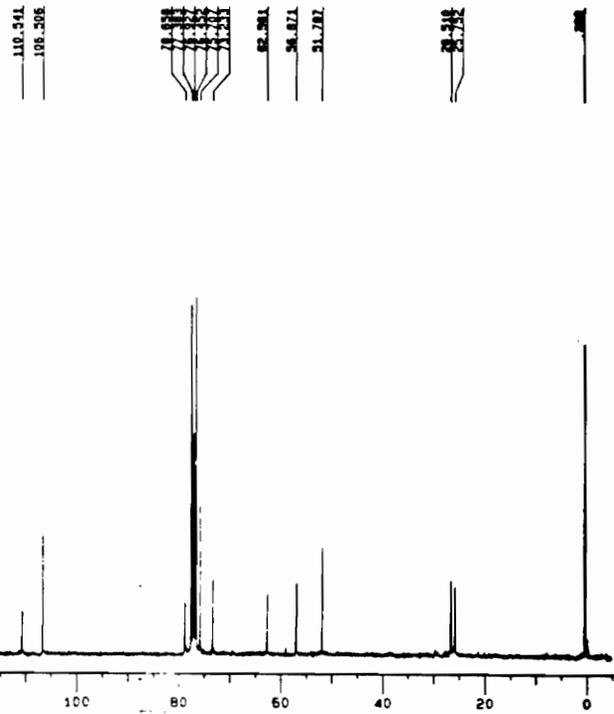
110.541 106.506   82.961 56.871 51.782   23.752

160 140 120 100 80 60 40 20 0

x1 Bgd=1   2-AUG-91 18:53+0:01:46 70E-HF CI+
BpA=0   I=10v Hm=0   TIC=1983789056   Acnt:TH   Sys:DICI
HL IV-024   PT= 0°   Cal:PFKAU2A

85 102 129 142 159 174 186 202 216 230 244 256 272

318 302 344 360

166
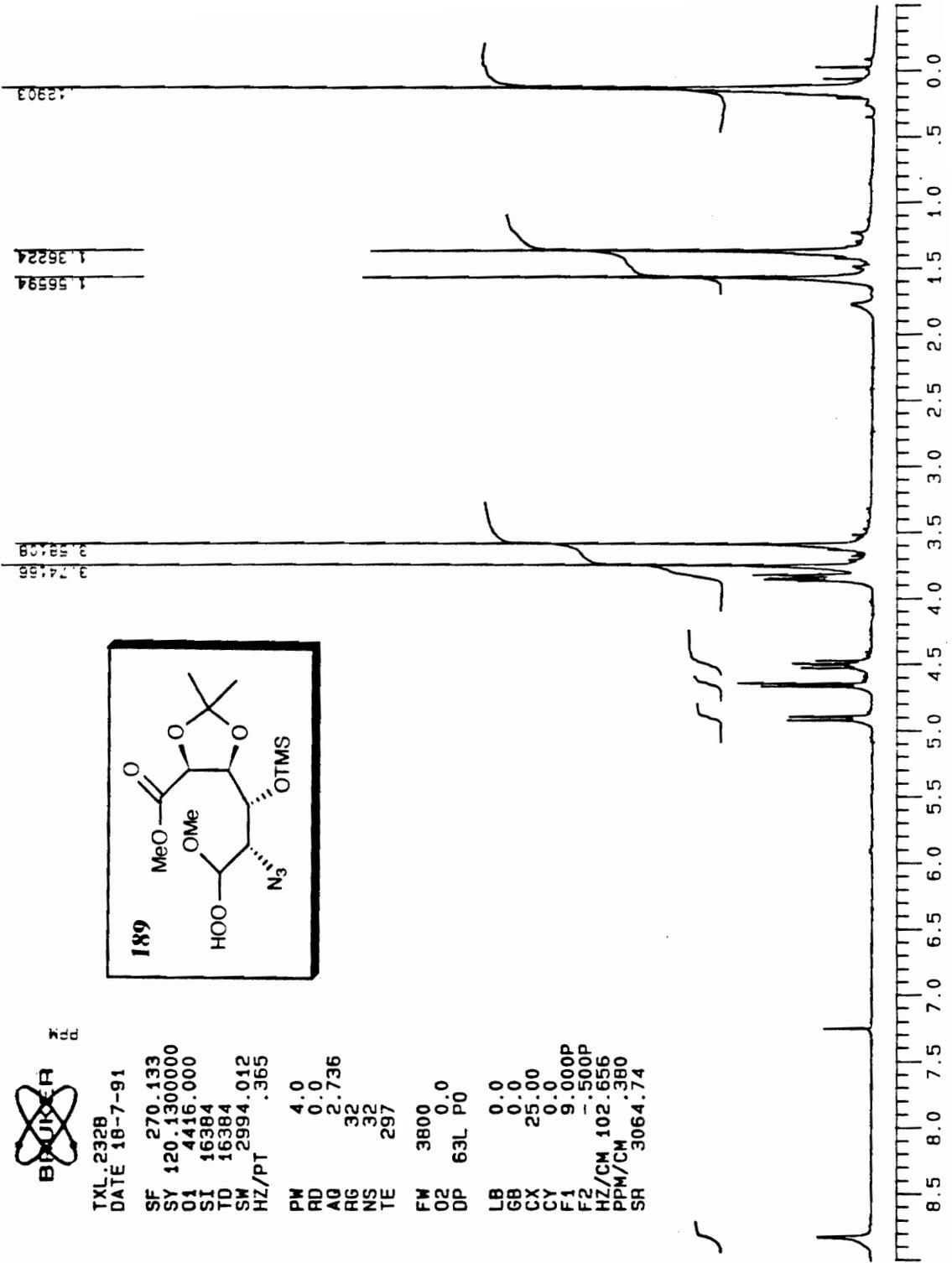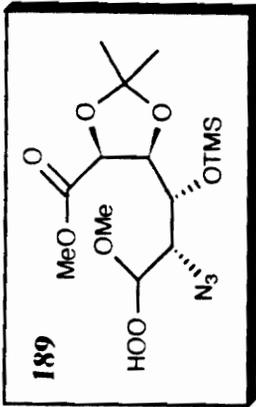
VI. REFERENCES.

1.   Newman, P. *Optical Resolution for Chiral Compounds* , vols. 1-3, Optical Resolution Center, New York.

2.   Schuring, V.; Nowotny, H.-P. *Angew. Chem. , Int. Ed. Engl.* **1990**, *29*, 939; Köning, W.A. *The practice of enantiomer Separation by Capillary Gas Chromatography*, Hünthig, Heidelberg, 1987; Pirkle, W.H.; Pochapsky, T.C. *Chem. Rev.* **1989**, *89*, 347; Däppen, R.; Arm, H.; Meyer, V.R. *J. Chromatogr.* **1986**, *373*, 1.

3.   Morrison, J.D.(Ed.), *Asymmetric Synthesis, vol. 1, Analytical Methods*; Academic Press, New York, 1983.; Allenmark, S.G. *Chromatographic Enantioseparation: Methods and Applications*; Ellis Horwood, Chichister, 1988.

4.   Coppola, G.M.; Schuster, H.F. *Asymmetric Synthesis, construction of chiral molecules using aminoacids*; Wiley, New York, 1987.

5.   Szabo, W.A.; Lee, H.T. *Aldichimica Acta* **1980**, *13*, 13

6.   C. Mori, K. *Tetrahedron Lett.* **1975**, *31*, 3011.

7.   a) Tomioka, K.; Ishiguro, T.; Koga, K. *Chem. Comunn.* **1979**, 652; b) Tomioka, K; Koga, K *Tetrahedron Lett.* **1979**, 3315, 8272.

8.   Stork, G.; Takahashi, I.; Suzuki, T. *J. Am. Chem. Soc.* **1978**, *100*, 8272.

9.   Hanessian, S.; Roy, R *J. Amer. Chem. Soc.* **1979**, *101,* 5839; Hanessian, S. *Total synthesis of Natural Products:The Chiron Approach*; Pergamon Press, New York, 1983.

10.   Schneider, M.P. (Ed.) *Enzymes as Catalyst in Organic Synthesis*, D. Reidel Publishing Co., Boston, 1986.

11.   Porter, R.; Clark, S. (Eds.) *Enzymes in Organic Synthesis* Ciba Foundation Symposium III, Pitman, London, 1985.

12.   Huang, F.C.; Lee, L.F.H.; Mittal, R.S.D.; Ravikumar, P.R.; Chau, J.A.; Sih, C.S. *J. Am. Chem. Soc.* **1976**, *97,* 4144

13.   Nara, T.; Okachi, R. *J. Antibiot.* **1971**, *24*, 321.

14.   Gibson, D.T.; Koch, J.R.; Schuld, C.L.; Kallio, R.E. *Biochemistry* **1968**, 7, 3195; Gibson, D.T.; Cardini, G.E.; Maseles, F.C.; Kallio, R.E. *Biochemistry* **1970**, *9*, 1631; Gibson, D.T.; Hensley, M.; Mabry, T.J. *Biochemistry* **1970**, *9*, 1626.

169

15. Storm, D.R.; Koshland, Jr, D.E. *Proc. Natl. Acad. Sci. USA* **1970**, *66*, 445.

16. Yamada, H.; Shimizu, S. *Angew. Chem., Inter. Ed. Engl.* **1988**, *27*, 622.

17. Rhem, H.; Reed, H. (Eds): a) *Biotechnology*, vol. 6A, Verlag Chemie Weinheim, 1984; b) *Biotechnology*, vol. 3, Verlag Chemie Weinheim, 1983; c) *Biotechnology*, vol. 4, VCH Verlangsgesellschaft, Weinhein, 1985, p.150.

18. Yamada,.H.; Shimizu S, in. *Ullmann's Encyclopedia of Industrial Chemistry* vol. 4A, Verlagsgesellschaft VCH.; Weinkein 1985, p. 150.

19. Tramper J.; Vander Plas H. C. , Linko P. (Eds.): *Biocatalysis in Organic Synthesis* (Studies in Organic Chemistry, 22) Elsevier, Amsterdan 1985

20. Rosazza, J. P. (ed): *Microbial Transformations of Bioactive Compounds* ; .vol. 1 and 2, CRC Press, Boca Raton, Fla; 1982.

21. Skryabin, G.R.; Golovelea, L.A.M. *Microorganims in Organic Chemistry*, Nauka, Moscow, 1976.

22. Demain, A.L. *Science*, **1981**, *214*, 987.

23. Chibata, I. (Ed.), *Inmobilized Enzymes-Research and Development*, Kodansha, Tokyo, 1978.

24. Klibanou, A.M. *Science*, **1983**, *219*, 722.

25. Rosazza, J.P. in *Anticancer Agents Based on Natural Products Models*; Cassady, J.M.; Douros, J.D. (Eds.); Academic Press, 1980, p. 437.

