Development of Chemoenzymatic Methods for the Preparation of Optically Pure Drugs and Drug Intermediates

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DOCTOR OF PHILOSOPHY IN CHEMISTRY

By

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CERTIFICATE

It is certified that the research work presented in this thesis titled "**Development of Chemoenzymatic Methods for the Preparation of Optically Pure Drugs and Drug Intermediates**" submitted by **Mr. Sandeep R. Ghorpade** has been carried out by the candidate under my supervision. This work is original and has not been submitted in part or full for any other degree or diploma to this or any other University or Institution. Such material as has been obtained from other sources has been duly acknowledged in the thesis.

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DECLARATION STATEMENT UNDER 0.771

The work presented in the thesis has been carried out by me under the guidance of Dr. T. Ravindranathan, Ex. Head and Deputy Director, Division of Organic Chemistry: Technology, National Chemical Laboratory, Pune. The experimental work, observations and interpretation of the data in connection with the studies are entirely my own. This work is original and has not been submitted in part or full for any other degree or diploma to this or any other University or Institution.

Research Guide **Candidate**

(T. Ravindranathan) (Sandeep R. Ghorpade)

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Not to forget the great help which I received from my dear friends, Rajagopal, Rajendra, Shinde, Vishal, Mandar, Dhananjay, Ramesh, Paresh, Manasi, Sachin,… (big list ahead) who were there for me in all conditions, be it joy or sorrow.

Last but not the least, I would like to dedicate this moment of joy to my parents and family; without their moral support and encouragement the goal would not had been accomplished.

SANDEEP R. GHORPADE

NCL, Pune - 411008 Feb., 2001

PREFACE

This thesis embodies a theme being pursued in the Division of Organic Chemistry: Technology (OCT) at the National Chemical Laboratory (NCL), since OCT's inception in 1991. The theme is making organic chemistry more useful and relevant and chemists more responsive to the needs of the society. This idea is summed up in OCT's logo: "Chemistry with Purpose". In this purposeful pursuit, we have often seen from our experience as well as from what is happening elsewhere that the interface with different disciplines is pregnant with strength, a panorama of possibilities and fruitful sweet results.*

In this direction, we have launched a program on screening of microbial cultures available at "National Collection of Industrial Microorganisms' (NCIM) NCL, Pune. Screening was taken up to identify a culture, which can effect particular biocatalysed reactions useful in creating an asymmetric synthon (say a biosynthon) or an asymmetric bioactive molecule of relevance to Pharmaceutical Industry. In this short time of our preoccupation with the theme, we have realized that the methods give access to asymmetry directly not conveniently possible through conventional chemistry. This approach also gives overall more efficient and economic routes to the 'biosynthons' mentioned earlier and has the additional advantage of the use of green chemistry for life saving drugs.

There was a murmur in some corners of the famed halls of science since almost a decade that Organic Synthesis a mature subject for long, has now become too old fashioned and sterile to serve much purpose including the needs of the society. **The interface mentioned above demonstrates that organic synthesis is still vibrant with possibilities; organic chemists can still identify problems and provide solutions, and direct their attention and target their goal to be purposeful. They can be relevant and useful and quite often play an enabling role for other disciplines to focus onto useful societal needs. The future of synthetic chemist will be therefore bright and full of excitement.***

We hope this thesis will be only the first offering among many to follow from OCT in this direction through the use of Microbial Transformations and is dedicated in spirit and good faith to the tribe of Organic Chemists including Prof. E. J. Corey the Global Grandmaster of Organic Synthesis.

Ravindranathan, Sandeep and Colleagues

*See the Letter of E. J. Corey Published in *SynLett*. **1997**, *5*, 401. (Reproduced in the next page)

3 Feb. 1997

To the readers of Synlett,

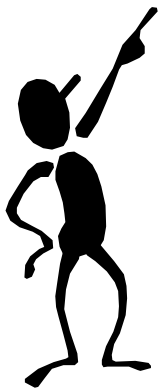
I am grateful to each of my former research students for their excellent contributions to this special issue of Synlett. Many thanks! Our "Corey group" (known in some countries as "the Corey School") is but a small part of the massive effort by many thousands of chemists the world over to advance the power of synthesis for the benefit of humankind. I follow their accomplishments with delight, satisfaction and pride.

It is a wonderful time to be working in the field of synthetic chemistry. This field has already proven itself as one of the most crucial disciplines of science by its signal contributions to the understanding of chemical reactions and mechanisms (the fundamental language of life), to the creation of new molecules of great medical value (antiviral, antibiotic compounds, and medicines for metabolic, organ and cellular disease states), and to the creation of valuable new materials (for electronics, medical devices, clothing, food, etc.).

The coming years will see an explosion in the stock of fundamental biological knowledge — from genetics of disease to genome structures (microbes to man), from receptors, enzymes and their ligands to chemical signaling between macromolecules, and structures of proteins from individual macromolecules to complex assemblies. The opportunities for synthetic chemists to design and synthesize bioactive molecules stagger the imagination. The challenges and the opportunities are greater than they ever have been. I believe that if bright and talented young people continue to devote their lives to synthetic chemistry, the future of the subject will be bright and full of excitement. The beauty and elegance of our subject will be unsurpassed.

E. J. Corey

GENERAL INTRODUCTION – STUDY OF CHEMOENZYMATIC METHODS



General Contents

1.0. General Introduction - Study of Chemoenzymatic Methods

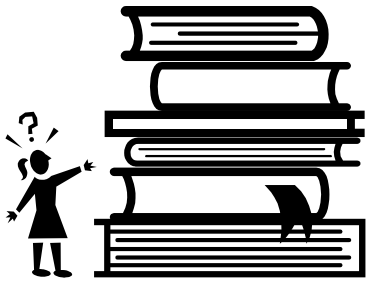
We in Division of Organic Chemistry: Technology (OCT) at National Chemical Laboratory (NCL), Pune, have initiated a biotransformation program to develop industrially applicable and environmentally safe techniques using biocatalysts for the production of commercially important bioactive molecules. Main emphasis is given to the development of chirotechnology using enzyme catalysts. As a part of the continuing efforts in this direction, the present work demonstrates some of successful applications of hydrolytic enzymes for the synthesis and process development of few biologically significant chiral compounds. Various commercial available and inexpensive enzymes e.g. Porcine Pancreatic Lipase, Lipozyme®/Chirazyme® etc. are used for the purpose. Also the large collection of microbial cultures available at NCL i.e. National Collection of Industrial Microorganisms (NCIM) is a major source of enzymes in the form of whole cell cultures. Various techniques to improve enantioselectivity of hydrolytic enzymes have been exploited.

Chapter I is an introductory chapter where importance of chirality in drugs and agrochemicals and various methods for the production of chiral materials have been summarized. Advantages and disadvantages of biocatalyst are stated. Relative merits of the use of whole cells and isolated enzymes, classification and sources of enzymes are described. Mechanism of enzyme action, kinetic reasons for enzyme enantioselectivity are discussed. Hydrolytic enzymes are reviewed. Their mechanistic and kinetic aspects, types of enantiodiscrimination, desymmetrization, kinetic resolution, sequential biocatalytic resolution and dynamic resolution are explained. Various methods to improve enantioselectivity of hydrolytic enzymes are described. Application of special enzymatic techniques viz., immobilization of enzymes, enzymes in organic solvents, membrane bioreactors, enzymes in supercritical fluids, cross-linked enzyme crystals are also discussed. Finally a few commercial applications of enzymatic processes and future prospects of enzymatic transformations in organic synthesis are mentioned and commented.

Chapter II describes our efforts towards development of cost effective chemoenzymatic processes for the production of prostaglandin intermediate component C i.e. 4-(*R*)-hydroxycyclopent-2-en-1-one and its silyl derivative. Initially, nomenclature of prostaglandins, important methods for the synthesis of prostaglandin are briefed. Various chemical and enzymatic methods for the production of component C are reviewed. Three enzymatic methods developed by us for the production of component C through desymmetrization technique and kinetic resolution are described. Our work here comprises screening of commercial enzymes and various microbial cultures from NCIM for the desired conversions and exploration of various techniques of parameter optimization to achieve desired level of selectivity.

Chapter III describes our efforts towards development of (1) chemoenzymatic route for 6-hydroxymethyl-4-(*tert*-butyldimethylsilyloxy)-(4*R*,6*S*)-tetrahydro-2*H*-2-pyranone (δ-lactone), which serve as an important intermediate for mevilinic acid analogues, fluvastatin, atorvastatin etc. which are potent antihyperlipidemic drugs. (2) Chemoenzymatic method for ketone intermediates for A-ring synthon of 19-norvitamin D_3. Initially various synthetic methods - chemical and chemoenzymatic - for the preparation of δ-lactone and its analogues have been reviewed. Our approach is based on utilization of all *cis*-hydroxy configuration of phlorogulcitol in stereo-controlled manner using enzymes. Hydrolytic enzymes have been utilized at the various stages of synthesis to perform chemoselective hydrolysis, enantioselective hydrolysis, etc. to obtain desired optically pure hydroxycyclohexanone. A highly regiospecific Bayer-Villiger reaction was developed, which afforded the desired lactone as a single isomer from hydroxycyclohexanone. Methods for the preparation of both the enantiomers are developed. The chiral intermediates developed in this scheme were further utilized for the simple synthesis of ketone intermediates of phosphine oxide A-ring synthon for 19-norvitamin D_3. The key steps in this synthesis are efficient Mitsunobu inversion followed by facile selective protection/deprotection of –OTBDMS and –OTHP groups and PCC oxidation.

Techniques and Practice in the Production of Chiral Materials using Biocatalysis



Chapter L

CHAPTER I

Contents

2.1. Importance of Chirality in Industry

The importance of obtaining optically pure materials hardly requires restatement. Manufacture of chemical products applied either for the promotion of human health or to combat pests that otherwise adversely impact on the human food supply is now increasingly concerned with the enantiopurity. A large proportion of such products contain at least one chiral center. To show importance of single-enantiomer drugs today, Sujan Ba, Director of Chiral Chemistry Consulting Services at the consulting firm Technology Catalyst International (TCI) measured their appearance among the top-selling drugs.[1] Of the top 100 drugs world wide, 50 are single enantiomers. Their sales were $42.8 billion in 1997 i.e. 51% of the total sales of $85.2 billion for these top 100. Single enantiomers remain important among the top 300, with 158 drugs accounting for $64.7 billion out of a total sales of $124.4 billion. The situation is the same where you consider the top 500 drugs, where single enantiomers number 269, with 1997 sale of $71.1 billion or 52% of the total $135.9 billion. There is a move towards increasing single enantiomer use, wherever possible as a matter of choice as well as by dictates of regulates in bioactive materials for different reasons including the biological ones (*vide infra*). TCI further estimates 1997 worldwide sales of dosage forms of all drugs at $310 billion.

The desirable reasons for producing optically pure materials include the following: (i) biological activity often associated with only one enantiomer (ii) enantiomers may exhibit very different types of activity, both of which may be beneficial or one may be beneficial and the other undesirable (Figure 2.1). Racemic thalidomide consumed by expectant mothers as sedative in the early sixties created a generation of maltransformed babies because of teratogenicity of the (*S*)-isomer[2] which made the company (Germany) to close down for ever and opened the eyes of scientist against the potential harm from the wrong isomer usage. Production of only one enantiomer allows the separation of the effects; (iii) the unwanted isomer is at best 'enantiomeric ballast'[3] gratuitously applied to the environment; (iv) the optically pure compound may be more than twice as active as the racemate because of antagonism, for example the pheromone of the Japanese beetle **1** where as little as 1% of the (*S*, *Z*)-isomer inhibits the (*R*, *Z*) isomer;[4] (v) registration consideration;[5] production of materials as the required enantiomer is now a question of law in certain countries, the unwanted enantiomer being considered as an impurity; (vi) where the switch from racemate to enantiomer is feasible, there is the opportunity effectively to double the capacity of an industrial process; alternatively, in some cases, where the optically active component of the synthesis is not very expensive, it may allow significant savings to be made in some other achiral but very expensive process intermediate; (vii) improved cost-benefit ratio; (viii) the physical characteristics of enantiomers versus racemates may confer processing or the formulation advantages.

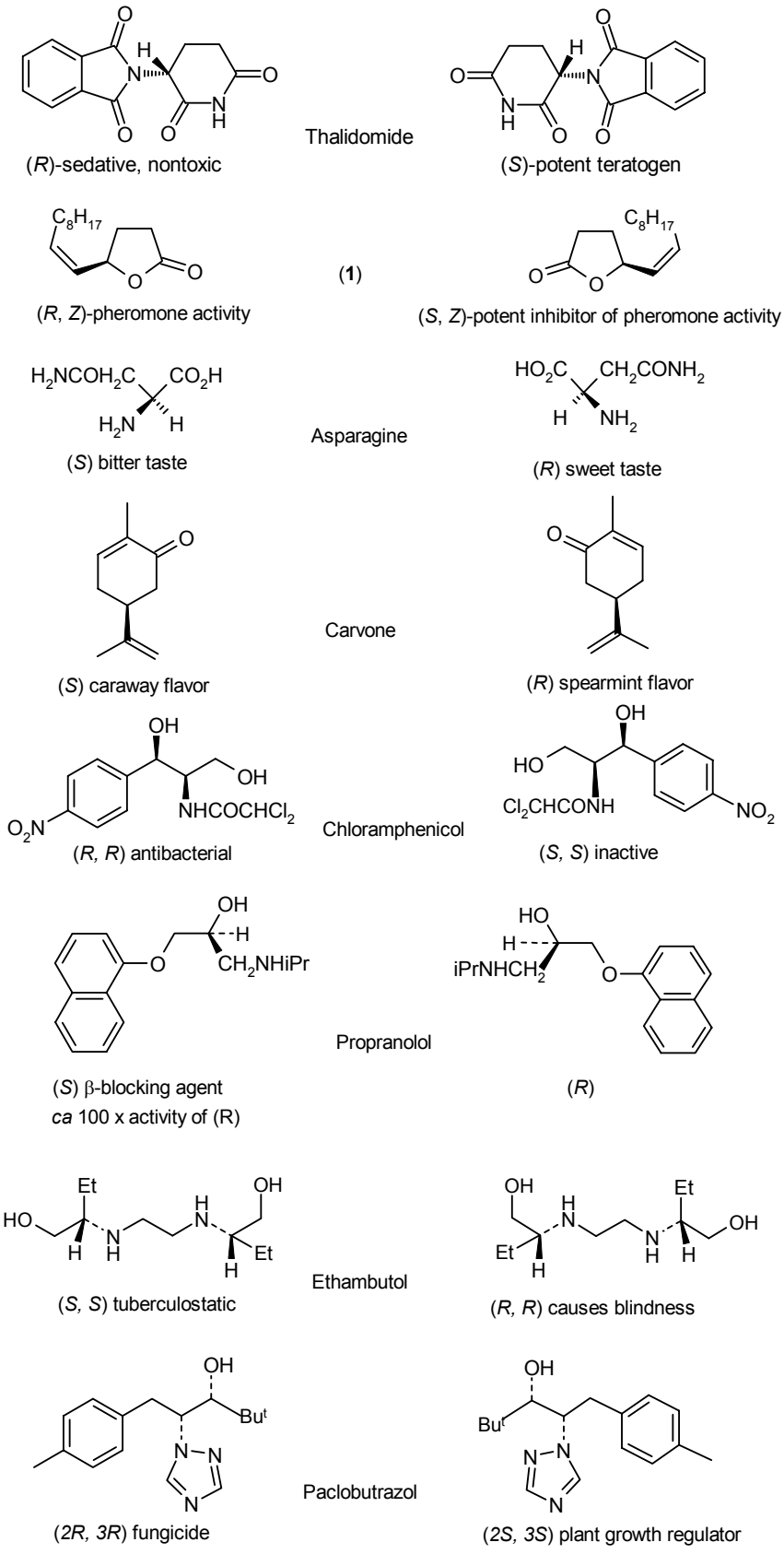

(R)-sedative, nontoxic
Thalidomide
(S)-potent teratogen
(1)
(R, Z)-pheromone activity
(S, Z)-potent inhibitor of pheromone activity
(S) bitter taste
Asparagine
(R) sweet taste
(S) caraway flavor
Carvone
(R) spearmint flavor
(R, R) antibacterial
Chloramphenicol
(S, S) inactive
(S) β-blocking agent
ca 100 x activity of (R)
Propranolol
(R)
(S, S) tuberculostatic
Ethambutol
(R, R) causes blindness
(2R, 3R) fungicide
Paclobutrazol
(2S, 3S) plant growth regulator

Figure 2.1.

All conceivable methods for the production of optically pure materials are being actively researched. All these methods are summarized in Figure 2.2. The enzymes can play their role in the production of single enantiomer either by kinetic resolution of racemates or by asymmetric synthesis from prochiral substrates. The use of natural catalyst - enzymes – for the transformation of non-natural man-made organic compounds is not at all new: they have been used for more than one hundred years, employed either as whole cells, cell organelles or isolated enzymes.[6] Certainly, the object of the early research was totally different from that of the present day. Thus elucidation of biochemical pathways and enzyme mechanisms was the main reason for research some decades ago. It was mainly during the 1980s that the enormous potential of applying natural catalysts to transform non-natural organic compounds was recognized. What started as trend in the late 1970s could almost be called a fashion in synthetic organic chemistry in the 1990s. As a result of this extensive, recent research, there have been an estimated 12,000 papers published on the subject.[7]

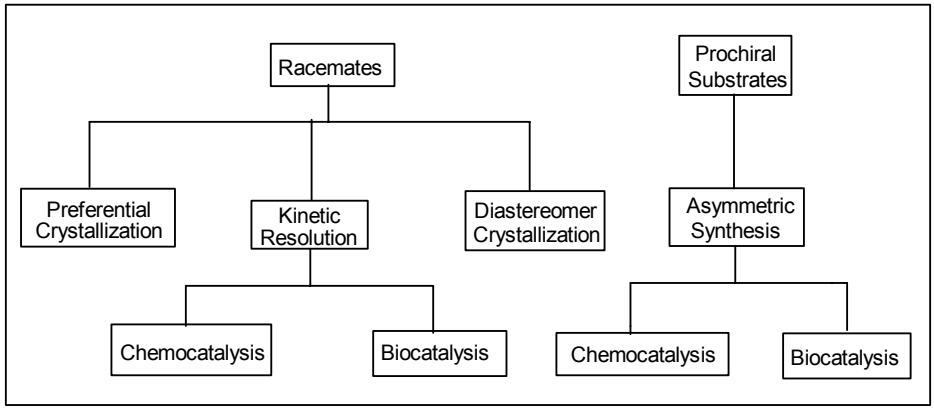


Figure 2.2.

2.2. Advantages and Disadvantages of Biocatalysts

Advantages of Biocatalysts

-Enzymes are very efficient catalysts.

Typically the rates of enzyme-mediated process are accelerated, compared to those of the corresponding nonenzymatic reactions, by a factor of 10^8-10^{10}. The acceleration may even exceed a value of 10^{12}, which is far above the values that chemical catalysts are capable of achieving.[8] Generally chemical catalysts are employed in concentrations of a mole percentage of 0.1-1% whereas most of enzymatic reactions can be performed at reasonable rates with a mole percentage of 10^{-3}-10^{-4}% of catalyst, which clearly makes them more effective by some orders of magnitude.

-Enzymes are environmentally acceptable.

Unlike heavy metals biocatalysts are completely degraded in the environment.

-Enzymes act under mild conditions.

Enzymes act in a pH range of about 5-8, typically around 7, and in a temperature range of 20–40^{o}C, preferably at around 30^{o}C. This minimizes problems of undesired side-reactions such as decomposition, isomerization, racemization and rearrangement, which often plague traditional methodology. Thus side/waste products are decreased with increased selectivity.

-Enzymes are compatible with each other.

Since enzymes generally function under the same or similar conditions, several biocatalytic reactions can be carried out in one flask (proteases are exception for obvious reasons). Thus sequential reactions can be performed by using multienzyme system in order to simplify reaction process, in particular, if the isolation of an unstable intermediate can be omitted. Furthermore, an unfavorable equilibrium can be shifted towards the desired products by linking two consecutive enzymatic steps onto each other.

-Enzymes are not bound to their natural role.

They exhibit a high substrate tolerance by accepting a large variety of man-made unnatural substances and often they are not required to work in water. If advantageous for a process, the aqueous medium can sometimes be replaced by an organic solvent.

-Enzymes can catalyze a broad spectrum of reactions.

Like all catalysts, enzymes only accelerate a reaction, but they have no impact on the position of the thermodynamic equilibrium of the reaction. Thus, in principle, some enzyme-catalyzed reactions can be run in both directions.

There is an enzyme-catalyzed process equivalent to almost every type of organic reaction:[9] for example

- Hydrolysis-synthesis of esters,[10] amides,[11] lactones,[12] lactams,[13] ethers,[14] acid anhydrides,[15] epoxides,[16] and nitriles.[17]
- Oxidation-reduction of alkanes,[18] alkenes,[19] aromatics,[20] alcohols,[21] aldehydes and ketones,[22, 23] sulfides and sulfoxides.[24]
- Addition-elimination of water,[25] ammonia,[26] hydrogen cyanide.[27]
- Halogenation and dehalogenation,[28] alkylation and dealkylation,[29] isomerization,[30] acyloin-[31] and aldol reactions.[32] Even Michael additions have been reported.[33]

Some major exceptions where equivalent reaction types cannot be found in nature are the Diels-Alder reaction[34, 35] and the Cope-rearrangement, although [3,3]-sigmatropic rearrangements such as the Claisen-rearrangement are known.[36, 37] On the other hand, there are some biocatalysts which can accomplish reactions impossible to emulate in conventional organic chemistry e.g. the selective functionalization of ostensibly non-activated positions in organic molecules, such as hydroxylation of aliphatics.

Enzymes display three major types of selectivities:

- Chemoselectivity

Since the purpose of an enzyme is to act on a single type of functional group, other sensitive functionalities, which would normally react to a certain extent under chemical catalysis, survive. As a result, reactions generally tend to be ‘cleaner’ and laborious purification of product(s) from impurities emerging through side reactions can largely be omitted. For instance, enzymatic ester hydrolysis does not show any propensity for acetal-cleavage.

-Regioselectivity and Diastereoselectivity

Due to their complex three-dimensional structure, enzymes may distinguish between functional groups, which are chemically situated in different regions of the same substrate molecule.[38, 39]

- Enantioselectivity

Last but not least, almost all enzymes are made from L-aminoacids and thus are chiral catalysts.[40] As a consequence, any type of chirality present in the substrate molecule is

'recognized' upon the formation of the enzyme-substrate complex. Thus a prochiral substrate may be transformed into an optically active product and both enantiomers of a racemic substrate may react at different rates, affording a kinetic resolution. These latter properties collectively constitute the 'specificity' of an enzyme and represent its most important feature for selective and asymmetric exploitation.[41] This key feature was recognized by E. Fischer as early as 1898![42]

Disadvantages of Biocatalysts

There are certainly some drawbacks worthy of mention for a chemist intent on using biocatalysts:

- Enzymes are provided by Nature in only one enantiomeric form.

Since there is no general way of creating mirror-image enzymes (from D-aminoacids) it is impossible to invert the chiral induction of given enzymatic reaction by choosing the 'other enantiomer' of the biocatalyst, a strategy which is possible when chiral chemical catalyst are involved. To gain access to the other enantiomeric product, one has to follow a longer path in search of an enzyme with exactly the opposite stereochemical selectivity. However, this is quite feasible.

- Enzymes require narrow operation parameters.

The obvious advantage of working under mild reaction conditions can sometimes turn into a drawback. If a reaction proceeds only slowly under given parameters of temperature or pH, there is only a narrow scope for alteration. Elevated temperatures as well as extreme pH lead to deactivation of the protein, as do high salt concentrations. The usual technique of lowering the reaction temperature in order to gain an increase in selectivity is of limited use with enzymatic transformations. The narrow temperature range for the operation of enzymes prevents radical changes, although positive effects from certain small changes have been reported.[43] Quite astonishingly, some enzymes remain catalytically active even in ice.[44, 45]

- Enzymes display their highest catalytic activity in water.

Due to its high boiling point and high heat of vaporization, water is usually the least desired solvent of choice for most organic reactions. Furthermore, the majority of organic compounds are only poorly soluble in aqueous media. Thus shifting enzymatic reactions from an aqueous to an organic medium would be highly desired, but the unavoidable price one has to pay is usually some loss of activity, which is often in the order of one magnitude.[46]

- Enzymes are prone to inhibition phenomena.

Many enzymatic reactions are prone to substrate or product inhibition, which causes the enzyme to cease to work at higher substrate and/or product concentrations, a factor, which limits the

efficiency of the process. Whereas substrate inhibition can be circumvented comparatively easily by keeping the substrate concentration at a low level through continuous addition, product inhibition is a more complicated problem. The gradual removal of product by physical means is usually difficult as is the engagement of another step to the reaction sequence in order to effect chemical removal of the product.

- Enzymes may cause allergies.

Enzymes may cause allergic reactions. However, this may be minimized if enzymes are regarded as chemicals and handled with the same care.

Isolated Enzymes Versus Whole Cell Systems

The physical state of biocatalysts, which are used for biotransformations, can be very diverse. The final decision as to whether one should use isolated, more or less purified enzymes or whole microorganisms – either in a free or immobilized form – depends on many factors, such as (i) the type of reaction, (ii) the requirement of cofactors to be recycled and (iii) the scale in which the biotransformation has to be performed. The general pros and cons of the situation are outlined in Table 2.1.

Table 2.1. Pros and cons of using Isolated enzymes and Whole cell systems

System	Advantages	Disadvantages
Isolated Enzymes	Simpler equipment, simpler work-up, less contamination from other enzymes, higher tolerance to organic solvents.	Expensive co-factor recycling necessary
Whole cell system	Cheap, no co-factor recycling necessary	Expensive equipment, tedious work-up due to large volumes and low tolerance of organic solvents

A significant degree of convergence of biochemistry, microbiology and biochemical engineering – biotechnology – has led to the development of routes to a number of specialty chemicals (ranging from aminoacids to penicillins) starting from cheap carbon sources (such as carbohydrates), cocktails of salts and using viable whole cells. Such syntheses requiring a multitude of biochemical steps are usually referred to as 'fermentation' processes since they constitute *de novo* syntheses in a biological sense. In contrast, the majority of microbially mediated biotransformations, often starting from relatively complex organic molecules, makes use of only a single (or a few) biochemical synthetic step(s) by using (or rather 'abusing'!) the microbe's enzymatic potential to convert a non-natural organic compound into a desired product. To distinguish these latter processes from typical fermentations where a multitude of enzymes are involved, the term 'enzymation' is often used.

2.3. Classification and Sources of Enzymes

Classification of Enzymes

At present almost 300 enzymes have been recognized by the International Union of Biochemistry[47] and if the speculation that there are about 25 000 enzymes existing in Nature is true,[48] about 90% of this vast reservoir of biocatalysts remains still to be discovered and used. However, only a minor fraction of enzymes already investigated (roughly 300, 10%) is commercially available. As depicted in Table 2.2, enzymes have been classified into six categories according to type of reaction they can catalyze. The importance of practical applications for organic synthesis is not at all evenly distributed amongst the different enzyme classes, as may be seen from the 'utility/ % R & D' column.[7]

Table 2.2. Classification of enzymes

Enzyme class	Number Classified	available	Reaction type	Utility*/ % R & D
1. Oxidoreductases	650	90	Oxidation-reduction: oxygenation of C-H, C-C, C=C bonds, or overall removal or addition of hydrogen atom equivalents.	+++ 25%
2. Transferases	720	90	Transfer of groups: aldehydic, ketonic, acyl, sugar, phosphoryl or methyl.	+ ~5%
3. Hydrolases	636	125	Hydrolysis-formation of esters, amides, lactones, lactams, epoxides, nitriles, anhydrides, glycosides.	+++ 65%
4. Lyases	255	35	Addition-elimination of small molecules on C=C, C=N, C=O bonds.	++ ~5%
5. Isomerases	120	6	Isomerizations such as racemization, epimerization.	$\pm$ ~1%
6. Ligases	80	5	Formation-cleavage of C-O, C-S, C-N, C-C bonds with concomitant triphoshpate cleavage.	$\pm$ ~1%

* The estimated 'utility' i.e. usefulness of an enzyme class for the transformation of non-natural substrates ranges from +++ (very useful) to $\pm$ (little use).[49] The values (%) indicate the percentage of research performed with enzymes from a given class for the 1987-96 period.

Coenzymes

A remarkable fraction of synthetically useful enzyme-catalyzed reactions require cofactors (coenzymes).[50] These are compounds of relatively low molecular weight compared to the enzyme [a few hundred Daltons (Da), in contrast to the general range of 15,000 to 1,000,000 Da for enzymes], which provide either 'chemical reagents' such as redox-equivalents, such as hydrogen, oxygen or electrons, and carbon-units. Alternatively, 'chemical energy' is stored in energy-rich functional group such as acid anhydrides, etc. As a rule of thumb, in general cofactors cannot be replaced by more economical man-made chemical substitutes. Some of the cofactors are gradually destroyed due to undesired side-reactions occurring in the medium, in particular

NAD(P)H and ATP. These cofactors are too expensive to be used in the stoichiometric amounts formally required. Accordingly, when coenzyme-dependent enzymes are employed, the corresponding coenzymes are used in catalytic amounts in conjunction with an efficient and inexpensive system for their regeneration in situ. Some methods for cofactor—recycling are already well developed, but others are still problematic. Fortunately, some coenzymes are tightly bound to their respective enzymes such that external recycling is not required.

Many enzymes require coordinated metals such as Fe, Ni, Cu, Co, V, Zn, Mg, or Mn. In many cases, chemists do not have to worry about supplying these metals since they are already present, tightly bound to the enzyme, if this is not the case, they can easily be supplied by enrichment of the medium with the respective metal ion.

Enzyme Sources

The large majority of enzymes used for biotransformations in organic chemistry are employed in a crude form and are relatively inexpensive. The preparations typically contain only about 1-30% of actual enzyme, the remainder being inactive proteins, stabilizers, buffer salts or carbohydrates from the fermentation broth from which they have been isolated. It should be kept in mind that crude preparations are often more stable than purified enzymes. Some of the main sources for enzymes and their uses at industrial scale are as follows:[51]

- The detergent industry produces many proteases and lipases in huge amounts. These are largely used as additives for detergents to effect the hydrolysis of proteinogenic and fatty impurities.
- The food industry uses proteases and lipases for meat and cheese processing and for the amelioration of fats and oils.[52]
- Numerous enzymes can be isolated from slaughter waste or cheap mammalian organs such as kidney or liver. Alternatively, microbial sources can be utilized. Only a small fraction of enzymes is obtained from plant sources.
- Pure enzymes are usually very expensive and thus are mostly sold by the unit, while crude preparations are often shipped by the kg. Since the techniques for protein purification are becoming easier, thus making their isolation more economically feasible, the use of pure enzymes in biotransformations is steadily increasing.

2.4. Mechanism of Enzyme Catalysis

Though the mechanism of enzyme action is not yet perfectly understood, it is believed that the action of an enzyme is equivalent to that of a chemical catalyst in that it promotes a given reaction, but itself remains unchanged at the end of the reaction. It is also known that only a portion of the enzyme, called the active center, is responsible for its catalytic property.

The first step in an enzymatic reaction is the formation of an enzyme substrate complex (ES). This process takes place in a confined region of the enzyme known as the active center or active site. In this part of the enzyme the substrate binds to a specific area called the substrate binding site by multiple weak interactions,[53] subsequently the catalytic group carry out the chemical transformation of substrate into product.

Several theories have been proposed over the years to explain the interaction between enzyme active site and substrate. Fischer suggested the "*Key and Lock*" model[54] to account for the high degree of enzyme specificity – i.e. the active site of an enzyme (lock) can only be filled by a substrate (key) with correct shape and size.

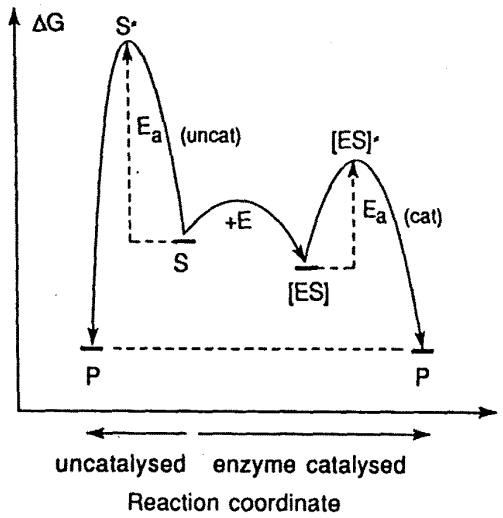
Although this assumption was quite sophisticated at that time, it assumes a completely rigid enzyme structure. Thus, it cannot explain why many enzymes do act on large substrates, while they are inactive on smaller similar counterparts. A more modern view is the "*Induced fit*" model suggested by Koshland[55] in 1958. According to it conformational changes induced by the substrate when it binds produce optimal alignment of the catalytic groups which in the absence of substrate, may lie at a considerable distance from each other. The most typical induced fit enzymes are the lipases.

Quite recently Dewar[56] developed a rationale called *desolvation theory* in attempting to explain the high conversion rates of enzyme reactions, which are often substantially faster than the chemically catalyzed equivalent processes. This theory assumes that the kinetics of the enzyme reaction have much in common with those of gas-phase reactions. If a substrate enters the active site of the enzyme, it replaces all the water molecules from the active site of the enzyme. Then a formal gas phase reaction can take place that mimics two reaction partners interacting, without disturbing solvent. This desolvation theory has recently been substituted by a "*Solvation – Substitution*" theory.[57] It is based on the assumption that the enzyme would not be able to strip off the water that is surrounding the substrate to effect desolvation, which would be energetically unfavoured. Instead, the solvent is displaced by another polar environment by a so called "*Solvation Substitution*".

The widely used rationale to explain the enantioselectivity of enzymes was suggested by Ogston.[58] According to this rationale, to get a high degree of enantioselection, a substrate must be held firmly in three-dimensional space. Therefore, there must be at least three different points of attachment of the substrate on the active site. If we assume that an optically active compound can be bound to the enzyme through a minimum of three points, then the "Fit" will occur with either D- or L-enantiomer, but not with both. For example, if the D-enantiomer fits, the L- will not and vice-versa. For example, reduction of pyruvic acid to lactic acid will occur on one side to produce one enantiomer of lactic acid. The pyruvic acid molecule fits into the enzyme in one way only and consequently hydrogen transfer must occur to one face only, thereby resulting in the formation of only one enantiomer of lactic acid.

Kinetic Reasons for Selectivity

As in every other catalytic reaction, an enzyme (E) accelerates the reaction by lowering the energy barrier between substrate (S) and product (P) i.e. the activation energy (E_a). The origin of this catalytic power of rate acceleration has generally been attributed to transition- state stabilization of the reaction by the enzyme, assuming that the catalyst binds more strongly to the transition state than to the ground state of the substrate, by a factor approximately equal to the rate of acceleration. All stereoselectivities of enzymes originate from the energy difference in the enzyme-transition-state complex (ES) (Figure 2.3).

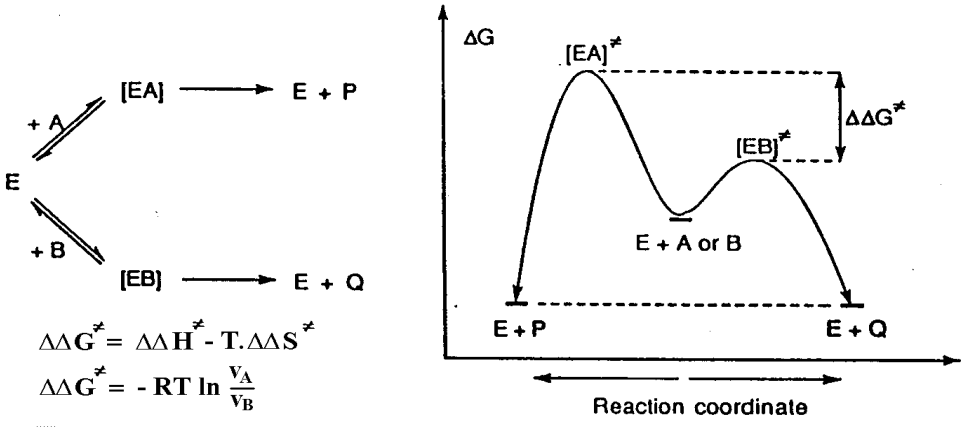


S = substrate, p = product, E = enzyme, [ES] = enzyme-substrate complex,
# denotes a transition state, E_a = activation energy.

Figure 2.3.

In an enantioselective reaction both the enantiomeric substrates A and B, or the two forms of mirror-image orientation of a prochiral substrate involving its enantiotopic groups or faces

compete for the active site of the enzyme. Due to the chiral environment of the active site of the enzyme, diastereomeric enzyme–substrate complexes (EA) and (EB) are formed, which possesses different values of free energy (G) for their respective transition states $(EA)^{\neq}$ and $(EB)^{\neq}$. The result is a difference in activation energy for both of the enantiomeric substrates or the enantiomeric orientations. As a consequence, one enantiomer will be transformed faster than the other. The value of this difference in free energy is expressed and $\Delta\Delta G^{\neq}$. It is a direct measure for the selectivity of the reaction, which in turn governs the optical purity of the product (Figure 2.4).



E = enzyme, A and B = enantiomeric substrates, P and Q = enantiomeric products, [EA] and [EB] = enzyme-substrate complexes, ≠ denotes a transition state, $\Delta\Delta G, \Delta\Delta H$ & $\Delta\Delta S$ = free energy, enthalpy and entropy difference, resp.; T = Temperature, v_A & v_B = reaction rates of A & B resp.

Figure 2.4.

Enzyme Inhibition

An important number of compounds have the ability to combine with certain enzymes, but do not serve as substrates, and therefore block catalysis by that enzyme. These compounds are called noncompetitive inhibitors.

☞ **Competitive Inhibition:** When a compound competes with a substrate or coenzyme for the active site on the enzyme protein and there by reduces the catalytic activity of the enzyme, the compound is considered as competitive inhibitor. The competitive inhibitor (I) binds reversibly with free enzyme and prevents substrate binding (S).

$$[ES] \overset{(S)}{\rightleftharpoons} (E) \overset{(I)}{\rightleftharpoons} [EI]$$

One of the classical examples of this type of inhibition is the inhibition of the enzyme succinic dehydrogenase by malonic acid. Because of the very similar arrangement of the two carbonyl groups of malonic acid, it binds very effectively to the succinic acid site of the enzyme. Since malonic acid has no carbon-carbon bonds to oxidize, it cannot act as a substrate. However, it prevents the formation of true enzyme-substrate complex form and there acts as an inhibitor. This inhibition can now be reversed by increasing in turn the concentration of the substrate succinic acid.

☞ **Noncompetitive Inhibition:** The type of inhibition that cannot be reversed by increasing substrate concentration is called noncompetitive inhibition. The inhibitor combines irreversibly with a site on the enzyme surface and cannot be displaced by increasing the substrate concentration.

$$[ES] \underset{}{\overset{(S)}{\rightleftharpoons}} (E) \xrightarrow{(I)} [EI]$$

A good example is the reaction of iodoacetamide on triose phosphate dehydrogenase, a sulfhydryl enzyme.

$$\textbf{Enzyme – SH + } ICH_2CONH_2 \longrightarrow \textbf{Enzymes-S-}CH_2CONH_2 \textbf{ + HI}$$

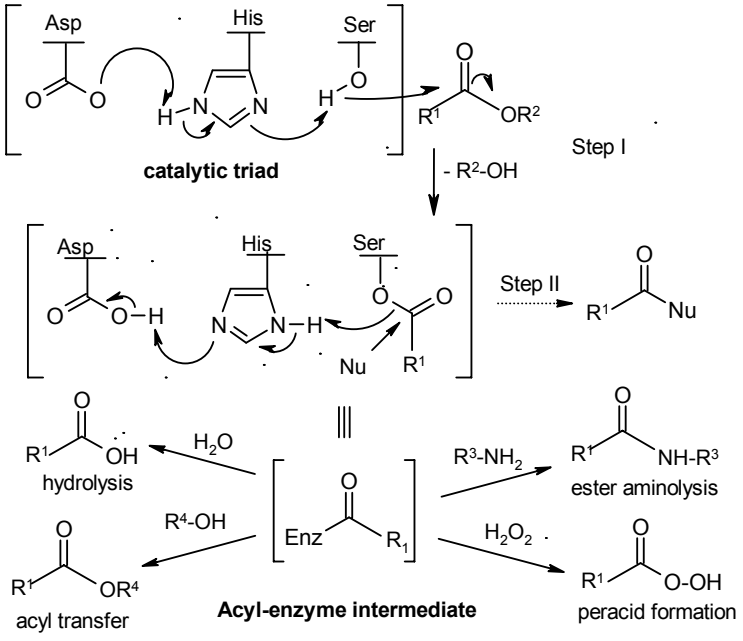
A considerable body of information supports the rationale in medicine that many drugs function because of specific inhibitory effect on a critical enzyme in a tissue. Thus, penicillin appears to block cell wall construction in microorganisms and the highly dangerous nerve poison diisopropylfluorophosphate strongly inhibits acetylcholine esterase, the enzyme intimately associated with the nerve function.

2.5. Hydrolytic Reactions

Of all the types of enzyme-catalyzed reactions, hydrolytic transformations involving amide- and ester-bonds are the easiest to perform using proteases, esterases or lipases. A lack of sensitive cofactors which would have to be recycled, and a large number of readily available enzymes possessing relaxed substrate specificities to choose from, are the main features which have made hydrolases the favorite class of enzyme for the organic chemists during the past decade. About two thirds of the total research in the field of biotransformations has been performed using hydrolytic enzymes of this type.[59] The reversal of reaction, giving rise to ester- or amide-synthesis, has been particularly well investigated using enzymes in solvent of low water activity.

Mechanistic and Kinetic Aspects

The mechanism of amide- and ester-hydrolyzing enzymes is very similar to that describing chemical hydrolysis by base. A nucleophilic group from the active site of the enzyme attacks the carbonyl group of the substrate ester or amide. This nucleophilic 'chemical operator' can be either the hydroxyl group of a serine (e.g. pig liver esterase, subtilisin and the majority of microbial lipases), a carboxy group of an aspartic acid (e.g., pepsin) or a thiol-functionality of a cysteine (e.g. papain).[60] The mechanism, which has been elucidated, is that of serine-hydrolases (Scheme 2.1).[61]



Nu = H_2O, R_4-OH, R_3-NH_2, H_2O_2

R_3 = alkyl, aryl, $-NR^5_2$

R_4 = alkyl, aryl, $-N=CR^5_2$

Scheme 2.1.