26. Lamba, S.S.; Wlker, C.A. (Eds.) *Antibiotics and Microbial Transformations*; CRC Press; 1987, p. 61.

27. Dunnill, P.; Wisman, A.; Blackebrough, N. (Eds.) *Enzymatic and non-Enzymatic Catalysis*; Ellis Horwood, Chichester, 1980.

28. Suckling, C.J. in Suckling, K.W.. (Eds.) *Enzyme Chemistry, Impact and Applications*; Chapman and Hall; 1984, p. 78; Suckling, C.J.; Suckling, K.E. *Chem. Soc. Rev.* **1974**, *3*, 387; Suckling, C.J.;Wood, H.C.S. *Chem. Ber.* **1979**, *15*, 243; Suckling, C.J. *Chem. Soc. Rev.* **1984**, *13*, 97; Suckling, C.J. *Angew. Chem., Int. Ed. Engl.* **1988**, *27*, 537.

29. Jones, J.B.; Sih, C.J.; Perlman, D. *Applications of Biochemical Systems in Organic Chemistry*, Wiley, New York, 1976.

30. Battersby, A.R. *Chem. Ber.*, **1984**, *20*, 611.

31. Porter, R.; Clark, S. (Eds.) *Enzymes in Organic Synthesis* (Ciba Foundation Symposium 111), Pitman, London, 1985.

32. Whitsides, G.M.; Wong, C.H. *Aldrichimica Acta* **1983**, *16*, 27; *Angew Chem*, **1985**, *97*, 617; *Angew. Chem., Int. Ed. Engl.*, 1985, 23, 570.

33. Sih, J.; Chen, C.S. *Angew. Chem.*, **1984**, *96*, 556; *Angew Chem., Int. Ed. Engl.*, **1984**, *23*, 570.

34. Roberts, S.M. *Chem. Ind.* **1988**, 384; and references therein.

35. Baum, R. *C&E News* **1990**, Nov. 12, 30.

36. International Union of Biochemistry : *Enzymes Nomenclature 1984*, Academic Press, New York, 1984.

37. Sigma Catalog (1990).

38. Nicolk, G.B. in *Microbial Technology*, Peppler, H.J.; Perlman, D. (Eds.)vol. 2, p. 155, Academic Press, New York, 1979.

39. Underwood, A.J.U. *Trans. Indt. Chem. Eng. (London)* **1935**, *13*, 34.

40. Commer, H.A.; Allegeier, R.J. *Adv. Appl. Microbiol.*, **1976**, *20,* 166.

41. Compere, A.L.; Griffith, W.L. *Dev. Ind. Microbiol.* **1979**, *20*, 509.

42. Underkofler, L.A.; Hickey, R.J. *Industrial Fermentation*, vol. 1, p.347, Chemical Publishing Co., New York, 1954.

43. Neuberg, C; Hirsh, J. *Biochem. Z.* **1921**, *282*, 115.

44. Peterson, D.H.; Murray, H.C. *J. Am. Chem. Soc.* **1952**, *74*, 1871.

45. Smith, R.V.; Rosazza, J.P. in *Microbial transformations of Bioactive Compounds*, , Rosazza, J.P. (Ed.), CRC Press, Boca Raton, 1982, vol. 2, p 1; Sih, C.J.; Rosazza, J.P. in *Applications of Biochemical Systems in Organic Chemistry*, Jones, J.B.; Sih, C.J.; Perlman, D (Eds.), Wiley, New York, 1976, 69; Rhem, H.J.; Reed, G, *Microbial Transformations* , vol. VI-B, in *Biotechnology: A comprehensive Treatise in Eigth Volumes*, Kieslich, K. (Ed.), Verlag-Chemie, Weinheim, 1984.

46. Yamamoto, Y.; Tosa, T.; Chibata, I. *Biochem. Bioeng.* **1980**, *22*, 2045.

47. Sato, T.; Nishida, Y.; Tosa, T.; Chibata, I. *Biochem. Biophys. Acta* **1979**, *570*, 179; Nishida, Y; Sato, T; Tosa, T.; Chibata, I. *Enzyme Microb. Technol.* **1979**, *1*, 95.

48. Linko, Y.-Y.; Pohjola, L.; Linko, P. *Process Biochem.* **1977**, *12*, 14.

49. Sato, T.; Tosa, T.; Chibata, I. *Eur. J. Appl. Microbiol.* **1976**, *2*, 153.

50. Huang, F.-C.; Hsu Lee, L.F.;Mittal, R.S.D.; Ravikumar, P.R.; Chan, J.A.; Sih, C.J. *J.Am.Chem.Soc.* **1976**, *97*, 4144.

51. Ohno, M.; Kobayashi, S.; Limori, T.; Wang, Y.-F.; Izawa, T., *J. Am. Chem. Soc.*, **1981**, *103*, 2405; Okano, K.; Izawa, T.; Ohno, M., *Tetrahedron Lett.*, **1983**, *24*, 217; Limori, T; Takahashi, Y.; Izawa, T.; Kobayashi, S.; Ohno, M., *J. Am. Chem. Soc.*,**1983**, *105*, 4049.

52. Ito, Y.; Shibata, T.; Arita, M.; Sawai, H.; Ohno, M., *J. Am. Chem. Soc.*, **1981**, *103*, 6739; Arita, M.; Adachi, K.; Ito, Y.; Sawai, H.; Ohno, M., *J. Am. Chem. Soc.*, **1983**, *105*, 4049.

53. Mori, K., *Tetrahedron*, **1981**, *37*, 1341; Mori, K.; Matsunaga, M. *Liebigs. Ann. Chem.* **1984**, 157.

54. Cohen, N.; Eitchel, W.F.; Lopersti, R.J.; Neukon, G.; Saucy, G., *J. Org. Chem.* **1976**, *41*, 3505.

55. Branca, Q.; Fischli, A., *Helv. Chim. Acta* **1977**, *60*, 925.

56. Hudlicky, T.; Luna, H.; Barbieri, G.; Kwart, L.*J. Am. Chem. Soc.* **1988**, *110*, 4735.

57. Klibanov, A.M. *Acc. Chem. Res.* **1990**, *23*, 114.

58. Pratt, J.A. *Chem. Britt.* **1989**, *25*, 282.

59. Napper, A.D.; Benkovic, S.B; Tramontano, A.; Lerner, R.A. *Science*, **1 987**, *237*, 1041; Lerner, R.A.; Tramontano, A. *Sci. Am.* **1988**, *258*, 58.

60. Hayaishi, O.; Katagiri, M.; Rothberg, S. *J. Am. Chem. Soc.* **1955**, *77*, 5441.

61. Clarke, P.H.; Richmond, M.H. (Eds.) *Genetics and Biochemistry of Pseudomonas*, John Wiley & Sons, New York, 1975

62. Gibson, D.T.; Subramanian, V. in Gibson, D.T. *Microbial degradation of Organic Compounds*. Marcel Dekker, Inc., New York, !984.

63. Dagley, S.; Chapman, P.J.; Gibson, D.T.; Wood, J.M. *Nature* **1962**, *202*, 775.C64. Gibson, D.T.; Zylstra, G.J.; Chauhan, S. in *Pseudomonas: Biotransformations, Pathogenesis, and Evolving Biotechnology* ,Silver, S. et.al., Eds.; p 121, 1990.

65. Gibson, D.T.; Koch, J.R.; Kallio, R.E. *Biochemistry* **1968**, *7*, 2653.

66. Gibson, D.T.; Koch, J.R.; Schuld, C.L.; Kallio, R.E. *Biochemistry*, **1968**, *7*, 3195.

67. Gibson, D.T.; Cardini, G.E.; Maseles, F.C.; Kallio, R.E. *Biochemistry* **1970**, *9*, 1631.

68. Ziffer, H.; Seeman, J.I.; Hihet, R.J.; Sokoloski, E.A. *J. Org Chem.* **1974**, *34*, 3698.

69. Kobal, V.M.; Gibson, D.T.; Davis, R.E.; Garza, A. *J. Am. Chem. Soc.* **1973**, *95*, 4420.

70. Ziffer,H.; Kabuto, K.; Gibson, D.T.; Kobal, V.M.; Jerina, D.M. *Tetrahedron* **1977**, *33*, 2491.

71. Ziffer, H.;Gibson, D.T.; Kobal, V.M.; Jerina, J.M. *J. Am. Chem. Soc.* **1973**, *95*, 4048.

72. Yeh, W.-K.; Gibson, D.T.; Liu, T.-N. *Biochem. Biophys. Res. Commun.* **1977**, *78*, 401.

73. Subramanian, V.; Lui, T.-N.; Yeh, W.-K.; Narro, M.; Gibson, D.T. *J. Biol. Chem.* **1981**, *256*, 2723.

74. Subramanian, V.; Lui, T.-N.; Yeh, W.-K.; Serdan, C.M.; Wackett, L.D.; Gibson, D.T. *J. Biol. Chem.* **1985**, *260*, 2355.

75. Gibson, D.T.; Yeh, W.-K.; Liu, T.N.; Subramanian, V. in *Oxygenases and Oxygen Metabolism*, Nozaki, M.; Yamamoto, S.; Ishimura, Y.; Coon, M.J.; Ernster, L.; Estabrook, R.W. (Eds.), Academis Press New York, 1982.

76. Finette, B.A.; Subramanian, V.; Gibson, D.T. *J. Bacteriol.* **1984**, *160*, 1003.

77. Wackett, L.P. *Methods Enzymol.* **1990**, *188*, 39

78. Geary, P.J.; Mason, J.R.; Joannou, C.L. *Methods Enzymol.* **1990**, *188*, 52.

79. Ensley, B.D.; Haigler, B.E. *Methods Enzymol.* **1990**, *188*, 46.

80. Finette, B.A; Gibson, D.T. *Biocatalysis* **1988**, *2*, 29.

81. W.R. McCombie, *Abstr. Annu. Meet. Am. Soc. Microbiol.* **1985**, K53, p. 155.

82. Ziffer, H.; Gibson, D.T. *Tetrahedron Lett..* **1975**, *25*, 2137.

83. Spain, J.C.; Gibson, D.T. *Appl. Envirom. Microbiol.* **1988**, *54*, 1399.

84. Gibson, D.T.; Hensley, M.; Yoshioka, H.; Mabry, T.J. *Biochemistry* **1970**, *7*, 3795.

85. Spain, J.C.; Zylstra, G.J.; Blake, C.K.; Gibson, D.T. *Appl. Environ. Microbiol.* **1989**, *55*, 2648.

86.   Alexander, M.; Lustigman, B. *J. Agric. Food Chem.* **1966**, *14*, 410.

87.   Zylstra, G.J.; Wackett, L.P.; Gibson, D.T. *Appl. Environ. Microbiol.* **1989**, *55*, 3162.

88.   Brazier, A.J.; Lilly, M.D.; Herbert, A.B. *Enzyme Microbiol. Technol.* **1990**, *12*, 90.

89.   Jerina, D.M.; Dly, J.W.; Jeffrey, A.M.; Gibson, D.T. *Arch. Biochem. Biophys.* **1971**, *142*, 394.

90.   Jeffrey, A.M.; Yeh, H.J.C.; Jerina, D.M.; Patel, T.R.; Davey, J.F.; Gibson, D.T. *Biochemistry* **1975**, *14*, 575.

91.   McMordie, A. Ph. D. Dissertation, 1989, Queens University, Belfast, U.K.

92.   Harries, C.D. *Ber. Dtsch. Chem.* **1903**, *36*, 105.

93.   Kadesch, R.G. *Prog. Chem. Fats Other Lipids* **1963**, *6*, 291.

94.   Rieche, A.; Meister, R. *Ber. Dtsch. Ges.* **1932**, *65*, 1274; Rieche, A.; Meister,

95.   Bailey, P.S. *Ozonation in Organic Chemistry*, vol I, Blomquist, A.T.; Wasserman, H.H. (Eds.), Academic Press, New York, 1978.

96.   Bailey, P.S. *Chem. Rev.* **1958**, *58*, 925.

97.   Griesbaum, K.; Zwick, G.; Agarwal, S.; Keul, H.; Pfeffer, B.; Murray, R.W. *J. Org. Chem.* **1985**, *50*, 4199.

98.   Odinokov, V.N.; Akhunova, V.R.; Bakeeva, R.S.; Galeeva, R.I.; Semenovskii, A.V.; Moiseenkov, A.M.; Tolstikov, G.A. *Zh. Org. Khim.* **1977**, 485.

99.   Bailey, W.J.; Lawson, L.B. *J. Am. Chem. Soc.* **1955**, *77*, 1606.

100.   Bailey, W.J.; Hudson, R.L. *J. Am. Chem. Soc.* **1956**, *78*, 670.

101.   Nazarov, I.N.; Kuznetsov, N.V. *Zh. Obshch. Khim.* **1959**, *29*, 767; *Chem. Abst.* **1960**, *54*, 1348e.

102.   Menyailo, A.T.; Pokrovskaja, I.E.; Yakoleva, A.K. *Neftekimiya* **1967**, *7*, 70; *Chem. Abstr.* **1967**, *67*, 2720s.

103.   Taylor, H.F.; Smith, T.A. *Nature*(London) **1967**, *215*, 1513.

104.   Schneider, H.; Griesbaum, K. *Tetrahedron Lett.* **1979**, 57.

105.   Odinokov, V.N.; Akhmetova, V.R.; Cheskis, B.A.; Moiseenkov, A.M.; Tolstikov, G.A. *Zh. Org. Khim.* **1986**, 246.

106.   Veysoglu, T.; Mitscher, L.A.; Swayze, J.K. *Synthesis* **1980**, 807.

107.   Griesbaum, K.; Keul, H.; Agarwal, S.; Zwick, G. *Chem. Ber.* **1983**, *116*, 409.

108.   Meister, M.; Zwick, G.; Griesbaum, K. *Can. J. Chem.* **1983**, *61*, 2385.

109.   Hudlicky, T.; Luna, H.; Barbieri, B.; Kwart, L.D. *J. Am. Chem. Soc.* **1988**, *110*, 4735.

110.   Griesbaum, K.; Mertens, H.; Jung, I.C. *Cand. J. Chem.* **1989**, *68*, 1369.

111.   Callighan, R.H.; Wilt, M. H. *J. Org. Chem.* **1961**, *26.* 5212.

112   Pappas,J. J.; Keaveney,W. P.; Berger, M.; Rush, R. V.. *J. Org. Chem.* **1968**.*33*, 787

113.   Griesbaum, K.; Zwick, G. *Chem. Ber.* **1985**, *119*, 229

114   Wang, Z.; Zvlichovsky, G. *Tetrahedron Lett.* **1990**, *31*, 5579.

115.   Schreiber, L.S. *J. Am. Chem. Soc.* **1980**, *102*, 6163.

116.   Prasad, K.; Pepic, O. *Tetrahedron Lett.* **1984**, *25*, 4889.

117.   Schreiber, S.L.; Claus, R.E.; Reagan, J. *Tetrahedron Lett.* **1982**, *23*, 3867.

118.   Schreiber, S.L.; Liew, W.-F. *J. Am. Chem. Soc.* **1985**, *107*, 2980.

119.   Neumeister, J.; Keul, H.; Saxena, M.P.; Griesbaum, K. *Angew. Chem., Indt. Ed. Engl.* **1978**, *17*, 939.

120   Griesbaum, K.; Volpp, W. *Chem. Ber.* **1988**, 1795.

121.   Griesbaum, K.; Jung, I.C.; Mertens, H. *J. Org. Chem.* **1990**, *55*, 6024.

122.   Criegee, R.; Schweickhart, C.; Knoche, H. *Chem. Ber.* **1970**, *103*, 960.

123.   Slagel, R.C. *J. Org. Chem.* **1966**, *31*, 593

124.   Criegee, R.; Huber, H. *Angew. Chem.* **1969**, *81*, 749.

125.   Franz, J.E.; Knowles, W.S.; Osuch, C.J. *J. Org. Chem.* **1965**, *30*, 4328.

126.   Pryor, W.A.; Gimiamalva, D.; Church, D.F. *J. Am. Chem. Soc.* **1983**, *105*, 6858.

127.   Dillen, J.L.M.; Meth-Cohn, O.; Moore, C.; van Rooyen, P.H. *Tetrahedron* **1988**, *44*, 3127.

128.   Griesbaum, K.; Hoffmann *J. Am. Chem. Soc.***1976**, *98*, 2877

129.   Griesbaum, K.; Keul, H. *Angew. Chem., Int. Ed. Engl.* **1975**, *14*, 716.

130.   Griesbaum, K.; Bandyopadhyay, S.R.; Meister, M. *Cand. J. Chem.* **1986**, *64*, 1553.

131.   Rånby, B.; Rabek, J.F. *Singlet Oxygen, Reactions with Organic Compounds and Polymers*, Wiley, 1978.

132.   Frimer, A.A. (Ed.), *Singlet Oxygen*, CRC Press, Boca Raton, 1985.

133. Foote, C.S.; Wexler, S. *J. Am. Chem. Soc.* **1964**, *86*, 3879.

134. Corey, E.J.; Taylor, W.C. *J. Am. Chem. Soc.* **1964**, *86*, 3881.

135. Kearns, D.R.; Khan, A.U. *Photochem. Photobiol.* **1969**, *10*, 193.

136. Gollnick, K.; Franken, T.; Schaade, G.; Dorhofer, G. *Ann. N.Y. Acad. Sci.* **1970**, *171*, 89.

137. Arnold, S.J.; Kubo, M.; Orgryzlo, E.A. *Adv. Chem. Serv.* **1968**, 77, 133.

138. Murray, R.W. in *Singlet Oxygen*, Wasserman, H.H.; Murray, R.W., Eds., Academic Press, 1979, p 59.

139. McKeon, W.E.; Water, W.A. *J. Chem. Soc.* **1966**, 1040.

140. Bartlett, P.D.; Mendenhall, G.D. *J. Am. Chem. Soc.* **1970**, *92*, 210.

141. Schaap, A.P.; Bartlett, P.D. *J. Am. Chem. Soc.* **1970**, *92*, 6055.

142. Schaap, A.P.; Kees, K.; Thayer, A.L. *J. Org. Chem.* **1975**, *40*, 1185.

143. Bergman, W.; McLean, M.J. *Chem. Rev.* **1941**, *28*, 367.

144. Margaretha, P., in *Metoden der Organischen Chemie* (Houben-Weyl), Kropf,H.,(Ed)., Geor Thieme Verlag, Stuttgart, 1988,Bd E/13, p 434-472.;145. Saito, I.; Tamato, K.; Katsumura, A.; Sigiyama, H.; Matsuura, T. *Chem. Lettl* **1978**, 127.

146. Mousseron-Canet, M.; Mani, J.C.; Dlle, J.P.; Olive, J.L. *Bull. Soc. Chim. Fr.* **1966**, 3874.

147. Narada, A.; Kawamoto,A.; Tokuma, U.; Uda, H. *J. Chem. Soc., Perkin Trans. 1* **1976**, 1796.

148. Schenck, G.O.; Wirtz, R. *Naturwissenschaften* **1953**, *40*, 581.

149. Barton, D.H.R.; Haynes, R.K.; Magnus, P.D.; Menzies, I.D. *J. Chem. Soc., Chem. Commun.* **1974**, 511; Barton, D.H.R.; Haynes, R.K.; Leclerc, R.K.;Magnus, P.D.; Menzies, I.D. *J. Chem. Soc., Perkin Trans 1* **1975**, 2055.

150. Haynes, R.K. *Aust. J. Chem.* **1978**, *31*, 131.

151. Haynes, R.K. *Aust. J. Chem.* **1978**, *31*, 121.

152. Hunig, S. ; Muller, H.R. Thier, W. *Angew Chem., Int. Ed. Engl.* **1965**, *4*, 275; Wolinsky, J.; Schultz, T. *J. Org. Chem.* **1965**, *30*, 3980.

153. Adam, W.; Eggelte, H.J. *Angew Chem., Int. Ed. Engl.* **1977** *16*, 713.

154. Garbich, A., Jr.; Schilderout, S.M.; Paterson, D.P.; Sprecher, C.M. *J. Am. Chem. Soc.* **1965**, *87*, 2932; Hunig, S. ; Muller, H.R.*Angew Chem., Int. Ed. Engl.* **1978** *17*, 954.

176

155. Schenk, G.O.; Dunlap, E.D. *Angew. Chem.* **1956**, *68*, 248.

156. Kaneko, C.; Sugimoto, A.; Tanaka, S. *Synthesis*, **1974**, 876.

157. Bartlett, P.D.; Baumstark, A.L.; Landis, M.E. *J. Am. Chem. Soc.* **1973**, *95*, 6486; Baumstark, A.L.; Wilson, T.; Landis, M.E.; Bartlett, P.D. *Tetrahedron Lett.* **1976**, 2397.

158. Bartlett, P.D.; Landis, M.E.; Shapiro, M.J. *J. Org. Chem.* **1977**, *42*, 1661.

159. Adam, W.; Erden, I. *Tetrahedron Lett.* **1979**, 2781.

160. Foote, C.S. *Acc. Chem. Res.* **1968**, *1*, 104.

161. Vogel, E.; Atenbach, H.J.; Sommerfield, C.D. *Angew Chem., Int. Ed. Engl.* **1972**, *11*, 939.

162. Forster, C.H.; Berchtold, G.A. *J. Am. Chem. Soc.* **1972**, *94*, 7939.

163. Forster, C.H.; Berchtold, G.A. *J. Org. Chem.* **1975**, *40*, 3743.

164. Vogel, E.; Altenbach, H.J.; Cremer, D. *Angew. Chem.* **1972**, *84*, 983; Altenbach, H.J.; Stegelmeier, H.; Vogel, E. *Tetrahedron Lett.* **1978**, 3333.