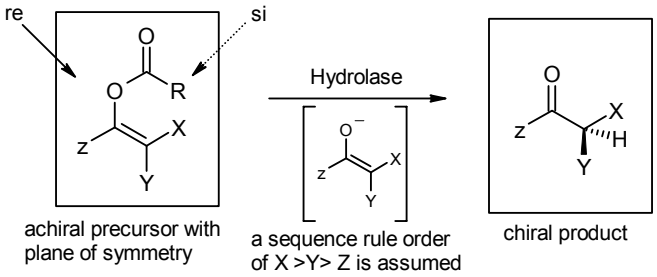
As shown in Scheme 2.1, two additional groups (Asp and His) located together with the serine residue (which is the actual reacting chemical operator in the active site) form the so-called '*catalytic triad'*.[62] The special arrangement of these three groups effects a decrease in the pK-value of the serine hydroxy group thus enabling it to perform a nucleophilic attack on the carbonyl group of the substrate R^1-CO-OR^2 (step I). Thus the acyl moiety of the substrate is covalently linked onto the enzyme, forming the '*acyl-enzyme intermediate*' by liberating the leaving group (R^2-OH). Then a nucleophile (Nu) – usually water – can in turn attack the acyl-enzyme intermediate, regenerating the enzyme and releasing a carboxylic acid R^1-CO-OH (step II). When the enzyme is operating in an environment of low water activity – in other words at low water concentrations - any other nucleophile can compete with the water for the acyl-enzyme intermediate thus leading to a number of synthetically useful transformations:

- Attack of another alcohol R^4-OH leads to another ester R^1-CO-OR^4. This is an inter-esterification reaction, called enzymatic 'acyl transfer',[63]
- An incoming amine R^3-NH_2 results in the formation of an amide R^1-CO-NH-R^3, yielding an enzymatic aminolysis of esters,[64] and
- Peracids of type R^1-CO-OOH are formed when hydrogen peroxides acting as the nucleophile.[65]
- Hydrazinolysis provides access to hydrazides,[66] and the action of hydroxylamine results in the formation of hydroxamic acid derivatives.[67] However, both of the latter transformations have not created a significant impact in the field.

During the course of all of these reactions, any type of chirality in the substrate is 'recognized' by the enzyme and this causes a preference for one of the two possible stereochemical pathways for a reaction. The value of this discrimination is a crucial parameter since it stands for the 'selectivity' of the reaction. In turn it is governed by the reaction kinetics. Following are the different types of chiral recognition found within the class of enzymes called hydrolases. The underlying principles of this recognition process and the corresponding kinetic implications are discussed below.[68]

Enantioface Differentiation

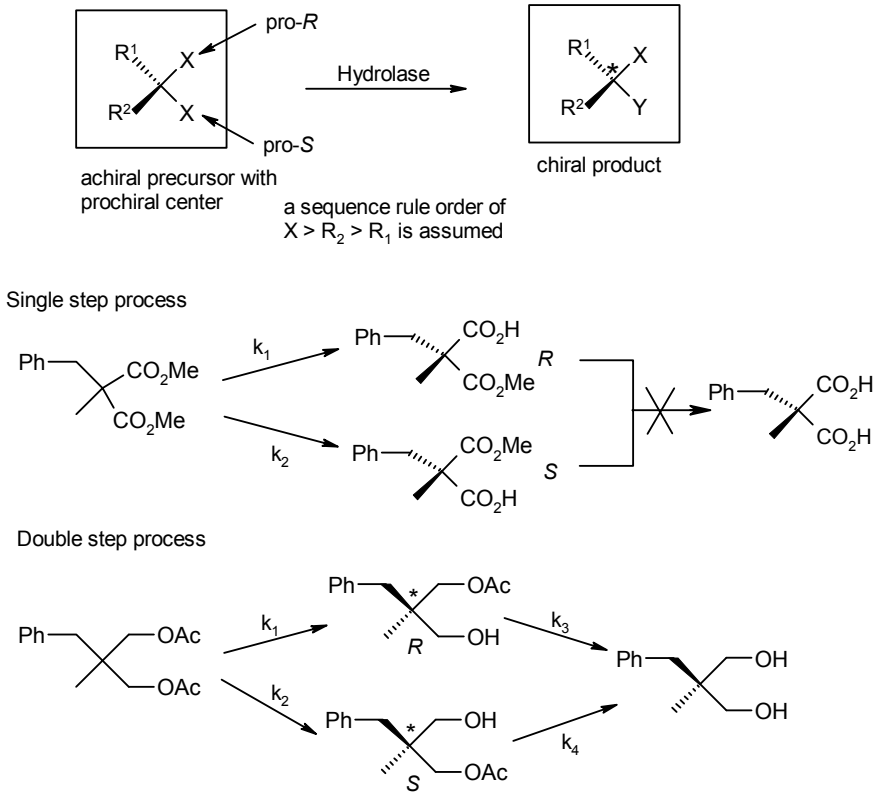
Hydrolases can distinguish between the two enantiomeric faces of achiral substrates such as enol esters possessing a plane of symmetry within the molecule. The attack of the enzyme's nucleophilic chemical operator predominantly occurs from one side, leading to an unsymmetric enolization of the unstable free enol towards one preferred side within the chiral environment of the enzyme's active site.[69] During the course of the reaction a new chirality is created in the product by this asymmetric protonation (Scheme 2.2).



Scheme 2.2.

Enantiotopic Differentiation

a) *Prochiral substrate:* If prochiral substrate possessing two chemically identical but enantiotopic reactive groups X (designated pro-*R* and pro-*S*) are subjected to an enzymatic transformation such as hydrolysis, a chiral discrimination between them occurs during the transformation of group X into Y, thus leading to a chiral product (Scheme 2.3).

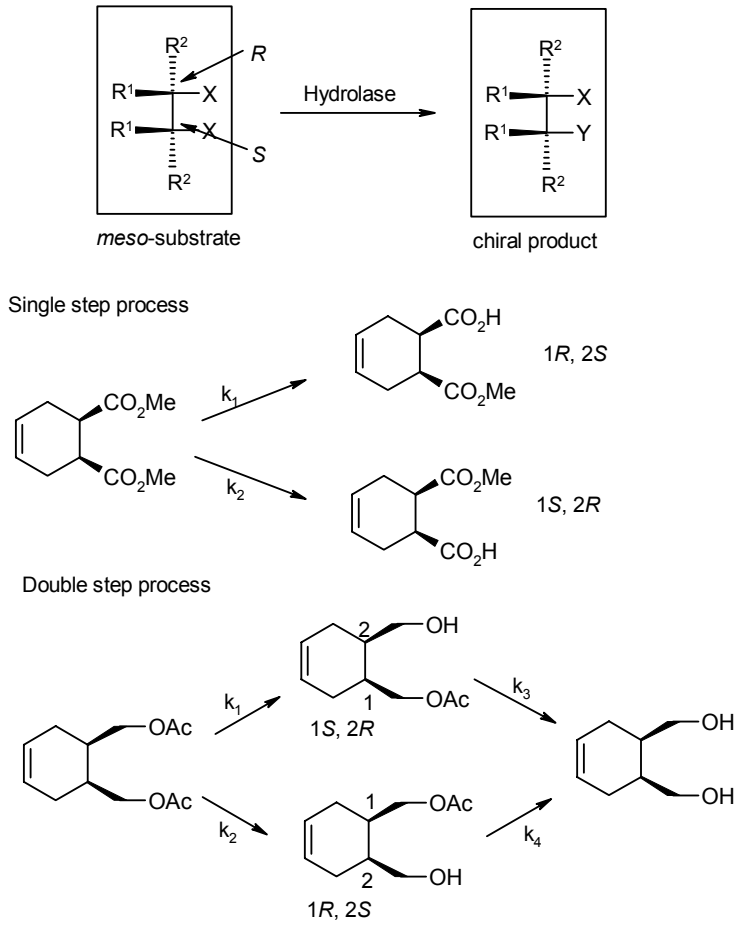


Scheme 2.3.

During the course of the reaction the plane of symmetry within the substrate is broken. The single-step asymmetric hydrolysis of a prochiral α,α-disubstituted malonic diester by pig liver esterase or α-chymotrypsin is a representative example.[70] Here, the reaction terminates at the

monoester stage since highly polar compounds of such type are generally not accepted by hydrolases.[71] On the other hand, when the substrate is a diacetate, the resulting monoester usually under goes further cleavage in a second step to yield an achiral diol (Scheme 2.3).[72]

b) *Meso-substrate:* Similarly, the two chemically identical groups X, positioned on carbon atoms of opposite configuration of a *meso*-substrate (*R*, *S*), can react at different rates in hydrolase-catalyzed reaction (Scheme 2.4). As a result, the optically inactive substrate is transformed into an optically active product due to the transformation of one of the reactive groups from X into Y going hand in hand with the destruction of the plane of symmetry within the substrate. This is known as desymmetrization technique. Numerous open chain or cyclic *cis-meso*-diesters have been transformed into chiral monoesters by this technique.[73] Again, for dicarboxylates the reaction usually stops after the first step, whereas two hydrolytic steps are observed with diacetoxy esters.[74] The theoretical yield of chiral product from single-step reactions based on an enantioface or –topos differentiation or an desymmetrization of *meso*-compounds is always 100%.



Scheme 2.4.

If required, the inter-conversion of a given chiral product into its enantiomer can be achieved by a simple two-step protection-deprotection sequence. Thus, regardless of the enantiomeric preference of the enzyme, which is used to perform the desymmetrization of the substrate, both enantiomeres of the product are available and no 'unwanted' enantiomer is produced. This technique has no generally applicable counterpart in conventional organic chemistry and is often referred to as the '*meso*-trick'.[75]

Since hydrolytic reactions are performed in an aqueous environment, they are virtually completely irreversible. The kinetics of all the single-step reactions shown above is very simple.[76] A prochiral or a *meso*-substrate S is transformed into two enantiomeric products P and Q at different rates determined by the apparent first-order rate constant k_1 and k_2, respectively (Schemes 2.2-2.4). The selectivity of the reaction (α) is only governed by the ratio of k_1/k_2, which remains constant throughout the reaction. As a consequence, the optical purity of the product ($e.e._p$) is not dependent on the extent of the conversion (Figure 2.5).

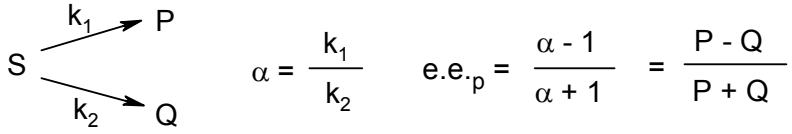


Figure 2.5.

On the other hand, in some cases a second successive reaction step cannot be avoided with bifunctional prochiral or *meso*-diesters (Scheme 2.3 and 2.4). For such types of substrates the reaction does not terminate at the chiral monoester stage to give the desired products P and Q (step 1), but rather proceeds via a second step (usually at a slower rate) to yield an achiral product (R) (Figure 2.6). Here, the reaction kinetics becomes more complicated.

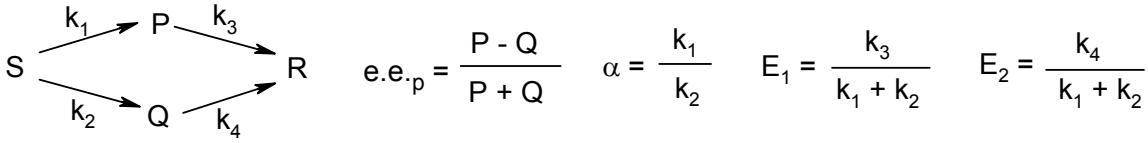
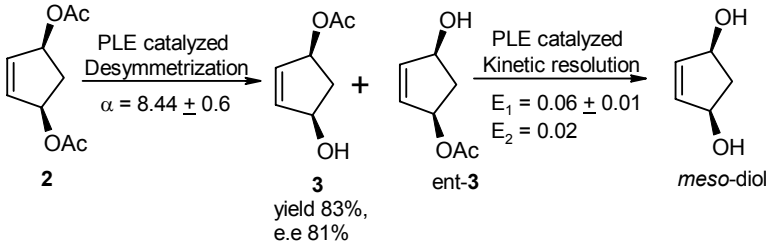


Figure 2.6.

As depicted in Figure 2.6, the ratio P and Q – in other words the optical purity of the product ($e.e._P$) – depends now on all four of the rate constants k_1 through k_4, since the second hydrolytic step cannot be neglected. From the fact that enzymes usually show a continuous preference for reactive groups with the same chirality, one may conclude that if S is transformed more quickly into P, Q will be hydrolyzed faster (into diol R) than P. Thus, the rate constants governing the

selectivity of the reaction are often in an order of $k_1 > k_2$ and $k_4 > k_3$. As an important consequence, the optical purity of the product monoester (e.e.$_P$) becomes a *function of the conversion* of the reaction and generally follows a sigmoid curve. In the early stages of the reaction the optical purity of the product is mainly determined by the selectivity of the first reaction step, which constitutes an enantiotopic or –face differentiation depending on the type of substrate. As the reaction proceeds, the second hydrolytic step, which actually constitute *per se* a kinetic resolution, starts to take place to a more significant extent due to increased formation of monoester P/Q, and its apparent 'opposite' selectivity compared to that of the first step leads to an enhancement of optical purity. The same analogues considerations are pertinent for the reverse situation-an esterification reaction.

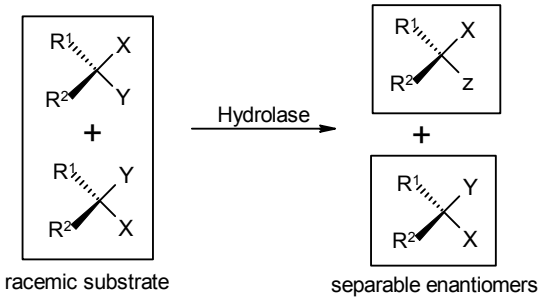
In addition to trial-and-error experiments (i.e. by stopping such double step reactions at various intervals and checking the yield and optical purity of the product) the e.e.-conversion dependence may be calculated. Determination of the amounts of substrate S and monoester P and Q and its optical purity at various intervals can be used to determine the kinetic constants k_1 through k_4 for a given reaction by using the computer program 'ScKiRe'.[77] Thus, the enantiomeric excess of the monoester may be predicted as a function of its percentage present in the reaction mixture. The validity of this method has been verified by C.J. Sih et al [78] by studying the desymmetrization of a prochiral *meso*-diacetate **2** using pig liver esterase (PLE) and porcine pancreatic lipase (PPL). The kinetic constants for the hydrolysis of **2** were $\alpha = 8.44 \pm 0.6$, $E_1 = 0.06 \pm 0.01$, and $E_2 = 0.02$. On the basis of computer-generated graph, the maximal recovery obtainable of the monoacetate fraction **3** was 83% with an e.e. of 81%. Experimental data was in good agreement with computer-generated curves for these kinetic constants (Scheme 2.5). The essential feature of this approach lies in the recognition of the importance of the inherent consecutive kinetic resolution step in enhancing the optical purity of chiral species during enantioselective hydrolysis of diesters.



Scheme 2.5.

Enantiomeric Differentiation

When a racemic substrate is subjected to enzymatic hydrolysis, chiral discrimination of the enantiomers occurs.[79] It should be noted that the chirality does not necessarily have to be of central type, but also can be axial or planar to be 'recognized' by enzymes. Due to the chirality of the active site of the enzyme, one enantiomer fits better into the active site than its counterpart and is therefore converted at a higher rate, resulting in a kinetic resolution of the racemate (Scheme 2.6). The vast majority of enzymatic transformations occur in a stereoselective manner and, interestingly, this potential of hydrolytic enzymes was realized as early as 1903.[80]



Scheme 2.6.

Of course, in contrast to the above-mentioned types of stereoselective transformations like desymmetrization showing a theoretical yield of 100%, a kinetic resolution of a racemate can lead to only 50% yield for each of the enantiomers.

In some ideal cases the difference in the reaction rates of both enantiomers is so extreme that the 'good' enantiomer is transformed quickly and the other is not converted at all. Then the enzymatic reaction will cease automatically at 50% conversion, when there is nothing left of the more reactive enantiomer.[81]

In practice, however, most cases of enzymatic resolution of a racemic substrate do not show this ideal situation i.e. in which one enantiomer is rapidly converted and the other not at all. The difference in – or better the ratio of – the rates of conversion of the enantiomers is not infinite, but measurable. What one observes in these cases, is not a complete standstill of the reaction at 50% conversion but a marked decrease in reaction rate at around this point. In these numerous cases one encounters some crucial dependences:

- The velocities of the transformation of each enantiomer vary with the degree of conversion, since the ratio of the two-substrate enantiomers does not remain constant during the reaction.

- Therefore, the optical purity of substrate ($e.e._S$) and product ($e.e._P$) is a function of the extent of conversion.

A very useful treatment of the kinetics of enzymatic resolution, describing the dependency of the conversion (c) and the enantiomeric excess of substrate ($e.e._S$) and product ($e.e._P$), was developed by C. J. Sih[82] in 1982 on a theoretical basis laid by K. B. Sharpless[83] and K. Fajans.[84] The parameter describing the selectivity of a resolution was introduced as the dimensionless 'Enantiomeric Ratio' (E), which remains constant throughout the reaction and is only determined by the 'environment' of the system. E corresponds to the ratio of the relative second order rate constants of the individual substrate enantiomers (v_A, v_B for the thermodynamic background see Figure 2.4).[85] Related alternative methods for the experimental determination of E-values have been proposed recently.[86]

☞ **Irreversible reaction**: Hydrolytic reactions are considered as being completely irreversible due the high 'concentration' of water in the aqueous environment (55.5 mol/l). The dependence of the selectivity and the conversion of the reaction is as follows:

For the product

$$E = \frac{\ln[1-c(1+e.e._P)]}{\ln[1-c(1-e.e._P)]}$$

For the substrate

$$E = \frac{\ln[(1-c)(1-e.e._S)]}{\ln[(1-c)(1+e.e._S)]}$$

c = conversion; e.e. = enantiomeric excess of substrate (S) or product (P);
E = Enantiomeric Ratio

The above mentioned equations give reliable results except for very low and very high extents of conversion, where accurate measurements is restricted by errors derived from sample manipulation. In such cases, the following equation is recommended instead, because only for the optical purities of substrate and product need be measured. The latter are relative quantities in contrast to conversion, which is an *absolute* quantity.[87]

$$E = \frac{\ln \frac{[e.e._P(1 - e.e._S)]}{(e.e._P + e.e._S)}}{\ln \frac{[e.e._P(1 + e.e._S)]}{(e.e._P + e.e._S)}}$$

Two examples of enzymatic resolutions showing a selectivity of E=5 and E=20, respectively, are depicted in Figure 2.7.

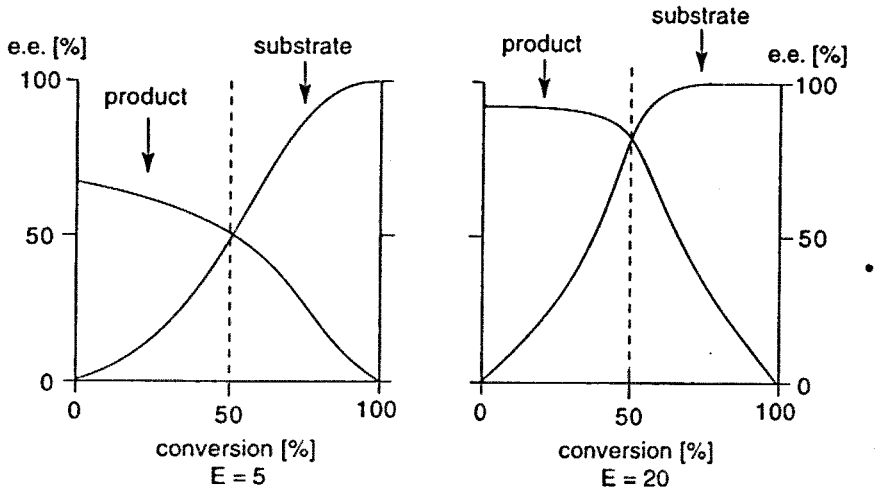


Figure 2.7.

As may be seen from the curves, the product can be obtained in its highest optical purities before 50% conversion, where the enzyme can freely chose the 'well-fitting' enantiomer from the racemic mixture. As a consequence, the 'well-fitting' enantiomer is gradually depleted from the reaction mixture during the course of the reaction, leaving behind the 'poor-fitting' counterpart. Beyond 50% of conversion, the high relative concentration of the 'poor-fitting' counterpart leads to its increased transformation by the enzyme. As a consequence, beyond 50% conversion the $e.e._p$ rapidly decreases.

Analogous trends are seen for the optical purity of the residual enantiomer of the substrate ($e.e._s$, as shown in the 'substrate-curve'). Its optical purity remains low before 40%, then climbs significantly at around 50% and reaches its maximum beyond the 60% conversion mark. It is evident that high optical purity of substrate can easily be reached by extending the reaction to the appropriate point of conversion (≥60%), but an attractive optical purity value for the product demands a very high selectivity for the enzyme-catalyzed reaction. Using the equations discussed above, the expected optical purity of substrate and product can be calculated for a chosen point of conversion and the Enantiomeric Ratio (E) can be determined as convenient constant value for the 'selectivity' for an enzymatic resolution. As a rule of thumb, Enantiomeric Ratios below 15 are unacceptable for practical purposes. They can be regarded as moderate to good from 15-30, and above this value they are excellent. It must be emphasized that values of E>200 cannot be accurately determined due to the inaccuracies emerging from the determination

of the enantiomeric excess (e.g. by NMR, HPLC or GLC) because in this range in a very small variation of $e.e._s$ or $e.e._p$ causes a significant change in the numerical value of E.

☞ **Reversible reaction**: The situation becomes more complicated when the reaction is reversible.[88] This is the case if the concentration of the nucleophile that attacks the acyl-enzyme intermediate is limited and is not in excess (cf. water in hydrolytic reaction). In this situation, the equilibrium constant of the reaction, neglected in the irreversible type of the reaction, plays an important role and therefore has to be determined. The equation linking the selectivity of the reaction [the Enantiomeric Ratio (E)], the conversion (c), the optical purity of substrate ($e.e._s$) and product ($e.e._p$) and also the equilibrium constant K are as follows:

For the product

$$E = \frac{\ln[1-(1+K)c(1+e.e._P)]}{\ln[1-(1+K)c(1-e.e._P)]}$$

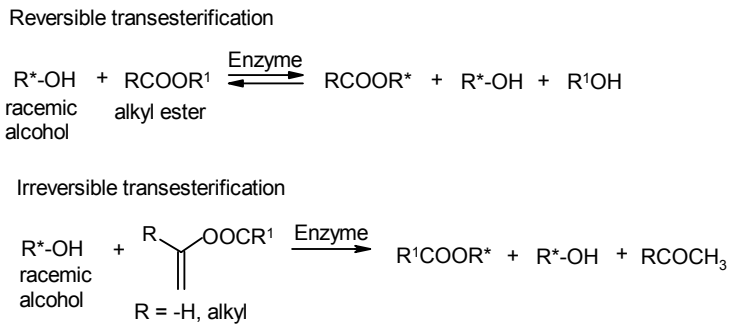
For the substrate

$$E = \frac{\ln[1-(1+K)(c+e.e._S\{1-C\})]}{\ln[1-(1+K)(c-e.e._S\{1-c\})]}$$

c = conversion; e.e. = enantiomeric excess of substrate (S) or product (P);
E = Enantiomeric Ratio, K = equilibrium constant of the reaction.

All the tricks to improve the optical purity of substrate and product of reversible enzymatic resolutions consist in shifting the reaction out of the equilibrium to obtain an irreversible type. The easiest way to achieve this is the use of an excess of co-substrate: to obtain an equilibrium constant of K>10, about 20 molar equivalents of nucleophile versus substrate are considered to be sufficient to obtain a virtually irreversible type of reaction in most cases.

In case of enzymatic transesterification reaction in organic medium, which is basically a reversible reaction, irreversible acyl donors are very effective to transform the reaction to irreversible one (Scheme 2.7).



Scheme 2.7.

Irreversible acyl donors viz. vinyl esters[89a], isoproenyl esters[89b], 2-haloethyl esters[89c], cyanomethyl esters,[89d] oxime esters,[89e] acid anhydrides[89f] etc. effectively transfer the acyl group onto racemic alcohol and their corresponding product alcohol immediately gets tautomerized to unreactive aldehyde, thus shifting equilibrium to right side (Scheme 2.7). Recently, 1-ethoxyvinyl esters have been invented as a new type of irreversible acyl donors for enzymatic resolution of racemic alcohols.[89g] These reagents exhibit high reactivity as well high enantioselectivity and more importantly generate ethyl acetate as a single product which does not affect any enzyme unlike aldehydes produced in other cases.

⊕ **Sequential Biocatalytic Resolutions**.

In the case wherein a racemic substrate has two chemically identical reactive groups, an enzymatic resolution proceeds through two consecutive steps via an intermediate monoester stage. During the course of such a reaction the substrate is forced to enter the active site of the enzyme twice, it is therefore 'double-selected'. Since each of the selectivities of both of the sequential steps determine the final optical purity of the product, exceptionally high selectivities can be achieved by using such a 'double-sieving' procedure (Figure 2.8).

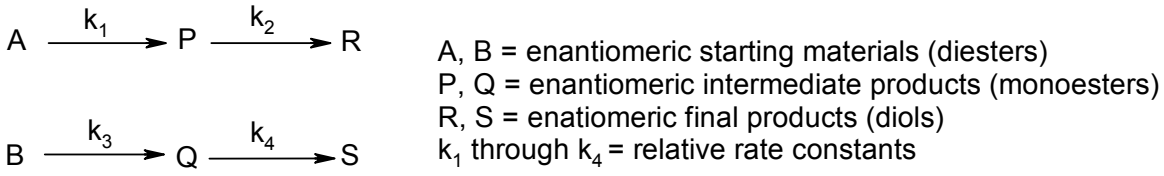
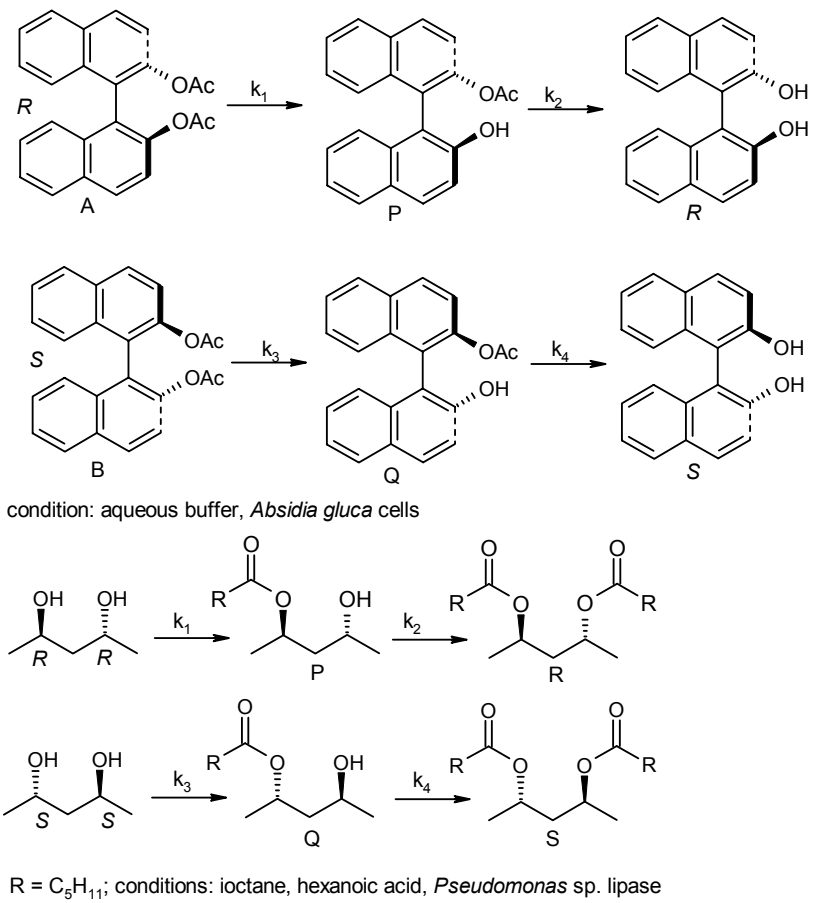


Figure 2.8.

It has been shown that the maximum overall selectivity (E_{tot}) of a sequential kinetic resolution can be related to the selectivity of each of the steps.[90] E_{tot} represents the enantioselectivity that a hypothetical single-step resolution would need to yield the enantiomeric purity of the two-step resolution.

$$E_{tot} \approx \frac{E_1 \cdot E_2}{2}$$

The technique has been proven to be highly flexible. It was shown to work successfully not only in hydrolytic reaction using cholesterol esterase[91] or microbial cells,[92] but also in the reverse esterification direction in an organic solvent catalyzed by *Pseudomonas* sp. lipase (see Scheme 2.8).



Scheme 2.8.

A special type of sequential enzymatic resolution involving a hydrolysis-esterification[93] or an alcoholysis-esterification sequence[94] has been reported recently (see Figure 2.9).

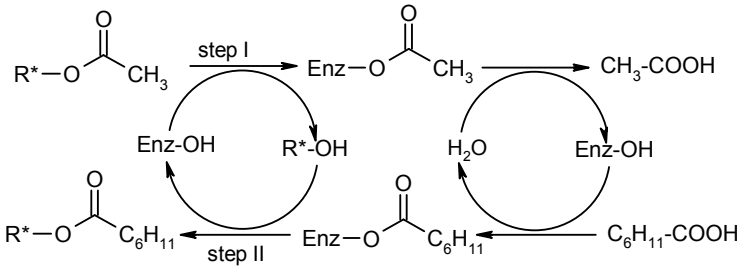
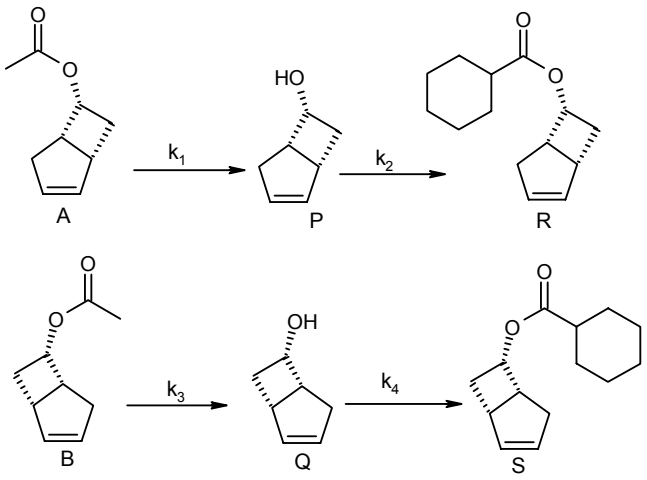


Figure 2.9.

The resolution of racemic alcohol can be effected by enantioselective hydrolysis of the corresponding ester or by esterification of the alcohol. As the biocatalyst displays the same stereochemical preference in both reactions, the desired product can be obtained with higher optical yields, if the two steps are coupled in a sequence. The basis of this approach parallels that

of product recycling in hydrolytic reactions. However, tedious chromatographic separation of the intermediates and the accompanying re-esterification is omitted.

As depicted in Scheme 2.9, the racemic starting acetate A and B is hydrolyzed to give alcohols P and Q in an organic medium containing a minimum amount of water, which in turn, by the action of the lipase, are re-esterified with cyclohexanoic acid present in the mixture. As a consequence, the alcohol moiety of the substrate has to enter the active site of the lipase twice during the course of its transformation into the final product cyclohexanoyl ester R and S. An apparent selectivity of E_{tot} = 400 was achieved in this way, whereas the corresponding isolated single-step resolutions of this process, which were carried out independently for reason of comparison, were E_1 = 8 for the hydrolysis of the acetate A/B, and E_2 = 97 for the esterification of alcohol P/Q with cyclohexanoic acid.



conditions: water-saturated hexane, cyclohexane carboxylic acid, *Mucor* sp. lipase

Scheme 2.9.

Convergent Resolutions

Despite its widespread use, kinetic resolution has several disadvantages for preparative applications, particularly on an industrial scale. After all, an ideal process should lead to a single enantiomer in 100% chemical yield. The drawbacks are as follows: (i) The theoretical yield of each enantiomer is limited to 50%. Furthermore, in general only one stereoisomer is desired and there is little or no use for the other. (ii) Separation of the product from the remaining substrate may be laborious; in particular when simple extraction or distillation fails.[95] (iii) As explained above, the optical purity of substrate and/or product is often less than perfect due to kinetic reasons. To overcome these disadvantages by avoiding the occurrence of the undesired 'wrong'

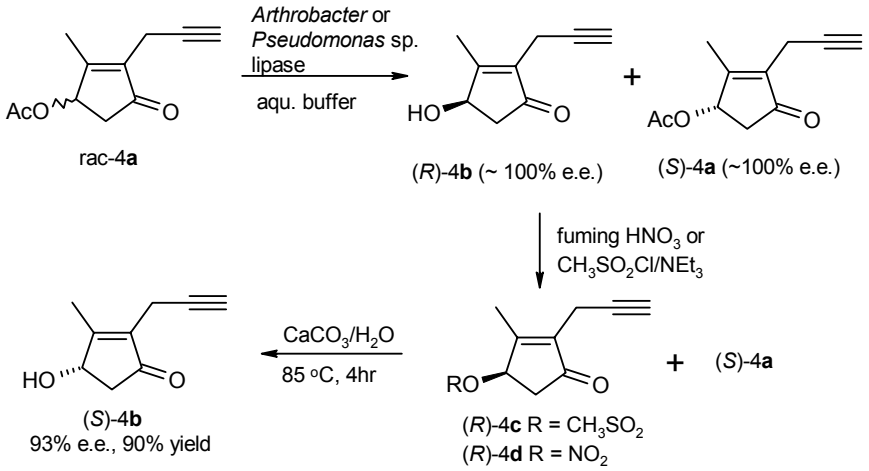
enantiomer, three strategies are possible. The result is single enantiomer production from the racemic substrate giving nearly 100% yield. Here both the isomers are converging by the techniques used to a single enantiomer of the product.

☞ Repeated Resolution

Racemization of the unwanted substrate enantiomer by chemical means (after its recovery from the resolution procedure) makes recycling a possibility. This process is far from being ideal since several steps are involved and overall yields are often low due to the harsh reaction conditions required for racemization.

☞ In-situ Inversion

The final product of a kinetic resolution of a racemate is a mixture of product and substrate. Separating them by physical or chemical means is often tedious and might pose a serious drawback to commercial application, especially if the mixture comprises an alcohol and an ester. However, if the molecule has only a single center of chirality, the alcohol may be inverted into its enantiomer *before* separating the products. Introduction of a good leaving group (L, e.g. tosylate, triflate or nitrate) yields an activated ester, which can be hydrolyzed with the inversion of configuration, while the stereochemistry of the remaining substrate ester is retained during hydrolysis. As a result, single enantiomer is obtained as the final product. A good example is shown in Scheme 2.10.



Scheme 2.10.

Compound **4b**, which serves as a building block for pyrethroids,[96] was resolved following a classical protocol via lipase-catalyzed hydrolysis of acetate (±)-**4a**. Both *Arthrobacter* and *Pseudomonas* sp. lipases hydrolyzed the (*R*)-ester, whereas only the alcohol (*S*)-**4b** is needed for

further synthetic purpose. To optimize this process, the mixture of (*R*)-**4b** and (*S*)-**4a** (both ~100% e.e.) obtained from the lipase-catalyzed reaction was subjected to chemical inversion of the alcohol. Thus, treating the mixture with mesyl chloride or fuming nitric acid led to the activated mesylate or nitrate ester (*R*)-**4c,d** respectively. The ester (*S*)-**4a** remained unaffected. Addition of strong base led to inversion of the activated intermediates with simultaneous hydrolysis of ester (*S*)-**4a**. Only a minor amount of racemization was observed and (*S*)-**4b** was obtained in 90% yield and 93% e.e. (Scheme 2.10). Alternatively, Mitsunobu-inversion conditions may likewise be employed.

☞ Dynamic Resolution

Dynamic resolution is a more elegant approach.[97] Such a process comprises a classic resolution having an additional feature, i.e. the resolution is carried out using conditions, under which enantiomers of the substrate are in a rapid equilibrium (racemizing) with each other. Thus, as the well-accepted substrate-enantiomer is depleted by the enzyme, the equilibrium is constantly adjusted by racemization of the poorly-accepted counterpart. To indicate the non-static character of such processes, the term 'dynamic resolution' has been coined.[98]

The kinetics of a dynamic resolution is outlined in the following example (Figure 2.10).[99]

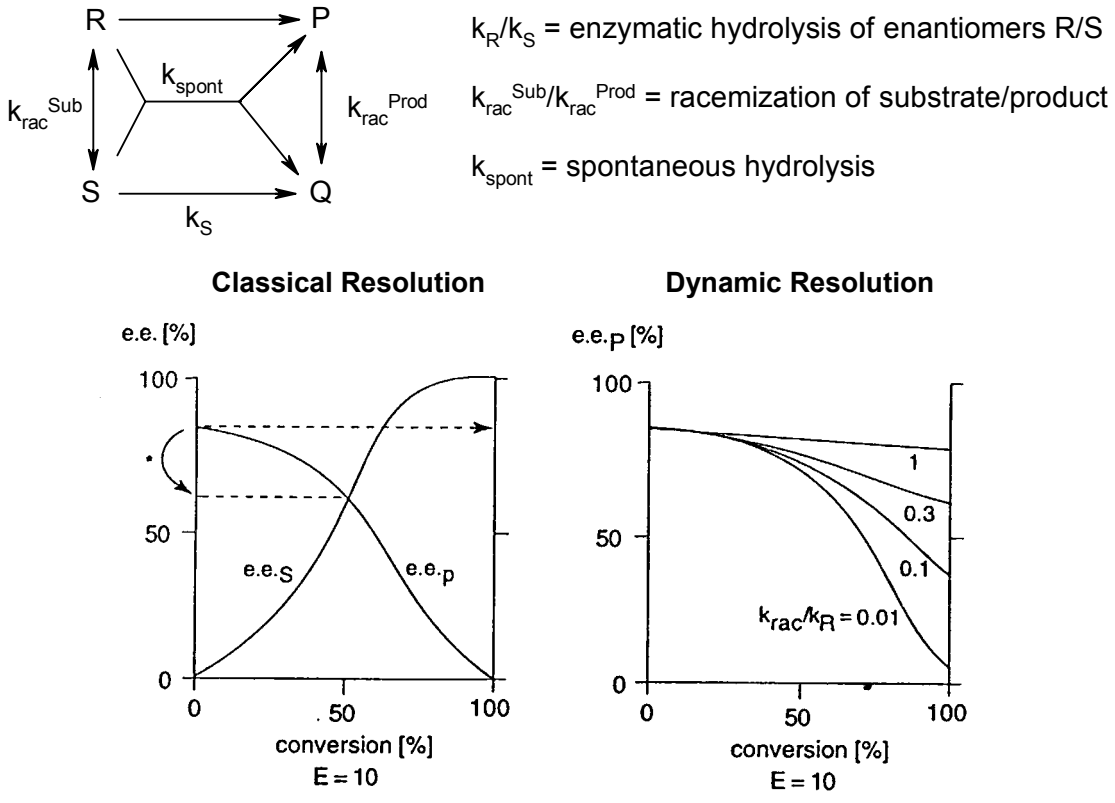


Figure 2.10.

Figure 2.10 shows the $e.e._s$ and product $e.e._p$ plotted for an E~ 10. In a classical resolution process, the product is formed in ~ 83% e.e. at the very beginning of the reaction, but this value rapidly decreases when the reaction is run towards ~50% conversion as indicated by the symbol '*' . In a dynamic process, this depletion does not occur, because the enzymes are always confronted with racemic substrate throughout the reaction since the 'well-fitting' enantiomer is not depleted but constantly formed from the 'poor-fitting' counterpart via the racemization. As a consequence, the $e.e._p$ remains at a constant level throughout the whole reaction. The selectivity of dynamic processes is related to the $e.e._p$ through the following formula:[100]

$$e.e.p = \frac{(E-1)}{(E+1)}$$

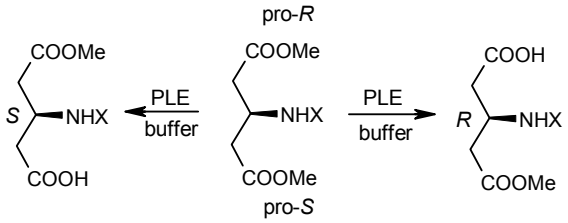
It is obvious, that a high $e.e._p$ for dynamic resolution can only be achieved for reactions displaying excellent selectivities. For example, E-values of ~19 and ~40 lead to an $e.e._p$ of 90 and 95% respectively, but for an enantiomeric excess of 98% an Enantiomeric Ratio of ~ 100 is required.

2.6. Optimization of Selectivity

Many enantiodifferentiating enzymatic hydrolyses of non-natural esters do not show a perfect selectivity, but are often in the range of 50-90% e.e., which corresponds to E-values which are considered as 'moderate' to 'good' (E = 3 to 20). In order to avoid tedious and material-consuming processes to enhance the optical purity of the product e.g. by crystallization techniques, there are some methods to improve the selectivity of an enzymatic transformation itself. Many, but not all of them, can be applied to other types of enzymes.

Substrate Modification

Substrate modification is one of the most promising techniques. This is applicable to all types of enzymatic transformations. As may be concluded from some of the foregoing examples, the ability of an enzyme to 'recognize' the chirality of a given substrate strongly depends on its steric shape. Thus by variation of the substrate structure, most easily performed by the addition or removal of protective groups of different size and/or polarity, a better fit of the substrate may be achieved which leads to an improved selectivity of the enzyme. Scheme 2.11 shows the optimization of a PLE-catalyzed desymmetrization of dimethyl 3-aminoglutarate esters using the 'substrate-modification' approach.[101]



X	Configuration	e.e. [%]
H	*R*	41
CH_3-CO	*R*	93
CH_2=CH-CO-	*R*	8
C_2H_5-CO-	*R*	6
n-C_4H_9-CO-	*S*	2
$(CH_3)_2$CH-CO-	*S*	54
C_6H_{11}-CO-	*S*	79
$(CH_3)_3$C-CO-	*S*	93
Ph-CH_2-O-CO-	*S*	93
(E)-CH_3-CH=CH-CO	*S*	>97

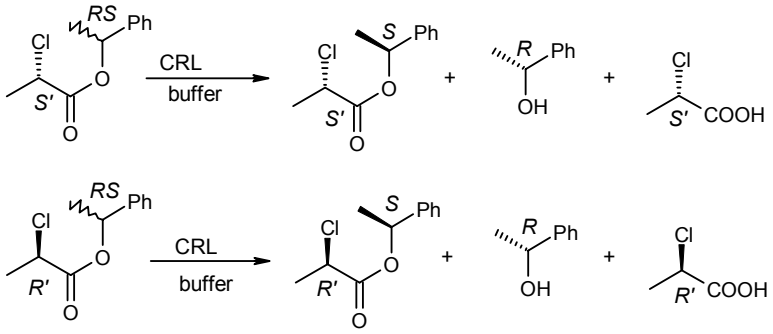
Scheme 2.11.

By varying the N-protecting group in size and polarity, the optical purity of the monoester could be significantly enhanced as compared to the unprotected derivative. In addition, a remarkable reversal in stereochemistry was achieved upon the stepwise increase of the size of the protective group X. This provides an elegant method for controlling the configuration of the product.

Bichiral–ester Method

The use of second acid- or alcohol moiety with a predetermined chirality in the substrate – leading to diastereomeric ester – can be helpful in the case of substrate modification. This

technique is called the “bichiral-ester method” and has been shown to be particularly useful for lipase-catalyzed resolutions. The asymmetric hydrolysis of the diastereomeric (*S’*)-2-chloropropionate esters of (*R/S*)-1-phenylethanol catalyzed by *Candida rugosa* (CRL) proceeds with low diastereoselectivity by producing the (*R*)-alcohol and by leaving the (*S’/S*)-diastereomeric ester untouched (Scheme 2.12). On the other hand, when the acid moiety of opposite (*R’*)-configuration was used, the chiral recognition for the alcoholic center was markedly increased.[102]

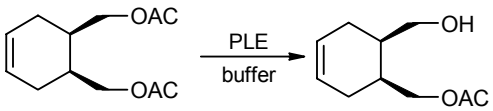


Reaction	Configuration of Acid	Selectivity (E)
S’/RS → *S’/S* + *R* + *S’*	*S’*	14
R’/RS → *R’/S’* + *R* + *R’*	*R’*	32

Scheme 2.12.

Medium Engineering

Variation of the solvent system by the addition of water-miscible organic cosolvents such as methanol, *tert*-butanol, acetone, dioxane, acetonitrile, dimethyl formamide (DMF) and dimethyl sulfoxide (DMSO) is a promising and quite frequently used method to improve the selectivity of hydrolytic enzymes, in particular with esterases (Scheme 2.13).[103]



Medium	e.e [%]
H_2O	55
H_2O/DMSO 6:4	72
H_2O/DMF 8:2	84
H_2O/t-BuOH 9:1	96

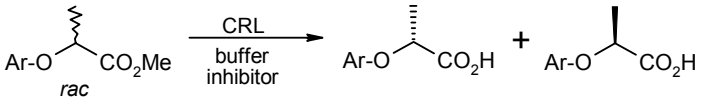
Scheme 2.13.

The concentration of co-solvent may vary from 10-50% of the total volume. At higher concentrations, however, enzyme-deactivation is unavoidable. The mechanistic action of such modified solvent systems on an enzyme is only poorly understood and predictions on the outcome of such a medium engineering cannot be made. The selectivity enhancement of PLE-

mediated hydrolyses upon the addition of methanol, *tert*-butanol and dimethyl sulfoxide to the reaction medium is exemplified in Scheme 2.13.[104]

Enantioselective Inhibition of Lipases

The addition of chiral bases functioning as non-competitive inhibitors has been found to have a strong influence on the selectivity of *Candida rugosa*[105] (Scheme 2.14) and *Pseudomonas* sp. lipase.[106]



Ar-	Inhibitor	Selectivity (E)
2,4-dichlorophenyl-	None	1
2,4-dichlorophenyl-	dextromethorphan[a]	20
2,4-dichlorophenyl-	levomethorphan	20
2,4-dichlorophenyl-	DMPA[b]	23
4-chlorophenyl-	none	17
4-chlorophenyl-	dextromethorphan	>100
4-chlorophenyl-	levomethorphan	>100

a: dextro- or levo-methorphan = D- or L-3-methoxy-n-methyl-methylmorphinane. b: N,N-Dimethyl-4-methoxyphenethylamine

Scheme 2.14.

The general applicability of this method – impeded by the high cost of morphinan alkaloids and their questionable use for large-scale synthesis – has been extended by the use of more simple amines such as N,N-dimethyl-4-methoxyphenethylamine (DMPA).[107]

Variation of pH

Due to the fact that the conformation of an enzyme depends on its ionization state, it is reasonable to assume that a variation in pH and the type of buffer may influence the selectivity of a given reaction. Such variations are facilitated by the fact that the pH-activity profile of the more commonly used hydrolytic enzymes is rather broad and thus allows pH-variations while maintaining a reasonable activity.[108]

Variation of Temperature

It is widely believed that enzymes, like other catalysts, generally exhibit their highest selectivity at low temperatures. This assumption has been supported by several experimental observations, not only with hydrolases[109] but also with dehydrogenases. Sakai et al, in their lipase PS catalyzed kinetic resolution studies,[110] examined temperature variation effects ranging from 30 to –60°C on the enantioselectivity of the reaction and have found a linear increase in enantioselectivity up to

-40^{o}C. Recently, Phillips et al proposed rational understanding of temperature effects on enzyme stereoselectivity based on so called 'racemic temperature' (T_{rac}), a temperature at which a given enzymatic reaction proceed without stereochemical discrimination.[111] At T_{rac} the activation energy is the same for both stereochemical forms participating in the reaction, hence there is no difference in free energy ($\Delta\Delta G^{\neq} = 0$, compare Figures 2.3 and 2.4, page 13 and 14)

$\Delta\Delta G^{\neq} = \Delta\Delta H^{\neq} - T \times \Delta\Delta S^{\neq}$ T_{rac} = Racemic Temperature

If $\Delta\Delta G^{\neq} = 0$ then $T = T_{rac} = \dfrac{\Delta\Delta H^{\neq}}{\Delta\Delta S^{\neq}}$

As may be deduced from the Gibb's-equation given above, only the entropy term (but not the enthalpy) is influenced by the temperature. Thus at temperature less that T_{rac} the contribution entropy becomes very small and the stereochemical outcome of the reaction is mainly dominated by the activation enthalpy difference ($\Delta\Delta H^{\neq}$). As a consequence, the optical purity of product(s) will *decrease with the increasing temperature*. On the other hand, at temperatures greater than T_{rac}, the reaction is controlled mainly by the activation entropy difference ($\Delta\Delta S^{\neq}$) and the enthalpy plays a minor role. Therefore the optical purity of product(s) will increase with increasing temperature. But the major product obtained at a temperature $T > T_{rac}$ will be the antipode to that at $T < T_{rac,}$ thus a temperature dependant reversal of stereochemistry is predicated. The validity of this rationale has been proven with the asymmetric reduction of ketones using a dehydrogenase from *Thermoanaerobium brockii*.[112] From the data available, it can be seen that upon lowering the temperature both an increase or a decrease in the selectivity of the hydrolase reaction may be observed. The outcome of the latter experiment depends on whether reaction has been performed above or below the racemic temperature.

Very recently, Cainelli et al demonstrated change in stereoselectivity of lipase catalyzed kinetic resolution according to change in substrate-solute interaction which is a temperature dependant phenomena.[113] This was proved by observing the correlation of 'inversion temperature' T_{inv} which is a intersection point between two opposite trends of change in enzyme selectivity upon linear variation in temperature and T_{NMR} which is similarly a intersection point between two opposite trends in change in chemical shift value in ^{13}C NMR of substrate in reaction solvent upon linear change in temperature. Thus, T_{inv} and T_{NMR} appear as two different experimental results due to the same phenomena of reorientation of solute-solvent clusters, which also act on diastereomeric transition states during enzymatic reaction leading to a different stereoselectivity below and above the T_{inv}. Thus, for the earlier example demonstrated by Sakai et al, T_{inv} was found to be 229 K till which there is progressive increase of enantioselectivity, but below this temperature enantioselectivity decreased.

2.7. Special Techniques

Immobilization of Enzymes[7]

The immobilization of isolated enzymes or whole cells provides for facile recovery and recycling of these often expensive catalysts. Moreover, immobilized biocatalysts are generally more stable and easier to handle than their free counterparts. Also, the stability is improved by maintaining the enzyme in its natural environment. There could be many advantages of using immobilized enzymes and some are listed below:

(i) Repeated and continuous use, (ii) Improved process control, (iii) More stability, (iv) High substrate-to-biocatalyst ratio, (v) Less contaminated product, (vi) Shortened reaction time, (vii) Saving in capital cost, (viii) Less labor intensive.

Immobilization methods are divided into three categories: Carrier Binding, Cross Linking and Entrapment.

☞ *Carrier binding* is commonly used with free enzymes. It consists of attaching a biocatalyst to a water-insoluble support by physical adsorption, or by ionic or covalent binding. Chibata and coworkers[114] were first to apply immobilized enzymes to industrial organic synthesis. Fungal amino acylase was immobilized on Sephadex by ionic binding. This catalyst forms the basis of the Tanabe process for the enantioselective hydrolysis of N-acyl aminoacids.

Enzymes immobilized by covalent binding are more stable towards leaching than those involving physical adsorption or ionic binding. It can be achieved by reaction with amino, hydroxyl or carboxyl groups in the polypeptide chain. For example, oxirane activated acrylic beads react with free amino groups.

☞ *Cross–linked* enzymes are insoluble macromolecules produced by reaction of enzymes with bifunctional compound glutaraldehyde, $OHC(CH_2)_3CHO$, whose aldehyde groups react with primary amino groups on proteins. They can be also prepared by cross-linking with other bifunctional reagents. Cross-linking produces stable immobilized enzyme particles that can be lyophilized and stored in dry form almost indefinitely at room temperature. They retain near maximum catalytic activity under harsh temperature and pH conditions, work in all kinds of solvents and solvent mixtures. They also find potential applications in chemical use.[115]

☞ *Entrapment* has the advantage that the biocatalyst is not subjected to structural modification and the catalysis is protected from proteases of high molecular weight. Several natural polysaccharides, such as alginate, agar, and k-carrageenan are excellent gel materials widely used for the entrapment of the biocatalyst. A broad range of gel materials have been screened by Chibata and coworkers[116] and found k-carrageenan to be the best.

Enzymes in Organic Solvents

Evolution has taken place in an environment in which water is one of the major components. It is not surprising therefore, that the biochemical reactions in living cells takes place in an aqueous medium. As a biomolecule it plays central role in many enzyme-catalyzed processes and is absolutely, directly or indirectly, involved in all non-covalent interactions-hydrogen bonding, hydrophobic and Van der Walls interactions-that maintains the native, catalytic active enzyme conformation.[117] For this reason, virtually all studies in enzymology, i.e. extraction, purification, characterization, reaction and industrial applications thus far have been carried out in aqueous medium. However, water is a poor solvent for nearly all reactions in preparative organic chemistry because most organic compounds are insoluble in this medium. Furthermore, the removal of water is tedious and expensive due to its high boiling point and high heat of vaporization. Side-reactions such as hydrolysis, racemisation, polymerization and decomposition are often facilitated in the presence of water. Further, recent developments in biotechnology demand wider applications of biocatalysts. For preparative chemistry, it would be better to carry out enzymatic conversions in organic solvents or mixed organic solvent systems instead of water for several reasons as follows:

(i) High solubility of most organic compounds in nonaqueous solvents.
(ii) Greater stability of enzymes in organic solvents.
(iii) Ability to carry out new reactions that are impossible in water because of kinetic or thermodynamic reactions.
(iv) Relative ease of product recovery from organic solvents compared to water.
(v) Insolubility of enzymes in organic solvents which permits their easy recovery and reuse thus eliminates the need for immobilization.

The most important advantage, however, is the possibility to shift thermodynamic equilibria to synthesis over hydrolysis. Thus, by using hydrolase enzymes esters, polyesters, lactones, amides and peptides can be synthesized in a chemo-, regio-, and enantioselective manner. Enzyme reactions performed in nearly anhydrous organic solvents is a particularly interesting and intriguing field of research. Klibanov and coworkers[118] have provided much of the initial impetus and continue to contribute actively to research into this area of enzyme catalysis. There has

recently been an exponential growth of interest in this field and has reflected by excellent reviews[119] on this aspect.

Membrane Bioreactors in Enzymatic Processes

The use of bioreactors, whereby the catalyst is entrapped within a membrane or separated from the environment by it, is a special example of the entrapment technique. The membrane bioreactor developed by Sepracor[120] is illustrated in Figure 2.11. The enzyme is physically entrapped within a microporous membrane, usually an asymmetric hollow fiber membrane. A slight pressure gradient on the outside of the membrane maintains the organic/aqueous phase boundary at the outside of the surface of the hydrophilic membrane. The enzyme is then effectively entrapped within the microporous membrane by taking advantage of two physical properties: its large size and insolubility in organic solvents.

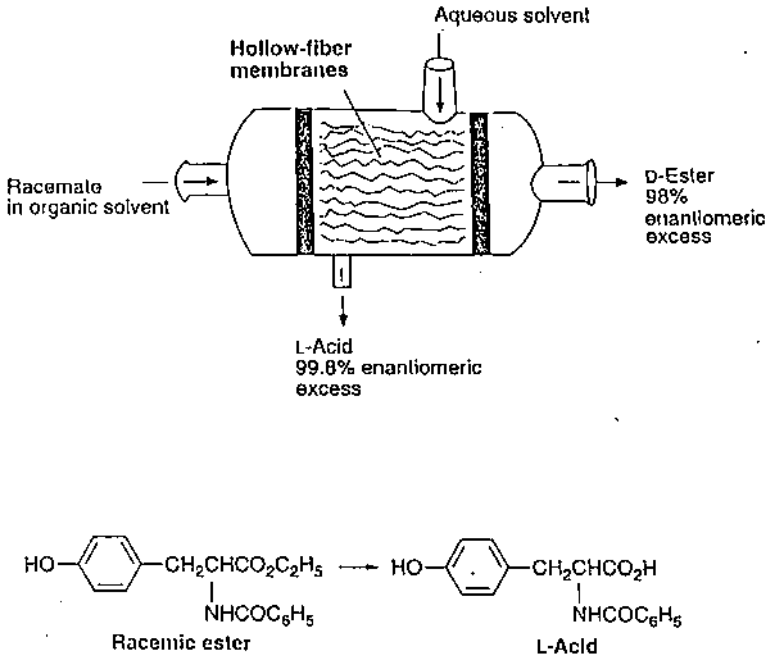


Figure 2.11.

Enzymes in Supercritical Fluids

Supercritical fluids (SCF), which been gaining recognition as suitable solvents for extraction of biomolecules have also shown some promise for use as novel media for enzyme reactions. They are currently receiving attention in biochemical reactions[121] as they offer advantages compared with organic solvents that include among others, easily controllable solubility components, high diffusivity, low toxicity and improved reaction rates. Enzyme-catalyzed reactions in supercritical fluids will be particularly useful because there will be no solvent contamination in the products of

the reaction after depressurization. Supercritical carbon dioxide is finding extensive use in biochemical reactions due to its availability, cost and low toxicity. Also ethylene, propane, fluroforms, sulfur hexafluoride and ethane can be used.[122] Sulfur hexafluoride is the only inorganic solvent other than water that has been shown to support biocatalytic activity.

Cross–linked Enzyme Crystals[123]

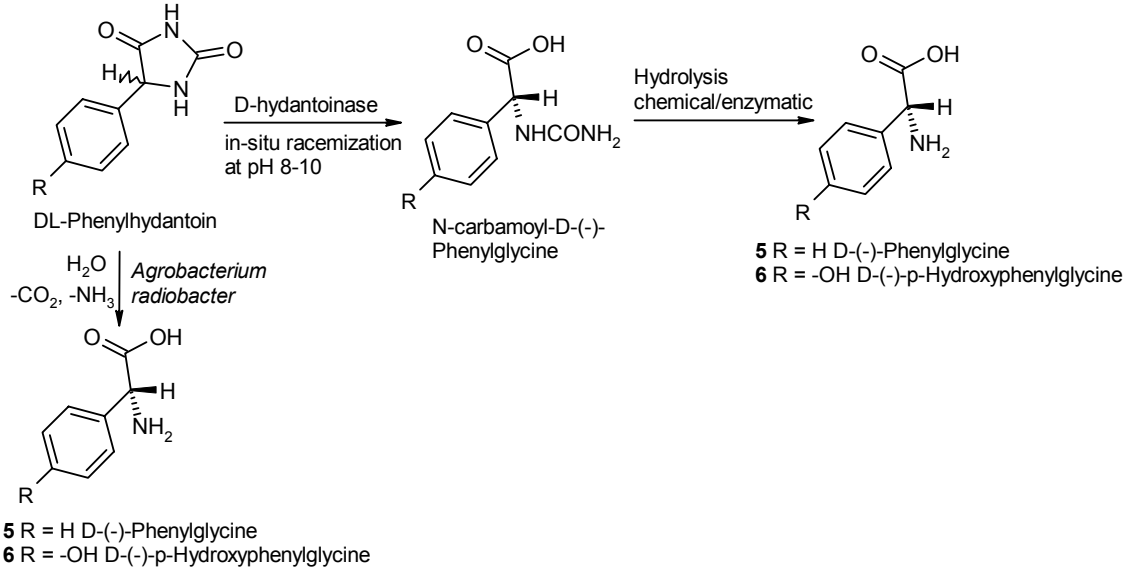
The basis of cross-linked enzyme crystal (trademarked CLEC) technology is the stabilization of enzyme catalysts by crystallizing enzymes, followed by chemical cross-linking. The catalytic activity and steroselectivity of an enzyme result from the precise orientation of reactive side chains through the thee-dimensional folding of the polypeptide backbone. Unraveling or rearrangement of the peptide chain most often results in a loss of catalytic activity (denaturation). When an enzyme molecule is in a crystal lattice, surrounded in three dimensions by other protein molecules, it is much more difficult to unravel the catalyst's tertiary structure than when the monomeric protein is dissolved in solution. Ionic, hydrogen-bonding and hydrophobic interactions between adjacent enzyme molecules within the crystal stabilize the crystalline protein. Moreover, intermolecular covalent cross-links provide an additional barrier to catalyst deactivation. CLEC maintain crystallinity in both water and organic media and can be considered "organic zeolites". CLEC offer the advantage of enhanced stability, recyclability and compared with impure biocatalyst mixtures, the highly purified CLEC catalysts can be many times more enantioselective.

2.8. Commercial Applications of Enzymatic Processes[124]

As discussed earlier, since the mid-1970s biotransformations have become a very well established tool in the fine chemical industry. Biocatalytic systems, including crude and purified enzymes as well as whole-cell systems performing highly selective reactions under mild conditions, are widely used, especially in synthesis and production of biologically active compounds in agrochemical and pharmaceutical sectors. Few commercially relevant examples are discussed below. They demonstrate the advantage of biocatalysts in the conversion of functional groups and configuration, resolution of racemates and asymmetric synthesis.

☞ D-(-)-Phenylglycine and D-(-)-p-Hydroxyphenylglycine

D-(-)-Phenylglycine (**5**) and D-(-)-p-hydroxyphenylglycine (**6**) have been produced industrially by resolving racemic aminoacids obtained by chemical synthesis. The chemical methods involve several complicated steps requiring costly resolving agents. A chemoenzymatic route for synthesis of various D-aminoacids has been developed by Takashi et al[125a] and Yamada et al (Scheme 2.15).[125b] In this process, DL-5-substituted hydantoin is asymmetrically hydrolyzed to N-carbamoyl-D-aminoacid using specific D-hydantoinase. This intermediate is either chemically converted to corresponding D-aminoacid using nitrous acid[125c] or enzymatically to D-aminoacid using second enzyme N-carbamoyl-D-aminoacid aminohydrolase found in *Agrobacterium* strains.[125d,e] The second enzyme has not been exploited commercially so far. Few organisms have been reported to posses both the enzymes capable of transforming DL-hydantoins directly to D- (-)-aminoacids.[125f,g]



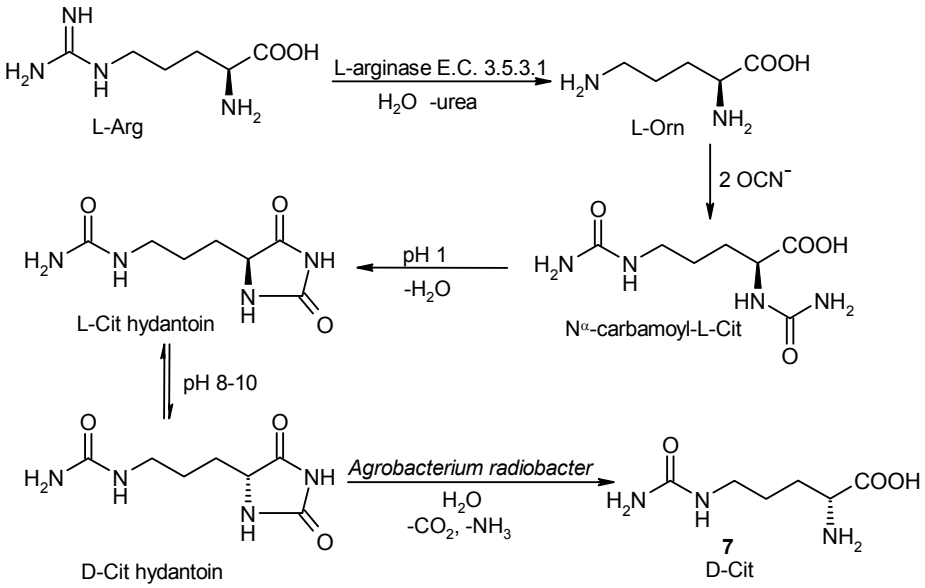
Scheme 2.15.

At present a chemoenzymatic process for production of D-(-)-phenylglycine is operated industrially by Kanegafuchi Chemical Co., Japan. A total microbiological process for the production of this aminoacid has been used on industrial scale by Recordati in which *Agrobacterium radiobacter* possessing both hydantoinase and N-carbamoylase catalyze the total conversion to D-(-)-p-hydroxyphenylglycine from the corresponding DL-hydantoin.

The work on screening of the microbes for hydantoinase activity was being carried out at NCL, Pune where a bacterial strain capable of converting DL-5-phenylhydantoin to N-carbamoyl-D-(-)-phenylglycine with high conversion efficiency was identified.[126] The advantages of this process are (i) identified strain grows in a cheaper substrate like molasses or corn steep liquor (ii) faster conversion of DL-5-phenylhydantoin to N-carbamoyl-D-(-)-phenylglycine; (iii) product concentration achieved up to 3-3.5%; (iv) convenient method for isolation of N-carbamoyl-D-(-)-phenylglycine; (iv) an easier method for decarbamoylation of N-carbamoyl-D-(-)-phenylglycine.[125c] This chemoenzymatic process was found to be comparable cost wise to purely chemical method developed at NCL.

☞ D-citrulline

The luteinizing hormone releasing hormone (LHRH) antagonist Cetrorelix®, first synthesized by A. Schally's group and further developed by Asta Medica,[127] contains five D-aminoacids, among which the ω–ureido aminoacid D-citrulline (D-Cit, **7**) is particularly difficult to synthesize. A hydantoinase/carbmolylase system from *Agrobacterium radiobacter* has been chosen for synthesizing D-Cit (**7**) starting from an easily obtainable precursor. (Scheme 2.16)

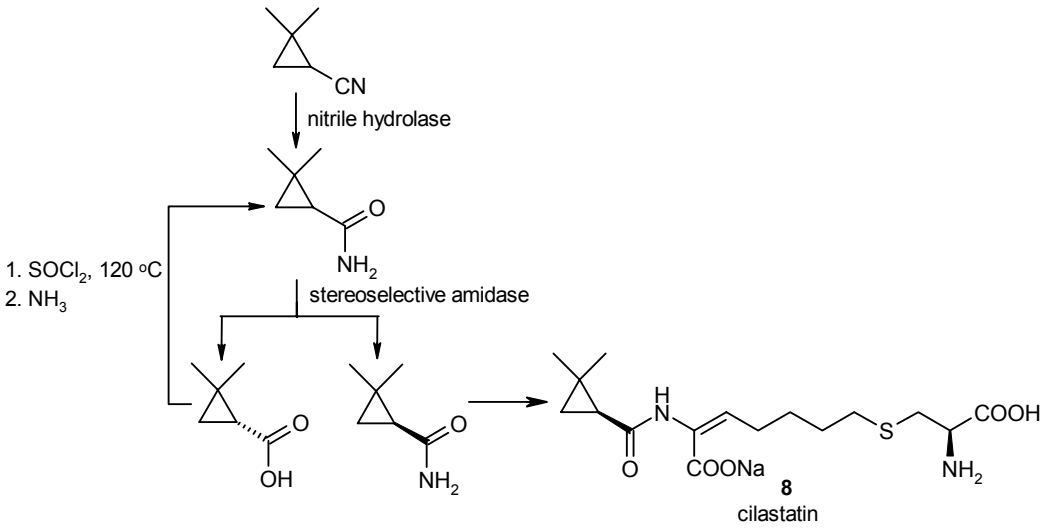


Scheme 2.16.

The synthesis starts from the inexpensive proteinogenic aminoacid, L-arginin (L-Arg), which has the appropriate carbon chain length and also a nitrogen atom attached in 5-position. With the help of arginase, L-arg is deguanidylated quantitatively to L-Ornithinine (L-Orni). This reaction is much more effective than any chemical hydrolysis. L-Orn then is reacted with two equivalents of cyanate, forming the biscarbamoylated L-Cit, which can be easily cyclized to the L-Cit hydantoin. When the *Agrobacterium* biomass with hydantoinase and carbomoylase activity is added to an aqueous alkaline solution of this hydantoin, complete transformation to D-Cit takes place. The e.e. of 99.8% demonstrates that the enzymes are working stereospecifically.[128] At Degussa D-Cit has been produced in quantities of several tens of kilograms. This hydantoinase /carbomoylase system offers an excellent method for producing D-aminoacids starting from racemic or suitable L-precursors without additional racemization or subsequent chemical steps. Especially for synthesis of side-chain functionalized D-aminoacids, this method is superior to other alternatives.[129]

☞ (S)-2,2-Dimethylcyclopropanecarboxylic Acid

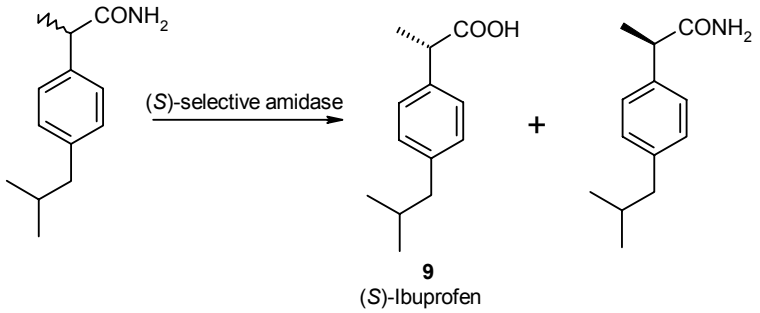
This chiral acid is an intermediate for the synthesis of cilastatin (**8**), a dehydropeptidase I inhibitor which is used in combination with β–lactam antibiotics to prevent degradation by microbial β-lactamases. Lonza has developed an industrial two-step biotransformation process, starting from racemic 2,2-dimethylcyclopropanenitrile (Scheme 2.17). The stereoselective amidase has been found in *Comomonas acidovorans* and cloned into a fast-growing *E. coli* strain which produces large amounts of biomass.[130]



Scheme 2.17

☞ (*S*)-Ibuprofen

For production of analgesic (*S*)-ibuprofen (**9**) from racemic ibuprofen amide, an (*S*)-selective amidase from *Brevibacterium* and *Corynebacterium* has been developed (Scheme 2.18).[131]

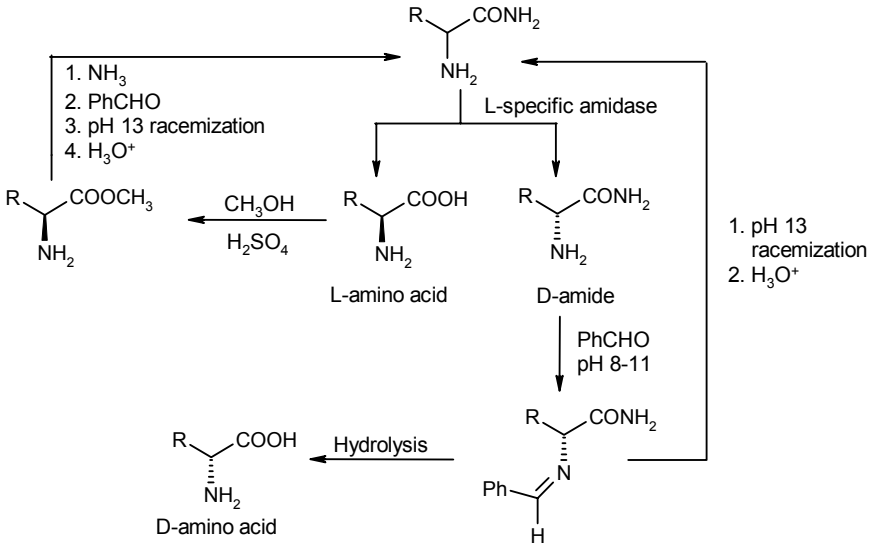


9
(*S*)-Ibuprofen

Scheme 2.18.

☞ Production of Enantiomerically Pure Aminoacids by amidases

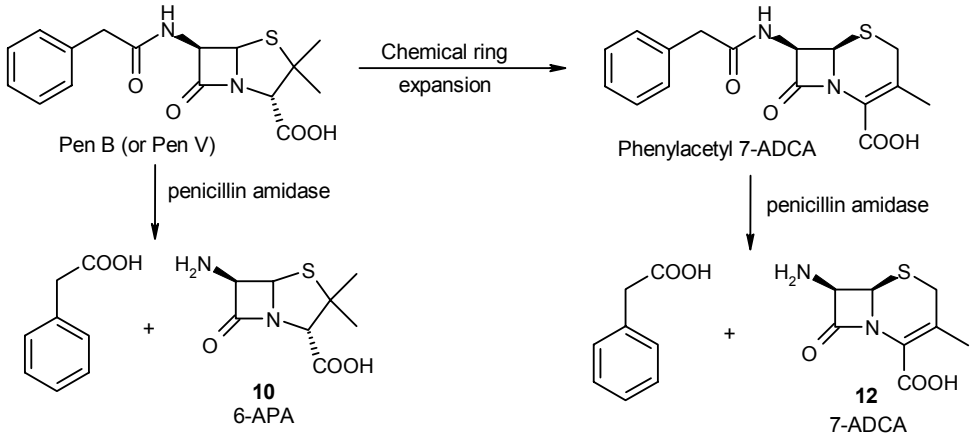
DSM has developed a widely applicable industrial process for production of enantiomerically pure aminoacids by enantioselective hydrolysis of racemic aminoacid amides. These precursor compounds can be easily obtained by alkaline hydrolysis of α–amino nitriles in presence of a ketone. The L-specific amidase (= aminopeptidase) has been found in *Pseudomonas putida* (ATCC 12633), and shows broad substrate specificity. Methods for racemizing the undesired enantiomer are also integrated in the process (Scheme 2.19).[132]



Scheme 2.19.

☞ Production of 6-APA, 7-ACDA and 7-ACA using Amidases

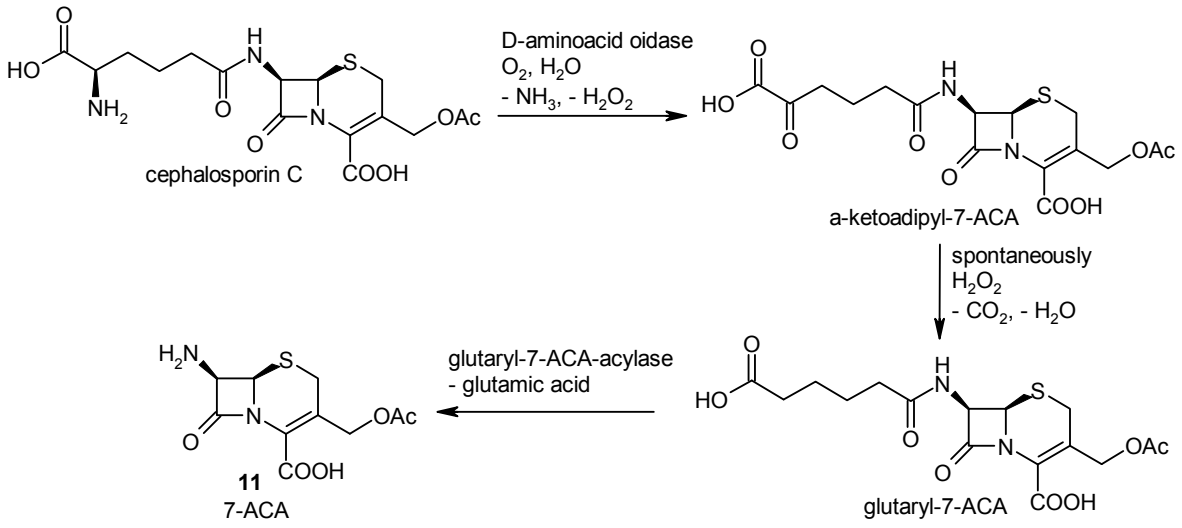
6-Aminopenicillinic acid (6-APA, **10**), 7-aminocephalosporonic acid (7-ACA, **11**), and 7-aminodesacetoxycephalosporonic acid (7-ADCA, **12**) are the most important precursors for the highly active semisynthetic β–lactam antibiotics. Before 1980, 6-APA and 7-ADCA were produced only by chemical deacylation of penicillin G and phenacetyl-7-ADCA, which could be obtained by chemical ring-expansion of penicillin G. Since 1980, enzymatic processes avoiding the use of environmentally critical reagents in large amounts have been developed. The most prominent enzyme is penicillin G amidase (Pen G amidase), which preferentially hydrolyzes penicillin G and penicillin V as well as phenacetyl-7-ADCA without affecting the β–lactam ring. Nowadays, Pen G amidase is applied in immobilized form (Scheme 2.20).



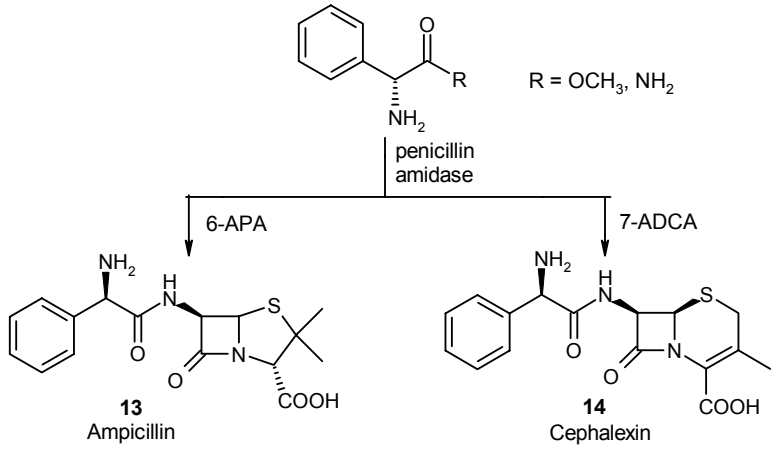
Scheme 2.20.

7-ACA is produced in a three-step process using two enzymes, starting from cephalosporin C (Scheme 2.21). After deamination by a D-aminoacid oxidase, the α-ketoadipyl-7-ACA spontaneously decarboxylates and the remaining glutary side chain is hydrolyzed by glutaryl-7-ACA.

Besides being used for the production of the precursors 6-APA and 7-ADCA, penicillin amidases are also able to couple 6-APA and 7-ADCA with D-phenyl glycine ester or amide in a kinetically controlled enzymatic peptide synthesis forming ampicillin (**13**) or cephalexin (**14**). These reactions (Scheme 2.22) have a great potential for being commercialized in the near future.[133]



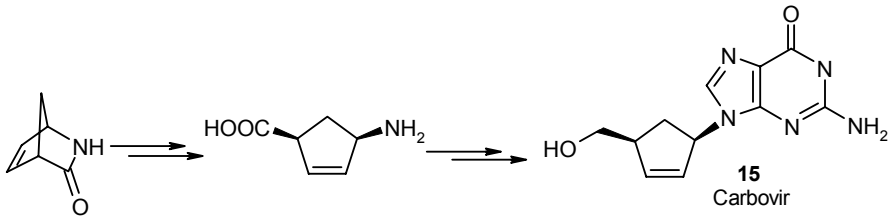
Scheme 2.21.



Scheme 2.22

☞ Enzymatic hydrolysis of Lactams

Recently, the enzymatic hydrolysis of racemic 2-azabicyclo[2.2.1]hept-5-en-3-one with specific lactamases from *Rhodococcus equi* and *Pseudomonas solanacerarum* has been described (Scheme 2.23).



Scheme 2.23.

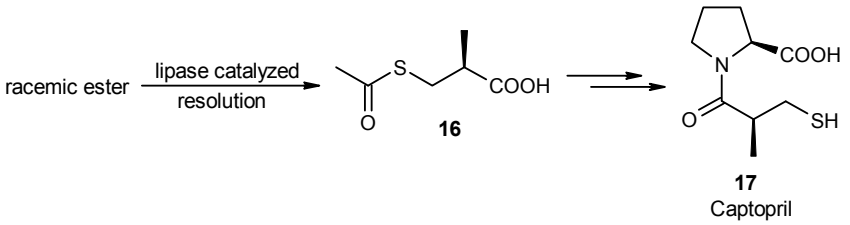
Optically active γ–aminoacids are versatile key intermediates for carbocyclic nucleosides such as carbovir (**15**).[134]

☞ Production of Enantiomerically Pure Aminoacids by Aminoacylases

As of now, enantiospecific hydrolysis of N-acylaminoacids by aminoacylase I from *Aspergillus oryzae* is the most established large-scale process for L-aminoacids. Both L- and D-aminoacids could be produced according to this technology. Tanabe has developed a process using an immobilized enzyme,[132] whereas at Degussa a process using native amino acylase I in homogeneous solution is applied. For this purpose, a new enzyme membrane reactor (EMR) has been designed, based mainly on the work of Kula and Wandrey.[136]

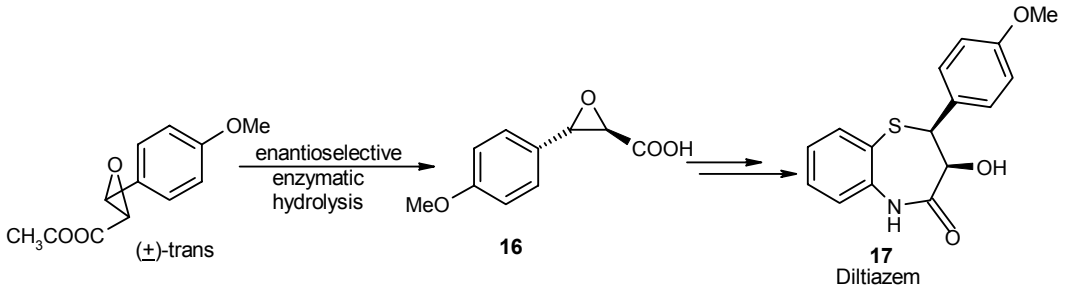
☞ Enzymatic Hydrolysis of C-O Bonds

Esterase, lipases and proteases are widely used in enzymatic resolution processes of racemic carboxy acid ester substrates. Industrially relevant examples are the manufacture of (D)-β-acetylmercaptoisobutyric acid (**16**), the optically active side chain of ACE-inhibitor captopril (**17**) (Scheme 2.24),[137] and synthesis and production of (*S*)-2-arylpropionic acids such as (*S*)-naproxen and (*S*)-ibuprofen.[138]



Scheme 2.24.

Also, in Andeno's Diltiazem synthesis (Scheme 2.25), an early enantioselective enzymatic hydrolysis of an epoxy ester by a lipase is the key step, creating the necessary optically active intermediate **16**.[139]



Scheme 2.25.

2.9. Future Prospects

Form the above examples it is clear that the applications of enzymes in organic synthesis especially in the developing field of chirotechnology is foreseeable. Attention has been focused on those methods that are most useful and accessible to synthetic chemists. More and more syntheses are now appearing that incorporate enzymatic or microbial steps, usually to set the stereochemistry of a key intermediate.

Advances in the following areas seem most promising

1. Elucidation of the active site and the reaction mechanism of enzymes, through a combination of X-ray analysis and computer-aided modeling, may allow precise prediction of enzyme-substrate interaction.
2. Screening of microorganisms from “exotic” environment may lead to discovery of promising new enzyme activities.
3. Using modern gene technology the catalytic properties of enzymes could be improved or custom-tailored for specific reactions. Even the design and engineering of synthetic catalysts may become feasible.

There is a strong need for new biocatalytic methods producing pure compounds which are the basis for synthesis and production of biologically active compounds in the agrochemical and pharmaceutical fields.

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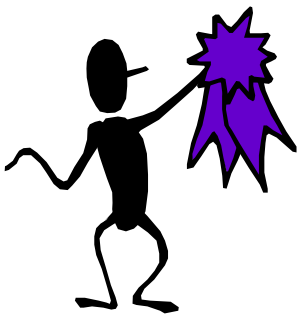
Development of Chemoenzymatic Methods for the Large-scale Preparation of Optically pure 4-(*R*)-*tert*-butyldimethylsilyloxy cyclopent-2-en-1-one



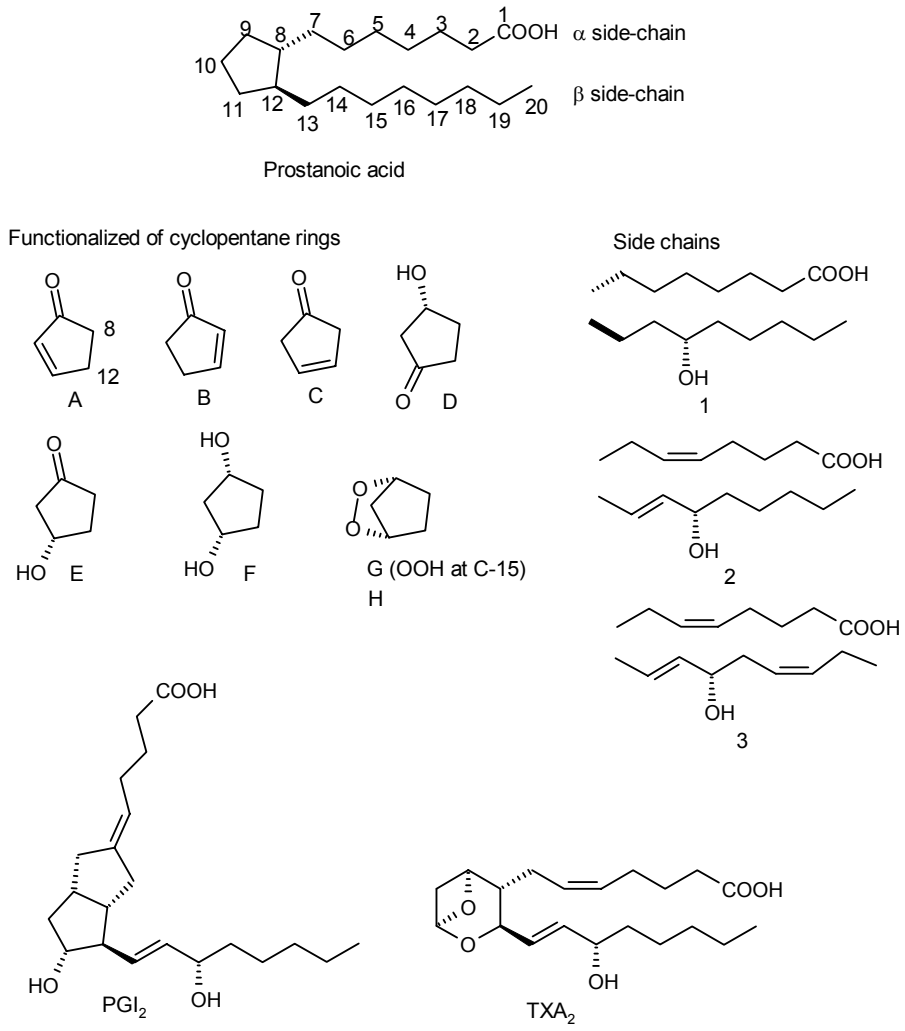
Chapter II

CHAPTER II

Contents

3.1. Introduction to Prostaglandins

Prostaglandins exhibit diverse pharmacological activities and are now recognized as local hormones that control a multitude of important physiological processes[1]. The chemical, biological and medicinal investigations being carried in this area stand in the forefront of the life sciences. Structural variations and classification of prostaglandins (PGs) and related compounds are explained in Scheme 3.1 below.

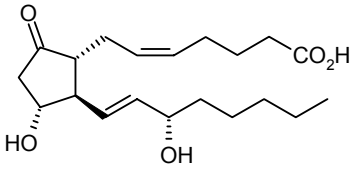


PGI_2 and TXA_2 are prostacyclin and thromboxane A_2 respectively.