165. Nelson, E.K. *J. Am. Chem. Soc.* **1911**, *35*, 1404; ibid **1913**, *35*, 84.

166. Boche, J.; Rungust, O. *J. Org. Chem.* **1968**, *33*, 4285.

167. Kearns, D.R. *J. Am. Chem. Soc.* **1969**, *91*, 6554.

168. Adam, W.; Balci, M. *Tetrahedron* **1980**, *36*, 833.

169. Adam, W.; Balci, M. *J. Am. Chem. Soc.* **1979**, *101*, 7542.

170. Takeshita, H.; Kanamori, H.; Hatsui, T. *Tetrahedron Lett.* **1973**, 3139.

171. Skorianetz, W.; Schulte-Elte, K.H.; Ohloff, G. *Angew. Chem., Int. Ed. Engl.* **1972**, *11*, 330; Havada, N.; Suzuki, S.; Uda, H.; Ueno, H. *J. Am. Chem. Soc.* **1972**, *94*, 1777.

172. Le Roux, J.P.; Goasdoue, C. *Tetrahedron.* **1975**, *31*, 2761.

173. Schaap, A.P.; Burns, P.A.; Zazlika, K.A. *J. Am. Chem. Soc.* **1977**, *99*, 1270.

174. McCapra, F.; Wrigglesworth, R. *J. Chem. Soc., Chem. Commun.* **1968**, 1256.

175. Natsume, M.; Sekine, Y.; Ogawa, M. *Tetrahedron Lett.* **1979**, 3473.

176. Natsume, M.; Wada, M. *Chem. Pharm. Bull.* **1975**, *23*, 2567; Natsume, M.; Sekine, Soyagimi, H. ibid. **1978**, *26*, 2188; Natsume, M.; Wada, M.; Ogawa, M. *ibid.* **1978**, *26*, 3364.

177. Lewrs, E.G. in *Comprenhensive Heterocyclic Chemistry*, vol. 7, Katritzky, A.R.; Rees, C.W. (Eds.), p. 114-119

178. Bartók, M.; Láng, K.L. in *The Chemistry of Heterocyclic Compounds*, vol. 42, part 3; Hassner, A. (Ed.); John Wiley & Sons; 1985.

179. Prileshaev, N. *Chem. Br.* **1990**, *42*, 4811.

180. Murray, R.U.; Youssefyeh, R.D.; Story, P.R. *J. Am. Chem. Soc.* **1967**, *89*, 2429.

181. Berti, G. , in *Topics in Stereochemistry*, Allinger, N.L.; Eliel, E.L., (Eds.), Wiley, New York, 1973.

182. Rosowsky, A. *The Chemistry of Heterocyclic Compounds,* 1964, **19-1**, 1-523.

183. Sheldon, R.A.; Kochi, J.K. *Advan. Catal.* **1976**, *25*, 272.

184. Hiatt, R., in *Oxidation Techniques and Applications in Organic Chemistry*, vol. 2; Agustine, R.,(Ed.); Marcel Dekker, New York, 1971, p. 133-138.

185. Doumaux, A. in *Oxidation*, Agustine, R. (Ed.); vol. 2, Marcel Dekker, New York, 1971, p. 141-185.

186. Lyons, J.E. *Aspects of Homogeneous Catalysis* **1977**, *3*, 1-136.

187. Sheldon, R.A. *J. Mol. Cat.* **1980**, *7*, 107.

188. Sharples, K.B.; Verhoeven, T.R. *Aldrichimica Acta* **1979**, *12*, 63.

189. Kropt, H.; Weickmann, A.; Zeller, K.-P in *Methoden der Organischen Chemie*, BdIV/1a, p 184-239, 1981.

190. Bartlett, P.A. *Tetrahedron* **1980**, *36*, 2

191. Wilkinson, S.G. *Int. Rev. Sci., Org. Chem., Ser. 2* **1975**, *2*, 111.

192. Armarego, W.L.F., in *Stereochemistry of Heterocyclic Compounds*, Part 2, Wiley, New York, 1977, p. 12-36.

193. Swern, D. *Organic Peroxides*, vol. 2, Wiley-Interscience, New York, 1971.

194. Prileshaev, E.N., *Prileshaev Reaction, Electrophilic Oxidation*, Nauka, Moscow, U.S.S.R., 1974.

195. Filippova, T.V.; Blymberg, E.A. *Uspekhi Khimii*, **1982**, *51*, 1017.

196. Allan, G.G.; Neogi, A.N. *J Catal.* **1970**, *19*, 256.

197. A.G. Hoechst, Neth. Appl., 75 08,894, through Chemical Abstracts **1976** *85*, 192531y.

198. Hildon, A.M.; David, T.D.; Jaggers, A.J. Ger. Offen., 2,747,762, through Chemical Abstracts **1978** *89*, 24123f.

199. Hildon, A.M.; Greenhalgh, P.F.Ger. Offen., 2,747,761, through Chemical Abstracts **1978** *89*, 24124g.

200. Mori, T.; Yang, K.H.; Kimoto, K.; Nozaki, H. *Tetrahedron Lett.* **1970**, 2419.

201. Matsumura, N.; Sonoda, N.; Tsutsumi, S. *Tetrahedron Lett.* **1970**, 2029.

202. Cocher, W.; Garson, D.H. *J. Chem. Soc., Perkin II* **1976**, 791.

203. Malinovskii, M.S.; Kmel, M.P.; Baranov, N.N.; Krtivosheeva, N.G. *Ukr. Khim. Zh.* **1975**, *41*, 1064.

204. Rebek, J.; Wolf, S.F.; Mossman, A.B. *J. Chem. Soc., Chem. Commun.* **1974**, 711.

205. Coates, R.M.; Williams, J.W. *J. Org. Chem.* **1974**, *39*, 3054.

206. Grieco, P.A.; Yokoyama, Y.; Gilman, S.; Nishizawa, M. *J. Org. Chem.* **1977**, *42*, 2034.

207. Kametani, T.; Nemoto, H.; Fukumoto, K. *Heterocycles* **1977**, *6*, 1365.

206. Ogata, Y.; Tomizawa, K. Ikeda, T. *J. Org. Chem.* **1979**, *44*, 2362.

207. Ibne-Rasa, K.M.; Pater, R.H.; Ciabattoni, J.; Edwards, J.O. *J. Am. Chem. Soc.* **1973**, *95*, 7894.

210. Pfenninger, A. *Synthesis* **1986**, 89; and references cited therein.

211. Wilkinson, S.G. *Int. Rev. Sci., Org. Chem., Serv 2* **1975**, *2*, 111.

212. Armarego, W.L.F., in *Stereochemistry of Heterocyclic Compounds*, Pergamon Press, Oxford, 1979.

213. Newman, M.S.; Chen, C.H. *J. Am. Chem. Soc.* **1972**, *94*, 2149.

214. Seeley, D.A.; McElwee, J. *J. Org. Chem.* **1973**, *38*, 1691.

215. Boigegrian, R.; Castro, B. *Tetrahedron Lett.* **1975**, 3459; *Tetrahedron* **1976**, *32*, 1283.

216. Butterham, T.J. *NMR Spectra of Simple Heterocycles*, Wiley, New York, 1973.

217. Corey, E.J.; Chaykovsky, M. *J. Am. Chem. Soc.* **1965**, *87*, 1353.

218. Van der Plas, H.C. *Ring Transformations of Heterocycles*, Academic Press, New York, 1973, vol. 1, p 364.

219. Cameron, T.B.; El-Kabbani, F.M.; Pinnich, H.W. *J. Am. Chem. Soc.* **1981**, *103*, 5414.

220. Adam, W.; Liu, J.; Rodriguez, O. *J. Org. Chem.* **1973**, *38*, 2269.

221. Menyalio, A.T.; Khcheyan, Kh. E. *Neth Appl.* **1975**, 73 12,323; *Chem. Abstr.* **1975**, *83*, 206084c.

222. Allan, G.G.; Neogi, A.N. *J. Phys. Chem.* **1969**, *73*, 2093.

223. Schirmann, J.P.; Delavarenne, S.Y. *Chem. Abstr.* **1978**, *89*, 59832p.

224. Allan, G.G.; Neogi, A.N. *J. Catal.* **1970**, *16*, 197.

225. Heumann, A.; Chaubel, F.; Waegell, B. *Tetrahedron Lett.* **1982**, *33*, 2767

226. Pralus, M.; Lecoq; Schirmann, New Ways in the Catalytic Epoxidation of Olefins by Hydrogen Peroxide in Tsutsui, M. (Ed.) *Fundamental Research in Homogeneous Catalysis*, vol. 3, Plenum, New York, 1977.

227. Watanabe, M.; Kano, Y.; Kasano, K.; Uemura, M. Jpn. Kokai Tokkyo Koho JP 01,128,976[89,128,976]; *Chem. Abstr.* **1990**, *112*, 55572z.

228. Neidleman, S.L.; Geigert, J. *Biochem. Soc. Symp.* **1983**, *48*, 39.

229. Wistuba, D.; Nowotny, H.-P.; Träger, O.; Schurig, V. *Chirality* **1989**, *1*, 127.

230. Barton, D.H.; Mousseron-Carnet, M. *J. Chem. Soc.* **1965**, 271.

231. Dittmann, W.; Stürzenhofecker, F. *Liebig. Ann. Chem.* **1965**, *688*, 57.

232. Portmann, R.E.; Ganter, G. *Helv. Chim. Acta* **1973**, *56*, 1962.

233. Heggie, W.; Sutherland, J.R. *J. Chem. Soc., Chem. Commun.* **1972**, 957.

234. White, R.F.; Birnbaum, J.; Meyer, R.T.; ten Broeke, J.; Chemerda, J.M.; Demain, A.L. *Appl. Microbiol.* **1971**, *22*, 55.

235. Barton, D.; Ollis, W.D. (Eds.) *Comprehensive Organic Chemistry*, Pergamon Press, New York, 1979, vol. 1.

236. Gunstone, F.D. *Adv. Org. Chem.* **1960**, *1*, 103.

237. Lloyd, W.D.; Navarrete, B.J.; Shaw, M.F. *Synthesis* **1972**, 610.

238. Hoffmann, K.A. *Chem. Ber.* **1912**, *45*, 3329; Hoffmann, K.A.;Erhart, O.; Schneider, O. *Chem. Ber.* **1913**, *46*, 1657.