Scheme 3.1.

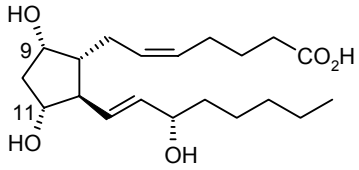
Characteristically PGs contain a functionalized cyclopentane or cyclopentene ring with two side chains (α and β) and adjacent carbon atoms and transdisposed at 8 and 12. The parent acid called prostanoic acid is a C-20 aliphatic acid derivative. There are series of prostaglandins generally abbreviated as PGSx, where 'S' denotes A, B, C to H and 'x' , the subscript for the three different series 1, 2 or 3 denoting the number, location and type of double bonds in α and β side

chains of the PGs structure as shown. Thus PGE_2 has the structure with cyclopentane ring functionalized as E; subscript 2 describes the number, location etc. of the double bonds. The structure should read then as follows:



PGE_2

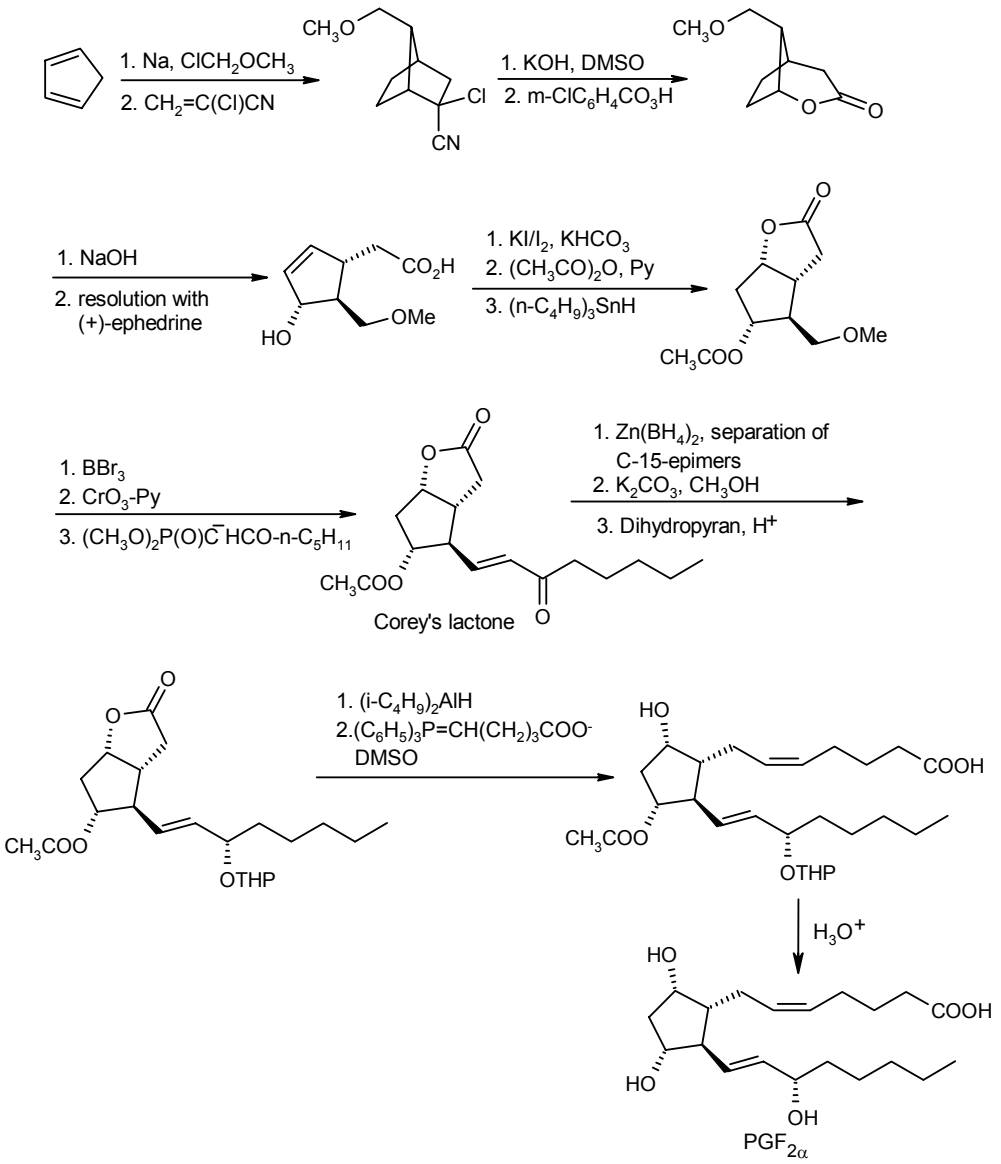
This is general for all PG family compounds except in PGF series another subscript often is used along with 'x' is α indicating that F series has 9, 11 α-OH function as a characteristic of this family. Thus, $PGF_{2\alpha}$ is as follows;



$PGF_{2\alpha}$

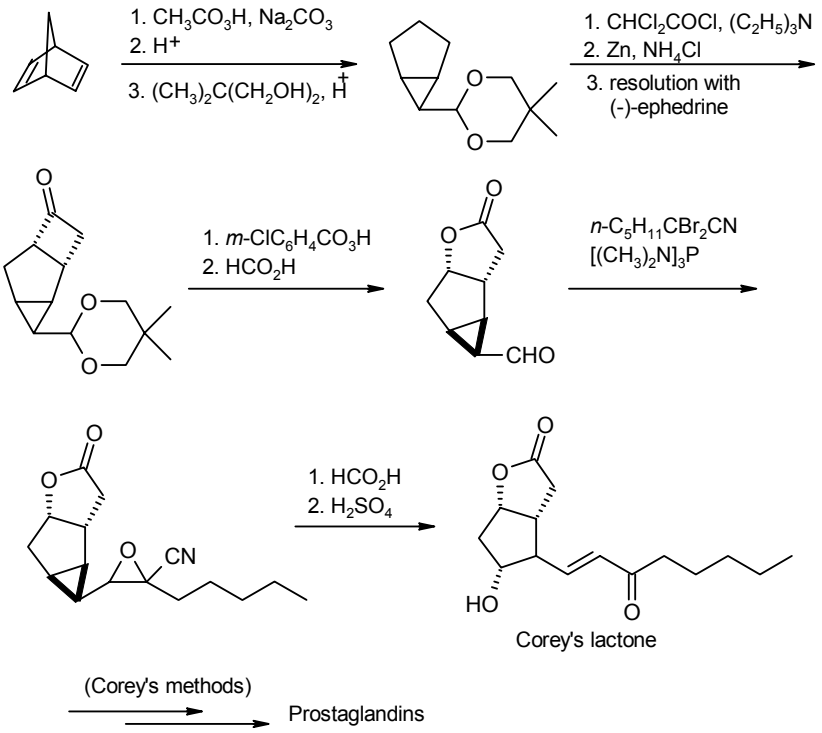
Another exception is the 'G' family with C-15 α-O-O- instead of α-OH functionality. Another two related families of extreme biological significance are PGI_2 and TXA_2, characterized by a bicycloctane and bicyclooxaheptane rings respectively instead of cyclopentane ring. They are called as prostacyclins and thromboxanes respectively.

Biosynthesis simply cannot meet the ever-increasing demand for prostaglandins[2] nor provide adequate amounts of precursors for the production of medicinally important compounds possessing modified structures. A total synthesis is the only means by which sufficient quantities of these physiologically bewildering but rare, naturally occurring substances can be made available. A tremendous number of chemists have so far been involved in the synthesis of prostaglandins[3]. Of the many elegant synthetic routes so far elaborated, the most practical are perhaps those outlined below: (1) Corey's method[4] [Scheme 3.2] (2) Just-Upjohn method[5] [Scheme 3.3] (3) Three component coupling method[6] [Scheme 3.4] and several of their variations.



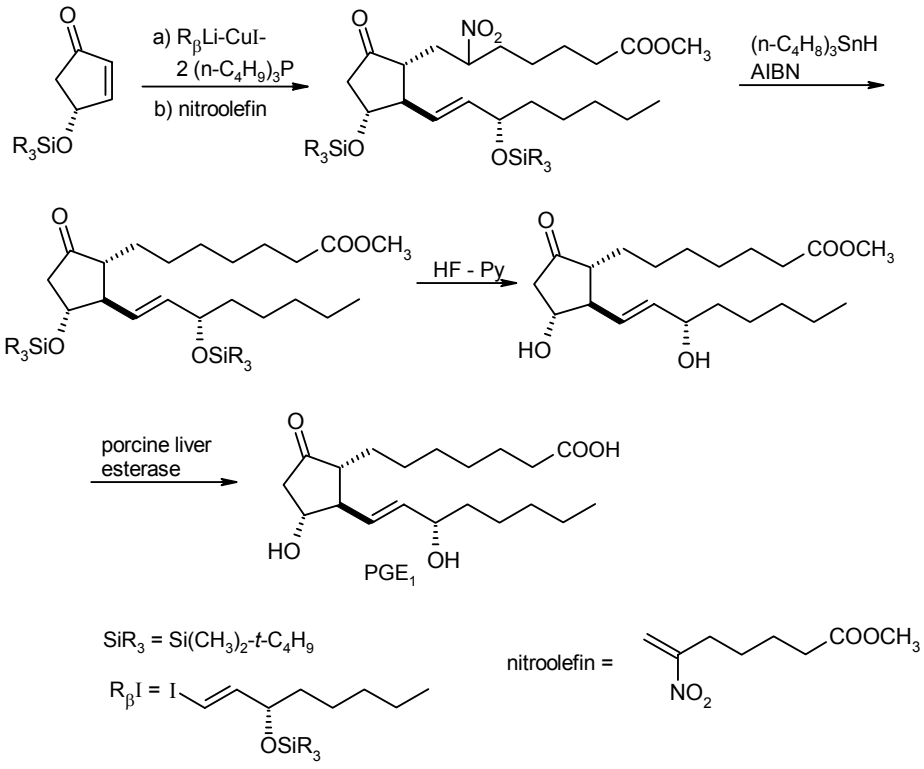
Scheme 3.2.

The approaches depicted in Scheme 3.2 and 3.3 are classified as linear or sequential synthesis; in which the whole structure is composed step-by-step. *Corey*'s route involves 17 steps for the preparation of (±)-$PGF_{2\alpha}$ from cyclopentadiene, and 19 steps for the synthesis of (±)-PGE_1. Four synthetic steps are required for the formation of the five carbon-carbon bonds, while the remaining 13 to 16 steps constitute functional group transformations such as oxidation, reduction, protection, deprotection, etc. The Just-Upjohn synthesis also proceeds *via* the Corey lactone and requires a total of 16 steps from norbornadiene to (±)-$PGF_{2\alpha}$, of which three are required for the creation of the carbon-carbon bonds. In addition, optical resolution is conducted at the stage of lactone intermediate so as to obtain the naturally occurring forms.



Scheme 3.3.

Although the above two procedures particularly with variations and modifications made time to time are clearly successful, shorter and highly convergent entries to prostanoids via a combination of suitably chosen building bocks are obviously much more desirable. Thus, Noyori et al has devised a one-pot synthesis of the complete prostaglandin skeleton by linking a chiral 4-oxygenated-2-cyclopentenone unit and two side-chain blocks.[6] This approach known as 'three component coupling method', is efficient and flexible and allows the direct production of all naturally occurring prostaglandins and a wide spectrum of analogues (Scheme 3.4). Obviously efficient methods are required for the production of all three components viz. α–side chain, β-side chain and chiral 4-hydroxycyclopentenone unit (**1**), also known as 'component C'. This chapter describes our efforts towards development of cost-effective method for large-scale preparation of component C through chemoenzymatic methods. Several methods providing access to chiral cyclopentenone intermediate **1** have been elaborated below.


a) R_βLi-CuI-
2 $(n\text{-}C_4H_9)_3P$
b) nitroolefin
$(n\text{-}C_4H_8)_3SnH$
AIBN
HF - Py
porcine liver
esterase
PGE$_1$
$SiR_3 = Si(CH_3)_2\text{-}t\text{-}C_4H_9$
$R_\beta I =$
nitroolefin =

Scheme 3.4.

3.2. Methods for the Preparation of Optically Pure 4-(*R*)-Hydroxycyclopentenone or its OH-protected derivatives

Large amount of literature is available for the preparation optically pure cyclopentenone building block **1**, its O-acetate derivative **2** and its O-silylated derivative **3**. Other cyclopentene building blocks useful for prostaglandin synthesis are 4-(*R*)-hydroxycyclopent-2-en-1-(*S*)-acetate (**6**) and its enantiomer (**ent-6**) which are mainly derived for *meso*-cyclopentene derivatives viz. *meso*-1,4-cyclopentenediol (**4**) and its diacetate (**5**) (Figure 3.1). Various methods reported for synthesis of **1** and its derivatives **2** and **3** are mainly of two types viz. chemical methods and enzymatic methods. Both the methods are described below in brief.

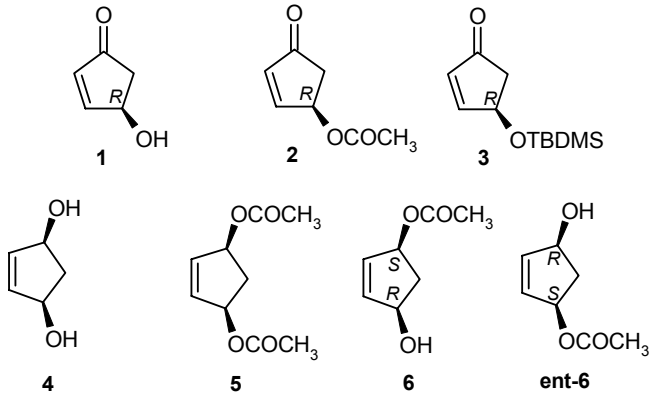


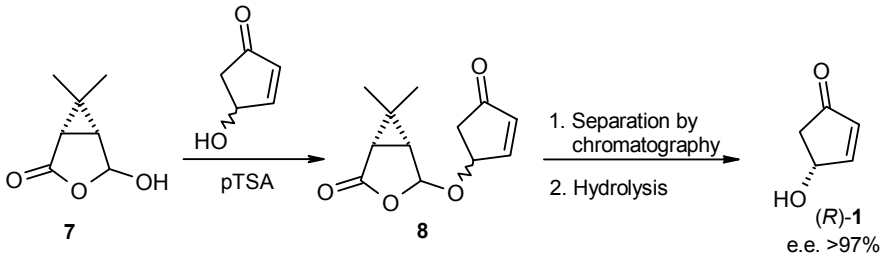
Figure 3.1.

Chemical Methods

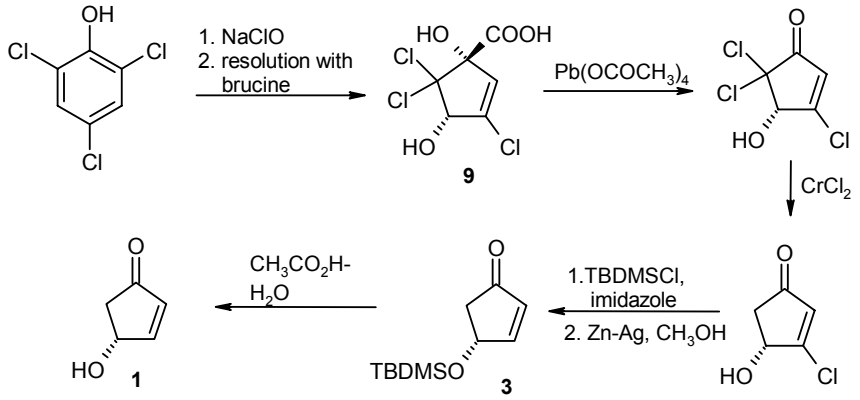
Chemical methods include classical resolution of the racemic **1**, preparation from chiral natural products or via resolved synthetic intermediates, by asymmetric synthesis and by kinetic resolution of (±)-**1** using chiral catalysts.

☞ The hydroxyenone **1** is a rather sensitive compound and hence the optical resolution is not easy by conventional methods. Fortunately, however the bicyclic species, caronaldehyde (**7**), derived from (1*S*, 3*S*)-*trans* crysanthemic acid proved to be an efficient resolving agent.[8] The diastereomeric adducts **8** of racemic **1** with caronaldehyde could be easily separated on a silica gel column. The desired (*R*)-**1** of 97% e.e. was obtained in 88% overall yield (Scheme 3.5).

☞ Gill and Rickards prepared **1** and **3** from phenol or 2,4,6-trichlorophenol as outlined in Scheme 3.6.[9] This route involves resolution of the hydroxy acid intermediates (±)-**9** with brucine (37% yield, 74% of theory). The (1*S*, 4*S*)-**9** was transformed into the silyl protected hydroxyenone **3** in four steps in 56% overall yield. Hydrolysis of **3** gave optically pure free hydroxyketone **1**.

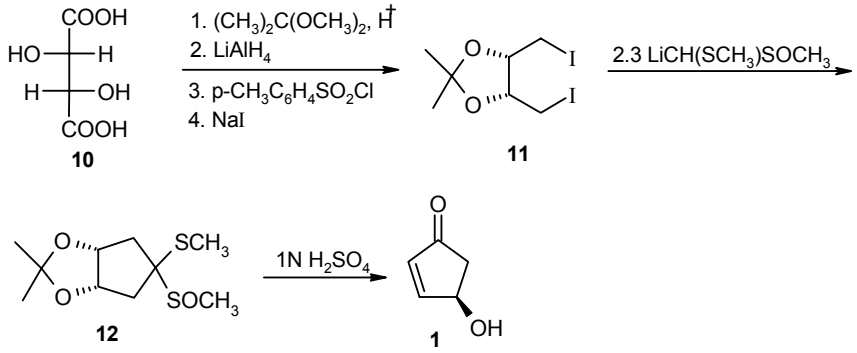


Scheme 3.5.



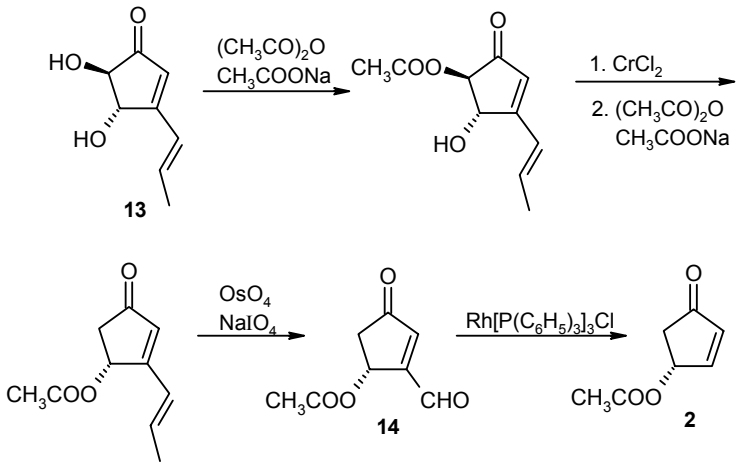
Scheme 3.6.

☞ Tsuchihashi et al used unnatural D-tartaric acid **10** as a chiral pool for the synthesis of the optically active **1**. (Scheme 3.7).[10] Using the known four-step procedure, **10** was converted into the diiodide **11** in 42% yield. Base-induced condensation of **11** with methyl methylthiomethyl sulfoxide afforded **12**, which on substituent acid-catalyzed hydrolysis gave the hydroxy enone **1** (85% e.e.) in 52.5% yield. Khanapure et al followed same methodology for preparation of (*S*)-**1** from natural L-tartaric acid.[11]



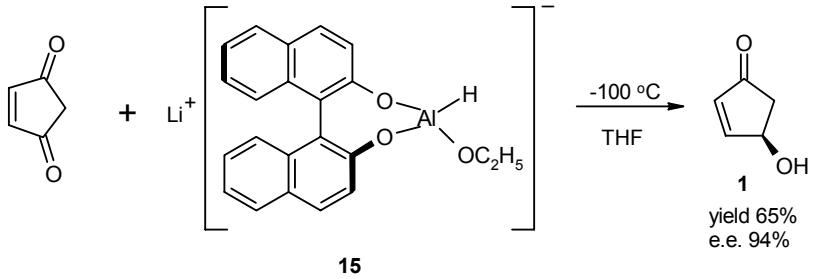
Scheme 3.7.

☞ As found by Mitscher et al,[12] naturally occurring terrein **13**, a metabolite of *Aspergillus fischerii*, can also serve as starting material. Scheme 3.8 illustrates the transformation of the highly functionalized chiral cyclopentenone into the protected hydroxyenone **2**. The olefinic side chain was removed by oxidative cleavage; giving the aldehyde **14** followed by rhodium (I)-catalyzed decarbonylation (56%). The overall yield of the five-step synthesis was 33%. **2** was converted into **1** by enzymatic hydrolysis.[20a]



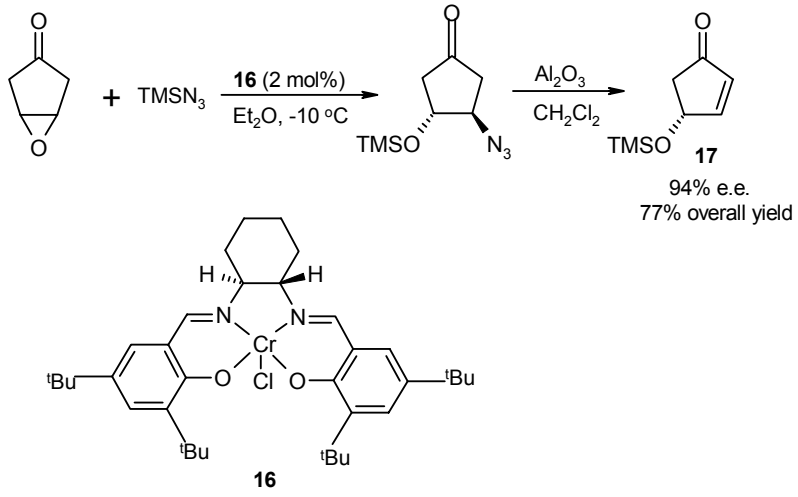
Scheme 3.8.

☞ Asymmetric synthesis is also employable (Scheme 3.9); reduction of 4-cyclopentene-1,3-dione with (*S*)-BINAL-H reagent **15** in THF at -100°C afforded the hydroxyenone **1** in 94% e.e. (65% yield).[13]



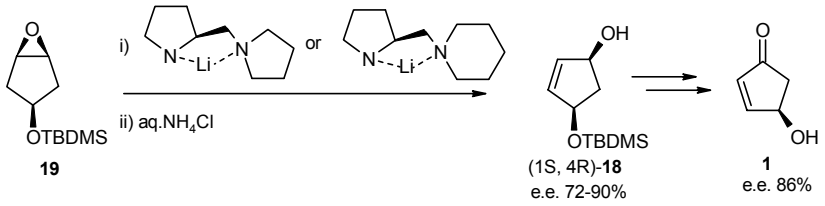
Scheme 3.9.

☞ Recently Eric N. Jacobsen et al reported chiral (salen)CrN_3 (**16**) complex catalyzed asymmetric epoxide opening approach for chiral synthesis of silylated **1** (Scheme 3.10).[14] Thus **1** of 94% e.e. was obtained in 77% overall yield from 3,4-epoxycyclopentanone.



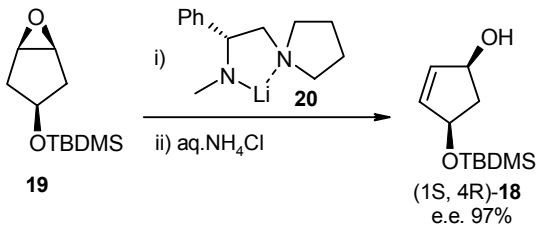
Scheme 3.10.

☞ Asami prepared chiral *cis*-4-*tert*-butyldimethylsilyloxy-2-cyclopenten-1-ol (**18**) by enantioselective deprotonation of *meso*-epoxide **19** derived from 3-cyclopenten-1-ol, using chiral lithium amide (Scheme 3.11).[15] Both (*R*) and (*S*)-4-hydroxy-2-cyclopenten-1-one were derived from (1*S*, 4*R*)-**18** stereospecifically.



Scheme 3.11.

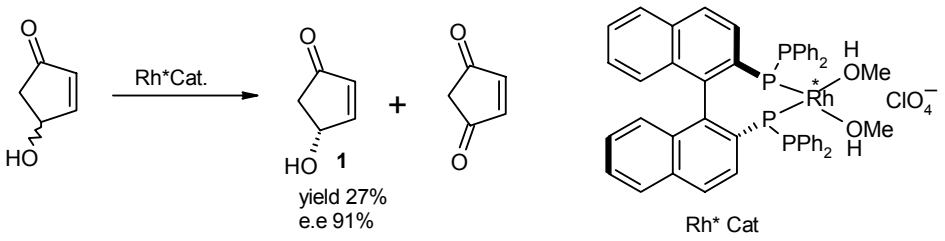
☞ V.K. Singh et al optimized the method by variation in chiral ligand. Thus use of ligand **20** afforded (1*S*, 4*R*)-**18** of 97% e.e. (Scheme 3.12).[16]



Scheme 3.12.

☞ R. Noyori et al reported kinetic resolution of (±)-**1** by rhodium-catalyzed asymmetric isomerization.[17] Thus cationic Rh-BINAP complex catalyzed isomerization of (±)-**1** to 1,3-

cyclopentanedione with 5:1 enantiomeric discrimination; thus affording (*R*)-**1** in 91% e.e. at 72% conversion (Scheme 3.13).



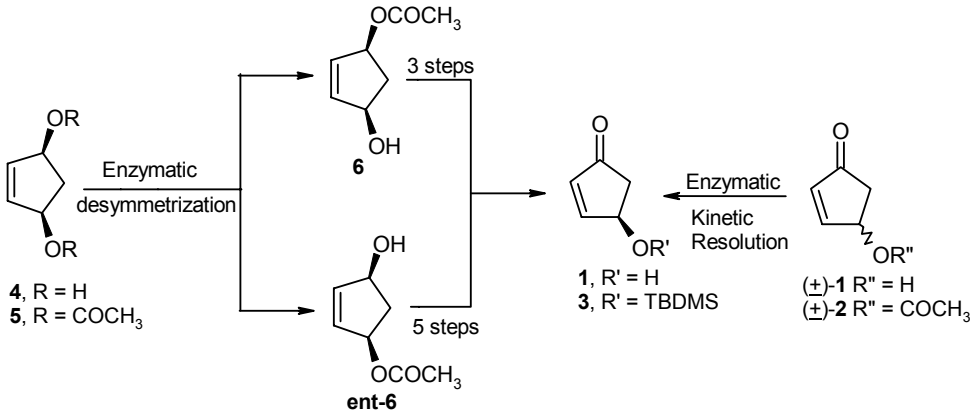
Scheme 3.13.

Enzymatic Methods

Enzymatic methods appear to be the preferred methods of choice in the preparation of enantiomerically pure **1**. There are two enzymatic approaches (Scheme 3.14).

a) Lipase/esterase catalyzed desymmetrization of *meso*-cyclopentene-1,4-diol (**4**) or its diacetate (**5**) to 4-(*R*)-hydroxycyclopent-2-en-1-(*S*)-acetate (**6**) or its enantiomer (**ent**. **6**), both of which can be efficiently converted to **1** and **3** by simple chemical transformations.

b) Lipase/esterase catalyzed kinetic resolution of racemic **1** and its acetate **2**, or of monoprotected cyclopentenediol derivatives.



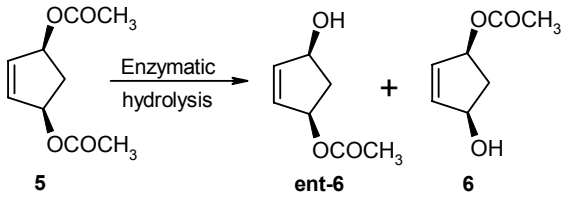
Scheme 3.14.

Desymmetrization Method

Desymmetrization methods is an attractive and extensively studied approach for the production of **1** since 100% yield of one of the enantiomer can be obtained theoretically whereas kinetic resolution suffers from the drawback of throwing away half of the material. The *meso*-cyclopentene derivatives required for desymmetrization are prepared by Pd(0) complex catalyzed

opening of cyclopentene monoepoxide[18] or by photochemical method which involves singlet oxygen addition to cyclopentadiene followed by *in situ* reduction of endoperoxide with thiourea.[19]

Various lipases and esterases are reported to catalyze enantioselective hydrolysis of *meso*-cyclopent-1,4-diacetate (**5**) in aqueous buffer medium to afford **ent-6** of high enantiopurity in high yields (Scheme 3.15, Table 3.1).[20]



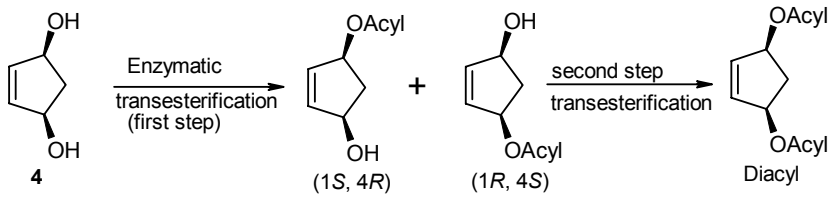
Scheme 3.15.

Table 3.1. Enzymatic hydrolysis of **5** catalyzed by different enzymes

Sr. No.	Enzyme	Product	% Yield	% e.e.	Ref.
1.	Porcine Pancreatic lipase	(1*R*, 4*S*)-**6**	86	95	20a
2.	Pig liver esterase	(1*S*, 4*R*)-**6**	83	81	20c
3.	*Pseudomonas* sp. lipase	(1*R*, 4*S*)-**6**	80	94	20f
4.	Acetyl choline esterase	(1*R*, 4*S*)-**6**	94	96	20g
5.	Lipozyme® (lipase from *Mucor miehei*)	(1*R*, 4*S*)-**6**	90	97	20f
6.	*Chromobacterium viscosum*	(1*R*, 4*S*)-**6**	80	93	20f
7.	NCIM 3574 (yeast sp.)	(1*R*, 4*S*)-**6**	85	99	20j

As can be seen from Table 3.1, most of the lipases reported for the hydrolysis of **5**, with the exception of PLE, have pro-*S* selectivity; thus, affording **ent-6** of high enantiopurity in high yields. Only PLE is having pro-*R* selectivity, which affords **6** of 81% e.e. in 83% yield.[20c] It has to be further crystallized (benzene-Skelly B 1:5) to enhance optical purity to >96%.

These enzymes can catalyze the reverse reaction i.e. transesterification of diol **4** in organic media with various acyl donors to afford the required product with (1*S*, 4*R*) configuration. Various enzymes have been reported to catalyze the conversion using variety of acyl donors, generally in the THF-Et_3N system (Table 3.2).[21] Monoacylated products with very high enantiopurity have been obtained in many cases in 50-60% yield along with remarkable quantities of the diacylated product indicating a low enantioselectivity in the first acylation step which was then compensated for in the second step (Scheme 3.16) (*vide supra* Scheme 2.4, page 19).



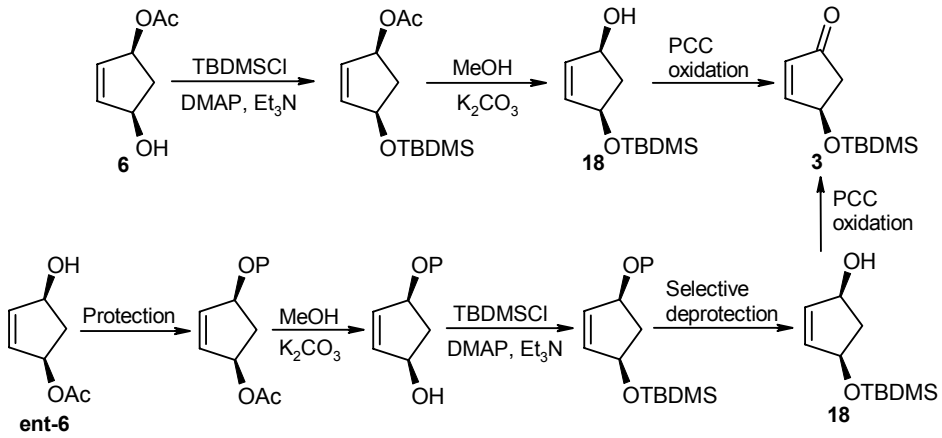
Scheme 3.16.

Table 3.2. Irreversible transesterification of *meso*-diol **4** catalyzed by various enzymes

Sr.No.	Acyl donor	Lipase	Yield %	e.e %	Config.	Ref.
1.	TCA[a]/THF-NEt_3	PPL	48	>99	*S*-OAc	21b, c
2.	TCB[b]/ THF-NEt_3	PPL	51	99	*S*-OAcyl	21c
3.	TCO[c]/ THF-NEt_3	PPL	53	80	*S*-OAcyl	21c
4.	VA[d]/ THF-NEt_3	PPL	65	>99	*S*-OAc	21c
5.	VB[e]/ THF-NEt_3	PPL	55	93	*S*-OAcyl	21c
6.	VA/ THF-NEt_3	*Mucor* sp	85	94	*S*-OAc	21d,e
7.	TCA/Py	PPL.	50	>99	*S*-OAc	21f
8.	IA[f]/BuOMe	SP 435[g]	48	>99	*S*-OAc	21g

a: 2,2,2-Trichlorethyl acetate, b: 2,2,2-Trichloroethyl butanoate c: 2,2,2-Trichoroethyl octanoate, d: Vinyl acetate, e: Vinyl butanoate, f: Isopropenyl acetate, g: Lipase from *Candida antartica*.

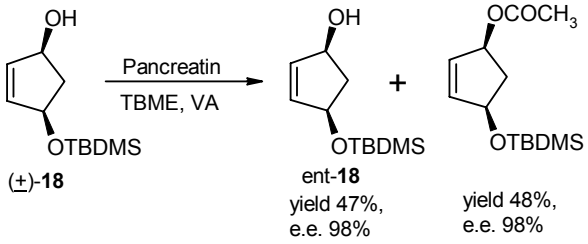
As it is clear from above discussion, desymmetrized product **6** is available in both enantiomeric forms in high optical purities by either of approaches viz. hydrolysis or transesterification. Both enantiomers, **6** and **ent-6** can be converted to component C with (4*R)*-configuration through *meso*-trick as shown in Scheme 3.17.[22] Compound **6** is converted to (*R*)-**3** through three steps viz. protection of (*R*)-hydroxy as TBDMS ether, methanolysis of acetate and PCC oxidation whereas **ent-6** is converted to (*R*)-**3** in five steps. Free (*S*)-hydroxy group in **ent-6** is initially protected as either THP ether[22b] or pivaloyl ester[22c] followed by methanolysis of acetate to obtain free (*R*)-hydroxy group, which is then protected as TBDMS ether. Next crucial step constitutes selective deprotection at (*S*)-hydroxy center followed by PCC oxidation of free –OH to afford desired compound **3** with (4*R*) configuration.



Scheme 3.17.

☞ Kinetic Resolution

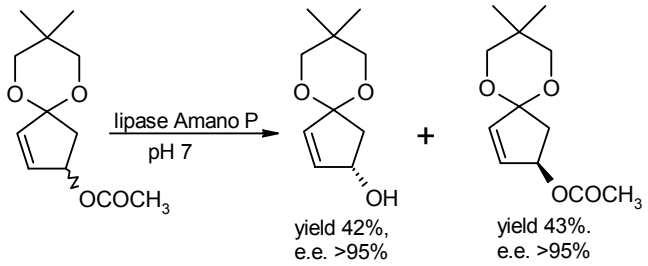
Few monoprotected cyclopentendiol derivatives are resolved efficiently be enzyme catalyzed transesterification in organic media.[23] These resolved derivatives can be converted to (*R*)-**3** by chemical conversion (Scheme 3.18).



Scheme 3.18.

As compared to desymmetrization method, direct kinetic resolution of racemic 4-hydroxycyclopentenone catalyzed by enzymes is very less studied approach. Even though, desymmetrization method is an attractive and most extensively studied approach for the production of **1** and **3** since 100% yield of one of enantiomer can be obtained theoretically whereas kinetic resolution suffers from the drawback of throwing away half of the material i.e. unwanted isomer. But at the large scale production, desymmetrization method becomes costlier because preparation of the required *meso*-cyclopentene derivatives involves either expensive and sensitive Pd(0) complexes as catalyst[18] or photochemical reaction[19] wherein yields are low and scale up is difficult. In addition, the method involves additional 3 to 5 chemical steps wherein *tert*-butyldimethylsilyl chloride which is a major cost component, is used at early stage of the synthesis.[22] Comparatively direct resolution of racemic 4-hydroxycyclopentenone, (±)-**1** would be a more attractive alternative as the large scale preparation of **2** is easier.[24] We have already scaled up the process using furfuryl alcohol even up to 3 kg scale. Also there are literature

reports wherein (*S*)-**1** has been inverted to (*R*)-**1** by simple chemical steps.[25] This resolved (*R*)-**1** can then be directly converted **3** using expensive *tert*-butyldimethylsilyl chloride as the silylating agent at the last step, saving on the cost. The classical resolution approach involving caronaldehyde as resolving agent is discouraging at large scale due to the exorbitant high cost of caronaldehyde. Other successful approach involves kinetic resolution by enantioselective hydrogenation using chiral catalyst developed by Noyori et al,[17] but yields are low and expensive chiral catalyst is required (Scheme 3.13). Although lipase catalyzed resolution of (±)-**1** would be an economic approach, very few reports are available on lipase catalyzed kinetic resolution by hydrolysis of its acetate (±)-**2** in aqueous media.[26] The limiting factors of the approach mainly are (i) high solubility of **1** in aqueous medium makes its isolation tedious, requires continuos extraction with ethyl acetate for 3 days and (ii) possibility of racemization in aqueous media which would deteriorate enantiopurity at least to some extend. Winterfeld et al have overcome the problem of racemization by derivatizing (±)-**2** to cyclic ketal prior to resolution (Scheme 3.19).[26b] But this protection step is very tricky and low yielding, hence not suitable to scale-up.

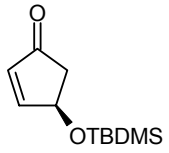


Scheme 3.19.

These problems can be circumvented by the judicious use of organic solvents for the conversion. But to our knowledge such an attempt is not reported.

3.3. Present Work

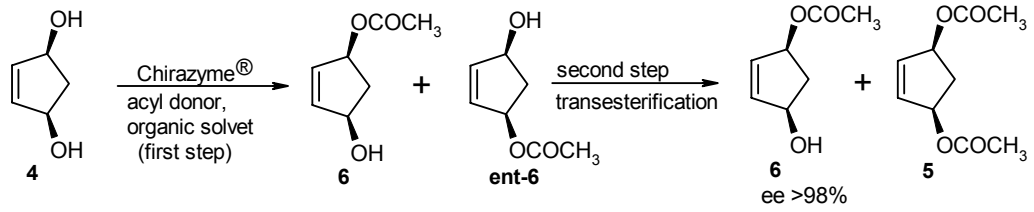
We have been interested in development of economically viable large-scale process for prostaglandins and their intermediates due to their varied biological activities. Development of cost-effective method for large-scale preparation of (*R*)-(+)-**3** has been the major goal of our group.



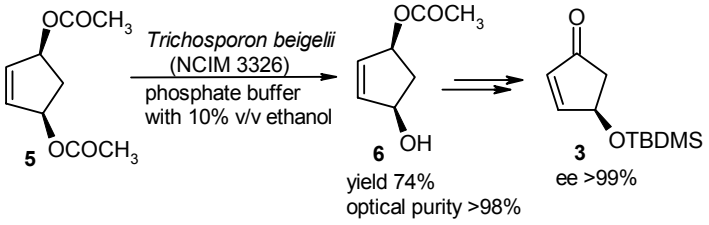
(*R*)-(+)-**3**

We attempted the process through well-studied desymmetrization method as well poorly studied direct enzymatic kinetic resolution of racemic cyclopentenone alcohol. The study is divided into three parts:

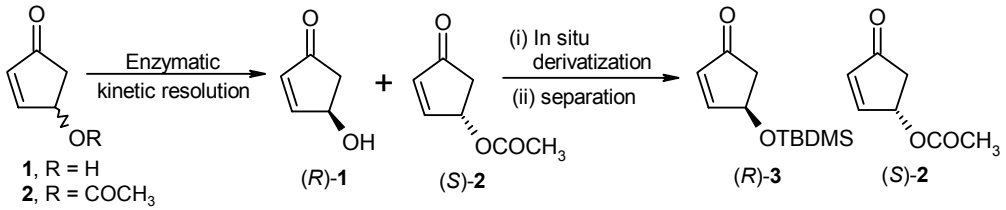
☞ 3.3.1. Desymmetrization of *meso*-cyclopenten-*cis*-1,4-diol to 4-(*R*)-hydroxyclopent-2-en-1-(*S*)-acetate by irreversible transesterification using Chirazyme®.



☞ 3.3.2. A practical and scalable process for 4-(*R*)-hydroxycyclopent-2-en-1-(*S*)-acetate by desymmetrization of *meso*-cyclopent-2-en-1,4-diacetate catalyzed by *Trichosporon beigelii* (NCIM 3326), an inexpensive Biocatalyst.



☞ 3.3.3. Enzymatic Kinetic Resolution Studies of Racemic 4-Hydroxycyclopent-2-en-1-one using Lipozyme IM®.

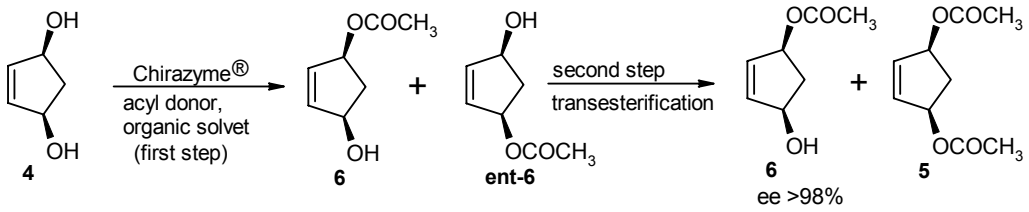


3.3.1. Desymmetrization of *meso*-Cyclopenten-*cis*-1,4-diol to 4-(*R*)-Hydroxy cyclopent-2-en-1-(*S*)-acetate by Irreversible Transesterification using Chirazyme®.

3.3.1.1. Introduction

As discussed earlier, desymmetrization of *meso*-cyclopentene-1,4-diol **4** through irreversible transesterification yields important intermediate **6**. Various enzymes have been reported to catalyze the conversion using variety of acyl donors, generally in the THF-Et_3N system yielding **6** of high enantiopurity in 50-60% yields (Table 3.2).[21] Unfortunately the lipase from *mucor* species which gave the best results (yield 85%, e.e. >98%)[21d,e] is not available commercially[21a] whereas the lipase from *Mucor meihei i.e.* Lipozyme IM®/Chirazyme® which is available commercially in bulk scale was found to be inefficient for the conversion (yield <5%) under the conditions attempted by author (THF, Et_3N).[21e]

Enhancement of enzyme efficiency through medium-engineering i.e. optimization of solvent system and optimization of other parameters as pH, temperature etc. has been well reported in several cases.[27] The availability of Chirazyme® in bulk scale prompted us to consider the study of enhancement of its selectivity towards desymmetrization of **4** by transesterification through medium engineering and optimization of other parameters (Scheme 3.20).



Scheme 3.20.