239. Milas, N.A.; Sussman, S. *J. Am. Chem. Soc.* **1936**, *58*, 1302; ibid **1937**, *59*, 2345.

240. Mundy, B.P.; Ellerd, M.G. *Named Reactions and Reagents in Organic Chemistry*, Wiley, New York, 1988; p. 170.

241. March, J. *Advanced Organic Chemistry*, 3rd Ed., Wiley, New York, 1985; p. 734.

242. Cambie, R.C.; Hayward, R.C.; Roberts, J.L.; Rutledge, P.S. *J. Chem. Soc., Perkin Trans 1.* **1974**, 1858.

243. Cambie, R.C.; Noall, W.I.; Potter, G.J.; Rutledge, P.S.; Woodgate, P.D. *J. Chem. Soc., Perkin Trans 1.* **1977**, 226.

244. Mangoni, L.; Adinolfi, M.; Barone, G.; Parrilli, M. *Gazz. Chim. Ital.* **1975**, *105*, 377.

245. Adinolfi, M.; Parrilli, M; Barone, G.; Laonigno, G.; Mangoni, L. *Tetrahedron Lett.* **1976**, 3661.

246. Jasserand, D.; Girad, J.P.; Rossi, J.C. Granger, R. *Tetrahedron Lett.* **1976**, 3661.

247. Glotter, E.; Schwartz, A. *J. Chem. Soc., Perkin Trans 1.* **1976**, 1660.

248. Freppel, C.; Favier, R.; Richer, J.-C.; Zador, M. *Cand. J. Chem.* **1971**, *49*, 2586.

249. Buddrus, J. *Angew. Chem., Int. Ed. Engl.* **1973**, *12*, 163.

250. Bachhawat, J.M.; Mathur, N.K. *Tetrahedron Lett.* **1971**, 691.

251. Wilkins, C. *Synthesis*, **1973**, 156.

252. Khuddos, M.A.; Swern, D. *Tetrahedron. Lett.* **1971**, 411.

253. Klein, J.; Levene, R.; Dunkelblum, E. *Tetrahedron Lett.* **1972**, 2845; Larson, G.L.; Hernandez, D.; Hernandez, A. *J. Organomet. Chem.* **1974**, *76*, 9.

254. Hentges, S. G.; Sharpless, K. B. *J. Am. Chem.Soc.* **1980**, 102, 4263;. Tomioka, K.; Nakajima, M.; Koga, K. *J. Am. Chem. Soc.* **1987**, 109, 6213.

256. Yamada, T.; Narasaka, K. *Chem. Lett.* **1986**, 131.

257. Tokles, M.; Snyder, J. K. *Tetrahedron Lett.* **1986**, 27, 3951.

258. Hirama, M.; Oishi, T.; Ito, S. *J. Chem. Soc., Chem. Commun.* **1986**, 665

259. Annunziata, R.; Cinquini, M.; Cozzi, F.; Raimondi, L.; Stefenelli, S. *Tetrahedron Lett.* **1987**, 28, 3139.

260. Sharpless, K.B; Amberg, W.; Beller, M.; Chen, H.; Hartung, J.; Kawanami, Y.; Lübben, D.; Manoury, E.; Ogino, Y.; Shibata, T.; Ukita, T. *J. Org. Chem.* **1991**, *56*, 4585.

261. Hirama, M.; Oishi, T.; Itô, S. *J. Chem. Soc., Chem. Commun.* **1989**, 665

262. Poli, G. *tetrahedron Lett.* **1989**, *30*, 7385

263. Corey, E.J.; Jardine, P.D.; Virgil, S.; Yen, P.-W.; Connell, R.D. *J. Am. Chem. Soc.* **1989**, *111*, 9243.

264. May, S. W.; and Schwartz, R. D.; *J. Am. Chem. Soc.* **1974**, 96, 4031.

265. Ziffer, H. and Gibson, D. T.; *Tetrahedron Lett.* **1975**, 2137.

266. Wallnofer, P. R.; Safe, S. and Hutzinger, O.; *J. Agric. Food Chem.* **1973** *21*, 502.

267. Mukherjee, B. B.; Kraidman, G.; and Hill I. D.; *Appl. Microbioil* **1973**. 25. 447.

267*. Barton ; Ollis *Comprehensive Organic Chemistry* , vol. 1, p 100.

268. Pihlaja, K. ; Taskinen, E. *Phys . Methods Heterocycl. Chem.* **1974**, *6*, 199.

269. Wilkinson, S.G., in *Aliphatic Compounds*, Chapman, N.B. (Ed.), Butterwoth, London, vol. 2, 1975; Rosowsky,A. *Chemistry of Heterocyclic Compound*, 1964, *19-1*, 17; Parker, R.E; Issacs, N.S. *Chem. Rev.*, **1959**, *59*, 737.

270. Fürst, A.; Plattner, P.A. *Helv. Chim. Acta* **1949**, *32*, 275.

271. Akhrem A. A.; Moiseenkov A. M.; and Dobrynin V. N., *Russ. Chem . Rev.* (Engl. transl.) **1968**, 37, 448.

272. Buchanan J. G.; and Sable H. Z. in *Selective Organic Transformations*, (Ed. B. S Thyagarajan) Wiley, N Y. **1972**, vol 2 p. 1-95.;Wilkinson S. G.; Int. *Rev. Sci, Org. Chem.*, Ser. 2, **1975**, 2, 111.;Armarego W. L. F. ; in *Stereochemistry of Heterecyclic compounds* part 2, Wiley, N.Y. **1977**, p. 12-36.

273. Lamaty G.; Maleq R.; Selve C.; Sivade, A.; Wylde J. *J. Chem. Soc. Perkin II*, **1975,** 1119.

274. Berti G.; Macchia B., and Macchia, *F. ,Tetrahedron.* **1972**, *28* , 1299

275. Berti G.; Calelani G.; Ferretti M. and Monti, *L. Tetrahedron* **1974,** *30*, 4013.

276. Langin M. T. and Huet, *J. , Tetrahedron Lett.* **1974**, 3115.; Morrison G.A.; Wilkinson, J.B. *Tetrahedron Lett.* **1975**, 2713.; Campion, T.A., Morrison, G.A.; Wilkinson J.B., *J. Chem. Soc. Perkin Trans. I*, **1976**, 2508.

277. Morrison G. A.; Wilkinson J. B. *J. Chem. Soc., Perkin Trnas. I* **1976**, 2508.

278. Kowollik, W.; Janairo, G.; Voelter, W. *J. Org. Chem.* **1988**, *53*, 3943.

279. Giuliano, R.M.; Kasperowics, S. *Carbohydr. Res.* **1988**, *183*, 277.

280. Grouillier, A.; Nonga, B.; Navarro, N.-L.; Moliére, P.; Pacheco, H. *J. Carbohydr. Chem.* **1988**, *7*, 507.

281. Hollingsworth, R.I.; Hrabak, E.M.; Dazzo, F.B. *Carbohydr. Res.* **1986**, *154*, 103.

282. Matsuda, K.; Tsuchiya, T.; Torii, T.; Umezama, J. *J. Bull. Chem. Soc. Japan* **1986**, *59*, 1397.

283. Drivas, I.; Picq, D.; Anker, D.; Pacheco, H. *J. Carbohydr. Chem.* **1984**, *3*, 243.

284. Kliegel, W.; *Liebig's Ann. Chem.* **1970,** 733, 192.

285. Cerny, I.; Trnka, T.; Cerny, M. *Coll. Czech. Commun.* **1984**, 49, 433.

286. Babu, H.; Frei, B. *Helv. Chim. Acta* **1986**, 69, 415.

287. Sinou, D.; Emziane, M. *Tetrahedron Lett.* **1987**, *37* , 4423.

288. Ho, T.-L. *Hard and Soft acids and bases principles in Organic Chemistry*, Academic Press, New York, 1977.

289. Olah, G.A.; Meidar, D. *Isr. J. Chem.* **1978**, *17*, 148.

290. Farges, G.; Kergomard, A. *Bull. Soc. Chim. France* **1975**, 315.

291. Brown, S.M. in *Organic Synthesis. Theory and Applications*, Hudlicky, T. (Ed.), JAI Press, 1992, vol.2, xxxx.

292. Ley, S.V.; Sternfeld, F.; Taylor, S. *Tetrahedron Lett.* **1987**, *28*, 225.

293. Carless, H. *Tetrahedron Lett.* **1989**, *30*, 3113.

294. Roberts, S.M.; Cotterill, I.C.; Williams, J.O. *J. Chem. Soc., Chem. Communn.* **1988**, 1628.

295. Carless, H.; Oak, O.Z. *Tetrahedron Lett.* **1989**, *30*, 1719.

296. Ley, S.V.; Sternfeld, F. *Tetrahedron* **1989**, *45*, 3463.

297. Roberts, S.M.; Pittol, C.A.; Pryce, R.J.; Ryback, G.R.; Sik, V.; Williams, J.O. *J. Chem. Soc., Perkin Trans. 1* **1989**, 1160.

298. Roberts, S.M.; Downing, W.; Latouche, R.;Pittol, C.A.; Pryce, R.J.; Ryback, Williams, J.O. *J. Chem. Soc., Perkin Trans. 1* **1990**, 2613.

299. Ley, S.V.; Parra, M.; Redgrave, A.J.; Sternfeld, F. *Tetrahedron* **1990**, *46*, 4995.

300. Carless, H. *J. Chem. Soc., Chem. Commun.* **1991**, 61.

301. Stephenson, G.R.; Howard, P.W.; Taylor, S.C. *J. Chem. Soc., Chem. Commun.* **1988**, 1603; *J. Chem. Soc., Chem. Commun.* **1991**, 127; *J. Organometal. Chem.* **1989**, *370*, 97.

302. Hudlicky, T.; Seoane, G.; Pettus, T. *J. Org. Chem.* **1989**, *54*, 4239.

303. Hudlicky, T.; Luna,H.; Price, J.D.; Rulin, F. *J.Org. Chem.* **1990**, *55*, 4683.

304. Hudlicky, T.; Price, J.D. *Synllet* **1990**, 159.

305. Hudlicky, T.; Price, J.D.; Rulin, F.; Tsunoda, T. *J. Am. Chem. Soc.* **1990**, *112*, 9439.

306. BeMiller, J.N.; *Adv. Carbohydr. Chem.***1976**, *22*, 22; *Adv. Carbohydr. Chem. Biochem.* **1970**, *25*, 544.

307. Capon, B. *Chem . Rev.* **1969**, *69*, 407.

308. Sinnott, M.J. in Page, M.I. (Ed) *The Chemistry of Enzyme Action (New Comprenhensive Biochemistry, vol. 6)* , Elservier, Amsterdam, **1984,** p. 389.

309. Lalégerie, P.; Legler, G.; Yon, J.M. *Biochimie*, **1982**, *64*, 977.

310. Sinnott, M.J., in Page, M.I.; Williams, D. (Eds) *Enzyme Mechanism,* The Royal Society of Chemistry, London, **1987**, p. 259.

311. Blake, C.C.F.; Johnson, L.N.; Mair, G.A.; North, A.T.C.; Phillips, D.C.; Sarma, V.R. *Proc. R. Soc., London, Ser. B*, **1967**, *167*, 378.