3.3.1.2 Results and Discussion

☞ *Effect of Solvent Variation*

The effect of solvent on activity and specificity of various enzymes has been well documented in literature.[27,28] Attempts have been made to derive correlation between enantioselectivity and physicochemical characteristics of the solvent such as hydrophobicity or dielectric constant etc.[28,29] Initially we carried out transesterification of *meso*-diol **4** with vinyl acetate in various organic solvents using Chirazyme® as catalyst. The results are indicated in Table 3.3. Though we cannot conclude on correlation of enzyme efficacy and physicochemical properties of solvent

with available data, nature of solvent was found to have profound effect on enzyme efficacy. In general enzyme activity was good in all the etheral solvents tested. Enzyme efficacy in terms of yields and enantiopurity of product **6** was maximum in diethyl ether (entry 5, yield 45%, e.e. 88%) and *tert*.butyl methyl ether (TBME) (entry 7, yield 35%, e.e. 93%). The reaction in ethyl acetate and butyl acetate afforded **6** of high optical purity, but in lower yields (entries 8 & 13 respectively). In case of dioxane and acetonitrile though second acylation step was inhibited, the enantioselectivity of monoacylation step was rather poor. TBME was found to have potentiating effect on enzyme activity, thus exhibiting fast reaction rates (2 hr only) and good enantioselectivity (e.e. of **6** = 93%).

Table 3.3. Various solvents used with vinyl acetate as acyl donor[a]

No.	Solvent	Time hr	Yield of **6** (%)	$[\alpha]_D$ of **6** [b]	e.e.[c] of **6**	Yield of **5** (%)
1.	-	2	40.0	-55.4	80.0	55
2.	THF	12	20.0	-62.0	89.0	65
3.	Dioxan	10	84.5	-15.5	22.0	-
4.	Dioxan	34	70.0	-26.4	38.0	Traces
5.	**Ether**	**10**	**45.0**	**-61.2**	**88.0**	**35**
6.	Diisoproyl ether	10	32.0	-15.1	23.8	66
7.	**TBME**	**2**	**35.0**	**-64.8**	**93.0**	**60**
8.	**Ethyl acetate**	**12**	**26.0**	**-68.0**	**>98.0**	**70**
9.	Acetone	28	30.0	-54.8	79.0	54
10.	Acetonitrile	60	36.0	-35.0	50.0	Traces
11.	Toluene	No reaction	-	-	-	-
12.	Hexane	"	-	-	-	-
13.	**Butyl acetate**	**12**	**22.0**	**-66.0**	**95.0**	**72**

a : 1mmol of diol **4** was reacted with 5 eqv. vinyl acetate & 0.2g enzyme in 5 ml of solvent
b: Lit[219] $[\alpha]_D$ = -69.3 (c =1, $CHCl_3$) e.e. > 99%, c: e.e. = enatiomeric excess

☞ *Effect of Acyl Donor*

The effect of acyl donor on the enantioselectivity of the lipase catalyzed transesterification reaction has been well demonstrated by T. Ema et al wherein change in structure of acyl donor has brought about considerable variations in enantioselctivity of an enzyme.[30] In order to study effect of acyl donor, we carried out the reaction with different acyl donors (Table 3.4). The

reaction proceeded slowly with low enantioselectivity with acyl donors other than vinyl acetate. The inefficient reaction with isopropenyl acetate may be attributed to steric reasons. Thus vinyl acetate was the only suitable acyl donor for the conversion and was used for further parameter optimization.

Table 3.4. Other acyl donors[a]

No.	Acyl donor	Solvent	Temp	Time	Yield of **6** (%)	$[\alpha]_D$ of **6**	e.e. of **6**	Yield of **5** (%)
1.	Isopropenyl acetate	-	RT	7 days	35.0	-33.3	48	16
2.	Isopropenyl acetate	TBME	RT	6 days	22.5	-42.8	64	10
3.[b]	Vinyl acetate	TBME	RT	2	35.0	-64.8	93.0	60
4.	Isopropenyl acetate	THF	RT	No reaction	-	-	-	-
5.	Ethyl acetate	-	RT	48 hr	21.0	-26.6	38	Traces
6.	Butyl acetate	-	RT	24 hr	46.4	-31.6	45	Traces
7.	Isopropyl acetate	-	RT	Very slow reaction	-	-	-	-
8.	n-Hexyl acetate	-	RT	"	-	-	-	-
9.[b]	Vinyl acetate	-	RT	2	40.0	-55.4	80.0	55

a: 1mmol of diol **4** was reacted with 5 eqv. acyl donor & 0.2g enzyme in 5 ml of solvent & in case of neat reactions 5 ml of acyl donor was used.
b: from Table 2.3 entries 7and 1

☞ *Effect of Temperature*

It is widely believed that enzymes, like other catalysts, generally exhibit their highest selectivity at low temperature. This assumption has been supported by several experimental observations, not only with hydrolases[27] but also with dehydrogenase. T. Sakai et al in their lipase PS catalyzed kinetic resolution studies examined the temperature variation effect ranging from 30 to -60°C on the enantioselectivity of the reaction and have found the linear increase in enantioselectivity till -40°C. [31] Therefore we studied the reaction at lower temperatures in several solvents (Table 3.5). Lower temperature was found to have very much beneficial effect on enzymes efficacy in terms of chemical yields and enantiopurity of product. Here again, diethyl ether and TBME turned out to be the best choice, affording **6** of >95% e.e. in 65% & 52% yield respectively (entries 3 & 6) at 4°C. Again reaction rates were much faster in TBME than any other solvent. Next we turned our attention to optimize the ratio of lipase to substrate and ratio of acyl donor to substrate.

Table 3.5. Variations in temperature[a]

No.	Solvent	Temp (°C)	Time (hr)	Yield of **6** (%)	$[\alpha]_D$ of **6**	e.e. of **6**	Yield of **5** (%)
1.	Ether	RT	10	45	-61.2	88.0	35
2.	Ether	15	15	52	-66.0	95.2	30
3.	**Ether**	**4**	**24**	**65**	**-67.0**	**96.7**	**37**
4.	TBME	RT	2	35	-64.8	93.0	60
5.	TBME	15	3	46	-68.6	>98.0	40
6.	**TBME**	**4**	**4**	**52**	**-69.0**	**>99.0**	**35**
7.	Ethyl acetate	RT	12	26	-68.0	98.0	70
8.	Ethyl acetate	4	22	63	-62.0	89.0	20
9.	THF	RT	12	20	-62.0	89.0	65
10.	THF	10	33	37	-36.5	52.0	62
11.	THF	0	29	67	-37.1	52.0	30

a: 1mmol of diol **4** was reacted with 5 eqv. vinyl acetate & 0.2g enzyme in 5 ml of solvent

☞ *Ratio of Enzyme to Substrate*

Table 3.6 indicates a surprising result that reduction in enzyme to substrate ratio from 2:1 to 1:1 has beneficial effect in both the solvents, thus affording product **6** of >99% e.e. in >40% yield in both the solvents, (entries 2 & 7). But at lower temperature, the yields and e.e. dropped in ether with 1:1 enzyme : substrate ratio (entry 3) a reason may be reaction is much slower at the lower temperature with lesser amount of enzyme as indicated by lower yields of monoacetate **6** as well as diacetate **5**; whereas reaction in TBME with 1:1 enzyme : substrate ratio at 4°C afforded **6** of >98% e.e. in >60% yield (entry 9).

☞ *Ratio of Vinyl acetate to Substrate*

Quantity of vinyl acetate acyl donor was varied from 5 eq. to 1.5 eq. using ether and TBME as solvent with 0.1g enzyme. Table 3.7 indicates that minimum of 5 eq. of acyl donor are required for efficient reaction. The reason may be explained as follows. The first acylation, which is desymmetrization step, would require some excess of acyl donor. The pro-*S* selectivity of the enzyme is not very high; thus **6** and **ent-6** are both formed in unequal quantities in first step, **6** being the major one. The second acylation is a kinetic resolution where enzyme is still more selective for pro-*S* -OH group, thus it acylates **ent-6** much faster than **6**, thus enriching the

enantiomeric excess of **6**. Thus, to have **6** of desired enantiomeric excess of >98% formation of diacetate **5** in sufficient quantity is required which in turn demands excess quantity of acyl donor.

Table 3.6. Variations in enzyme:substrate ratio[a]

No.	Solvent	Enzyme : substrate ratio	Temp (^oC)	Time (hr)	Yield of **6** (%)	$[\alpha]_D$ of **6**	e.e. of **6**	Yield of **5** (%)
1.	Ether	2:1	RT	10.00	45	-61.2	88.0	35
2.	**Ether**	**1:1**	**RT**	**10.00**	**43**	**>-69.0**	**>99.0**	**45**
3.	Ether	0.5:1	RT	10.00	51	-59.4	85.7	30
4.	Ether	1:1	15	15.00	40	-62.3	89.8	25
5.	Ether	1:1	4	26.00	52	-51.0	73.6	25
6.	TBME	2:1	RT	2.00	35	-64.8	93.0	35
7.	**TBME**	**1:1**	**RT**	**2.00**	**46**	**-69.3**	**>99.0**	**50**
8.	TBME	0.5:1	RT	2.00	43	-61.3	88.4	40
9.	**TBME**	**1:1**	**4**	**4.75**	**64**	**-68.1**	**>98.0**	**28**

a: 1mmol of diol **4** was reacted with 5 eqv. vinyl donor in 5 ml of solvent

Table 3.7. Variations in ratio of vinyl acetate[a]

No.	Solvent	Equi. of Vinyl acetate	Temp ^0C	Time (hr)	Yield of **6** (%)	$[\alpha]_D$ of **6**	e.e. of **6** (%)	Yield of **5** (%)
1.	Ether	5.0	RT	10.0	43.0	-69.0	>99.0	45.0
2.	Ether	3.0	RT	12.0	30.0	-40.0	58.0	traces
3.	Ether	1.5	RT	24.0	17.6	-30.0	43.0	traces
4.	TBME	5.0	RT	2.0	46.0	-69.3	>99.0	50.0
5.	TBME	3.0	RT	3.0	46.5	-66.3	95.7	44.3
6.	TBME	1.5	RT	5.5	52.8	-50.0	72.0	28.3

a: 1mmol of diol **4** was reacted with denoted eqv. vinyl acetate & 0.1g enzyme.

☞ *Effect of various Additives*

Addition of certain additives as water, amines, DMF, DMSO in small percentage have been reported to improve selectivity of hydrolytic enzymes in several cases.[32] Especially the intrinsic

water content (more precisely, the water activity, a_w) has been found to have an influence on the enzyme selectivity in several cases.[28,32g,33] Our results with various additives in the reaction are presented in Table 3.8. Unfortunately, none of the additives attempted were found to have beneficial effect on enzyme efficacy, on the contrary yields and e.e. were deteriorated badly in most of the cases. The commercial enzyme preparation has 2-3% moisture content which seems to be optimal in this case. Added extra water was found to be detrimental to the reaction.

Table 3.8. Effect of various additives[a]

No.	Solvent	Additive	Quantity of additive (%)	Time	Yield of **6** (%)	$[\alpha]_D$ of **6**	e.e.of **6** (%)	Yield of **5** (%)
1.	THF	Water	1	5 days	20	-24.0	34.6	-
2.[b]	THF	-	-	12 hr	20	-62.0	-89.0	65
3.	Dioxan	Water	1	5 days	51	-28.0	40.0	-
4.[b]	Dioxan	-	-	34 hr	70	-26.4	38.0	traces
5.	THF	Et_3N	10	15 hr	50	-43.0	62.0	traces
6.	TBME	Et_3N	10	24 hr	35	-52.0	75.0	traces
7.	TBME	DMF	10	24 hr	31	-43.0	62.0	traces
8.	TBME	DMSO	10	no rxn				
9.	TBME	CH_3CN	50	10 hr	45	-43.4	62.0	30
10.[b]	TBME	-	-	2 hr	35	-64.8	93.0	60
11.	Ether	CH_3CN	50	19 hr	53	-48.7	70.0	27
12.[b]	Ether	-	-	10 hr	45	-61.2	88.0	35
13.	TBME	Et_3N	20	43 hr	43	-37.0	53.0	traces

a: 1mmol of diol **4** was reacted with 5 eqv. acyl donor & 0.g enzyme in 5 ml of solvent
b: from Table 3.3 entries 2, 4, 5 and 7

3.3.1.3. Conclusion

*Desymmetrization of mesocyclopentene-1,4-diol (**4**) has been demonstrated successfully through irreversible transesterification using Chirazyme® by parameter optimization approach. Thus was **4** monoacylated with vinyl acetate in presence of Chirazyme® in TBME at 4^0C to afford* 4-(R)-*hydroxyclopent-2-en-1-(S)-acetate (**6**), an important prostaglandin intermediate in 64% yield with >98% e.e.. Further studies regarding enzyme recycling and scale-up of the process are in progress. The results indicate strong possibility of exploiting Chirazyme® for the development of economically viable technology for the large-scale production of **6.***

3.3.1.4. Experimental

☞ *General*

All the reagents were purchased from *Aldrich* and were used without further purification. NMR spectra were recorded on *Brucker NMR* (200 MHz) spectrometer. IR spectra were recorded on *Research Series FTIR* spectrometer. Optical rotations were recorded on a *Jasco Dip-181* and *Jasco P-1020* polarimeter using sodium vapor lamp. Enantiomeric excess (e.e.) were determined by comparing the specific rotation value $[\alpha]_D$ with the literature value. Chirazyme® was obtained as gift sample from Boehringer Mannheim, Germany.

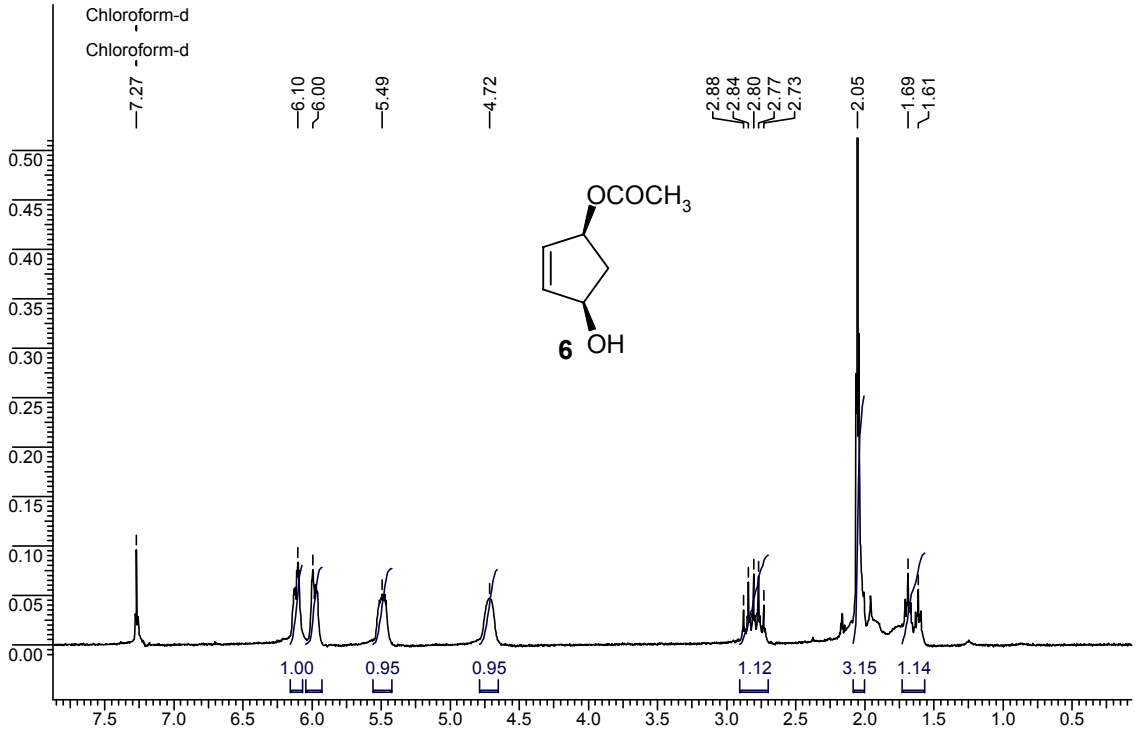
☞ *A typical example of Desymmetrization experiment*

In a typical experiment*, meso*-diol **4** (0.1 g, 1mmol), vinyl acetate (0.430g, 5 mmol, 0.46 ml) in 5 ml TBME was stirred at 4^oC for half an hour. Chirazyme® (0.1g) was added to the reaction mixture. The mixture was stirred at 4^oC for 4.75 hr. Filtered, solvent was evaporated under reduced pressure. Residue was chromatographed on silica gel (# 60-120) column using ethyl acetate-petroleum ether as eluent to separate diacetate **5** (yield = 0.051g, 28%) and monoacetate **6** (crystalline needles, yield = 0.091g, 64%)

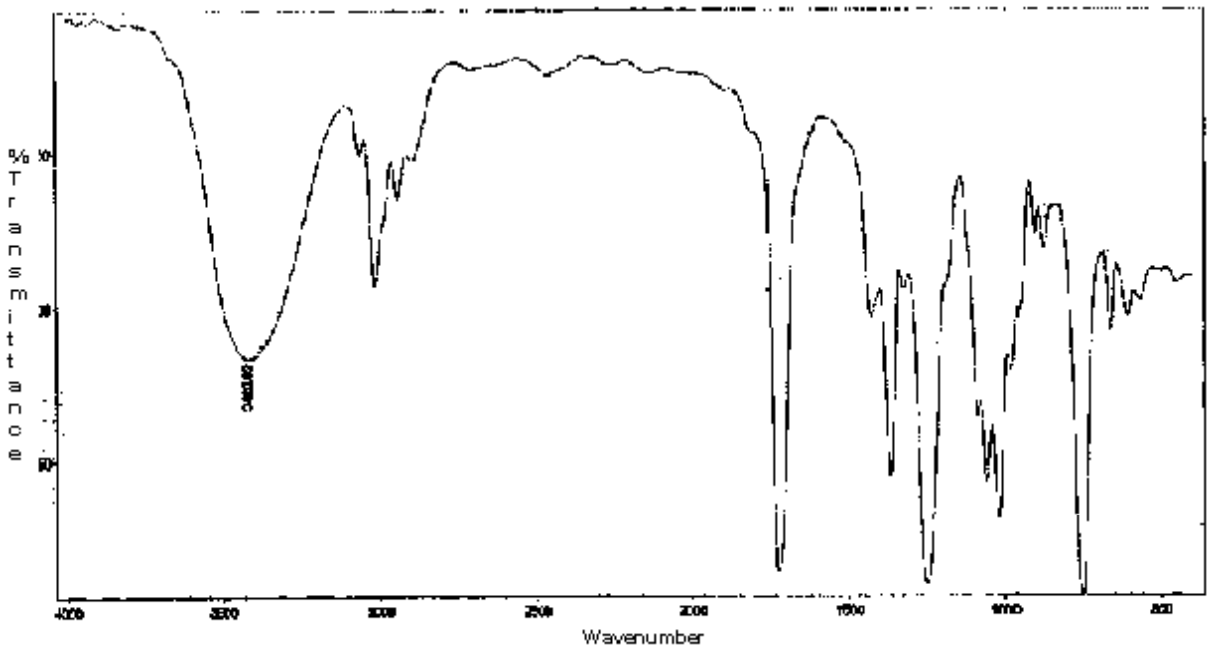
^1H NMR ($CDCl_3$): δ 1.63 (md, 1H), 2.05 (3H, s), 2.80 (qn, 1H), 4.72 (m, 1H), 5.49 (m, 1H), 5.49 (m, 1H), 6.10 (m,1H).
^{13}C NMR ($CDCl_3$): δ 21.15, 40.38, 74.27, 77.25, 132.02, 138.74, 171.05
IR ($CHCl_3$): cm^{-1} 756.77, 1018.61, 1057.61, 1087.15, 1250.97, 1367.22, 1728.74, 3420.89
Specific rotation: $[\alpha]_D$ = -68.1 (c =1, $CHCl_3$), e.e. >98%, Lit.[21g] -69.3 (c=1, $CHCl_3$), e.e. >99%).

3.3.1.5. Spectra

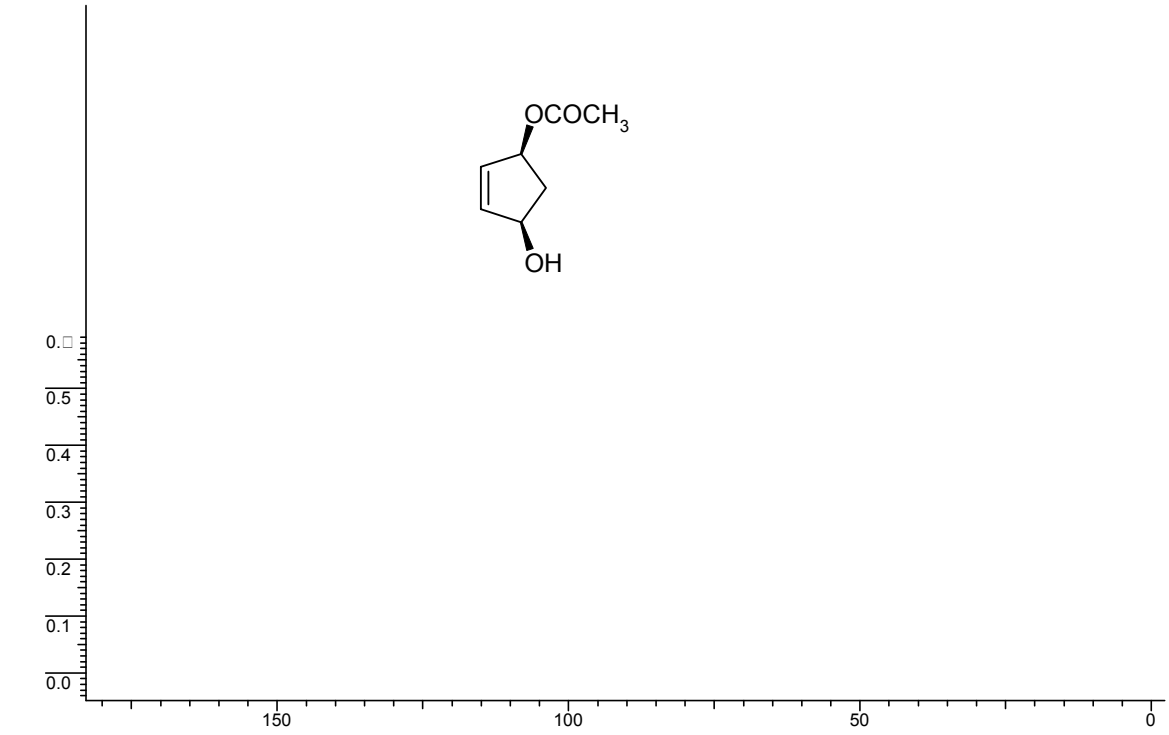
☞ *^{1}H NMR spectrum of **6***

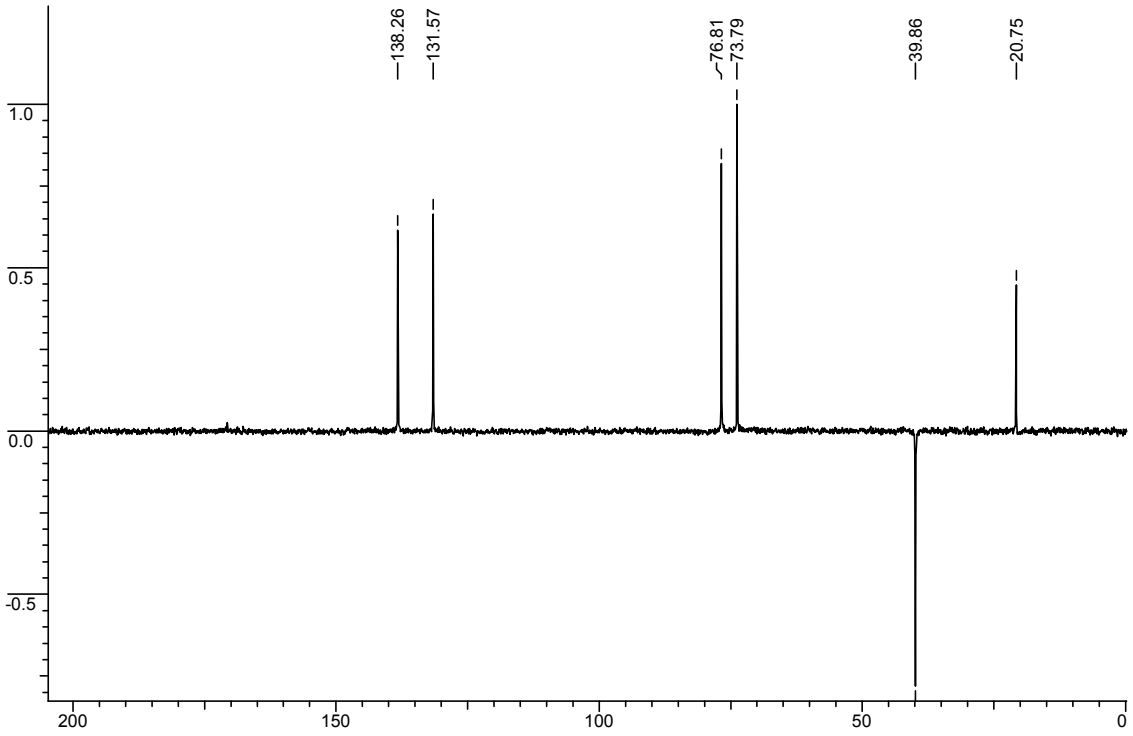


☞ IR spectrum of **6**



☞ *^{13}C NMR spectra of **6***





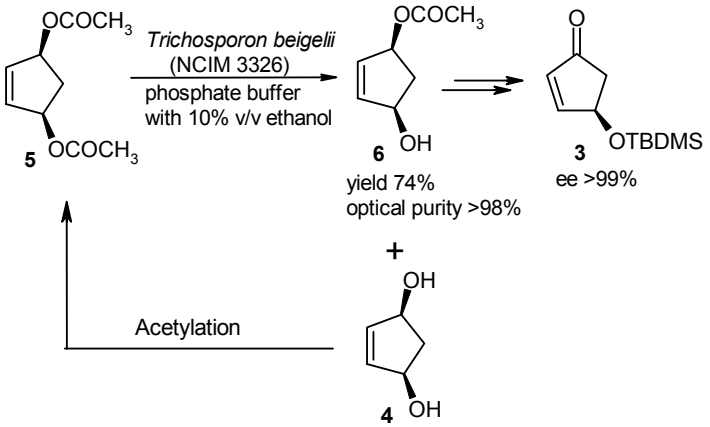
3.3.2. A Practical and Scalable Process for 4-(*R*)-hydroxycyclopent-2-en-1-(*S*)-acetate by Desymmetrization of *meso*-Cyclopent-2-en-1,4-diacetate catalyzed by *Trichosporon beigelii* (NCIM 3326), an inexpensive Biocatalyst

3.3.2.1. Introduction

In the case of desymmerization of **5** through enzymatic hydrolysis in aqueous medium, most of the efficient enzymes reported, with the exception of Pig Liver Esterase (PLE),[20c] show pro-*S* preference yielding **ent-6** in high enantiomeric excess (Table 3.1). It is possible to get desired (4*R*)-hydroxy configuration through manipulation of this (4*S*)-hydroxy enantiomer (*meso*-trick).[22] But this adds to the steps and involves use of diethylaluminium chloride or DIBALH which are costly and hazardous; hence not recommended for large scale. Therefore direct desymmetrization of **5** to the right enantiomer, **6** through aqueous hydrolysis is very much desirable.

PLE having pro-*R* preference for the hydrolysis of **5** yields **6** on hydrolysis and is currently used as a method of preparation. But PLE is expensive and also gives **6** of only 81% e.e. which has to be recrystallized to enhance e.e. to >96%; resulting in overall low yields.[20c] Hence replacement by other cheaper enzyme of microbial origin is required for the reasons of economy.

The large collection of over 300 yeast and fungal cultures at NCIM, Pune, India offered us a great opportunity to explore possibility of hunting a microbial culture of desired selectivity for the conversion of **5** to **6**. Herein we report our successful efforts in finding out the yeast culture having desired pro-*R* selectivity for the conversion of **5** to **6** through aqueous hydrolysis wherein the desired level of efficiency of the conversion in terms of enantiopurity and yield of the product was achieved through medium-engineering. (Scheme 3.21)



Scheme 3.21.

3.3.2.2. Results and discussion

Various cultures grown on oil media were tested for hydrolytic activity on **5** in 0.1 M phosphate buffer. Reactions were monitored by TLC. Where monoacetate was the hydrolysis product, its configuration and enantiomeric excess (e.e.) were determined from sign and value of optical rotation respectively. Most of the cultures having hydrolytic activity for **5** were pro-*S* selective affording ent-**6** in varying e.e.. Only few cultures belonging to *Trichosporon* species were having pro-*R* selectivity; however the optical purities obtained were poor (Table 3.9). Since we could not find any culture having high enantioselectivity for pro-*R* acetate, we decided to optimize the conversion using this poorly selective *Trichosporon* species of good hydrolytic activity with pro-*R* preference.

Table 3.9. Results of preliminary screening[a]

No.	Culture Name	NCIM No.	Yield of **6** (%)	$[\alpha]_D$ of **6**[b]	e.e. of **6**
1.	***Trichosporon beigelii***	**3326**	**88**	**-20.2**	**29.1**
2.	*Trichosporon sp.*	3369	75	-5.8	8.4
3.	*Trichosporon sp.*	3382	78	-5.5	7.9
4.	*Trichosporon beigeii*	3404	76	-15.6	22.5
5.	*Trichosporon captatum*	3412	78	-16.4	23.7

a: **5** (0.050g, 0.27mmol) was reacted with wet cell cultures in 0.1 M phosphate buffer (5ml) for 18 hr at 30°C on orbital shaker. *b*: rotations were measured in chloroform using 1% concentration. Lit.[21g] $[\alpha]_D$ = -69.3 (c =1, chloroform).

Enhancement of enzyme efficiency through medium engineering i.e. optimization of the solvent system has been well documented in several cases.[27] Addition of co-solvents, such as alcohols, acetone, DMF, DMSO etc. in small percentages are reported to have beneficial effect on hydrolytic reactions.[32] *Trichosporon beigelii* (NCIM 3326) was considered as the best candidate for selectivity enhancement studies as it showed the best results (88% yield, 29% e.e. entry 1, Table 3.9) in preliminary screening studies. Reactions were carried out by adding various water-miscible co-solvents in 10% v/v in buffer and the results are presented in Tables 3.10, 3.11 and 3.12.

The study indicates strong effect of ethanol on the selectivity of the whole cell culture NCIM 3326. Thus, by using 10% ethanol in solvent system, e.e. of product **6** shot up from 29.1 to 82.6% (entry 2, Table 3.10). But high concentrations of ethanol (>50%) were inhibitory for the reaction. Similar effect was observed with the cultures belonging to same species (Table 3.12). It was observed that the effect of 10% ethanol as co-solvent is even more pronounced in the case of culture NCIM

3404 (entry 4, Table 3.12, a boost from 22.5% e.e. to 85.9% e.e. of **6**). But considering the significant difference in yield, we decided to concentrate on NCIM 3326.

Table 3.10. Medium engineering effect on the hydrolysis of **5** catalyzed by NCIM 3326[a]

No	Co-solvent	Yield of **6** %	$[\alpha]_D$ of **6**	e.e. of **6** %
1.	Methanol	76	-39.5	57.0
2.	**Ethanol**	**83**	**-57.2**	**82.6**
3.	2-Propanol	79	-42.9	61.9
4.	1-Butanol	81	-14.4	20.8
5.	Acetone	84	-45.3	65.4
6.	Dimethylsulphoxide	77	-21.4	30.9
7.	Dimethylformamide	75	-34.9	50.4

a: All the reactions were carried out at 30°C on orbital shaker for 18 hr using 1% concentration of substrate **5** in 0.1 M phosphate buffer (pH 7) containing 10% v/v of co-solvent.

Table 3.11. Effect of variation of ethanol concentration on the hydrolysis of **5** catalyzed by NCIM 3326.

No.	Ethanol concentration %	Yield of **6** %	$[\alpha]_D$ of **6**	e.e. of **6** %
1.	4	81	-48.6	70.1
2.	**10**	**83**	**-57.2**	**82.6**
3.	20	79	-54.5	78.6
4.	50	No reaction	-	-
5.	80	No reaction	-	-

Table 3.12: Effect of 10% v/v ethanol in buffer media on the hydrolysis of **5** catalyzed by cultures from *Trichosporon* species

No.	Culture (NCIM No.)	Yield of **6** %	$[\alpha]_D$ of **6**	e.e. of **6** %
1.	3326	83	-57.2	82.6
2.	3369	78	-54.4	78.6
3.	3382	75	-45.3	65.4
4.	**3404**	**74**	**-59.5**	**85.9**
5.	3412	79	-53.5	77.3

Even though, enantiopurity of **6** can be enhanced from 85 to >98% by low temperature crystallization which is a tedious process; the yields became low (65%). C.J. Sih and coworkers have demonstrated the importance of inherent consecutive kinetic resolution step[20c] in enhancing the enantiomeric excess of chiral species obtained during enantioselective hydrolysis of *meso*-diesters (see section 2.5. page 21). In the case studied, desymmetrization produces a mixture of **6** and ent-**6**, enriched in **6**. This mixture would further undergo kinetic resolution catalyzed by the same enzyme system. Here enzyme having pro-*R* selectivity would preferentially catalyze hydrolysis of ent-**6** to corresponding diol; thereby enhancing the enantiomeric excess of remaining monoacetate **6**. This was demonstrated by carrying out the hydrolysis reaction for various time intervals and by checking the yield and enantiomeric excess of product **6** (Table 3.13). Thus, at 26 hr product **6** was obtained in >98% enantiomeric excess with 74% isolated yield (entry 4).

Table 3.13. Effect of reaction time on the hydrolysis of **5** catalyzed by NCIM 3326 in 0.1M (pH 7) buffer with 10% v/v ethanol

No.	Reaction time hr	Yield of **6** %	$[\alpha]_D$ of **6**	e.e. of **6** %
1.	15	85	-56.0	80.8
2.	18	83	-57.2	82.6
3.	20	80	-58.9	85.0
4.	**26**	**74**	**-69.0**	**>98.0**

Before scaling-up the process, it was required to optimize the substrate concentration tolerated by the enzyme. We found that substrate concentration of 3.33% w/v was well tolerated by enzyme whereas at concentrations above it enzyme inhibition was observed. Thus, the process was successfully scaled-up to 100 g scale using 3.33% substrate concentration and product **6** of greater than 98% enantiomeric excess was isolated in 74% yield. Remaining 26% is *meso*-diol **4** which can be recovered and recycled by acetylating to afford the starting material **5**. Thus with one recovery yields can be boosted to 90% from 74%. Compound **6** was further converted to 4-(*R*)-*tert*-butyldimethylsilyloxycyclopent-2-en-1-one (**3**) by known chemical transformations[3b, 8] and its e.e. was determined to be >99% by chiral HPLC method

3.3.2.3. Conclusion

*Thus, our study has revealed Trichosporon beigelii (NCIM 3326),[34] as new very effective biocatalyst which is a significantly cheaper alternative to PLE for hydrolysis of meso-diacetate **5** to the expensive intermediate **6** in excellent enantiomeric excess and has led to the discovery of a new process for **6**. Another strength of this process is beneficiating the side product diol **4** by conversion to meso-diacetate **5** the starting material by acetylation (see scheme 3.21). This can*

make the yield based on recovery nearly 100%! The study also demonstrated the importance of medium-engineering approach for optimization of the enzymatic process for an important chiral drug intermediate which could be scaled up.

3.3.2.4. Experimental

☞ *General*

All the reagents were purchased from *Aldrich* and were used without further purification. NMR spectra were recorded on *Brucker NMR* (200 MHz) spectrometer. IR spectra were recorded on *Research Series FTIR* spectrometer. Optical rotations were recorded on a *Jasco Dip-181* and *Jasco P-1020* polarimeter using sodium vapor lamp. Enantiomeric excess (e.e.) were determined by comparing the specific rotation value $[\alpha]_D$ with the literature value.

☞ *General procedure for cell biomass preparation (Whole Cell Enzyme)*

The inoculum (5-10 ml) was developed by growing the microorganisms in a medium containing malt extract (0.3%), glucose (1%), yeast extract (0.3%) and peptone (0.5%) at pH 6.6-7.0 for 48 hours with shaking at 150-180 rpm. This inoculum was transferred to medium containing $K_2H_2PO_4$ (0.2%), yeast extract (0.1%), peptone (0.5%). KCl (0.05%), $NaNO_3$ (0.05%), $MgSO_4.7H_2O$ (0.05%), olive oil (1%) at pH 5.5 and incubated at 28-30^oC for 36-48 hours on rotary shaker (180-200 rpm). The grown cells were separated by centrifugation and the wet biomass was used for the reaction.

☞ *General procedure for enantioselective hydrolysis of meso-Cyclopent-2-en-1,4-diacetate (**5**) to 4-(R)-Hydroxycyclopent-2-en-1-(S)-acetate (**6**)*

Meso-diacetate **5** (100 g, 0.543 mole) was dissolved in ethanol (300ml) in 5 lit. three necked round bottom flask equipped with overhead stirrer, pH electrode and a dropping funnel. To the solution 0.1 M sodium phosphate buffer (pH 7, 2.7 lit.) was added and stirred vigorously using overhead stirrer. To the stirred reaction mixture wet biomass (50 g) of *Trichosporon beigelii (*NCIM 3326) was added with small amount of buffer. The whole mixture was stirred vigorously to yield a uniform emulsion. pH of reaction was monitored regularly and was maintained at 7 by adding 1 M aqueous sodium hydroxide solution through dropping funnel. Progress of the reaction was monitored by TLC (30% ethyl acetate in pet.ether). Reaction was continued for 26 hours. Then reaction mixture was filtered through celite bed. Filtrate was extracted with ethyl acetate (3 x 2 lit). Organic extracts were combined and washed with brine. Aqueous layer was separated and organic layer was dried on anhydrous sodium sulphate. Solvent was removed on rotary

evaporator and dried at high vacuum. Product **6** was obtained as white crystalline needles. It was melted on water bath and was stirred vigorously with pet. ether. Mixture was allowed to stand at room temperature for few hours. Compound **6** separated as fine crystalline needles. Pet. ether was decanted off and washing with pet. ether was repeated once again. These washings remove any unreacted diacetate **5** and other non-polar impurities. Yield of **6** was 57.40 g. (74%). $[\alpha]_D$ = -68.9 (c = 1, chloroform) Lit.[21g] $[\alpha]_D$ = -69.3 (c =1, chloroform) enantiomeric excess >98%.

☞ Compound **6** of >98% e.e. was processed by known chemical transformations[3b, 8] to 4-(*R*)-*tert*-butyldimethylsilyloxycyclopent-2-en-1-one (**3**) of >99 e.e.(as determined by HPLC analysis on chiral column OD 1, see page 84).

^{1}HNMR ($CDCl_3$): δ 0.12, 0.13 (2s, 6H), 0.91 (s, 9H), 2.24 (dd, 1H). 2.71 (dd, 1H), 4.99 (m, 1H), 6.18 (m, 1H), 7.46 (m, 1H)

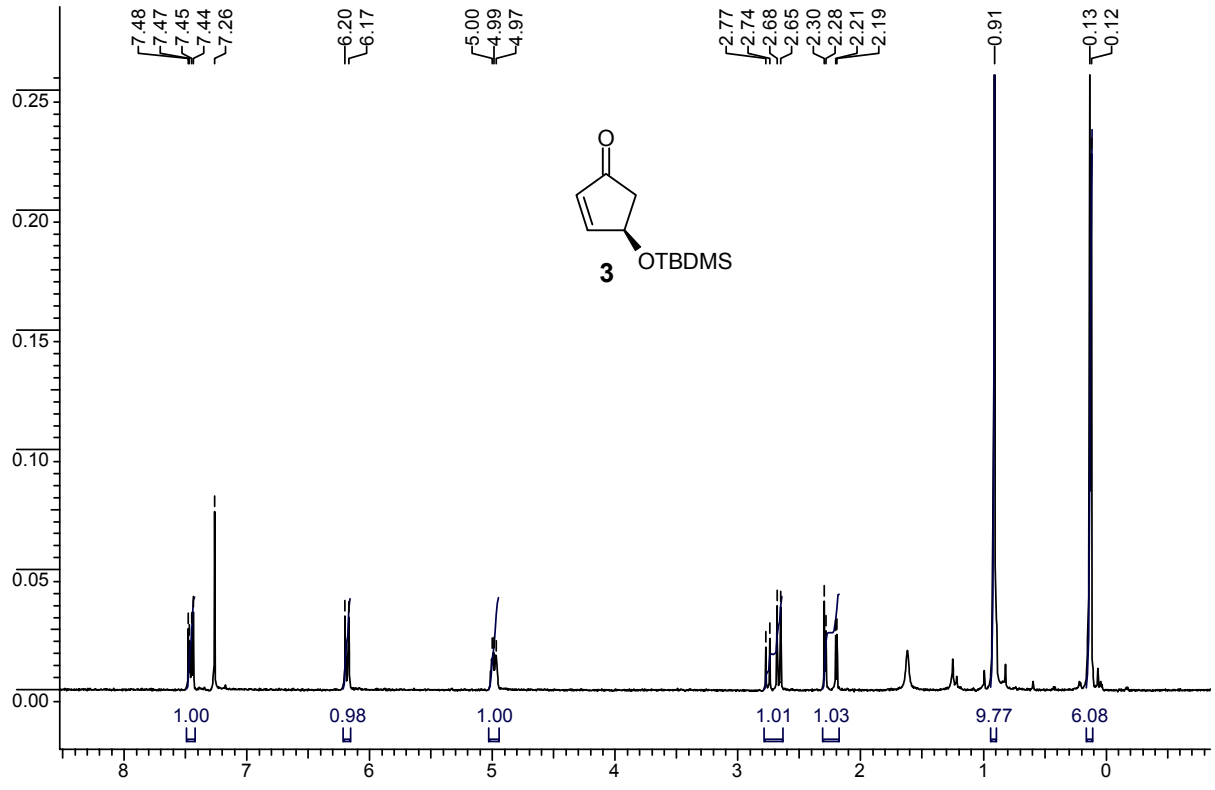
^{13}C NMR ($CDCl_3$): δ 4.72, 18.05, 25.74, 44.94, 70.88, 134.39, 163.63, 206.11

IR ($CHCl_3$): cm^{-1} 668.85, 758.78, 813.40, 837.57, 898.88, 1073.50, 1110.44, 1187.49, 1215.99, 1258.20, 1355.35, 1720.05, 2858.92, 2886.22, 2931.50, 2955.70, 3019.45

Specific rotation: $[\alpha]_D$ = +66.1 (c = 1, methanol) Lit.[17] $[\alpha]_D$ = +66.3 (c = 1, methanol)

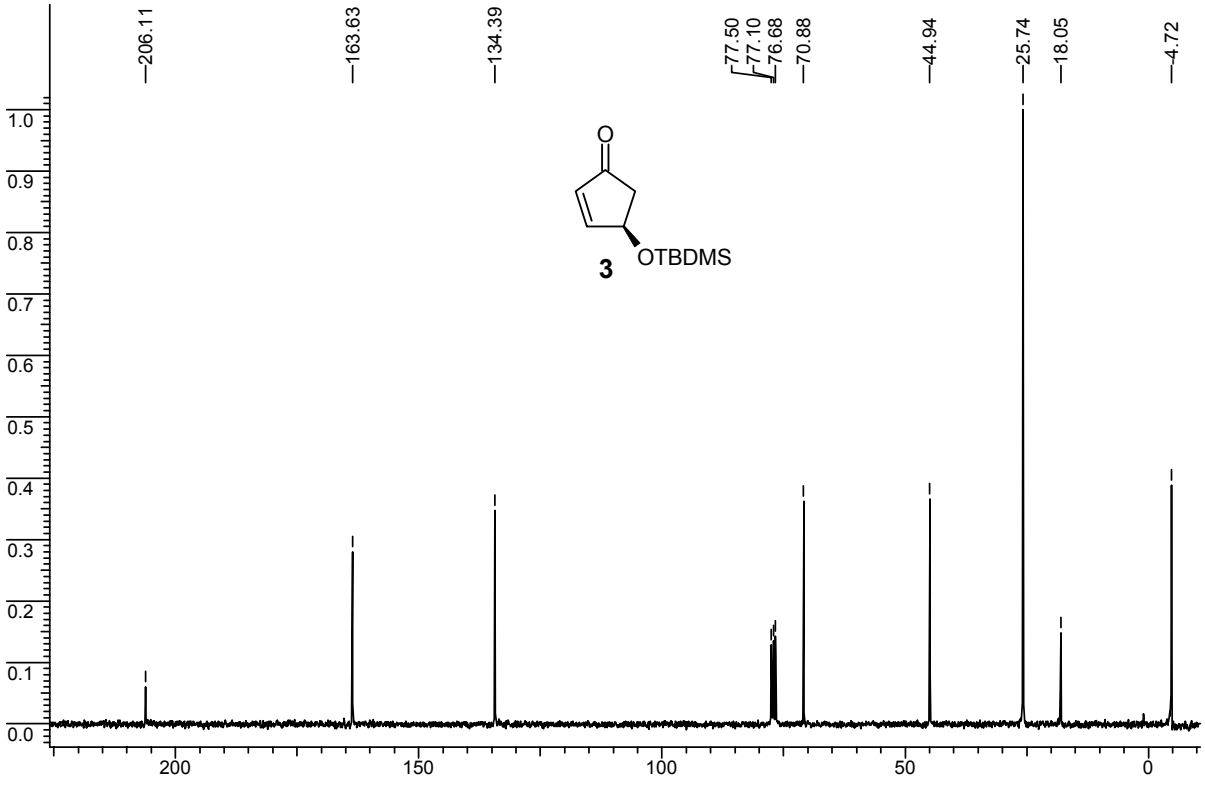
3.3.2.5. Spectra

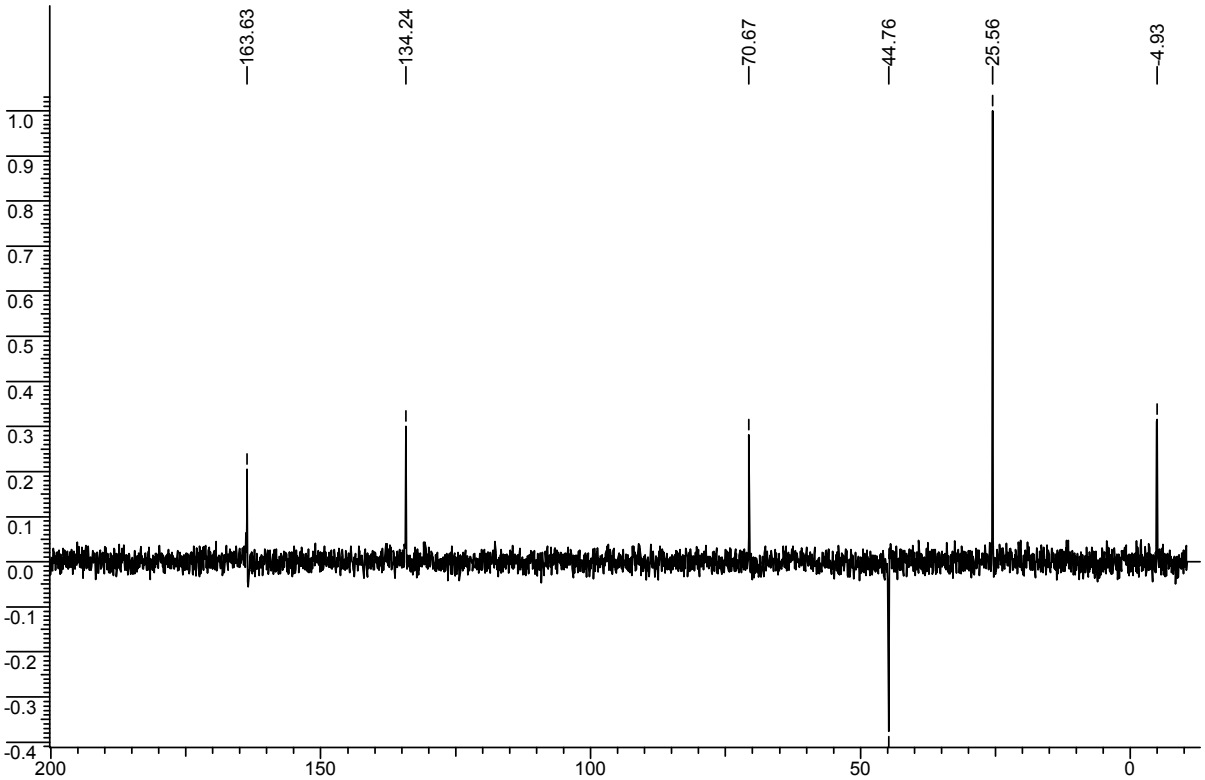
☞ *^{1}H NMR spectrum of compound **3***



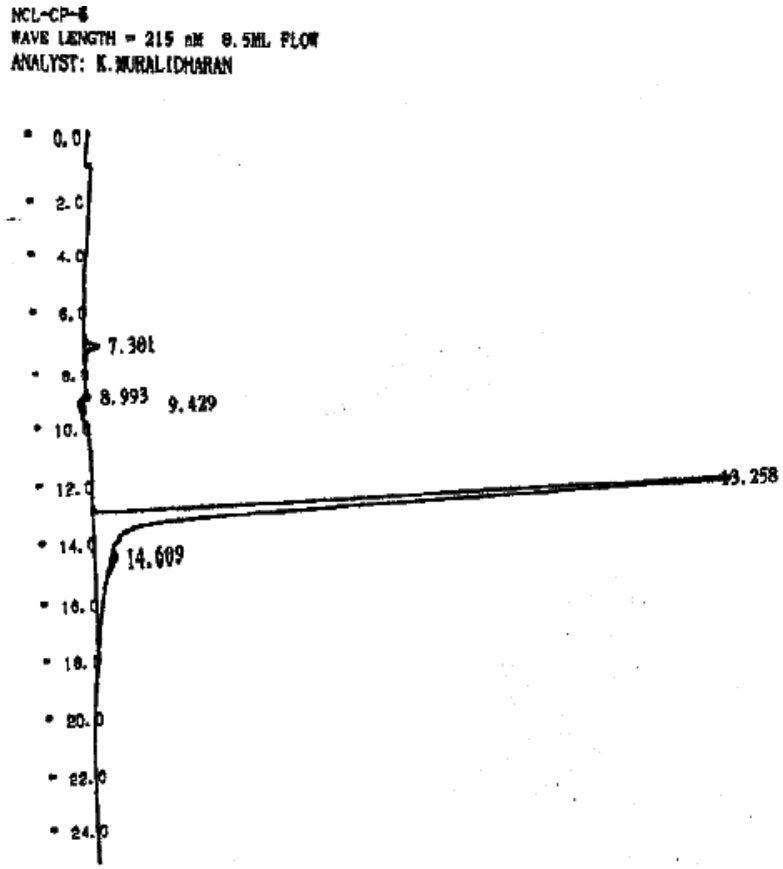
☞ *IR spectrum of compound **3***

☞ *^{13}C NMR spectra of compound **3***





☞ *Chiral HPLC chart for compound **3***



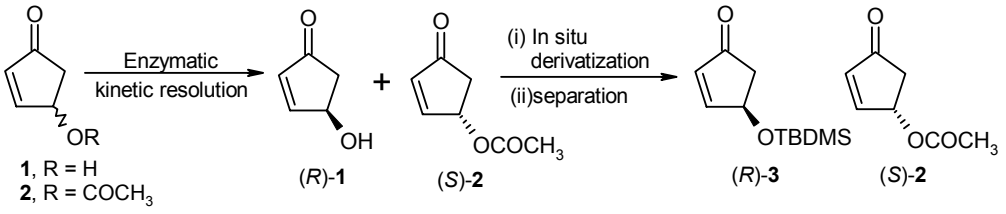
** CALCULATION REPORT **

CH	PKNO	TIME	AREA	HEIGHT	MK	IDNO	CONC	NAME
1	2	7.301	8191	714	V		1.2134	
	3	8.993	3681	287			0.5452	
	4	9.429	3338	198			0.4944	
	5	13.258	655830	33573	S		97.1512	
	6	14.609	4022	269	T		0.5957	
		TOTAL	675061	35040			100	

3.3.3. Enzymatic Kinetic Resolution Studies of Racemic 4-Hydroxycyclopent-2-en-1-one

3.3.3.1. Introduction

As discussed earlier in section 3.2 (page 64), direct enzymatic kinetic resolution of racemic 4-hydroxycyclopentenone (±)-**1** would be a more economic and easier approach for the large-scale preparation of (*R*)-**1** and (*R*)-**3**. We have already scaled-up the method for preparation of (±)-**1** up to 3 Kg scale. Thus, successful enzymatic kinetic resolution of it using some commercially available, cheap enzyme would provide a good opportunity for development of cost-effective large-scale process for the preparation of **1** and **3**, as an efficient alternative to enzymatic desymmetrization method. Therefore we decided to study kinetic resolution of (±)-**1** using few commercially available enzymes and various cultures from NCIM (Scheme 3.22). Main emphasis was given on development of kinetic resolution process in organic media for the various reasons discussed earlier. The study is divided in three parts (i) Enzymatic hydrolysis of (±)-4-oxocyclopenten-2-yl acetate (±)-**2**. (ii) Transesterification of (±)-4-hydroxycyclopentenone (±)-**1** with vinyl acetate catalyzed by Lipozyme IM®. (iii) Alcoholysis of (±)-4-oxocyclopenten-2-yl acetate (±)-**2**.



Scheme 3.22.

3.3.3.2. Enzymatic Hydrolysis of (±)-4-Oxocyclopenten-2-yl acetate (±)-2.

Various enzymes viz. Porcine Pancreatic Lipase (PPL), Pig Liver Acetone Powder (PLAP), Chicken Liver Acetone Powder (CLAP), Lipozyme® and NCIM cultures were screened for the enantioselective hydrolysis of racemic **2** (Table 3.14). Reactions were monitored by TLC. At sufficient conversion, aqueous layer was subjected to thorough extraction with ethyl acetate. Since **1** is rather sensitive compound, it is advantageous to convert it to silyl derivative **3**, which is more stable and easy to handle. Hence, residue was subjected for -OTBDMS derivatization, thus converting **1** to **3** without affecting acetate **2**. Both **2** and **3** were separated on column and their e.e. was determined by comparison of optical rotation with literature reported value. Enantioselectivity of the reaction i.e. Enantiomeric ratio E was determined using equation 1[35] (see page 23).

Table 3.14. Screening of various enzymes and NCIM cultures for hydrolysis of (±)-(**2**)[a]

No	Enzyme/ Culture	Time	Conversion % C	$[\alpha]_D$ of **3**[b]	e.e. of **3** ($e.e._p$)	$[\alpha]_D$ of **2**[d]	e.e. of **2** ($e.e._s$)	E
1.	NCIM K-310	24 hr	50.0	0	-	0	-	0
2.	PPL	4 days	-	-	-	0	-	-
3.	NCIM 880	4 days	37.1	-10.56	18.52	+10.95	10.90	1.60
4.	NCIM 881	4 days	20.3	-1.36	2.38	+1.69	1.60	1.15
5.	NCIM 3467	3 days	47.4	-11.63	20.40	+18.37	18.37	1.78
6.	NCIM 3462	3 days	55.0	-6.16	10.80	+13.17	13.00	1.39
7.	NCIM 2445	24 hrs	>80.0	0.00	0.00	-1.00	1.00	0.00
8.	Lipozyme®	22 hr	37.6	-16.12	28.28	+17.21	17.00	2.11
9.	PLAP[c]	10 hr	44.4	-17.16	30.10	+24.04	24.00	2.32
10.	CLAP[c]	10 hr	35.2	+14.7	25.80	-14.20	14.00	1.93

a :1.5 mmol of (±)-**2** was reacted with 0.25g enzyme in 10 ml 0.1 M phosphate buffer pH 7 at 30°C
b: Lit[22a] $[\alpha]_D$= +57 (c = 1, chloroform) e.e. >99% c: 0.5g of PLAP & CLAP were used for the reaction
d: Lit.[26] $[\alpha]_D$ = +100 (c = 2.9, methanol)

Table 3.15. Effect of temperature variation on the hydrolysis of (±)-(**2**) catalyzed by CLAP, PLAP and Lipozyme®[a]

No	Enzyme	Temp °C	Time hr	Conversion C %	$[\alpha]_D$ of **3**[a,b]	e.e. of **3** ($e.e._p$)	$[\alpha]_D$ of **2**[d]	e.e. of **2** ($e.e._s$)	E
1.	CLAP	30	10	35.2	+14.70	25.80	-14.20	14.20	1.93
2.	**CLAP**	**12**	**24**	**41.8**	**+30.70**	**53.86**	**-38.70**	**38.70**	**4.80**
3.	CLAP	6	48	34.5	+24.50	43.00	+23.60	23.60	3.28
4.	PLAP	30	10	44.4	-17.16	30.10	+24.00	24.00	2.32
5.	PLAP	12	24	36.4	-32.00	56.00	+32.00	32.00	4.79
6.	PLAP	6	48	37.8	-30.00	52.60	+32.00	32.00	4.36
7.	Lipozyme®[c]	30	22	37.6	-16.12	28.28	+17.21	17.20	2.11
8.	Lipozyme®[c]	12	40	31.3	-30.25	53.00	+24.25	24.20	4.10
9.	Lipozyme®[c]	6	72	22.6	-34.00	59.60	+17.40	17.40	4.67

a : 1.5 mmol (±)-**2** was reacted with 0.5g enzyme in 10 ml of 0.1 M phosphate buffer pH 7
b: Lit[22a] $[\alpha]_D$= +57 (c = 1, chloroform) e.e. >99% c: 0.25g of Lipozyme® were used.
d : Lit.[26] $[\alpha]_D$ = + 100 (c = 2.9, methanol)

As discussed in earlier sections, temperature has significant effect on enzyme selectivity. In order to examine the temperature effect we carried out the reactions at 6 and 12°C using CLAP, PLAP and Lipozyme® (Table 3.15) and observed that at lower temperatures enantioselectivity is increased by factor of 2. For practical convenience further reactions were carried out at 12°C. Since CLAP gives desired *R*-isomer (entry 2), it was used for further parameter optimization studies. To improve the enantioselectivity of reaction, we decided to carry out medium engineering studies. We attempted several water-miscible as well water immiscible cosolvents for CLAP catalyzed hydrolysis of (±)-**2** in phosphate buffer (pH 7) at 12°C (Table 3.16). Among all the cosolvents attempted, methyl isobutyl ketone (MIBK) (entry 10 &11, Table 3.16) was found to have some beneficial effect on enzyme enantioselectivity. E value improved up to 7.53 using MIBK as cosolvent at 20% concentration (entry 10). In case of dichloromethane and dichloroethane, E value improved up to 6.83 and 6.03 respectively (entry 14, 16, Table 3.16).

Table 3.16. Medium engineering studied on hydrolysis of (±)-(**2**) catalyzed by CLAP at 12°C[a]

No	Cosolvent	% of cosolvent	Time hr	C %	$[\alpha]_D$ of **3**[b]	e.e. of **3** (e.e.$_p$)	$[\alpha]_D$ of **2**[c]	e.e. of **2** (e.e.$_s$)	E
1.	Methanol	20	23	38.2	+30.70	53.86	-33.23	33.0	4.55
2.	2-propanol	20	47	9.5	+5.40	9.50	-1.00	1.0	1.22
3.	1-butanol	20	30	-	+2.20	3.85	0.00	0.0	-
4.	Isobutanol	20	30	-	0.00	0.00	0.00	0.0	-
5.	1-pentanol	20	30	29.4	+13.70	24.00	+10.00	10.0	1.79
6.	Acetone	20	24	19.0	+34.00	59.60	-14.34	14.0	4.54
7.	Acetonitrile	20	24	43.1	+11.30	19.80	-15.00	15.0	1.71
8.	DMSO	20	24	27.5	+30.00	52.60	-20.00	20.0	3.90
9.	DIPE	20	30	46.1	+25.30	44.38	-38.20	38.0	3.70
10.	**MIBK**	**20**	30	**38.6**	**+38.00**	**66.67**	**-42.30**	**42.0**	**7.53**
11.	**MIBK**	**10**	**30**	**44.5**	**+36.10**	**63.30**	**-50.80**	**50.8**	**7.29**
12.	Diethyl ether	20	30	33.1	+35.00	61.40	-30.40	30.4	5.60
13.	Chloroform	10	24	28.8	+35.22	61.80	-25.10	25.0	5.38
14.	Dichloromethane	10	24	23.0	+39.67	69.60	-20.80	20.8	6.83
15.	Carbon tetrachloride	10	24	40.8	+20.29	35.60	-24.50	24.5	2.64
16.	Dichloroethane	10	24	36.1	+35.57	62.40	-35.21	35.2	6.03

a: 1.5 mmol of (±)-**2** was reacted with 0.5g enzyme in 10 ml solvent (buffer + co-solvent). C = conversion
b: Lit[22a] $[\alpha]_D$= +57 (c = 1, chloroform) e.e. >99% c: Lit.[26] $[\alpha]_D$ = +100 (c = 2.9, methanol)

For the practical purposes expected E value should be at least 20 or higher which indicates moderate enantioselectivity. In our studies of kinetic resolution of (±)-**1**, through enzyme catalyzed hydrolysis of its acetate **2** under aqueous conditions, E value achieved was not satisfactory. As discussed earlier in section 3.2, the problems associated with hydrolysis of **2** viz. high water-solubility of **1** and possibility of racemization can be circumvented by the judicious use of organic solvents for the conversion. Therefore we focused our attention towards Lipozyme IM® (immobilized Lipozyme®) catalyzed transesterification of (±)-**2** in organic solvents.

3.3.3.3. Transesterification of (±)-1 with vinyl acetate catalyzed by Lipozyme IM®

Transesterification of (±)-**1** was attempted using vinyl acetate as acyl donor and Lipozyme IM® as a catalyst (Table 3.17). Since, the enantioselectivity of the enzyme is higher at lower temperatures, reactions were carried out at 12°C. The initial experiment in dry diisopropyl ether (DIPE) exhibited high reaction rates (80% conversion in 4 hr) but the enantioselectivity of the reaction was rather poor. (E = 4, entry no.1, Table 3.17).

Table 3.17. Transesterification of (±)-**1** with vinyl acetate catalyzed by Lipozyme IM® in various solvents at 12°C[a]

No	Acyl donor	Solvent	Water content %	Time hr	C %	$[\alpha]_D$ of **3**[b]	e.e. of **3** ($e.e._s$)	$[\alpha]_D$ of **2**[c]	e.e. of **2** ($e.e._p$)	E
1.	Vinyl acetate	DIPE	-	4	80.0	+59.4	89.2	-22.2	22.2	4
2.	**“**	**DIPE**	**1**	**19**	**28.0**	**+20.5**	**30.8**	**-78.2**	**78.2**	**11**
3.	“	TBME	1	11	46.5	+33.0	49.5	-56.9	56.9	6
4.	“	Dibutyl ether	1	11	17.0	+0.9	1.3	-75.0	75.0	7
5.	“	Toluene	1	16	22.9	+11.2	16.9	-56.8	56.8	4
6.	Isopropenyl acetate	DIPE	-	7	39.2	+30.0	45.0	-29.0	29.0	3
7.	“	DIPE	1	7	only	traces	of	product	-	-
8.	“	-	-	7	“	“	“	“	-	-
9.	Ethyl acetate	-	-	7	No	reaction	-	-	-	-
10.	Isopropyl acetate	-	-	7	No	reaction	-	-	-	-
11.	Trichloro-ethyl acetate	DIPE	-	7	No	reaction	-	-	-	-

a: 2 mmol of (±)-**1** was reacted with 5 equi. of acyl donor in 10 ml solvent. C = conversion b: rotations in methanol Lit.[17] $[\alpha]_D$ = + 66.3 (c = 1, methanol) e.e.>99% d : Lit.[26] $[\alpha]_D$ = +100 (c = 2.9, methanol)

Since the intrinsic water content (more precisely, the water activity, a_w) as low as 1% is reported to be beneficial for enzyme selectivity in several cases,[27, 33] the conversion was attempted in diisopropyl ether containing 1% water. Now the reaction rate was much slower (28% conversion in 19 hr, entry no.2, Table 3.17), the enantioselectivity improved significantly (E = 11) by 2.8 times. Such kind of beneficial effect of water content is reported previously in few cases, the reason though not very clear, is attributed to increase in enzyme flexibility by added water in dry system. The reaction was attempted in few other solvents containing 1% water (entry no. 3-5 Table 3.17), but E value more than 11 could not be achieved.

The effect of an acyl donor on enantioselectivity of lipase-catalyzed transesterification reaction has been well demonstrated by Ema et al.[30] Therefore we tested few other acyl donors for the conversion. With isopropenyl acetate the enantioselctivity as well as rate of reaction were poor (39.2% conversion in 1 week, E = 3, entry 6, Table 3.17). Addition of 1% water did not prove to be beneficial, rather inhibited reaction. Reaction did not proceed with acyl donors like isopropyl acetate, ethyl acetate etc (Table 3.17).

Thus the highest value obtained of enantiomeric ratio was 11 in kinetic resolution of (±)-**1** with vinyl acetate using Lipozyme IM® as catalyst which is not good enough for practical purposes. Therefore the resolution of (±)-**2** was planned by alcoholysis in organic solvents.

3.3.3.4. Alcoholysis of (±)-4-Oxocyclopenten-2-yl acetate (2)

Alcoholysis of (±)-**2** was attempted initially in toluene using various alcohols catalyzed by Lipozyme IM® (Table 3.18). Reaction rates of alcoholysis reactions were good enough, but enantioselectivity was not satisfactory (E = 3 to 5). Among all alcohols, 2-propanol which is a moderately hindered secondary alcohol reacted much slowly (C = 29.3% in 45 hr, entry 5, Table 3.18) in toluene, but when DIPE and methyl isobutyl ketone (MIBK) were used as solvents, reaction rates improved considerably (C = 31.5% in 15 hr & C = 42.5% in 15 hr respectively entry 6, 7 Table 3.18) with some improvement in enantioselectivity (E = 6 & 4 respectively).

We observed that when 2-propanol containing some water was used for alcoholysis, rate of reaction improved considerably with some improvement in E ratio as compared to the anhydrous reaction. Subsequently effect of water content was studied in DIPE using 2-propanol for alcoholysis (Table 3.19). The water content of 1% was found to enhance reaction rates as well as enantioselectivity considerably. Thus 37.3% conversion was achieved with E value of 17 (entry 3, Table 3.19) i.e. 2-fold increase in enantioselectivity at 1% water content. To further study water effect, several reactions were conducted using different alcohols in different solvents containing 1% water (Table 3.20). For 2-propanol reaction (entry 5, 7, Table 3.20), enantioselectivity was

maximum in DIPE (E = 17) and dibutyl ether (DBE) (E = 19). Beneficial effect of water was observed with most other alcohols.

Table 3.18. Preliminary screening of various alcohols in different solvents at 12°C for alcoholysis of (±)-**2** catalyzed Lipozyme IM®[a]

No	Alcohol	Solvent	Time hr	C %	$[\alpha]_D$ of **3**[b]	e.e. of **3** ($e.e._p$)	$[\alpha]_D$ of **2**[c]	e.e. of **2** ($e.e._s$)	E
1.	1-Butanol	Toluene	10	51.2	-25.0	37.7	+39.6	39.6	3
2.	Isobutyl alcohol	Toluene	17	45.0	-32.4	48.8	+40.0	40.0	4
3.	*t*-Butyl alcohol	Toluene	14	42.6	-31.1	54.6	+40.5	40.5	5
4.	Methanol	Toluene	19	20.5	-30.3	45.7	+11.8	11.8	3
5.	2-propanol	Toluene	45	29.3	-30.8	46.3	+19.2	19.2	3
6.	2-propanol	DIPE	15	31.5	-35.5	53.5	+22.5	22.5	6
7.	2-propanol	MIBK	15	42.5	-32.6	49.0	+36.2	36.2	4
8.	Isoamyl alcohol	Toluene	16	50.8	-25.0	37.7	+39.0	39.0	3
9.	Benzyl alcohol	Toluene	6	46.0	-35.0	52.8	+45.0	45.0	5
10.	Cyclohexanol	Toluene	6	35.2	-25.0	37.7	+20.5	20.5	3

a: 2 mmol of (±)-**2** was reacted with 5 equi. of alcohol in 10 ml solvent. C = conversion
b: rotations in methanol Lit.[17] $[\alpha]_D$ = + 66.3 (c = 1, methanol) e.e.>99%
c: Lit.[26] $[\alpha]_D$ = +100 (c = 2.9, methanol)

Table 3.19. Effect of water content on the alcoholysis of (±)-**2** by 2-propanol catalyzed by Lipozyme IM® in diisopropyl ether at 12°C[a]

No	Water content (%)	Time (hr)	C (%)	$[\alpha]_D$ of **3**[b]	e.e. of **3** ($e.e._p$)	$[\alpha]_D$ of **2**[c]	e.e. of **2** ($e.e._s$)	E
1.	-	12	25.0	-34.1	51.3	+17.3	17.3	4
2.	0.80	12	41.4	-46.1	69.2	+48.9	48.9	9
3.	**1**	**12**	**37.3**	**-55.1**	**82.7**	**+49.5**	**49.5**	**17**
4.	1.25	12	39.4	-43.9	65.9	+42.9	42.8	7
5.	1.43	12	39.4	-47.2	70.9	+46.1	46.1	9

a: 2 mmol of (±)-**2** was reacted with 5 equi. of alcohol in 10 ml solvent. C = conversion
b: rotations in methanol Lit.[17] $[\alpha]_D$ = + 66.3 (c = 1, methanol) e.e.>99%
c: Lit.[26] $[\alpha]_D$ = +100 (c = 2.9, methanol)

Enantioselectivity is found to be associated with alcohol structure. Thus, E value of >10 was observed with all secondary alcohols, 2-butanol (entry 9, Table 3.20) showing the best results (E = 24.28). Such kind of influence of alcohol structure on the enatioselectivity of enzyme during alcoholysis is somewhat analogous to the effect of structure of acyl donor on enzyme enantioselectivity in transesterification reaction studied by Ema et al.[30] Thus moderate steric hindrance provided by 2-butanol is most suitable for enzyme active site during reaction; giving highest enantiodiscrimination. Further decrease or increase in the hindrance by addition or deletion of $-CH_3$ groups deteriorates enantiodiscrimination in the case studied. With E value of approximately 25, substrate of >95% e.e. can be obtained at 60% conversion whereas product of 85% e.e. can be obtained near 40% conversion (*vide infra* eqn.1 & reference 36)

Table 3.20. Alcoholysis of (±)-**2** with various alcohols in different organic solvent containing 1% water at 12°C[a]

No.	Alcohol	Solvent	Time (hr)	C (%)	$[\alpha]_D$ of **3**[b]	e.e. of **3** ($e.e._p$)	$[\alpha]_D$ of **2**[c]	e.e. of **2** ($e.e._s$)	E
1.	Isobutyl alcohol	DIPE	12	49.2	-44.3	66.7	+64.5	64.5	9
2.	t-Butyl alcohol	DIPE	12	45.1	-45.6	68.6	+56.4	56.4	9
3.	Methanol	Toluene	24	very	slow	reaction	-	-	-
4.	2-Propanol	Toluene	24	33.0	-42.1	63.2	+31.3	31.3	6
5.	2-Propanol	DIPE	12	37.0	-55.1	82.7	+49.5	49.3	17
6.	2-Propanol	TBME	16	35.0	46.2	69.4	+37.4	37.0	10
7.	2-Propanol	DBE	12	39.8	-55.5	83.3	+55.0	55.0	19
8.	2-Propanol	Ether	18	18.6	-51.0	76.6	+17.5	17.5	9
9.	**2-Butanol**	**DIPE**	**12**	**43.3**	**-56.8**	**85.2**	**+65.0**	**65.0**	**24**
10.	4-Methyl-2-pentanol	DIPE	12	46.5	-50.6	76.0	+66.1	66.0	14
11.	2-Pentanol	DIPE	12	43.0	-50.4	75.7	+5.7	57.0	13
12.	3-Pentanol	DIPE	18	47.6	51.8	77.8	+70.6	70.0	17

a: 2 mmol of (±)-**2** was reacted with 5 equi. of alcohol in 10 ml solvent containing 1% water
C = conversion b: rotations in methanol Lit.[17] $[\alpha]_D$ = + 66.3 (c = 1, v) e.e.>99%
c: Lit.[26] $[\alpha]_D$ = +100 (c = 2.9, methanol)

3.3.3.5. Conclusion:

*Thus we could resolve (±)-**1** in moderate enantioselectivity (E = 24) by alcoholysis of its acetate (±)-**2** in organic solvents using commercially available enzyme Lipozyme IM®. Our study demonstrates strong effects of solvent, alcohol structure and water content on the enzymatic alcoholysis reaction in organic media. Even though the enantioselectivity obtained is not very high, study has set the guidelines for further optimization work. We hope that this methodology would provide a more economic alternative to the desymmetrization method for the large-scale production of **1** and **3**.*

3.3.3.6. Experimental

☞ *General*

All the reagents were purchased from *Aldrich* and were used without further purification. NMR spectra were recorded on *Brucker NMR* (200 MHz) spectrometer. IR spectra were recorded on *Research Series FTIR* spectrometer. Optical rotations were recorded on a *Jasco Dip-181* and *Jasco P-1020* polarimeter using sodium vapor lamp. Enantiomeric excess (e.e.) were determined by comparing the specific rotation value $[\alpha]_D$ with the literature value. Lipozyme IM® was obtained as gift sample from Arun & Co., Mumbai. PLAP and CLAP were made by literature reported method.[35] Enantiomeric ratio E was calculated using the following formula (equ. 1),[36] where c, $e.e._s$, $e.e._p$ are conversion, enantiomeric excess of substrate and enantiomeric excess of product respectively.

$$E = \frac{(1-c)\,(1-\text{e.e.}_s)}{(1-c)\,(1+\text{e.e.}_s)} \quad \text{(equ.1)} \qquad \text{conversion, } c = \frac{\text{e.e.}_s}{\text{e.e.}_s + \text{e.e.}_p}$$

☞ *Typical laboratory scale procedure for preparation of (±)-4-Hydroxycyclopent-2-en-1-one (**1**)*

A 3 lit. three-necked round bottom flask equipped with long air condenser, thermometer pocket and a bubbler was charged with furfuryl alcohol (25g, 0.255 mole), potassium dihydrogen orthophosphate (6.3g, 0.022 mole) and distilled water (1.5 lit.). The reaction mixture was purged with slow stream of nitrogen along with magnetic stirring. It was heated to 95°C for 48 hrs while maintaining effective stirring and purge with nitrogen. The solution develops brownish insoluble impurities during reaction. It was cooled to room temperature and the washed twice with ethyl acetate. Aqueous layer was concentrated almost to dryness under reduced presser. The residue was then thoroughly extracted with ethyl acetate. The combined organic extracts are then dried

on anhydrous sodium sulphate and concentrated under vacuum. Residue was distilled under high vacuum using fractionating column. Product **1** was obtained as lemon yellow colored liquid distilling at 95 -100^{o}C at 0.5 mm vacuum. Yield = 10g (40%). **1** was stored in refrigerator (*ca* -5^{o}C).

1H NMR: δ 2. (dd, 1H), 2.8 (dd, 1H), 3.2-3.6 (bs,1H), 5.0 (m,1H), 6.2 (dd, 1H), 7.6 (dd, 1H).

☞ *Preparation of (±)-4-oxocyclopenten-2-yl acetate **(2)***

In a 100 ml two necked round bottom flask equipped with two-way stopcock and a dropping funnel (±)-**1** (5g, 51 mmol) was placed. Assembly was evacuated and flushed with argon. Dry dichloromethane (50 ml) was added and the solution was cooled below 0^{o}C using ice-salt bath along with magnetic stirring. To the cold solution dry pyridine (7.9g, 0.1 mole) was added and stirred further at 0^{o}C for 10 minutes. To the stirred solution, acetic anhydride (7.8g, 76.5 mmol) was added drop-wise while maintaining the temperature below 0^{o}C. Reaction mixture was then stirred at room temperature for 15 hrs. Reaction was quenched by adding cold, dilute hydrochloric acid. Organic layer was washed four times with 1:4 mixture of brine and dilute hydrochloric acid followed by wash with cold water, 10% sodium bicarbonate solution and finally with brine. Organic layer was dried on anhydrous sodium sulphate and was concentrated under vacuum. Residue was distilled under high vacuum. Product **2** was obtained as colorless liquid (b.p. 59-61^{o}C at 0.2 mm vacuum), which solidifies as colorless crystals on cooling. Yield = 5.71g (80%).

1H NMR: δ 2.0 (s, 3H), 2.3 (dd, 1H), 2.8 (dd, 1H), 5.8 (m, 1H), 6.3 (dd, 1H), 7.55 (dd, 1H).

☞ *General procedure for Enzymatic hydrolysis of (±)**-2***

Compound (±)-**2** (1.5 mmol, 0.2g) was stirred vigorously with 0.25 g enzyme in 10 ml of 0.1 M phosphate buffer (pH 7). Reaction was monitored by TLC (40% ethyl acetate in petroleum ether) by withdrawing samples at time intervals. When sufficient conversion was observed, reaction was quenched by adding excess sodium chloride. Filtered through celite bed. Filtrate was extracted several times with ethyl acetate. Organic layers were combined, dried over anhydrous sodium sulphate, filtered and solvent was removed under vacuum. The residue was treated with *tert*-butyldimethylsilyl chloride (1.5 mmol, 0.225g), p-dimethylaminopyridine (0.15 mmol, 0.018g) and triethylamine (2 mmol, 0.2 g, 0.28ml) under argon atmosphere in dry dichloromethane at 0^{o}C. Reaction mixture was further stirred at RT for 3 hrs. It was then washed with cold dil. hydrochloric acid followed by wash with water, 10% bicarbonate and finally with brine. Organic layer was dried on sodium sulphate and solvent was removed under vacuum. This derivatised residue contained OTBDMS derivative **3** and acetate **2**. Both were separated on silica gel column using pet. ether and ethyl acetate (gradient elution). The e.e. of both the compounds were determined by

comparing value of specific rotation with literature reported value and Enatiomeric ratio was calculated using equation 1.

☞ *General procedure for Enzymatic transesterification of (±)-**1***

Compound (±)-**1** (1.5 mmol, 0.15g) was stirred with 5 equivalent acyl donor and Lipozyme IM® (0.3g) in 10ml organic solvent at 12^{o}C. The reaction was monitored by TLC. At sufficient conversion reaction mixture was filtered, dried on anhydrous sodium sulphate and solvent was removed under vacuum. Residue was treated as described in hydrolysis experiment and E value was calculated.

☞ *General Procedure for Enzymatic Alcoholysis of (±)-**2***

(±)-**2** (1.5 mmol, 0.2 g) was stirred with 5 eqiv. alcohol and Lipozyme IM® (0.3 g) in 10 ml organic solvent at 12^{o}C. The progress of reaction was monitored by TLC. At sufficient conversion reaction mixture was filtered, dried on anhydrous sodium sulphate and solvent was removed under vacuum. Residue was treated as described in hydrolysis experiment and E value was calculated.

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cis-Phloroglucitol to Chiral Drug Intermediates through Chemoenzymatic methods

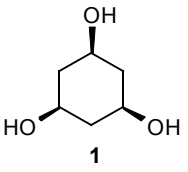
Chapter III

CHAPTER III

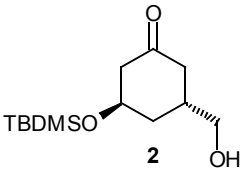
Contents

4.1. Introduction

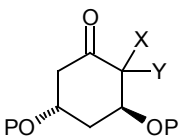
Cis-1,3,5-cyclohexantriol i.e. phloroglucitol (**1**) is readily available by hydrogenation of phloroglucinol.[1] All *cis*-hydroxy configuration of **1** can be utilized for the synthesis of various natural products.[2] Its symmetric structure offers an opportunity for the preparation of *meso*-compounds, which can be further desymmetrized and explored for the synthesis of various important optically active compounds.[3] We planned to develop such strategy based on enzymatic desymmetrization method for the preparation of certain optically pure intermediates which are useful for the synthesis of chiral drugs. We decided to develop chemoenzymatic route for lactone **2**, which is an important chiral synthon for the 'statin' group of drugs such as fluvastatin, compactin, mevinolin etc. Also, we planned to apply the same strategy for synthesis of intermediates **3** and **4** of optically pure A-ring phosphine oxide synthon required for 1α, 25-dihydroxyvitamin D_3 and its 19-nor analogue.



1



2



3 X, Y = CH_2
4 X = Y = H

4.2. Chemoenzymatic route for the Optically Pure lactone 6-Hydroxymethyl-4-*tert*-butyldimethylsilyloxy-(4*R*, 6*S*)-tetrahydro-2*H*-2-pyranone (2)

4.2.1. Introduction

In 1976, Endo et al, at the Sankyo Co. and Brown et al at Beecham Pharmaceuticals isolated a potent competitive inhibitor of hydroxymethylglutaryl coenzyme A reductase (HMG CoA reductase) from the metabolites of *Penicillium citrinum* and *P*. *brevicompactum*, respectively.[4,5] The new compound, shown to have structure **5** was named ML236B by the Japanese group and compactin by the British workers. In 1980, Alberts et al at Merck, Sharp & Dohme, reported the isolation of a relative of compactin from *Aspergillus terrus*.[6] The Merck compound was named as mevinolin and shown to have stereostructure **6**. An identical fungal metabolite was isolated from Monascus rubber and named monacolin K.[7]

In humans, more than one half of total body cholesterol is derived from *de novo* synthesis. The rate-limiting step in cholesterol biosynthesis is the reduction of HMG CoA to mevalonic acid (Figure 4.1).[8] Because of their potent inhibitory activity on this key enzyme; compactin and related compounds act as hypocholesterolemic agents. Various synthetic analogues of compactin are found to act as inhibitors of HMG CoA reductase. Amongst these, fluvastatin[9] (**7**) and atorvastatin[10] (**8**) have emerged as leading antihyperlipoproteinemic agents and are used in treatment of arteriosclerosis and hyperlipoproteinemia. Fluvastatin is sold under the brand name **Lescol®** whereas atorvastatin sold as **Lipitor®** is a blockbuster from Parke-Davis Pharmaceuticals.

In all these compounds, a β-hydroxy-δ-lactone portion or its open chain form is essential for the activity. A retrosynthetic analysis for these compounds traces to a common lactone moiety **2** (P = -TBDMS). We planned to develop a chemoenzymatic route for **2** utilizing phloroglucitol **1** as starting material (Scheme 4.1). Before presenting this work and its discussion, a brief review on various reported methods for the preparation of **2** and analogues is presented.

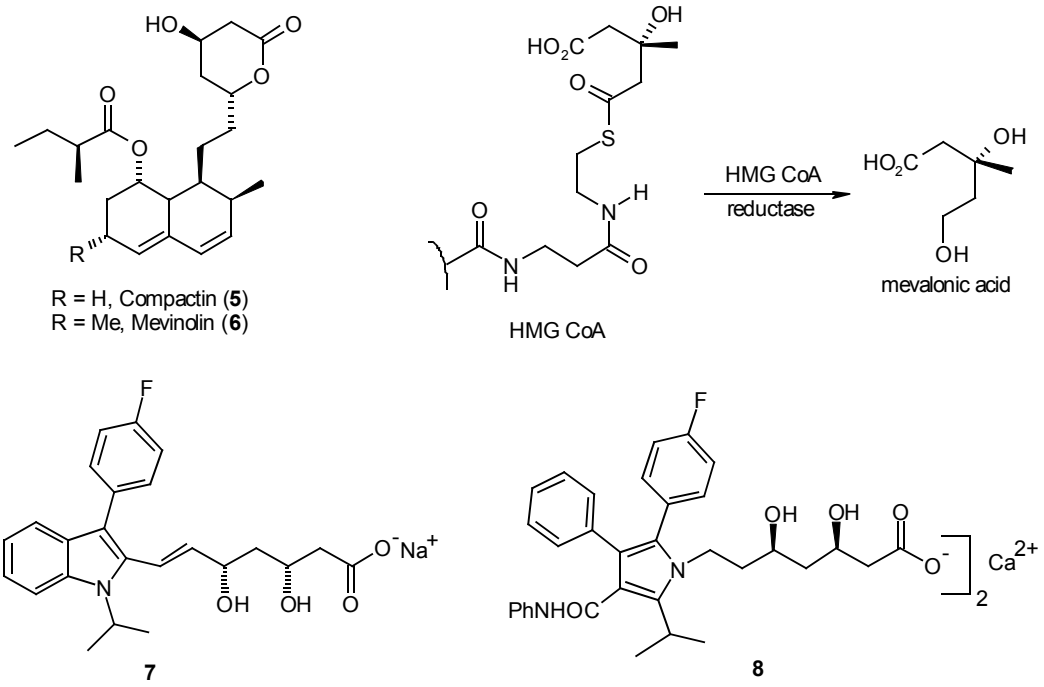
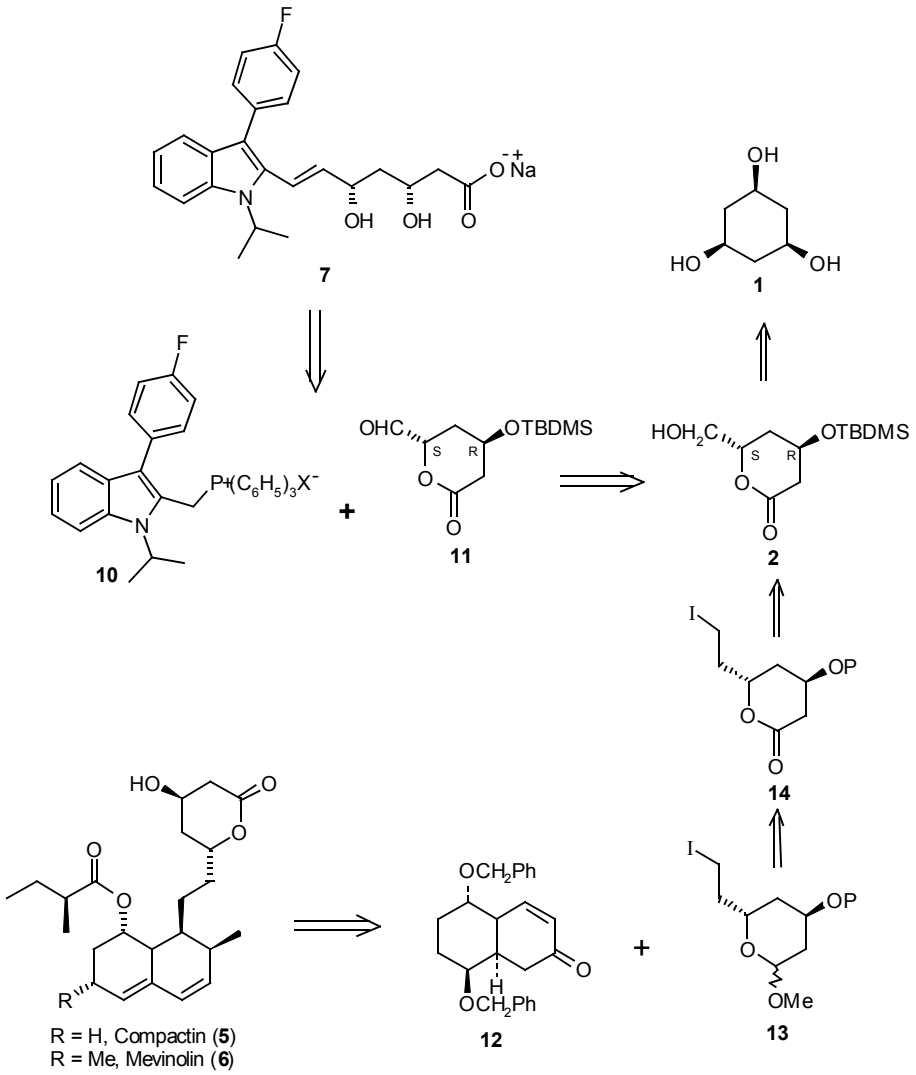

HO
O
O
O
O
R
R = H, Compactin (5)
R = Me, Mevinolin (6)
OH
HO2C
O
S
N
H
O
N
H
O
HMG CoA
HMG CoA
reductase
OH
HO2C
OH
mevalonic acid
F
N
O-Na+
OH
OH
O
7
F
OH
OH
O
N
O-
Ca2+
2
PhNHOC
8

Figure 4.1.