312. Matsuura, Y.; Kusunoki, M.; Harada, W.; Kakudo, M. *J. Biochem. (Tokyo)*, **1984**, *95*, 697.

313. Legler, B. *Adv. Carbohydr. Chem. Biochem.* **1990**, *48*, 319.

314. Sinnott, M.L. *Chem. Rev.* **1990**, *90*, 1171.

315. Ezaki, S.J. *J. Biochem. (Tokyo)* **1940**, *32*, 104.; Horikoshi, K. *J. Biochem.(Tokyo)*, **1942**, *35*, 39.

316. Levvy, G.A.; Snaith, S.M. *Adv. Enzymol.* **1972**, *36*, 151.

317. Leaback, D.H. *Biochem. Biophys. Res. Commun.* **1968**, *32*, 1025.

318. Fleet, G.W.J.; Smith, P.W.; Evans, S.V.; Fellows, L.E. *J. Chem. Soc. Chem. Commun.***1984**, *38*, 1240.

319. Sinnott, M.L.; Souchard, I.J.L. *Biochem. J.* **1973**, *133*, 89.

320. Inouye, S.; Tsuruoka, T.; Ito, T. Niida, T. *Tetrahedron* **1968**, *23*, 2125.

321. Dale, M.P.; Ensley, H.E.; Kern, K.; Sastry, K.A.R.; Byers, L.D. *Biochemistry* **1985**, *24*, 3530

322. Lai, Y.-L.; Axelrod, B. *Biochem. Biophys. Res. Commun.* **1973**, *54*, 463.

323. Legler, G. *Biochim. Biophys. Açta* **1978**, *524*, 94.

324. Legler, G.; Sinnott, M.L; Withers, S.G. *J. Chem. Soc., Perkin Trans. 2*, **1980**, 1376.

325. Legler, G.; Herrchen, M. *Carbohydr. Res.* **1983**, *116*, 95.

326. Liedtke, H.; Ph. D. Thesis, University of Cologne; 1987.

327. Inouye, S.; Tsuruoka, T.; Ito, T. Niida, T.*J. Antibiot.* **1966**, *19*, 288.

328. Schmidt, D.D.; Formmer, W.; Müller, L.; Truscheit, E. *Naturwissenschaften* **1979**, *66*, 584.

329. Hanozet, G.; Pircher, H.P.; Vnni, P.; Oesch, B.; Semenza, G. *J. Biol. Chem.* **1981**, *256*, 3703.

330. Paulsen, H.; Todt, K. *Adv. Carbohydr. Chem.* **1968**, *23*, 116.

331. Legler, G.; Jülich, E.. *Carbohydr. Res.* **1984**, *128*, 61

332. Legler, G.; Pohl, S. *Carbohydr. Res.* **1986**, *155*, 119.

333. Kappes, E.; Legler, G. *J. Carbohydr. Chem.* **1989**, *8*, 371.

334. Evans, S.V.; Fellows, L.E.; Shing, T.K.; Fleet, G.W.J. *Phytochemstry* **1985**, *24*, 1953.

335. Furhmann, U.; Bause, E.; Legler, G.; Ploegh, H. *Nature* **1984**, *307*, 755.

336. Legler, G. *Pure Appl. Chem.* **1987**, *59*, 1457; Fuhrmann, U.; Bause, B.; Plough, H. *Biochim. Biophys. Acta* **1985**, *825*, 95; Elbein, A. *Annu. Rev. Biochem.* **1987**, *56*, 497.

337. Kameda, Y.; Horii, S. *J. Chem. Soc., Chem. Commun.* **1972**, 746.

338. Kameda, Y; Asano, N.; Teranishi, M.; Matsui, K. *J. Antibiot.* **1980**, *33*, 1573.

339. Kameda, Y; Asano, N.; Yoshikawa, M. Matsui, K. *J. Antibiot.* **1980**, *33*, 1575.

340. a) Itoh, J.; Omoto, S.; Shomura, T.; Ogino, H.; Iwamatsu, K.; Inouye, S.; Hadaka, H. *J. Antibiot.* **1981**, *34*, 1424; b) Omoto, S.;.Itoh, J.; Ogino, H.; Iwamatsu, K.; Nishizawa, N.; Inouye, S. *J. Antibiot.* **1981**, *34*, 1429.

342. Colgate, S.M.; Dorling, P.R.; Huxtable, C.R. *Aust. J. Chem.* **1979**, *32*, 2257.

342. Molyneux, R.J.; James, F. *Science*, **1982**, *216*, 190.

343. Dorling, P.R.; Huxtable; Vogel, P. *Neuropathol. Appl. Neurobiol.* **1978**, 285.

344. Schneider, M.J.; Ugenachi, F.S.; Brosquist, H.P.; Harris, T.M. *Tetrahedron* **1983**, *39*, 29.

345. Dorling, P.R.; Huxtable, C.R.; Colgate, S.M. *Biochem. J.* **1980**, *191*, 649.

346. Tulsiani, D.R.P.; Bronquist, H.P.; James, L.F.; Touster *Arch. Biochem. Biophys.* **1984**, *232*, 76.

347. Hohenschutz, L.D.; Bell, E.A.; Jewess, P.J.; Leworthy, D.P.; Pyrce, R.J. Arnold, E.; Clardy, J. *Phytochemistry*, **1981**, *20*, 811.

348. Saul, R.; Chambers, J.P.; Molyneux, R.J.; Elbein, A.D. *Arch. Biochem. Biophys.* **1983**, *221*, 593.

349. Bashyal, B.P.; Fleet, G.W.J.; Cough, M.J.; Smith, P.W. *Tetrahedron* **1987**, *43*, 3083.

350. Fleet, G.W.J.; Smith, P.W.; Evans, S.V.;Fellows, L.E.; *J. Chem. Soc., Chem. Commun.* **1884**, 1240; Palamarczyk, G.; Mitchell, M.; Smith, P.W.; Fleet, G.W.J.; Elbein, A.D. *Arch. Biochem. Biophys.* **1985**, *243*, 35; Daniel, P.F.; Newburg, D.S.; O'Neil, N.E.; Smith, P.W.; Fleet, G.W.J. *Glycoconjugate J.* **1989**, *6*, 229; Cenci di Bello, I.; Fleet, G.W.J.; Namgoog, S.K.; Tadano, K.-T.; Winchester, B. *Biochem. J.* **1989**, *259*, 855.

351. Fleet, G.W.J.; Smith, P.W. *Tetrahedron Lett.* **1985**, *26*, 1469.

352. Austin, G.N.; Baird, P.D.; Fleet, G.W.J.; Peach, J.M.; Smith, P.W.; Watkin, D.J. *Tetrahedron* **1987**, *43*, 3108.

353. Fleet, G.W.J., Son, J.C.; Green, D.C.; Cenci di Bello, I.; Winchester, B. *Tetrahedron* **1988**, *44*, 2649.

354. Setoi, H.; Kayakiri, H.; Takeno, H.; Hashimoto, M. *Chem. Pharm. Bull.* **1987**, *35*, 3995.

355. Guillerm, G.; Varkados, V.; Auvin, S.; Le Goffic, F. *Tetrahedron Lett.* **1987**, *28*, 535.

356. Reitz, A.B.; Baxter, E.W. *Ttrahedron Lett.* **1990**, *31*, 6777.

357. Wehner, V.; Jäger, V. *Angew. Chem., Int. Ed. Engl.* **1990**, *29*, 1169; Jäger, V.; Hümmer, H.J. *Angew. Chem., Int. Ed. Engl.* **1990**, *29*, 1171.

358. Fleet, G.W.J.; Nicholas, S.J.; Smith, P.W.; Evans, S.V.; Fellows, L.E.;

359. Winkler, D.A.; Holand, G. *J. Med. Chem.* **1989**, *32*, 2084.

360. Eis, M.J.; Rule, C.J.; Wurzburg, B.A.; Ganem, B. *Tetrahedron Lett.* **1985**, *26*, 5397

361. Koyama, M.; Sakamura, S. *Agr. Biol. Chem.* **1974**, *38*, 1111.

362. Evans, S.V.; Hayman, A.R.; Fellows, L.E.; Shing, T.K.M.; Derome, A.E.; Fleet, G.W.J. *Tetrahedron Lett.* **1985**, *26*, 1465.

363. von der Osten, C.H.; Sinskey, A.J.; Barbas, C.F.III; Pederson, R.L.; Wang, Y-F.; Wong, C-H. *J. Am. Chem. Soc.* **1989**, *111*, 3924.

364. Sunkara, P.S.; Bowlin, T.L.; Liu, P.S.; Sjoerdsma, A. *Biochem. Biophys. Res. Commun.* **1987**, *148*, 206.

365. Molyneux, R.J.; Roitman, J.N.; Dunnheim, G.; Szumilo, T.; Elbein, A.D. *Arch. Biochem. Biophys.* **1986**, *251*, 450.

366. Hamana, H.; Ikota, N.; Ganem, B. *J. Org. Chem.* **1987**, *52*, 5494.

367. Bashyal, B.P.; Chow, H-F.; Fellows, L.E.; Fleet, G.W.J. *Tetrahedron* **1987**, *43*, 422.

368. Bruce, I.; Fleet, G.W.J.; Cenci di Bello, I.; Winchester, B. *Tetrahedron Lett.* **1989**, *30*, 7257.

369. Niwa, T.; Tsuruoka, T.; Goi, H.; Kodama, Y.; Itoh, J.; Inouye, S.; Yamada, Y.; Niida, T.; Nobe, M.; Ogawa, Y. *J. Antibiot.* **1984**, *37*, 1579.

370. Fleet, G.W.J.; Ramsden, N.G.; Witty, D.R. *Tetrahedron Lett.* **1988**, *29*, 2871.

371. Fleet, G.W.J.; Ramsden, N.G.; Witty, D.R. *Tetrahedron* **1989**, *45*, 319.

372. Shing, T.K.M. *J. Chem. Soc. Chem., Commun.* **1988**, 1221.

373. Miyake, Y.; Ebata, M. *J. Antibiot.* **1987**, *40*, 122.

374. Fleet, G.W.J. *Chem. Brit.* **1989**, 287.

375. Fleet, G.W.J.; Shaw, A.N.; Evans, S.V.; Fellows, L.E. *J. Chem. Soc., Chem. Commun.* **1985**, 841.

376. Manning, K.S.; Lynn, D.G.; Shabanowitz, J.; Fellows, L.E.; Shing, M.; Schrire, B.D. *J. Chem. Soc., Chem. Commun.* **1985**, 127.