F
OH
HO
OH
1
7
F
OHC
OTBDMS
HOH2C
OTBDMS
P+(C6H5)3X-
10
11
2
I
OP
14
HO
OCH2Ph
I
OP
OMe
R = H, Compactin (5)
R = Me, Mevinolin (6)
12
13

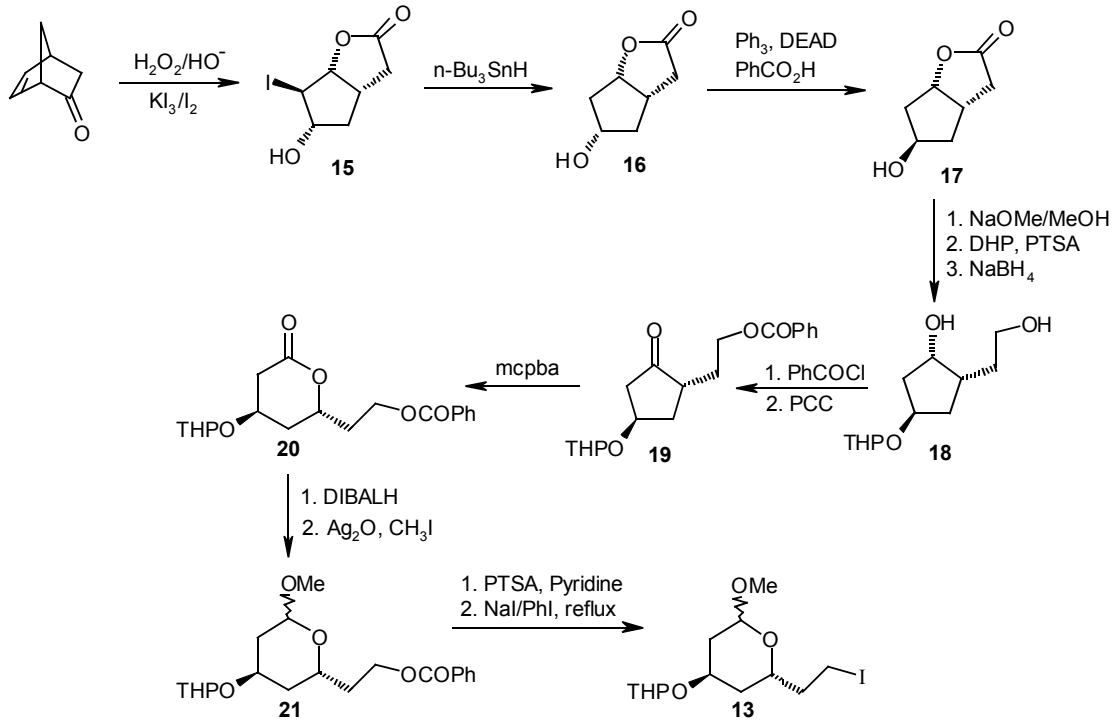
Scheme 4.1.

4.2.2. Reported Syntheses of lactone 6-Hydroxymethyl-4-*tert*-butyldimethyl-silyloxy-(4*R*, 6*S*)-tetrahydro-2*H*-2-pyranone (2) and related lactones

Numerous reports on the synthesis of lactone **2** and related lactones are reported in literature.[11] These methods can be broadly divided into two types viz. (1) Chemical methods (2) Chemoenzymatic methods

Chemical Methods

☞ Sih and coworkers[12] reported the first total synthesis of compactin wherein racemic iodide lactone **13** was synthesized as shown in Scheme 4.2.

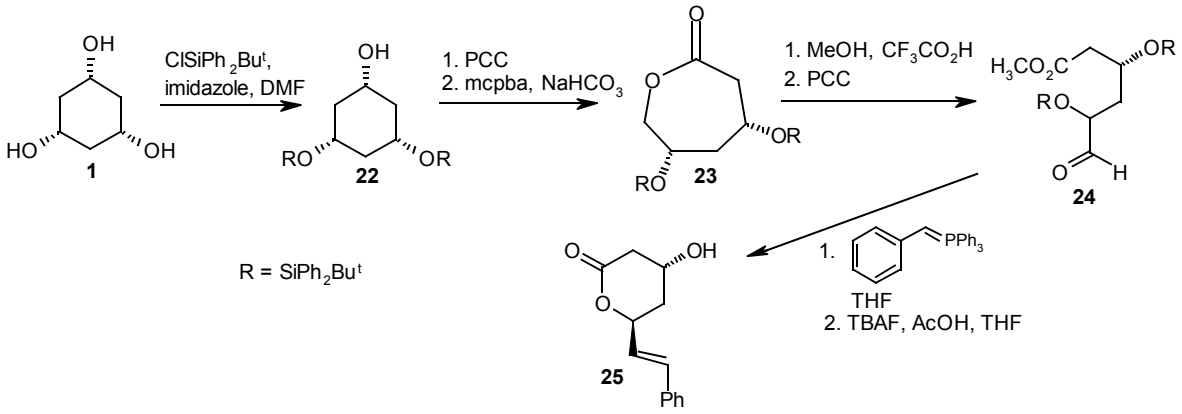


Scheme 4.2.

Conversion of 5-norbornen-2-one to iodolactone **15** and subsequent reductive deiodination with tributyltin hydride afforded lactone **16**. Mitsunobu inversion at hydroxy center followed by saponification, THP protection and borohydride reduction gave diol tetrahydropyranyl ether **18**. Selective benzoylation of primary alcohol and oxidation of the secondary hydroxyl group afforded ketone **19**, which was transformed to lactone **20** by Baeyer-Villiger oxidation. Reduction of **20** with DIBALH and treatment of the resulting hydroxyacetal with silver oxide and methyl iodide gave

methyl glycoside **21** as an anomeric mixture. Tosylation of **21** and displacement with iodide furnishes **13** in 18% overall yield from 5-norbornen-2-one.

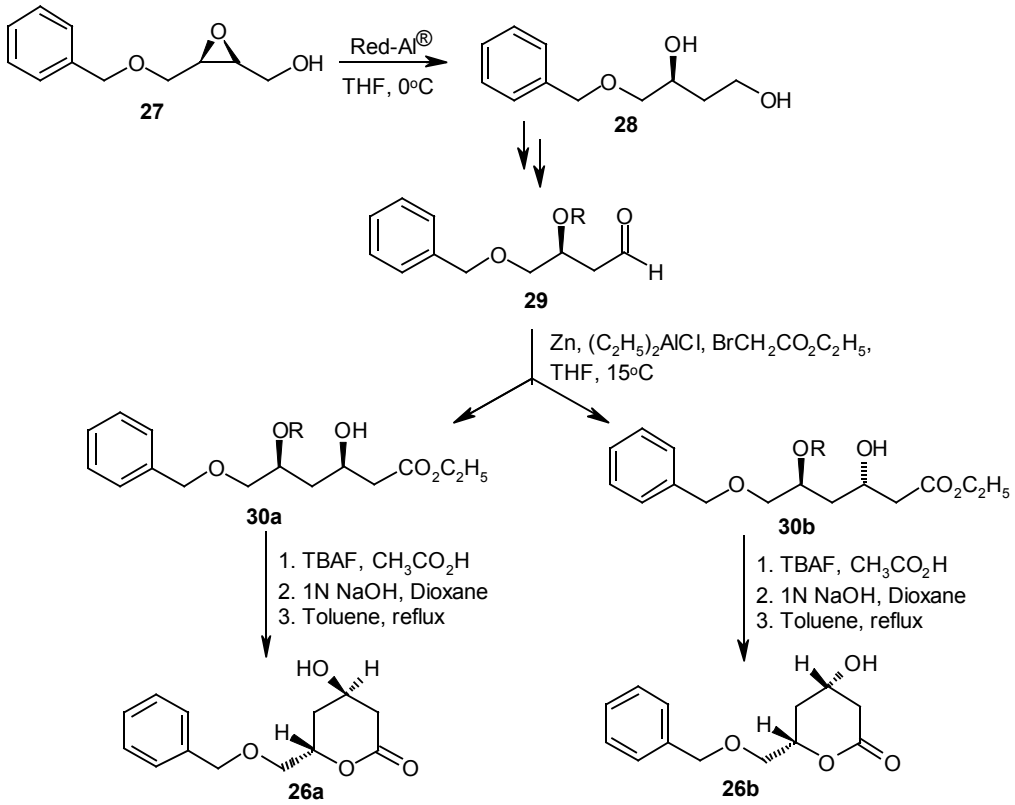
☞ Prasad and Repic have published an approach to the lactone system that begins with **1** (Scheme 4.3).[13] Conversion of triol **1** to bis-silyl ether **22** (40% yield) followed by PCC oxidation (93% yield) and Baeyer-Villiger oxidation afforded lactone **23** in 77% yield. Methanolysis and oxidation of the resulting hydroxyester provided aldehyde **24** (95% yield). Wittig coupling (77% yield) and desilylation provided the unmasked lactone **25** in 45% yield.



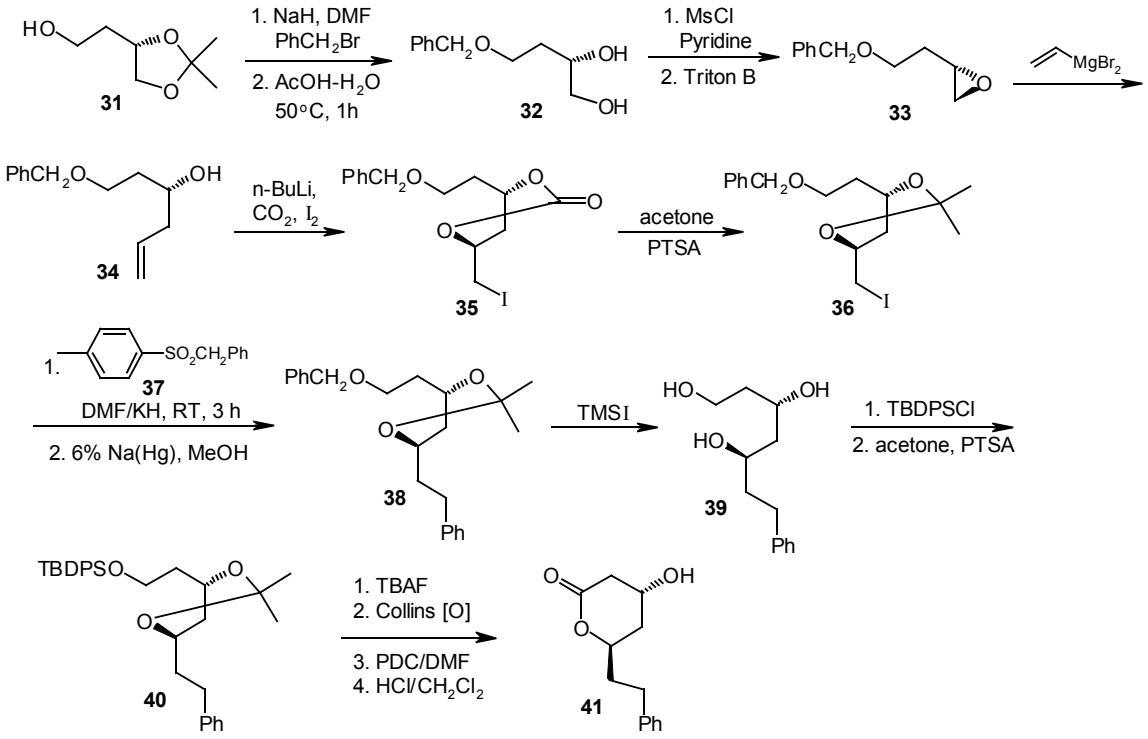
Scheme 4.3.

☞ Kapa Prasad and coworkers reported the asymmetric synthesis of lactone **26** using optically active epoxide **27** (Scheme 4.4).[14] Epoxide **27** was opened with Red-Al® to give diol **28**. Diol **28** was further converted to chiral aldehyde **29**, which is the key intermediate in the scheme. Aldehyde **29** was converted to 1:1 diastereomeric mixture of aldol products **30a** and **30b** through modified Reformatsky condition. These diastereomers were separated by HPLC and were converted to lactones **26a** and **26b** through deprotection and cyclization.

☞ Clive and coworkers utilized L-malic acid and derivative **31** as a precursor to lactone **41** (Scheme 4.5).[15] Benzylation of **31** and hydrolysis of the acetonide afforded monoprotected triol **32** in 86% yield. Mesylation and subsequent treatment with Triton B provides optically active epoxide **33**. The epoxide **33** was converted to ketal **36** through few steps, which was purified by column chromatography to afford single isomer. Coupling of **36** and sulfone **37** followed by desulfonylation affords adduct **38** (78% yield), which was deprotected to give triol **39**. Triol **39** through a mutistep procedure was converted to the desired lactone **41** in 33% overall yield from **39**.

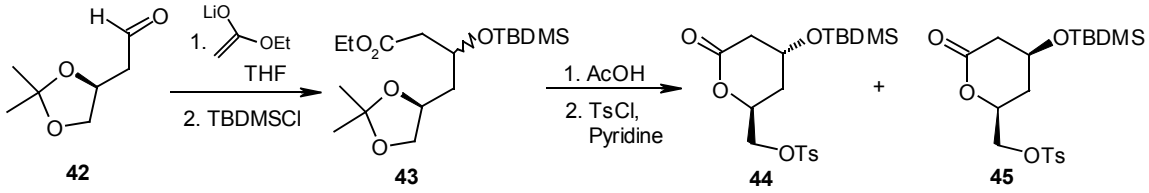

Red-Al®
THF, 0°C
27
28
29
Zn, (C2H5)2AlCl, BrCH2CO2C2H5,
THF, 15°C
30a
30b
1. TBAF, CH3CO2H
2. 1N NaOH, Dioxane
3. Toluene, reflux
26a
26b

Scheme 4.4.



Scheme 4.5.

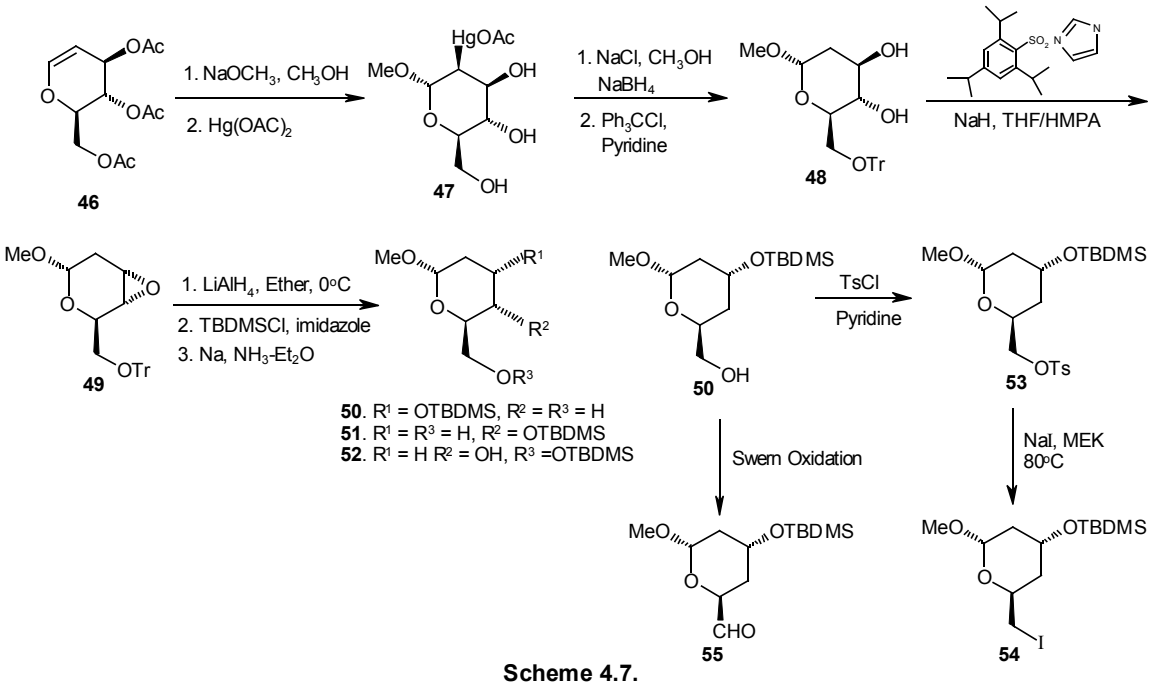
☞ Heathcock and coworkers investigated the use of optically active aldehyde **42**, derived from L-malic acid as the chiral building block for elaboration of the lactone synthon (Scheme 4.6).[16] Addition of the lithium enolate of ethyl acetate to **42** and silylation of the resulting mixture of epimeric alcohols furnishes **43** in 88% yield. Lactonization (60% yield) and subsequent tosylation provides **44** and **45**, which are separated by chromatography (91% combined yield).



Scheme 4.6.

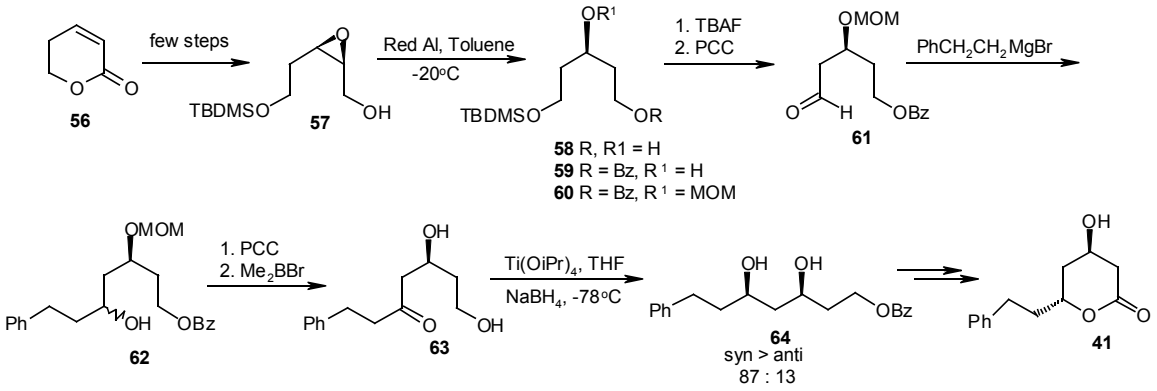
☞ Due to the inefficiency of having to perform an isomer separation, Heathcock and coworkers persuaded an approach based on a carbohydrate precursor, tri-O-acetyl-D-glucal (**46**) (Scheme 4.7).[16] Glucal **46** was converted to epoxide **49** through Corey's procedure.[17] Lithium aluminium hydride epoxide opening (90% axial selectivity), silyl protection followed by removal of trityl

protection afforded alcohol **50** and an inseparable mixture of **51** and **52**. Alcohol **50** is converted to tosylation **53** in the standard manner (90% yield) and this material was transformed into iodide **54** (95% yield). Alcohol **50** is also oxidized by Swern's method to obtain aldehyde **55** in 93% yield. Same method was followed by Kathawala for the preparation of aldehyde **55**, required for the synthesis of fluvastatin.[9]



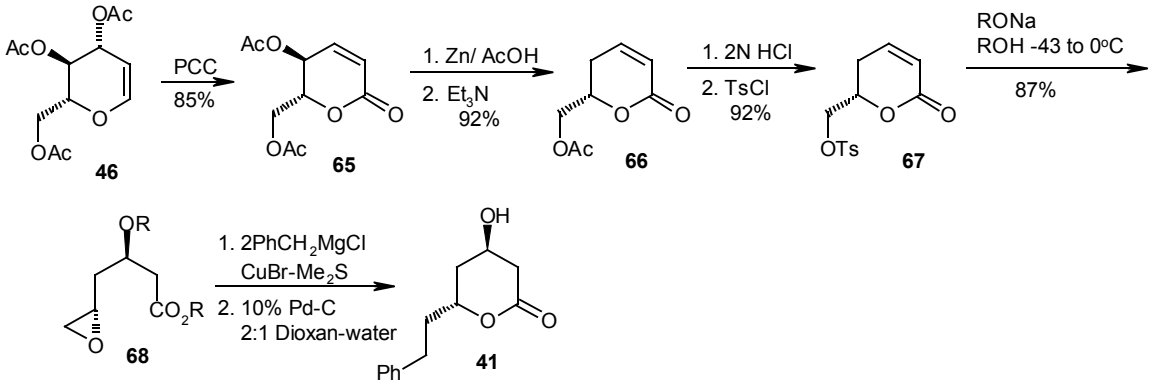
Scheme 4.7.

☞ C. Bonini and coworkers developed the asymmetric synthesis of lactone **41** by asymmetric epoxidation of the appropriate allylic alcohol and subsequent introduction of the second chiral center via a $Ti(OiPr)_4$ mediated reduction of β–hydroxyketones (Scheme 4.8).[18] The chiral epoxy alcohol **57** was prepared from the commercially available lactone **56**. Regioselective opening of **57** (Red/Al, 2hr, -20°C,) afforded exclusively diol **58**, which was transformed by standard procedure into compound **60**. Compound **60** was then desilylated and oxidized to aldehyde **61** (78% yield). Addition of Grignard reagent, ethylphenylmagnesium bromide on **61** afforded a diastereomeric mixture of alcohol, which was converted to β–hydroxyketone **63**. $Ti(OiPr)_4$ mediated $NaBH_4$ reduction of **63** affords required syn diol **64** (syn:anti > 87:13) which is further lactonized to lactone **41** in few steps.



Scheme 4.8.

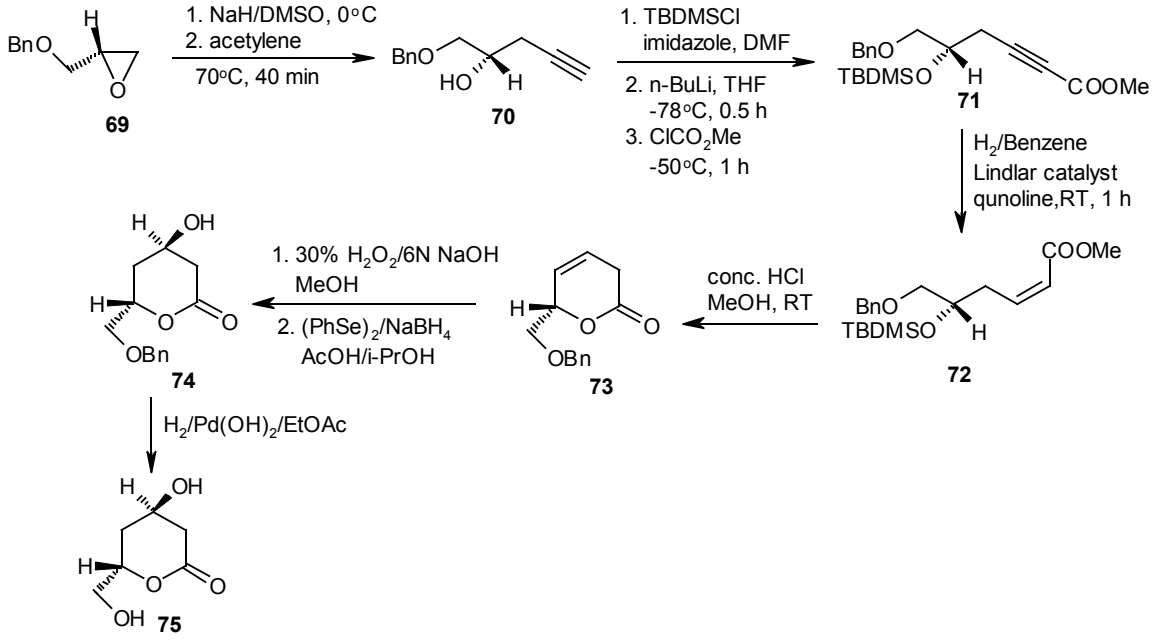
☞ Roath and Roark developed an asymmetric route for lactone **41** starting from commercially available tri-O-acetyl-D-glucal **46** (Scheme 4.9).[19] Treatment of **46** with PCC afforded unsaturated lactone **65** which is further converted to tosylate **67** in few steps. Tosylate **67** undergoes stereoselective Michael addition on treatment with sodium allyl alcoholate in allyl alcohol at low temperature (-43 to 0°C) to afford a key chiral synthon **68**, which is easily converted to lactone **41** in two steps



Scheme 4.9.

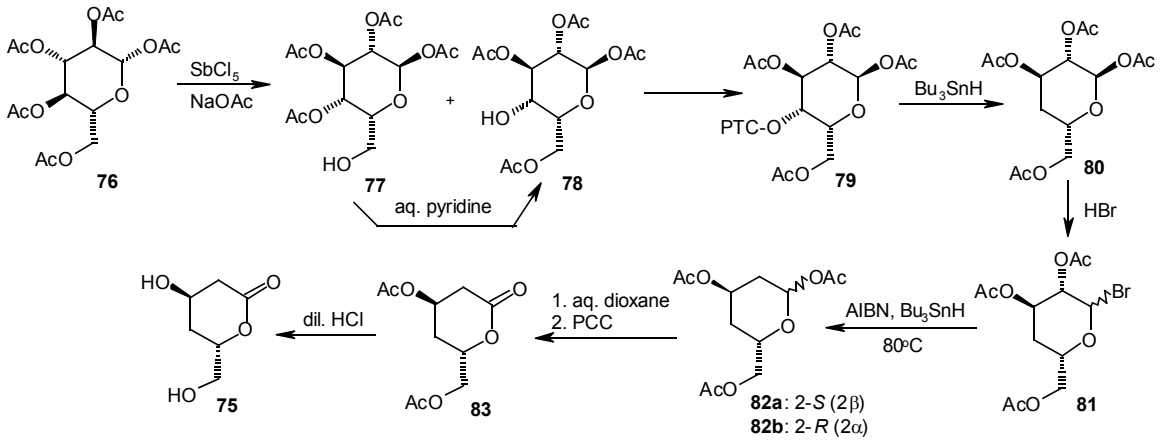
☞ S. Takano and coworkers developed a facile chiral synthesis for lactone moiety using (*R*)-O-benzylglycidol (**69**) as starting material (Scheme 4.10).[20] Treatment of sodium acetylide generated in situ with **69** affords the terminal acetylene **70**. Protection of free –OH as silyl ether and further methoxycarbonylation gives the ester **71** in 87% yield. Hydrogenation of **71** on Lindlar catalyst followed by brief exposure of the resulting olefin **72** to acid furnishes the α,β–unsaturated δ–lactone **73** in 86% overall yield. Epoxidation of **73** using alkaline H_2O_2 followed by

regioselective cleavage of epoxide using sodium phenylseleno(triisopropyloxy)borate affords β–ketol **74** in 87% yield. Finally, the benzyl group of **74** is removed by hydrogenolysis to afford the lactone **75** in 81% yield.



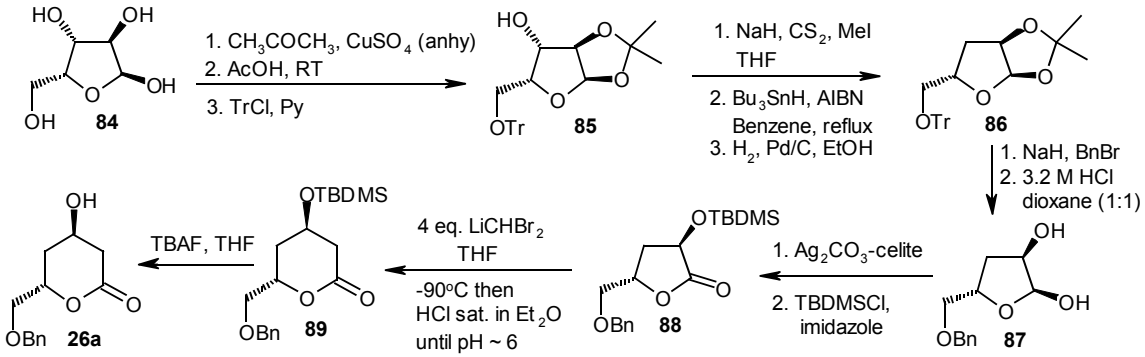
Scheme 4.10.

☞ Y. Maki and coworkers developed route to lactone **75** starting from glucose (Scheme 4.11).[21] Acetylated glucose **76** was rearranged to D-iodose derivative (**77** and **78**) by the modified Paulsen procedure ($SbCl_5$, NaOAc) followed by treatment of aqueous pyridine which converted **77** to **78** by acyl migration. After conversion of **78** to the 4-O-phenoxythiocarbonyl derivative (**79**), the reaction with tributyltinhydride resulted in deoxygenation at C-4 of **78** to give the 4-deoxy derivative (**80**) which was treated with HBr to give 1-bromoderivative **81**. Radical rearrangement on **81** by heating at 80°C with AIBN and tributyltinhydride afforded 1,3,6-tri-O-acetyl-2,4-dideoxy-β-D-iodopyranose (**82a**) which was hydrolyzed in aqueous dioxane and subsequently oxidized with bromine to give the lactone **83**. Deprotection of **83** to lactone **75** was achieved by dil. hydrochloric acid.



Scheme 4.11.

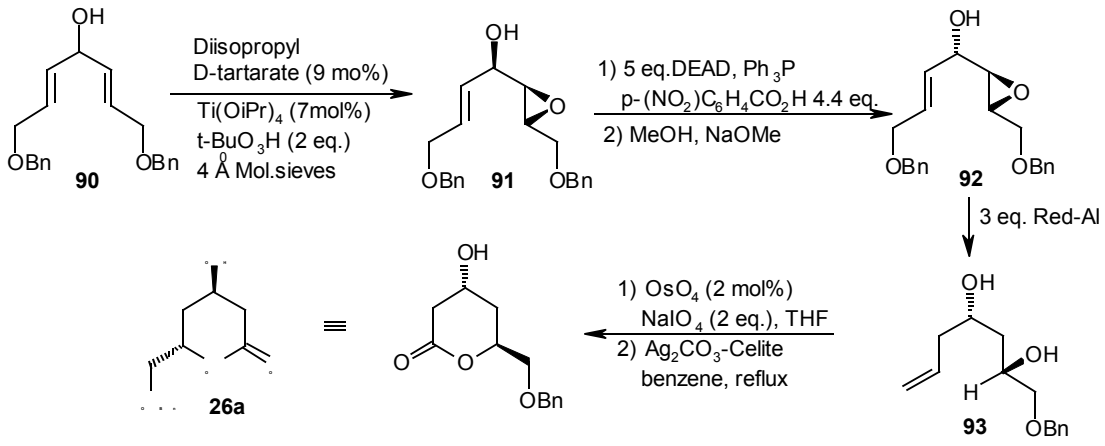
☞ M. Petrini and coworkers developed a chiral route for lactone **26a** using D-xylose (**84**) as starting material (Scheme 4.12).[22] Selective protection-deprotection of **85** afforded a diol which was selectively tritylated at the primary hydroxy group to give compound **85**. This was further transformed into xanthanate and deoxygenated by the method of Barton to deoxy product **86**, which on benzylation and acetonide deprotection afforded lactol **87**. The lactol **87** was oxidized to the corresponding lactone with Fetizon's reagent and resulting α–hydroxy lactone was protected as TBDMS ether **88**. Homologation of the lactone **88** afforded **89**, which was desilylated to lactone **26a**.



Scheme 4.12.

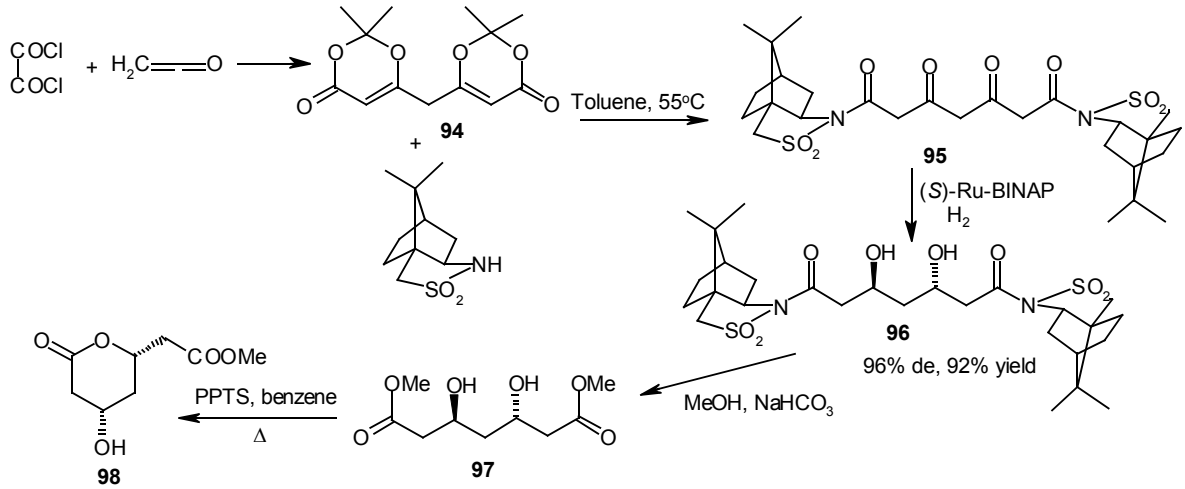
☞ S. Takano and coworkers developed asymmetric synthesis for lactone **26a** based on Red-Al promoted intramolecular reductive cleavage of benzyl 4-hydroxy-2-butenyl ether structures (Scheme 4.13).[23] Thus, **92** which was obtained from **90** by Sharpless epoxidation and Mitsunobu

inversion, on treatment with 3 eq. Red-Al afforded syn-diol **93**. It could be further converted into lactone **26a** in two steps.



Scheme 4.13.

☞ J. Kiegiel and coworkers developed asymmetric route to δ–valerolactone **98** based on synthesis and asymmetric hydrogenation of 3,5-dioxoheptanedionates (Scheme 4.14).[24] Bisdioxinonone **94** was obtained by the action of ketene on oxalyl chloride in acetone. Bisdioxinonone **94** and (1*S*)-bornane-10,2-sultam were heated in toluene at 55ºC to obtain **95**. Compound **95** was hydrogenated by using (*S*)-Ru-BINAP as catalyst to afford anti-diol **96** in 96% de. Diol **96** was then transformed into enantiomerically pure dimethyl ester **97** by applying mild methanolysis in the presence of $NaHCO_3$, which on refluxing in benzene in the presence of pyridinium p-toluenesulfonate furnished enantiomerically pure lactone **98**.

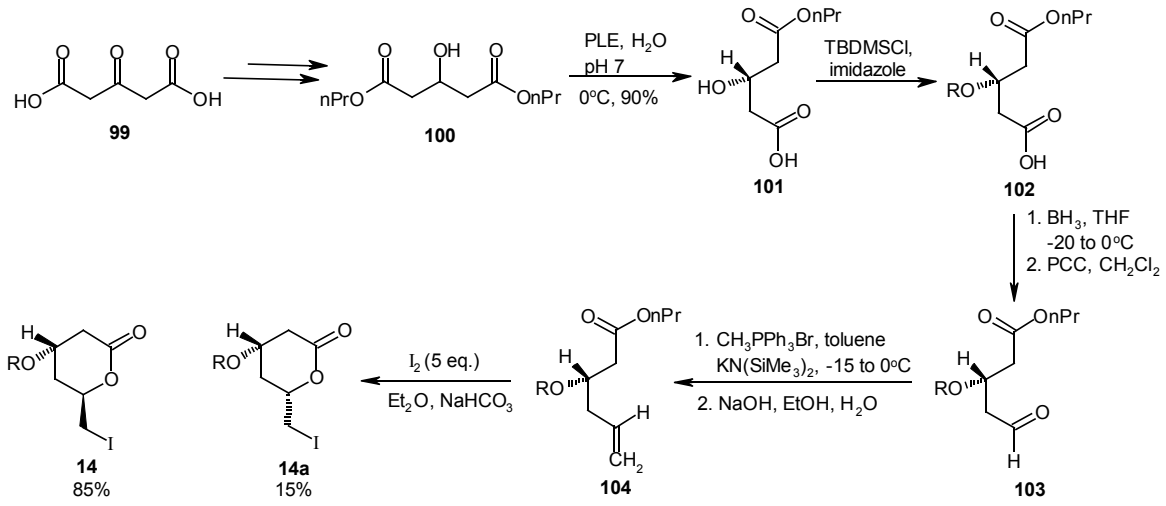


Scheme 4.14.

Chemoenzymatic Methods

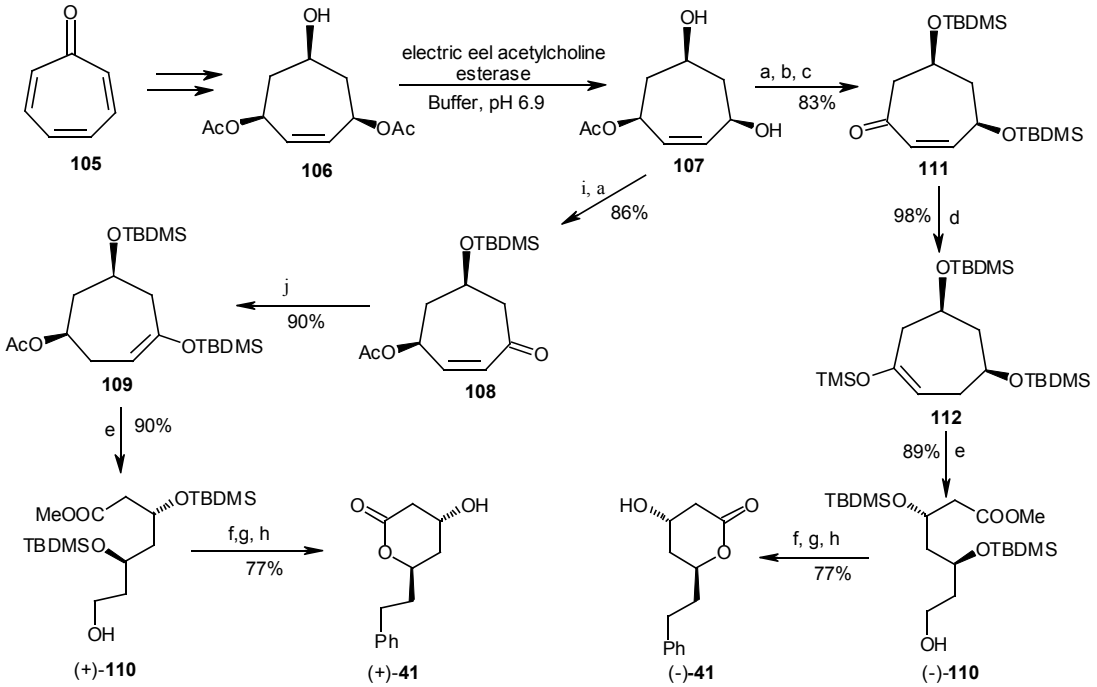
Various chemoenzymatic methods are reported for the synthesis of chiral lactone synthon of compactin and mevinolin. In most of the reports, hydrolytic enzymes viz. lipases/esterases have been utilized to afford optically active intermediates which are further elaborated by chemical methods to the desired lactone moiety. A brief account of these methods is presented below.

☞ Badder et al developed a chemoenzymatic route for enantiomer of iodolactone **14** (R = TBDMS). Key steps in the synthesis are enzyme PLE catalyzed saponification of prochiral di-n-propyl-3-hydroxyglutarate **100** and iodolactonization of **104** (Scheme 4.15).[25]



Scheme 4.15.

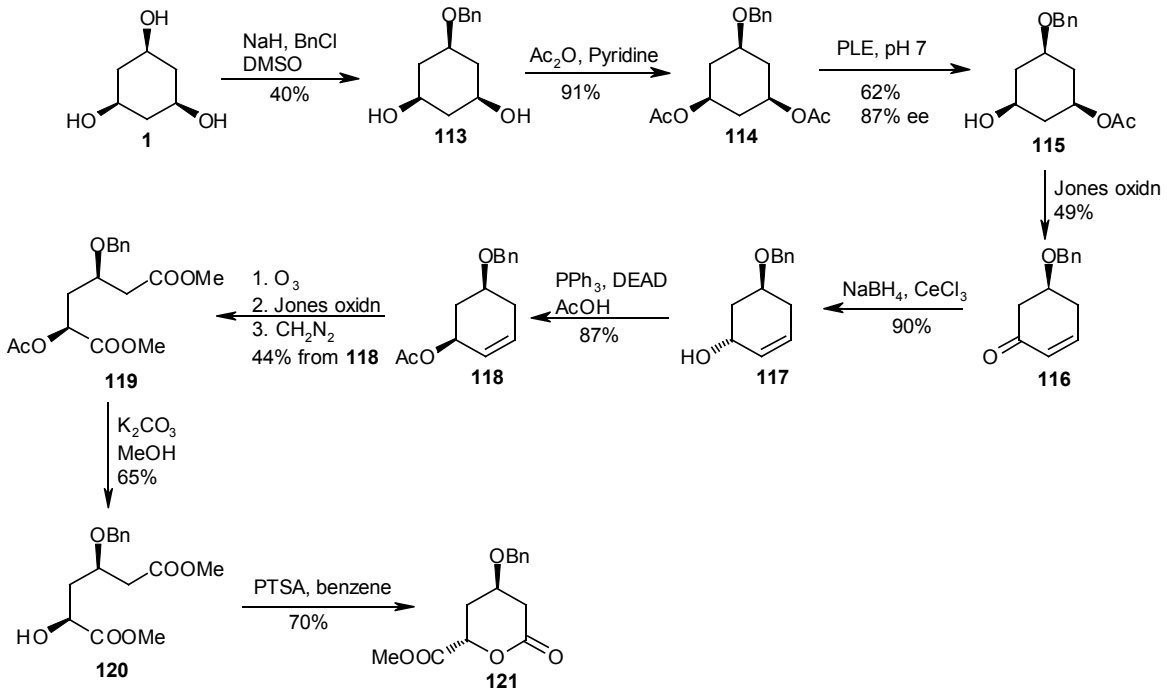
☞ C. R. Johnson reported a chemoenzymatic route for both enantiomers of compactin analogue lactone **41** (Scheme 4.16).[26] Seven membered *meso*-diacetate **106**, that is obtained from troponone **105**, was desymmetrized by enzymatic hydrolysis catalyzed by electric eel acetylcholine esterase to afford chiral diol **107** in >95%. Compound **107** was further elaborated into both enantiomers of **41**. Main chemical steps involved protection-oxidation sequence to get pseudoenantiomeric enones **108**/**111** followed by MeCu catalyzed enolization of enone to form enol ether **109**/**112**. Ozonolysis of enol ethers followed $NaBH_4$ reduction and esterification afforded enantiomers of diprotected triol **110**, which could be converted to (+)-**41** or (-)-**41** in three steps.