377. Murao, S.; Miyata, S. *Agric. Biol. Chem.* **1980**, *44*, 219.

378. Ziegler, T.; Straub, A.; Effenberger, F. *Angew. Chem., Int. Ed. Engl.* **1988**, *27*, 716.

379. Fellows, L.E.; Bell, E.A.; Lynn, D.G.; Nakanishi, K. *J. Chem. Soc., Chem. Commun.* **1979**, 977.

380. Flett, G.W.J.; Gough, M.J.; Shing, T.K.M. *Tetrahedron* **1984**, *25*, 1853.

381. Fleet, G.W.J.; Ramsden, N.G.; Witty, D.R. *Tetrahedron* **1989**, *45*, 327.

382. Fellows, L.E.; Flett, G.W.J., Alkaloidal Glycosidase Inhibitors from Plants, ch. 13 in *Natural Product Isolation*, (Wagman, G.H; Cooper, R., Eds.), p 540-560, Elservier, 1988.

383. Fleet, G.W.J.; Shing, T.K.M. *Tetrahedron Lett.* **1984**, *25*, 4029.

384. Fleet, G.W.J.; Ramsden, N.G.; Dwek, R.A.; Rademacher, T.W.; Fellows, L.E.; Nash, R.J.; Green, D.C.; Winchester, B. *J. Chem. Soc., Chem. Commun.* **1988,** 483.

385. Fleet, G.W.J.; Ramsden, N.G.; Molyneux, R.J.; Jacob, G.S. *Tetrahedron Lett.* **1988**, *29*, 3603.

386. Legler, G.; Bieberich, E. *Arch. Biochem. Biophys.* **1988**, *260*, 437.

387. Fairbanks, A.J.; Fleet, G.W.J.; Jones, A.H.; Bruce, I.; Daher, S.A.; Cenci di Bello, I.; Winchester, B. *Tetrahedron* **1991**, *47*, 131.

388. Parr, R.A.; Pett, N.P.; Kang, M.S. *Tetrahedron Lett.* **1990**, *31*, 7109.

389. Kino, T.; Inamura, N.; Nakahara, K.; Kiyoto, S.; Goto, T.; Terano, H.; Koshaka, M.; Aoky, H.; Imanaka, H. *J. Antibiot.* **1985**, *38*, 936.

390. Broquist, H.P.; Mason, P.S.; Hagler, W.M.; Harris, T.M. *Appl. Envirom. Microbiol.* **1984**, *48*, 386.

391. Elbein, A.D. *Ann. Rev. Biochem.* **1987**, *56*, 497.

392. Arends, J.; Willms, B.H.L. *Horm. Metab. Res.* **1986**, *18*, 761.

393. Humphries, J.M.; Matsumoto, K.; White, S.L.; Olden K. *Cancer Res.* **1986**, *46*, 5215.

394. Hanozet, G.; Pircher, H.-P.; Vanni, P.; Oesch, B. Semenza, G. *J. Biol. Chem.* **1981**, *256*, 3703.

395. Truscheit, E.; Formmer, W.; Junge, B.; Müller, L.; Schmit, D.; Wingender, W. *Angew. Chem. Int Ed. Engl.* **1981**, *20*, 744.

396. Nash, R.J.; Evans, S.V.; Fellows, L.E.; Bell, E.A. *Plant Toxicol., Proc. Aust.-USA Poisonous Plant Symp. 1984* **1985**, 309

397. Evans, S.V.; Gatehouse, A.M.R.; Fellows, L.E. *Entomol. Exp. Appl.* **1985**, *37*, 257.

398. Nash, R.J.; Fenton, K.A.; Gatehouse, A.M.R.; Bell, E.A *Entomol. Exp. Appl.* **1986**, *42*, 71

399. Blaney, W.M.; Simmons, M.S.J.; Evans, S.V.; Fellows, L.E. *Entomol. Exp. Appl.* **1984**, *36*, 209

400. Schelinger, S.; Koyama, A.H.; Malfer, C.; Gee, S.L.; Schlensinger, M.J. *Virus Res.* **1985**, *2*, 139.

401. Gruters, R.A.; Neefjes, J.J.; Tersmette, M.; de Goede, R.E.Y.; Tulp, A.; Huisman, H.G.; Miedema, F.; Ploegh, H.L. *Nature*, **1987**, *330*, 74.

402. Fellows, L.E. *Chem. Brit.* **1987**, 842.

403. Blough, H.A.; Pauwels, R.; De Clerq, E.; Cogniaux, J.; Sprecher-Goldberger, B.; Thiry, L. *Biochem. Biophys. Res. Commun.* **1986**, *141*, 33.

404. Schwarz, R.T.; Datema, R. *Trend Biochem. Sci.* (Pers. Ed.) **1984**, *9*, 32.

405. Winkler, D.A.; Holan, G. *J. Med. Chem.* **1989**, *32*, 2084.

406. Inami, M.; Nakayama, O.; Terano, H.; Kohsaka, M.; Aoki, H.; Imanaka, H. *J. Antibiot.* **1987**, *40*, 612.

407. Elbein, A.D.; Tropea, J.E.; Mitchell, M.; Kaushol, G.P. *J. Biol. Chem.* **1990**, *265*, 15599.

408. Kayakiri, H.; Takase, S.; Shibata, T.; Okamoto, M.; Terano, H.; Hashimoto, H. Titada, Sh.K. *J. Org. Chem.* **1989**, *54*, 4015.

409. Kayakiri, H.; Kasahara, C.; Oku, T.; Hashimoto, M. *Tetrahedron Lett.* **1990**, *31*, 225.

410. Kayakiri, H.; Oku, T.; Hashimoto, H. *Chem. Pharm. Bull* . **1990**, *38*, 293.

411. Stainer, et.al. *J. Gen. Microbiol.* **1966**, *43*, 159.

412. Murray, M.-Y. (Ed.), *Comprehensive Biotechnology*, Pergamon Press, New York, vol. 2.

413. Multigen Bench Fermentor from New Brunswick Scientific Co., Inc.

414. Biostat E Fermentation System from B. Braum Biotech Inc.

415. Hudlicky, T.; Luna, H.; Barbieri, G.; Kwart, L.D. *J. Am. Chem. Soc.* **1988**, *110*, 4735.

416. Hudlicky, T.; Luna, H.; Olivo, H.O.; Andersen, C.; Nugent, T.; Price, J.D. *J. Chem. Soc., Perkin Trans. 1* **1991**, xxxx

417. Hudlicky, T.; Luna, Andersen, C.; Price, J.D. *Isr. J. Chem.* **1991**, xxxx.

418. Vane, J.R. *Angew. Chem. Int. Ed. Engl.* **1983**, *22*, 741.

419. Weinhemer, A.J.; Spraggins, R.L. *Tetrahedron Lett.* **1969**, 5185.

420 Garcia de la Mora, G.; Perez, G.; Maldonado, L.A.; Grillasca, Y.; Korkowski, I.; Luna, H. Final Report to CONACYT, 1977.

421. Bundy, G.L.; Scheiner, W.P.; Lincoln, F.H.; Pike, J.E. *J. Am. Chem. Soc.* **1972**, *94*, 2123; Scheiner, W.P.; Bundy, G.L.; Lincoln, F.H. *Chem. Commun.* **1973**, 254; Corey, E.J.; Ensley, H.E. *J. Org. Chem.* **1973**, *38*, 3187; Scheiner, W.P.; Bundy, G.L.; Lincoln, F.H.;Daniels, E.G.; Pike, J.E. *J. Am. Chem. Soc.* **1977**, *99*, 1222.

422. Just, G.; Simonovitch, C. *Tetrahedron Lett.* **1967**, 2093; Just. G.; Simonovitch, C.; Lincoln, F.H.; Schneider, W.P.; Axen, U.; Spero, G.B.; Pike, J.E. *J. Am. Chem. Soc.* **1969**, *91*, 5364.

423. Corey, E.J.; Weinshenker, N.M.; Schaaf, T.K.; Huber, W. *J. Am. Chem. Soc.* **1969**, *91*, 5675.

424. Noyori, R.; Maseaki, S. *Angew. Chem., Int. Ed. Engl.* **1984**, *23*, 847.

425. Johnson, C.R.; Pennig, T.D. *J. Am. Chem. Soc* **1986**, *108*, 5655.

426. Cocu, F.G.; Postenak, T. *Helv. Chim. Acta* **1972**, *55*, 2838; Ohuri, H.; Konno, M.; Meguro, H. *Agric. Biol. Chem.* **1987**, *51*, 625; Bocherding, D.R.; Scholtz, S.A.; Borchardt, R.T. *J. Org. Chem.* **1987**, *52*, 5457.

427. Greene, T.W. *Prtotective Groups in Organic Synthesis*; Wiley; New York; 1981.

428. The author wants to thank the help of Dr. Graciel Barbieri in completing the results presented in Tables 5 and 6.

429. Griesbaum, K.; Volpp, W.; Greinert, R.; Greunig, H.-J.; Schid, S.; Henke, H. *J. Org. Chem.* **1989**, *54*, 383.

430. Ayer, W.A.; Brwne, L.M.; Fung, S. *Can. J. Chem.* **1976**, *54*, 3276.

431. Maryanoff, B.E.; Mollinari, A.J.; Wooden, G.P.; Olofson, B. *Tetrahedron Lett.* **1982**, *23*, 2829.

432. Muzart, J. *Synthesis* **1982**, 60; Posner, G.H. *Angew. Chem. Int. Ed. Engl.* **1978**, *17*, 487.

433. Hudlicky, T.; Natchus, M.; Nugent, T. *Synth. Commun.* **1992**, *21*, xxxx.

434. Deardorff, D.R.; D.R.; Shambayati, S.; Dvid, C.M.; Heerding, D. *J. Org. Chem.* **1988**, *53*, 3614; Borchardt, R.T.; Borcherding, D.R.; Scholtz, S.A. *J. Org. Chem.* **1987**, *52*, 5457; Borchardt, R.T.; Wolfe, M.S.; Anderson, B.L.; Borcherding, D.R. *J. Org. Chem.* **1990**, *55*, 4712; Betsmann, H.J.; Roth, D. *Synlett* **1990**, 751; *Angew. Chem.* **1990**, *102*, 99; Ali, S.M.; Ramesh, K.; Borchardt, R.T. *Tetrahedron Lett.* **1990**, *31*, 1509; Belanger, P.; Prasit, P. *Tetrahedron Lett.* **1988**, *29*, 5521; Flann, J.C.; Mash, E.A. *Synth. Communn.* **1988**, *18*, 391.

435. Hudlicky, T.; Luna, H.; Price, J. D.; Rulin, F. *Tetrahedron Lett.* **1989**, *30*, 4053.

436. Hudlicky, T.; Luna, H.; Price, J.D.; Rulin, F. *J. Org. Chem.* **1990**, *55*, 4683.

437. Keul,H.; Griesbaum, K. *Can. J. Chem.* **1980**, *58*, 2049.

438. **¹H NMR of 135** : (CHCl₃) δ 9.6 (d, J= 2.0 Hz, 1H), 4.88 (d, J= 5.4 Hz, 1H), 4.59 (dd, J= 7.3, 2.1 Hz, 1H), 3.77 (s, 3H), 1.63 (s, 3H), 1.45 (s, 3H);

439. **¹H NMR of 136** : (CHCl₃) δ 5.34 (s, 1H), 4.81 (d, J= 5.4 Hz, 1H), 4.5 (d, J= 5.4 Hz, 1H), 3.53 (s, 3H), 1.46 (s, 3H), 1.38 (s, 3H).

440. Chaudaria, C.G.; Heathcock, C.H. *J. Org. Chem.* **1975,** *40,* 2970; Clark, R.D.; Heathcock, C.H. *J. Org. Chem.* **1976,** *41,* 1396.

441. Estimated from TLC analysis

442. Murray, R.W.; Kaplan, M.L. *J. Am. Chem. Soc.* **1969,** *91,* 5358; *J. Am. Chem. Soc.* **1968,** *90,* 537.

443. Adam, W.; Balci, M.; Pietrzak, B.; Rebollo, H. *Synthesis,* **1980,** 820.

444. Oda, M.; Kitahara, Y. *Tetrahedron Lett.* **1990,** *31,* 1617.

445. Yamamoto, Y.; Niki, E.; Kamiya, Y. *J. Org. Chem.* **1981,** *46,* 250.

446. Posternak, T. *The Cyclitols*; Hermann, Paris, 1962.

447. Legler, G; Bause, E. *Carbohydr. Res.* **1973,** *28,* 45.

448. Kubler, K. *Arch. Pharm. Ber. Stsch. Pharm.* **1908,** *246,* 620. Manni, P. E.; Sinshelmer, J. E. *J. Pharm. Sci.* **1965,** *54,* 1541.

449. Plouvier, V. CR Hebd. *Séances Acad. Sci.* **1962,** *255,* 360. Kindi, H.; Hoffmann-Ostenhof, O. *Phytochemistry* **1966,** *5,* 1091; ibid **1967,** *6,* 77.;Fortschr. *Chem. Org. Naturts.* **1966,** *24,* 149; *Monatsh. Chem.* **1970,** *101,* 1704. Kindi, H. *Ann . N.Y. Acad . Sci.* **1968,** *165,* 615.

450. Hudlicky, T.; Price, J. D.; Rulin, F.; Tsundda, T. *J. Am. Chem. Soc.* **1990,** *112,* 9434.

451. Sutbeyaz, Y.; Secen, H.; Balci, M. *J. Chem. Soc. Chem. Commun.* **1988,** 1330.

452. The author wants to acknowledge the help of C. Andersen in the study of the different reducing agents.

453. Hudlicky, T.; Price, J. D.; Luna, H.; Andersen, C.; *M. Synlett.* **1990,** 309.

454. Vogel, P.; Vieira, E.; LeBrian, C.L. *Helv. Chm. Acta* **1989,** *72,* 338.

455. Nakajima, M.; Tomida, I.; Takei, S. *Chem. Ber.* **1959,** *92,* 11.

456. William, N.R. (S. Reporter) Carbohydr. Chemistry **1986,** vol. 20, part 1; p. 182; .Spohr, U.; Meyer zu Reckendorf, W. *Justus Liebigs Ann. Chem.* **1982,** *115,* 946.

457. Nakajima, M.; Hasegawa, A.; Kurihara, N. *Chem. Ber.* **1962,** *95,* 2708

458. Chini, M.; Crotti, P.; Flippin, L.A.; Macchia, F. *J. Org. Chem.* **1990**, *55*, 4265.

459. Saito, S.; Bunya, N.; Inaba, M.; Moriwake, T.; Torii, S. *Tetrahedron Lett.* **1985**, *26*, 5309.

460. Evans, D.A.; Truesdale, L.K. *Tetrahedron Lett.* **1973**, 4929.

461. VanderWerf, C.A.; Heisler, R.Y.; McEwen, W.E. *J. Am. Chem. Soc.* **1954**, *76*, 1231.

462. Sinou, D.; Emziane, M. *Tetrahedron Lett.* **1986**, *27*, 4423; Chong, J.M. Sharpless, K.B *J. Org. Chem.* **1985**, *50*, 1557.

463. Hudlicky, M. *J. Org. Chem.* **1974**, *39*, 3460.

464. NMR analysis of the product showed to be in agreement with the structure
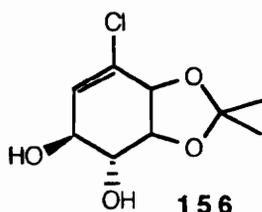
156; this compound has been prepared by straightforward methods to corroborate its structure.

465. Yamamoto, Y.; Asao, N. *J. Org. Chem.* 1990, **55**, 5303.

466. Kagan, J.; Firth, B.E.; Shih, N.Y.; Boyajian, C.G. *J. Org. Chem.* **1977**, *42*, 343.; Fleet, G.W.J.; Ramsden,N.G.; Witty, D.R. *Tetrahedron* **1989**, *45*, 319.

467. (a) $ZnCl_2$: Birkofer, L.; Kaiser, W. *Ann. Chem.* **1975**, 226; (b) $MgCl_2$: Schweisenger, R.; Breuniger, M.; Gallenkamp, B.; Müller, K-H ; Hunkler , D.; Prinzbach, H. *Chem Ber.* **1980**, *113*, 3127; (c) Et_3Al: Mereyala, H.B.; Frei, B. *Helv. Chim. Acta* **1986**, *69*, 415; (d) Et_2AlF: Markova, K.; Sano, H.; Yamamoto, H. *Chem. Lett.* **1985**, 599; (e) Ti(OPr^i)$_2$, VO(OPr^i)$_3$, CpVCl$_2$: Blady, C.; Choukron, R.; Gervais, G. *Tetrahedron Lett.* **1983**, *24*, 4189.

468. Kagan, J; Firth, B.E.; Shih, N.Y.; Boyajian, C.G. *J. Org. Chem.* **1977**, *42*, 343.

469. Niwa, T.; Tsuruoka, T.; Goi, H.; Kodama, Y.; Toh, J.; Inouye, S.; Yamada, Y.; Nida, T.; Nobe, M.; Ogawa, Y. *J. Antibiot.* **1984**, *37*, 1579.

470. Robindon, R.P.; Matsuyama, H.; Wasserman, H. *Tetrahedron Lett.* **1980**, *21*, 3493.

471. Matsuya, H.; Kobayashi, M. Wasserman, H. *Heterocycl.* **1987**, *26*, 85.

472. d'Angelo, J.; Maddaluno, J. *J. Am. Chem. Soc.* **1986**, *108*, 8112.

473. Combret, J.C.; Klein, J.L.; Mouslouhouddine, M. *Synthesis* **1984**, 493; *Tetrahedron Lett.* **1984**, *25*, 3449.

474. Ricci, A.; Degl'Innocenti, A.; Borselli, G. Reginato, G. *Tetrahedron Lett.* **1987**, *28*, 4093.

475. Köll, P.; Dürrfeld, R.; Wolfmeier, U.; Heyns, K. *Tetrahedron Lett.* **1972**, 5081.

476. Fleet, G.W.; Ramsden, N.G.; Witty, D.R. *Tetrahedron Lett.* **1989**, *45*, 319.

477. Griesbaum, K. *Cand. J. Chem.* **1980**, *58*, 2049.

478. Abudarham, J.P.; Meyet, J.; Smadje, W.; Levisalles, J. *Org. Mag. Res.* **1977**, *10*, 192.

479. Soai, K.; Yokoyama, S.; Dokawa, A. *Synthesis* **1987**, 48.

480. Yamamoto, Y.; Asao, N. *J. Org. Chem.* **1990**, *55*, 5303.

481. Berry, J.M.; Hall, L.D. *Carbohydr. Res* **1976**, *47*, 307.

482. Hwang, C.K.; Li, W.S.; Nicolaou, K.C. *Tetrahedron Lett.* **1984**, *25*, 2295.

483. Blandy, C.; Choukroun, R.; Gervais, D. *Tetrahedron Lett.* **1983**, *24*, 4189; *Tetrahedron Lett.* **1987**, *27*, 4423.

484. Andrews, G.C.; Crawford, T.C.; Contillo, Jr., L.G. *Tetrahedron Lett.* **1981**, *22*, 3803.

485. Kagan, J.; Firth, B.E.; Shih, N.Y.; Boyajian, C.G. *J. Org. Chem.* **1977**, *42*, 343.

486. Sweeley, C.C.; Bentley, R.; Makita, M.; Wells, W.N. *J. Am. Chem. Soc.* **1963**, *85*, 2497.

487. Chaundria, C.G.; Heathcock, C.H. *J. Org. Chem.* **1975**, *40*, 2970.

488. Greenwood, F.L. *J. Org. Chem.* **1955**, *20*, 803.

489 Saito, I.; Nagata, R.; Kotsuki, H.; Matsuura, T. *Tetrahedron Lett.* **1982**, *23*, 1717.

490. Maiti, S.N.; Singh, M.P.; Micetich, R.G. *Tetrahedron Lett.* **1986**, *27*, 1423.

491. Krishnamurthy, S. *J. Org. Chem.* **1981**, *46*, 4629.

492. Bayley, H.; Standring, D.N.; Knowless, J.R. *Tetrahedron Lett.* **1978**, 3633.; Voigth, B.; Adam, G. *Z. Chem.* **1983**, *23*, 177.

493. Fleet, G.W.J.; Gough, M.J.; Shing, T.K.M. *Tetrahedron Lett.* **1984**, *25*, 4029.

494. Rao, K.R.; Nageswar, Y.U.D.; Srinivasan, T.N.; Sattur, P.B.; Dhar, D.N.; Murthy, K.S. *Synthesis* **1986**, 437.

495. Reckendorf, W.M.; Weber, R.; Hechenberger, H. *Chem . Ber.* **1981**, *114*, 1306.

496. Kwasnik, H.R.; Oliver, J.E. Brown, R.T. *J. Med. Chem.* **1972**, *1*, 1429.

497. Goerdeler, J.; Sapplet, R. *Chem. Ber.* **1967**, *100*, 2064.

VII. VITA

  Hector Luna Contla was born in Mexico City, Mexico on August 12, 1950 to Ana Maria Contla Carbajal and Hector Luna Cendejas. He received his B.S.degree in Pharmaceutical Chemistry from the National University of Mexico (UNAM) in 1975, and his M.S.degree in Organic Chemistry from the same University in 1981. He was an Assistant Professor in the School of Chemistry at UNAM for eleven years, teaching experimental Pharmacology. He was also named Associate Professor at the Metropolitan University of Mexico for six years, teaching Pharmacology and Organic Chemistry. He joined Dr. Tomas Hudlicky's group in January 1986, as a Research Assistant and started graduate studies at Virginia Tech in 1987 under the guidance of Dr. Hudlicky.