Reaction and conditions: (a)TBSOTf, lutidine, CH_2Cl_2, 0°C; (b) KOH, MeOH, 0°C; (c) PDC, CH_2Cl_2;(d) cat. MeCu, DIBALH-HMPA, -50°C, then at -78 °C, MeLi, TMSCl; (e) O_3, MeOH, CH_2Cl_2, -78°C, then $NaBH_4$ followed by CH_2N_2; (f) TsCl, Et_3N, DMAP; (g) Ph_2CuLi, Et_2O, 0°C; (h) HF, CH_3CN; (i) MnO_2, CH_2Cl_2; (j) cat. MeCu, 2.5 equ. of DIBALH in HMPA/THF, -50 °C, then at -78°C, MeLi (2.5 equ.), TBSOTf (2.5 equv.)

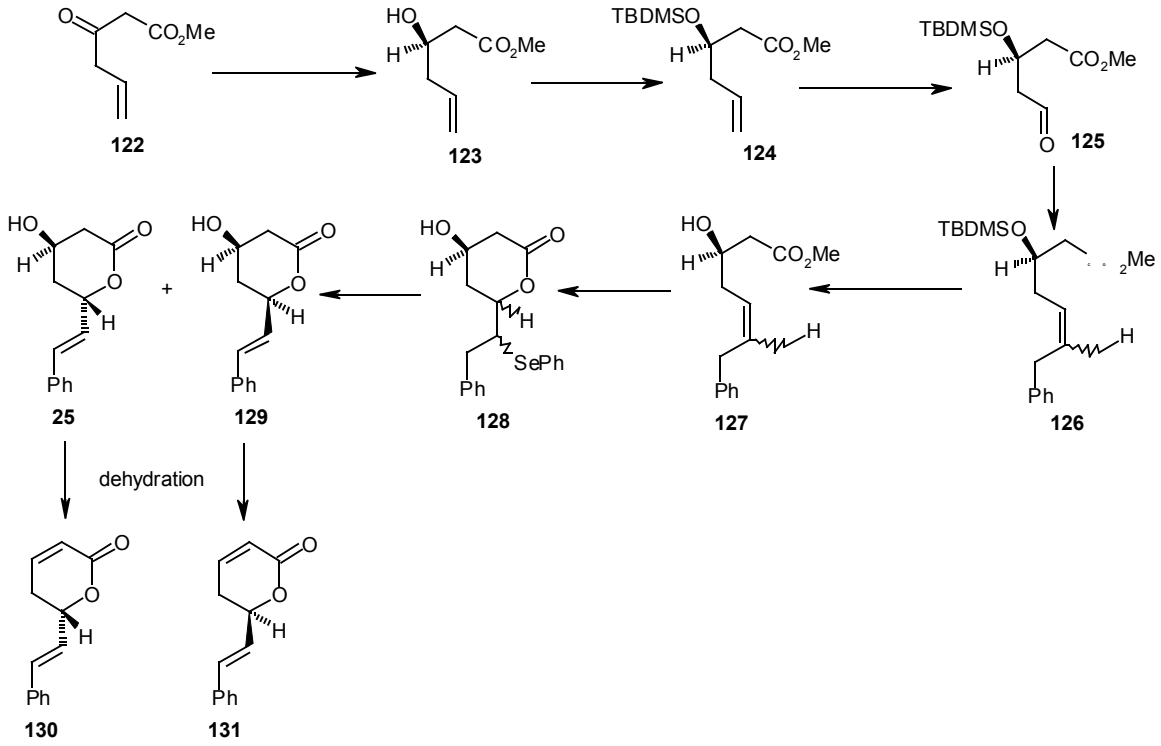
Scheme 4.16.

☞ K. Sakai and coworkers developed a chemoenzymatic synthesis for compactin lactone moiety utilizing phloroglucitol (**1**) as starting material (Scheme 4.17).[3b] *Meso*-diacetae **114**, prepared from phloroglucitol was desymmetrized by enzymatic hydrolysis catalyzed by PLE to afford optically active cyclohexanol **115** of 87% e.e. in 62% yield. Jones oxidation of **115** afforded the enone **116**, which on diastereloselective reduction using $NaBH_4$-$CeCl_3$ afforded 3,5-*trans* isomer **117**. Compound **117** was converted to the acetate **118** with desirable configuration by Mitsunobu method. The diester **119** was obtained from **118** via ozonolysis, Jones oxidation and subsequent esterification with CH_2N_2. After hydrolysis of acetate, lactonization of **120** in the presence of p-TsOH afforded the lactone moiety **121** of compactin. Thus, phloroglucitol **1** was converted to lactone **121** in overall 1.73% yield.



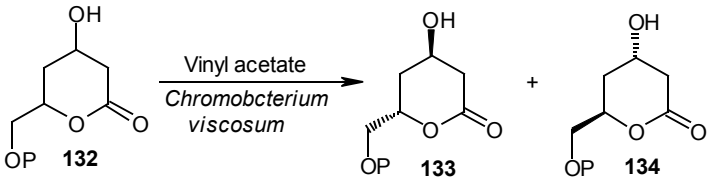
Scheme 4.17.

☞ D.W. Knight and coworkers developed a chemoenzymatic route for optically active lactone **25** and its epimer (Scheme 4.18).[27] These were further converted to both enantiomers of the natural product goniothalamin **130** and **131**. Ozonolysis of the 3-silyloxyhexanoate **124**, derived from the yeast reduction product (3*R*)-(-)-3-hydroxyhex-5-enoate **123** (e.e. 78%), followed by Wittig homologation and selenolactonization leads to the lactones **25** and **129**. Subsequent dehydration gives **130** and **131**.



Scheme 4.18.

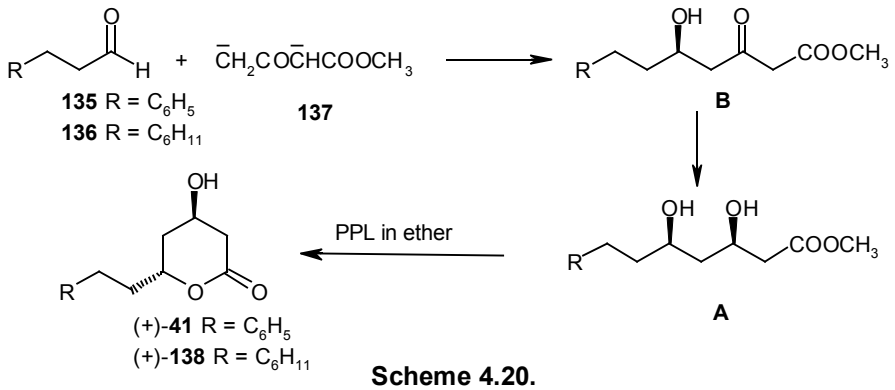
☞ Crobs et al have patented the enzymatic resolution of lactone **132** (Scheme 4.19).[28] Racemic lactones with formula **132** were resolved by enzymatic transesterification with vinyl acetate in THF using *Chromobacterium viscosum* lipase as catalyst at 40°C. Optically active lactone **133** with free secondary –OH was recovered with e.e. >95%.



Scheme 19.

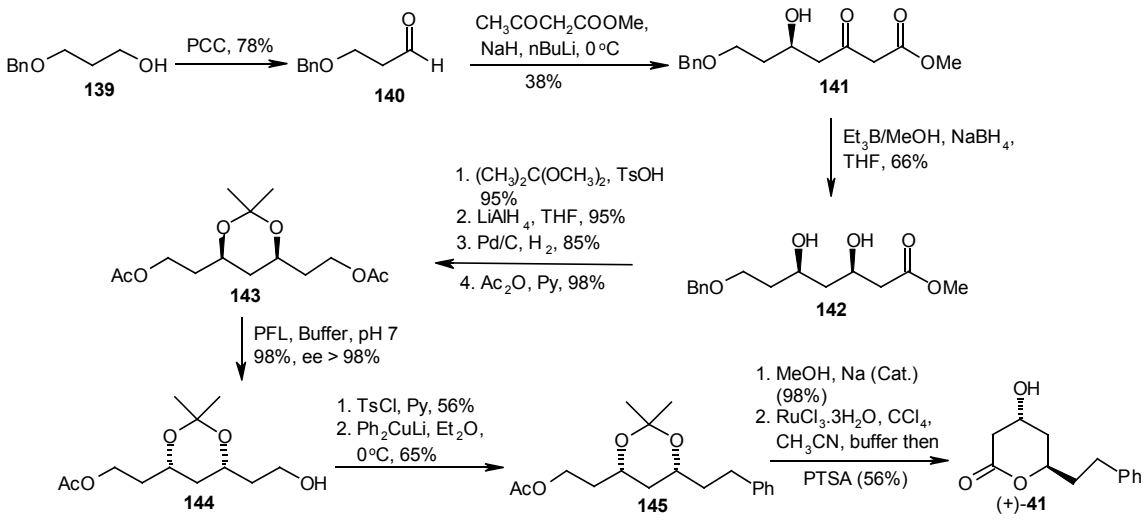
☞ C. Bonini and coworkers reported a short synthesis of optically active mevinic acid analogues by biocatalytic lactonization of syn-3,5-dihydroxy esters (Scheme 4.20).[29] The starting syn-3,5-dihydroxyesters of type **A** were prepared respectively from phenylpropionaldehyde **135** and cyclohexylpropionaldehyde **136** with the dianion of methyl acetoacetate **137** followed by diastereoselective reduction of resulting aldols **B**. Biocatalytic lactonization of these syn-

dihydroxyesters was performed using PPL in ether solution. Corresponding lactones (+)-**41** and (+)-**138** of >98% e.e. were obtained in 35% yield.



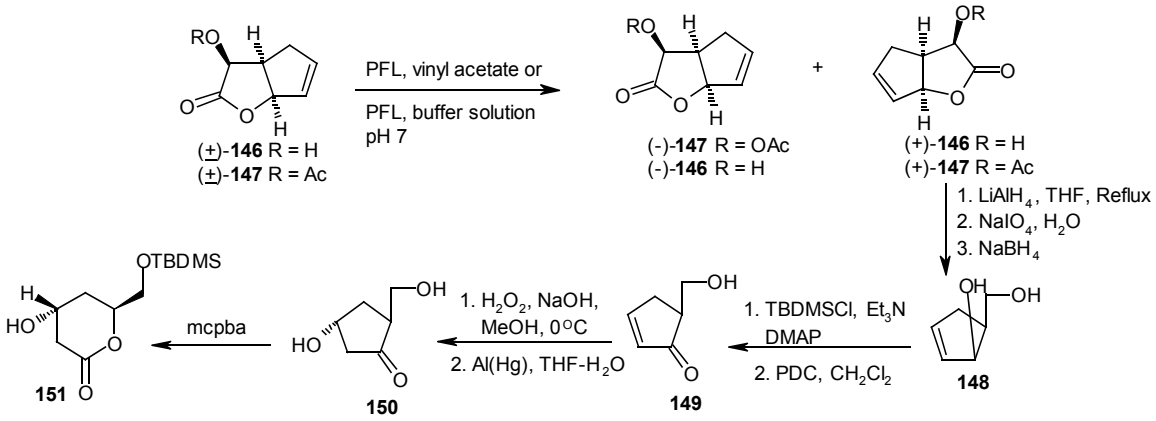
Scheme 4.20.

☞ In another chemoenzymatic route for lactone (+)-**41**, C. Bonini and coworkers obtained a new seven-carbon polyhydroxylated chiral synthon **144** as single enantiomer, in nearly quantitative yield by enzymatic desymmetrization of the *meso*-compound **143** (Scheme 4.21).[30] The compound **143** was prepared by the aldol condensation of aldehyde **140** with methylacetoacetate and subsequent diastereoselective reduction of the aldol **141** to syn 1,3-diol **142** followed by acetonoide protection, LAH reduction, debenzylation and acetylation to afford *meso*-diacetate **143**. *Meso*-diacetate **143** was desymmetrized to **144** with high degree of enantiotopic discrimination by *Pseudomonas fluorescens* lipase (PFL) catalyzed hydrolysis in phosphate buffer. Compound **144** could be further elaborated to lactone (+)-**41** in four chemical steps.



Scheme 4.21.

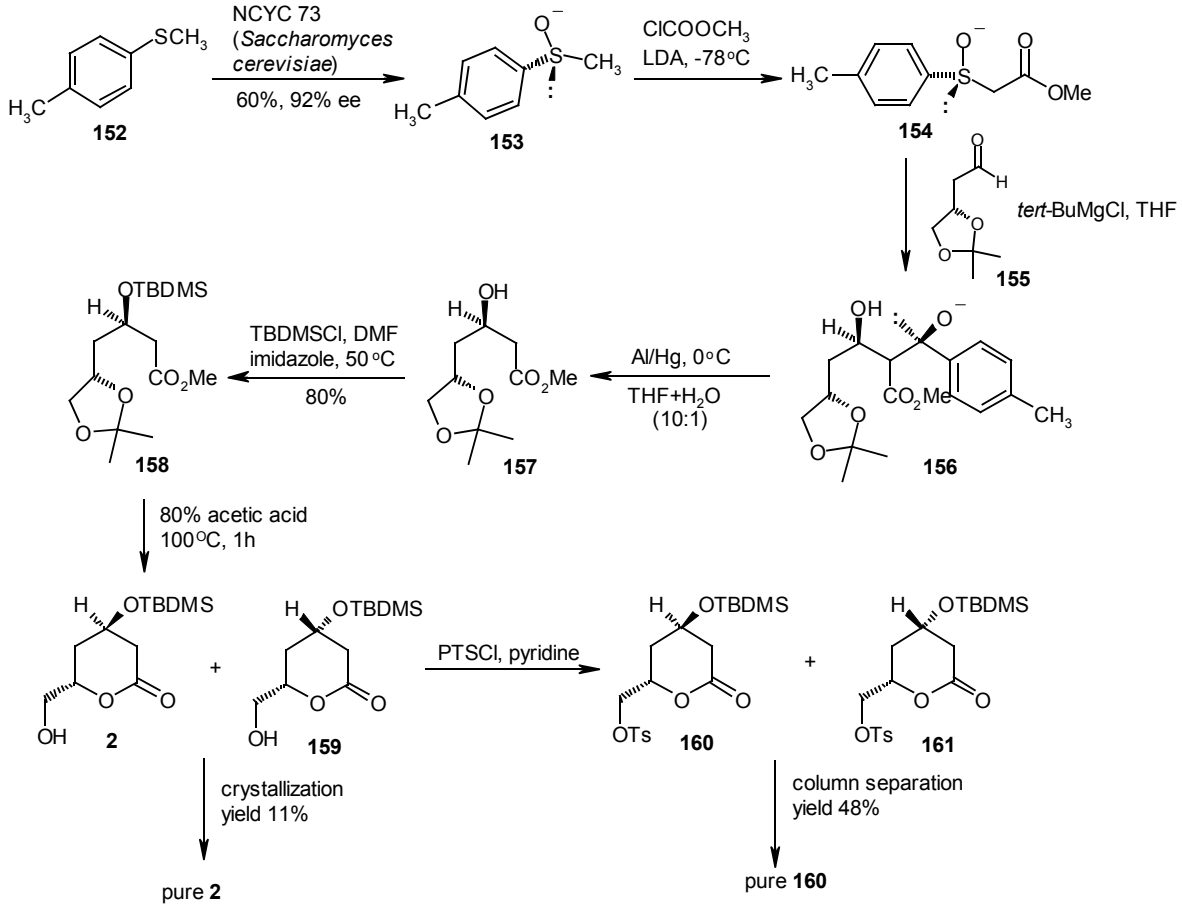
☞ R. McCague et al reported a chemoenzymatic synthesis of mevinic acid lactone **151** starting from endohydroxylactone (+)-**146** or ester (+)-**147** which is obtained by enzymatic resolution of racemic endo-hydroxylactone **146** using *Pseudomonas flurorescens* lipase (PFL) either by esterification in an organic solvent, or by hydrolysis of the acetyl ester (±)-**147** in a buffer solution (Scheme 4.22).[31] Hydroxylactone (+)-**146** or ester (+)- **147** was reduced to diol **148** in three steps- $LiAlH_4$ reduction, sodium periodate diol clevage, and $NaBH_4$ reduction. Cyclopentenone **149** was obtained by selective monoprotection of diol **148** with TBDMSCl followed by PCC oxidation. Compound **148** was converted to β–hydroxycyclopentanone by stereoselective epoxidation followed by epoxide opening using Kect's aluminium amalgam conditions. Baeyer-Villiger oxidation of cyclopentanone **150** provided the desired lactone **151** which posses the correct stereogenic center in the mevinic acids.



Scheme 4.22.

☞ S. M. Roberts and coworkers developed an asymmetric synthesis of a α–silyloxy-δ-lactone (**2**, R = TBDMS) using (*R*)-methyl paratolylsulfoxide as a chiral auxillary (Scheme 4.23).[32] The chiral sulfoxide **153** was obtained via a straightforward biooxidation of methyl paratolylsufide **152** using Baker's yeast (*Saccharomyces cerevisiae* NCYC 73) in 60% yield and 92% e.e.. The enolate ion of **153** was generated using LDA and reacted with methyl chloroformate to afford the α-sulfinylester (**154**), which on treatment with *tert*-BuMgCl and aldehyde **155** underwent stereocontrolled aldol condensation to afford ester **156**. Desulphurization of **156** yielded C-3 epimers of **157** in ratio of 4:1, which were protected as TBDMS ethers **158**. Lactonization of **158** by heating to reflux in 80% acetic acid afforded the desired lactone **2** and **159**. Successive recrystallizations from hexane gave **2** in pure form. Alternatively, tosylation of a mixture of **2** and

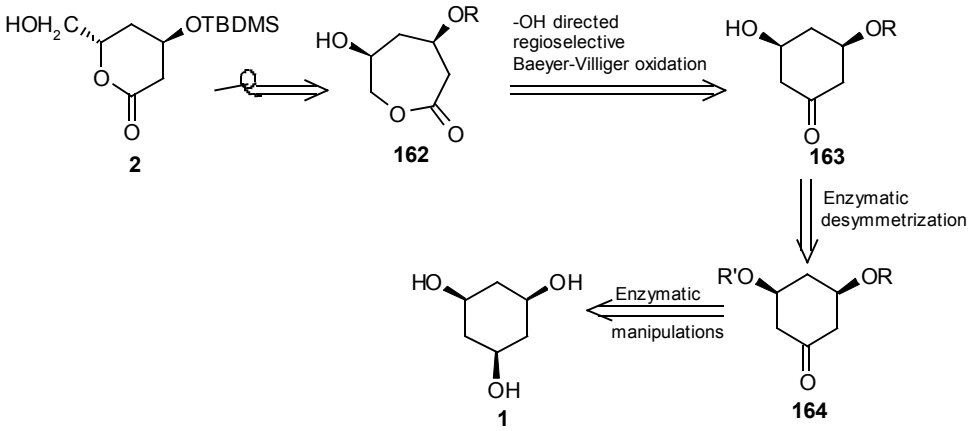
159 afforded tosyl derivatives, **160** and **161**, which could be separated by flash chromatography. Thus pure lactone **2** was obtained from **152** in 2% overall yield.



Scheme 4.23.

4.2.3. Present Work and Discussion

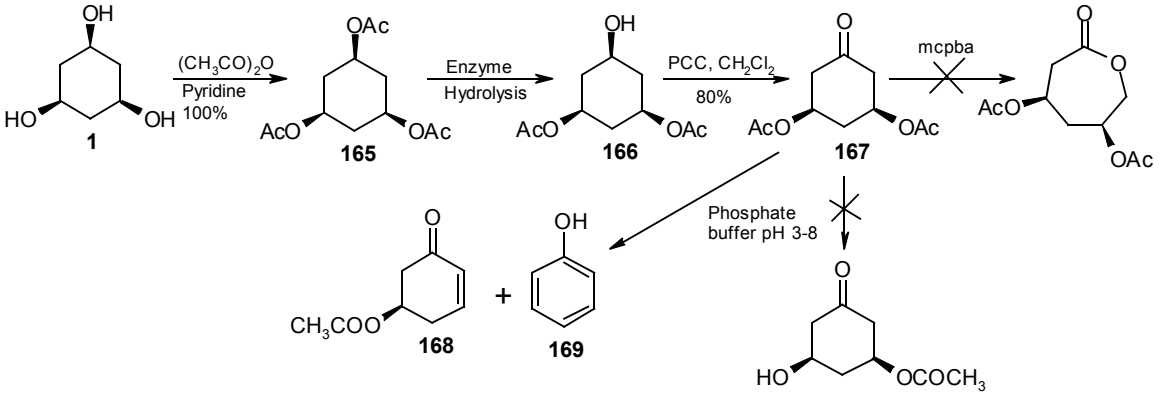
We planned to develop chemoenzymatic route for the synthesis of lactone **2** using phloroglucitol as starting material. A retrosynthetic plan is shown in Scheme 4.24.



Scheme 4.24.

Thus our retrosynthetic plan exploits the all *cis*-geometry of hydroxy groups in phloroglucitol **1** to prepare 1,4-*cis*-disubstituted *meso*-cyclohexanone derivative through enzymatic assistance. *Meso*-compound **164** can be desymmetrized enzymatically to afford optically pure monohydroxyketone **163**, which is proposed to undergo -OH directed regioselective Baeyer-Villiger oxidation to give seven-membered lactone **162** which will readily undergo rearrangement to furnish optically pure six-membered lactone **2**.

As per the above plan following enzyme-assisted sequence was attempted (Scheme 4.25).



Scheme 4.25.

It is tedious to prepare diacetate **166** selectively through direct acylation by chemical methods.[1c] Even using limited quantities of acyl donor (acetic anhydride/acetyl chloride in pyridine) it was not possible to control acylation because mono/di acylated derivatives are competing nucleophiles for acylating agent, thus yielding mixture of monoacetyl, diacetyl and triacetyl derivatives as end product. Therefore we planned to exploit enzyme selectivity to prepare selectively diacetyl derivative **166**. There is only one report for enzymatic preparation of diacetate **166** from **1** wherein lipase-catalyzed transesterification of **1** using vinyl acetate neatly stops at diacetate stage affording **166** in 78% yield.[33] But, it is a very lengthy procedure, requires 10 days at 35°C. Therefore we planned to attempt an alternative enzymatic protocol.

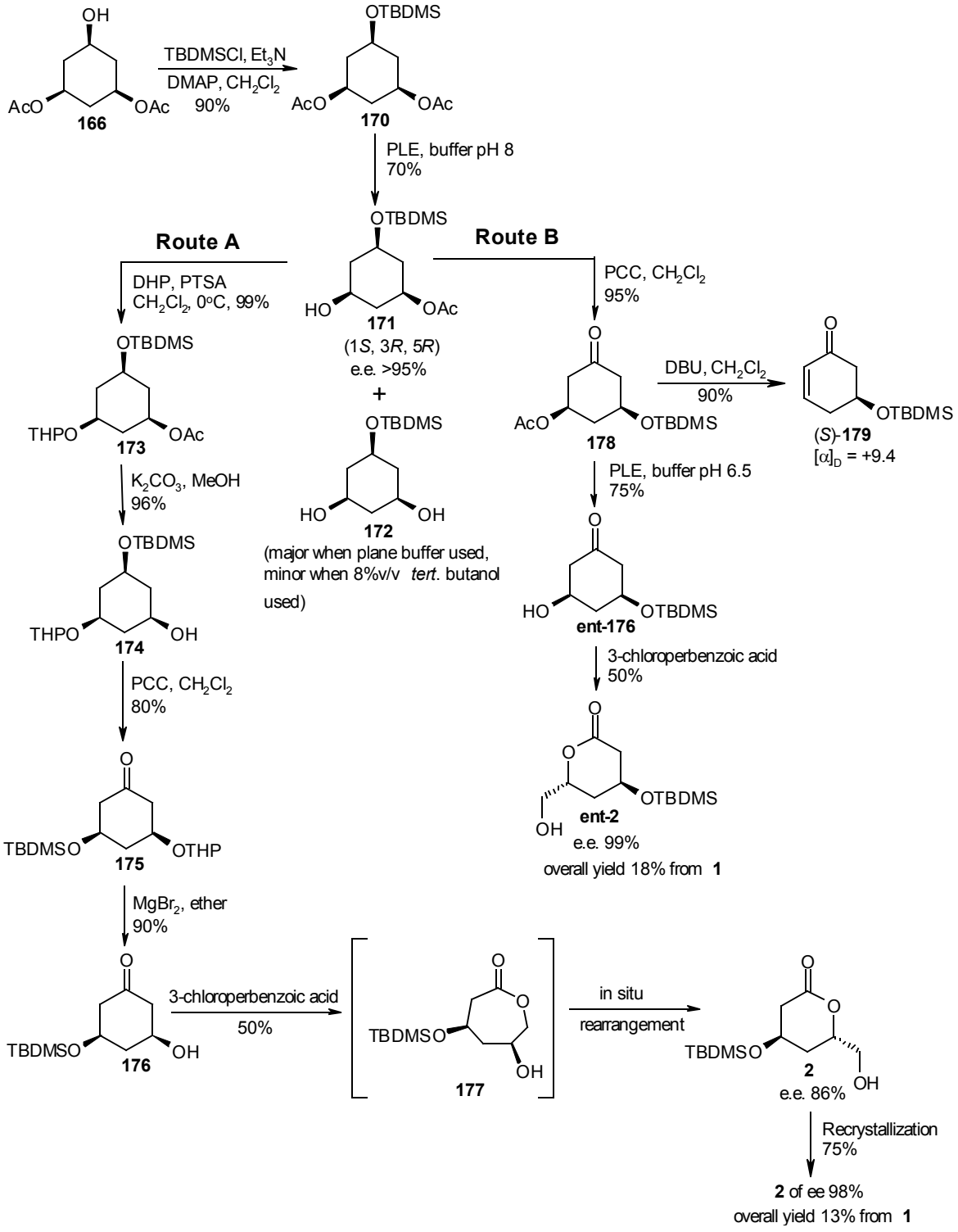
Triacetate **165** was prepared from **1** in quantitative yields by treatment with acetic anhydride/pyridine. Triacetate **165** was subjected to enzymatic hydrolysis using few commercially available lipases as PPL, PLE, Lipozyme®, PLAP, CLAP. Among these, PLE gave very good results; hydrolysis stopped neatly at diacetate stage and pure diacetate **166** could be isolated in 90% yield. Reaction was complete within 24 hr, thus desired diacetate **166** could be prepared in higher yield from **1** within 48 hr as compared to reported 10 days procedure.

Diacetate **166** was oxidized using PCC to afford *meso*-1,4-diacetoxycyclohexanone isolated as crystalline solid in 80% yield. *Meso*-**167** was subjected to enzymatic hydrolysis, but β-elimination was found to be major reaction at pH 7. To suppress β-elimination, we used acidic conditions for enzymatic hydrolysis using lipases active at acidic pH, but elimination was still the major reaction. Therefore, we had to abandon the scheme. It compelled us to design a system devoid of β-ketoacetate functionality as shown in Scheme 4.26.

Free –OH in diacetate **166** was protected as TBDMS ether to afford *meso*-diacetate **170**. *Meso*-diacetate **170** was subjected to enzymatic hydrolysis at pH 7 using few commercial enzymes viz. PPL, PLE, PLAP, CLAP and Chirazyme®. Only PPL and PLE were able to accept compound **170** as substrate for hydrolysis. PPL hydrolyzed diacetate slowly to afford optically active **171** in 50% yield whereas hydrolysis with PLE was very fast mainly affording diol **172**. We tried to scale up the desymmetrization process using PPL, but it was not reproducible at larger scale. Large amount of enzyme was required for hydrolysis and results were inconsistent. We tried to improve the results through medium engineering approach, but no improvement could be achieved.

It is reported that activity and selectivity of PLE can be improved considerably using medium engineering approach.[34] We carried out few probe experiments using PLE as catalyst for

hydrolysis of **170** in phosphate buffer media containing 10% of co-solvent (Table 4.1). Among various co-solvents tested, *tert*.-butanol was found to be most effective as a co-solvent in controlling hydrolysis of **170** catalyzed by PLE. Thus, optically active **171** could be obtained in 80% yield using *tert*.-butanol as cosolvent in 8% v/v at pH 7 (compared to 70% yield with higher e.e. at pH 8, *vide infra*). The process could be scaled up to 10g scale.



Scheme 4.26.

Table 4.1. Medium engineering for the hydrolysis of **170** using enzyme PLE[a]

10.[d]	***t*-Butanol**	**8**	**4**	**87**	**0**	**25**

a: All the reactions were carried out in 0.1 M phosphate buffer (pH 7) with specified amount of co-solvent using 2% substrate concentration. b: Yields are based on consumed starting material. c: e.e. = 90%, d: at pH 8, e.e. = 96%

Amount of co-solvent	%Time hr	Yield of **171**	% Yield of diol **172**

Since compound **171** is not reported next important tasks were (i) to assign absolute configuration to **171** (ii) to determine e.e. of **171**.

Absolute configuration could be determined by correlation method. Compound **171** was converted to enone **179** (Scheme 4.26), absolute configuration and rotation of which is known.[35a] The $[\alpha]_D$ of **179** was found to be +9.4 corresponding to *S*-configuration. Thus, by correlation absolute configuration of **171** was assigned as (1*S*, 3*R*, 5*R*). Very recently, chemoenzymatic synthesis of **ent-171** was reported.[35b] Its $[\alpha]_D$ value was reported as +5 (c = 1, chloroform) e.e. = 99% whereas $[\alpha]_D$ of **171** was found to be –4.8 (c = 1, chloroform) e.e. = 96%. Thus, configuration of **171** is further confirmed.

It was essential to obtain racemic sample of **171** to follow any method of e.e. determination. It was prepared by controlled methanolysis of diacetate **170** using limited quantity of methanol in THF using K_2CO_3 as catalyst. We could obtain racemic **171** in 75% yield from **170** using this method. Following methods were attempted for the determination of e.e..

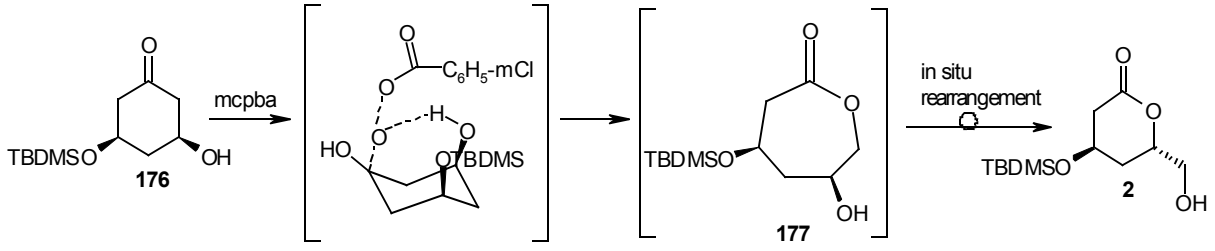
NMR chiral shift reagent: Tris[3-(triflоromethylhydroxymethylne)-(+)-camphorato]$_3$ europium (II) was used as chiral shift reagent and its effect on the NMR spectra of racemic as well optically enriched **171** was studied. Few overlapping peaks in the spectra were shifted and resolved, but separation of enantiomeric peaks was not observed at various concentrations of shift reagent used. Therefore it was not useful.

Mosher ester method: Mosher ester of racemic as well as optically enriched **171** was prepared by treating with (*S*)-Mosher acid chloride and pyridine in dichloromethane. The ^{1}H spectra of both Mosher esters were studied. Peak of –OCOCH$_3$ resolved partially, but separation was not up to the baseline; therefore was not useful for analytical purpose. ^{19}F spectra could not be obtained due to some technical reasons. Therefore we decided to study resolution of diastereomeric Mosher esters by chiral HPLC method. On achiral stationary phases used we could not achieve resolution. Few chiral stationary phases were attempted. Satisfactory resolution was achieved on Lichrocart (*R*, *R*) Whelk-O 1 5μm column. By comparison of peak areas e.e. of **171** was determined to be 90%.

Further we optimized the desymmetrization process at pH 8 to obtain compound **171** of >95% e.e. in 70% yield (see page no. 158 for chiral HPLC chart).

Compound **171** of >95% e.e. was subjected to further chemical transformations as shown in route A of Scheme 4.26. Free –OH in **171** was protected as THP ether **173** at low temperature, which on solvolysis in methanol afforded cylcohexanol **174**. PCC oxidation of **174** afforded 3,5-disubstituted cyclohexanone **175** in 80% yield. Now, selective deprotection of –OTHP group was carried out to obtain desired hydroxyketone **176**. Treatment of **175** with PPTS in methanol afforded –OTBDMS deprotected ketal as major product! Therefore, we used anhydrous $MgBr_2$.etherate in dry ether, which is reported to be selective for -OTHP deprotection in the presence of –OTBDMS group.[36] The reaction was smooth; afforded desired hydroxycyclohexanone **176** in 90% yield.

The next important step was regioselective Bayer-Villiger oxidation of **176** to give lactone **177**. Here high regioselectivity was postulated through directive effect of free hydroxy group. It was assumed that free –OH would bind with the oxidizing agent during transition state, thus would assist delivery of oxygen from the side of the ring holding –OH, affording exclusively single regioisomer (Scheme 4.27).



Scheme 4.27.

We subjected cyclohexanone **176** to Baeyer-Villiger oxidation under standard conditions using 3-chloroperbenzoic acid in dichloromethane for several hours, but the substrate **176** was found to be unreactive under these conditions. Various reaction conditions were attempted (Table 4.2).

Table 4.2. **Various reaction conditions attempted for BV-oxidation**

No.Reaction conditionResults1.3-chloroperbenzoic acid, CH_2Cl_2, RTNo reaction2.3-chloroperbenzoic acid, CH_2Cl_2, $NaHCO_3$, RTNo reaction3.3-chloroperbenzoic acid, CH_2Cl_2, $NaHCO_3$, refluxNo reaction4.Trifluoroethanol, 50% H_2O_2Decomposition5.Trifluoroacetic acid, 50% H_2O_2Decomposition6.Peracetic acidDecomposition7.Permaleic acid (in situ)Decomposition8.30% H_2O_2, NaOHDecomposition9. 30% H_2O_2, $NaHCO_3$Decomposition10.*t*-butylhydroperoxide (TBHP)No reaction11.$Ti(OiPr)_4$, diethyl tartarate, TBHPNo reaction12.3-chloroperbenzoic acid, $NaHCO_3$No reaction**133-chloroperbenzoic acid (neat)45% lactone**

Hydroxyketone **176** was either unreactive or unstable under conditions attempted. Fortunately, it reacted with 3-chloroperbenzoic acid under neat conditions to afford lactone in 45% yield. To our delight, ^{1}H NMR, ^{13}C NMR and ^{1}H NOE spectrum (for NOE data see page no. 147) matched exactly with that of 6 membered lactone **2** previously reported by some other method.[31a] Other regioisomer i.e. lactone arising from the delivery of the oxygen from the side opposite to that having free –OH group could not be detected by NMR of crude reaction mixture. Thus our speculation of free –OH directed regioselective Bayer-Villiger oxidation was found to be correct and the corresponding seven membered lactone was found to undergo rearrangement to thermodynamically more stable six membered lactone **2** under reaction conditions.

Optical rotation of the lactone obtained was found to be +3 (c = 1, $CHCl_3$) whereas reported value is –7 (c = 1, $CHCl_3$).[31a] To clear the ambiguity, which perhaps arose out of impurity in the sample/s or inadequate purification methods employed, we prepared tosyl derivative of **2**. The rotation of tosyl derivative was found to + 7 (c = 2, $CHCl_3$) which is in agreement with the reported value of +5 (c = 1, $CHCl_3$). Thus our configuration assignment was found to be correct. Enatiomeric excess of the lactone **2** was determined by the chiral HPLC of its benzoate [Lichrocart (*R*, *R*) Whelk-O 1 5μm column, see page 159]. It was determined to be 86%. There could have been epimerization during THP deprotection stage, resulting in deterioration of e.e.. We attempted to improve the e.e. by crystallization. Lactone **2** was recrystallized from pet.ether + $CHCl_3$ mixture (yield 75%). The e.e. of recrystallized lactone was found to be 98% by chiral HPLC method (see page no. 159) whereas its specific rotation was found to be +1.9 (c = 1, $CHCl_3$)! There could have been some diastereomeric impurity responsible for higher rotation value before recrystallization, which was removed during recrystallization. Thus, synthesis of enantiopure lactone **2** was achieved in overall 13% yield from **1**.

Since we encountered problem of epimerization in above discussed scheme, we decided to develop synthesis of **ent-2** starting from same intermediate **171** wherein we can bypass protection-deprotection sequence and avoid epimerization. As shown in route B of Scheme 4.26, compound **171** was oxidized efficiently with PCC to afford β-ketoacetate **178**. Now a mild hydrolytic condition for hydrolysis of **178** was required to avoid β-elimination. It could not be achieved by chemical methods e.g. methanol-K_2CO_3, methanol-NH_3 etc. Therefore we decided to attempt enzymatic hydrolysis under neutral pH condition. We attempted hydrolysis of **178** using PLE in phosphate buffer media of pH 7. When hydrolysis was carried out in buffer media containing 10% DMSO, we could isolate desired hydroxyketone **ent-176** in 50% yield. Its optical rotation was found to be –22.5 (c = 1, $CHCl_3$) whereas that of **176** obtained in route A was only +16.8 (c = 1, $CHCl_3$). It indicates that enzymatic hydrolysis is not only mild enough to minimize β-elimination and avoid epimerization, there is likely kinetic resolution of **178**, further enhancing e.e. of it. This assumption was confirmed by carrying out hydrolysis of racemic **178** under same reaction conditions wherein we could isolate **178** showing optical rotation of -20 (c = 1, $CHCl_3$). To improve the yields of reaction we attempted hydrolysis under slightly acidic conditions of pH 6.5

whereby β-elimination can be further suppressed. We could isolate **178** of same enantiopuirty as indicated by its optical **rotation**, in 75% yield by carrying out enzymatic hydrolysis at pH 6.5. **Ent-178** reacted with 3-chloroperbenzoic acid under neat conditions to afford lactone **ent-2** in 45% isolated yield. Optical rotation of **ent-2** was found to –1.96 (c = 1, $CHCl_3$) and its e.e. was found to be 99% by chiral HPLC method (see page no. 160). The structure of **ent-2** was also elucidated by single crystal X-ray analysis (see page 156 & 157). As expected, **ent-2** has a six-membered flattened chair conformation with substituents at 4 and 5- position trans disposed to each other. Thus synthesis of **ent-2** was accomplished from phloroglucitol **1** in 18% overall yield.

4.2.4. Conclusion

*Thus we could successfully achieve chemoenzymatic synthesis of both enantiomers of lactone **2** in good yields and very high optically purity. Following are the salient features of the syntheses:*
All the steps are simple and easy to scale up
No extreme reaction conditions are involved.
Synthesis starts from achiral staring material, which is easily available.
High regioselectivity is demonstrated, thus avoiding tedious separation of isomers.
Products are obtained in very high enantiopurity >98%

We hope that addressing on some of the key cost factors like cost of enzyme, cost of oxidizing agent, protecting groups etc. would lead to industrially applicable process. The desired optimization would include following considerations:

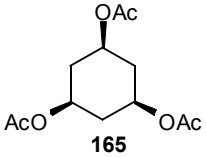
Screening of microbial culture from NCIM to replace PLE, which is a major cost center.
*Screening of a culture with opposite enantioselectivity so that **ent-171** can be obtained which can be converted to lactone **2** by route B, which is more efficient.*
To change the TBDMS protection which is also a major cost center with cheaper benzyl or some other suitable protection.
To improve yields of Baeyer-Villiger oxidation
To replace 3-chloroperbenzoic acid with some other non-hazardous, commercial oxidizing agent as magnesium monoperphthalate.[37]

4.2.5. Experimental Section

General

All the reagents were purchased from *Aldrich* and were used without further purification. NMR spectra were recorded on *Brucker NMR* (200 MHz) spectrometer. IR spectra were recorded on *Research Series FTIR* spectrometer. Optical rotations were recorded on a *Jasco Dip-181* and *Jasco P-1020* polarimeter using sodium vapor lamp. Enantiomeric excess (e.e.) were determined by comparing the specific rotation value $[\alpha]_D$ with the literature value. PPL and PLE were purchased from *Sigma*.

Preparation of cis, cis-3,5-di(methylcarbonyloxy)cyclohexylacetate ***(165)***



165

Phloroglucitol (dried at 110°C to remove water of crystallization, 6.6g, 50 mmol) was mixed with pyridine in a 100 ml two necked round bottom flask fitted with pressure equalizing dropping funnel and calcium chloride guard tube. Mixture was cooled to 0°C in ice-salt mixture. To the cold, stirred mixture, acetic anhydride (17.34g, 0.17 mole) was added dropwise while maintaining temperature below 0°C. After the addition was over, reaction mixture was stirred at RT for 10 hr. Then it was cooled to 0°C in ice-salt mixture and reaction was quenched by adding slowly ice-cold dil. HCl. Mixture was taken up in separating funnel and was extracted with ethyl acetate (3 x 50 ml). Organic extracts were mixed and washed several times with cold, dilute HCl till free from pyridine, followed by aqueous $NaHCO_3$ wash and final brine wash. Organic layer was then dried on anhydrous sodium sulphate and solvent was evaporated under vacuum. Residue was crushed and dried under high vacuum to afford pure, crystalline *cis*, *cis*-3,5-di(methylcarbonyl- oxy)cyclohexylacetate (**165**, yield 12.9g, 100%, m.p. 78°C)

^{1}H NMR($CDCl_3$): δ 1.33-1.51 (dd, 3H), 2.03 (s, 9H), 2.33 (m, 3H), 4.79 (m, 3H)

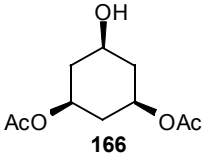
^{13}C NMR ($CDCl_3$): δ 21, 36, 67, 169

IR (KBr): cm^{-1} 759.04, 1022.91, 1235.12, 1436.33, 1739.71, 2094.56, 2252.00, 2873.59, 2946.00

Mass: Base m/e = 139, 96 other m/e: 238,198, 156, 78, 67, 61

Elemental analysis: calculated for $C_{12}H_{18}O_6$ C 55.81%, H 6.98% Found C 55.53%, H 7.01%

Preparation of cis, cis-3-hydroxy-5-methylcarbonyloxycyclohexylacetate ***(166)***



166

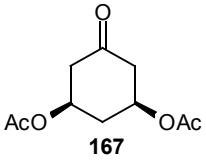
Finely powdered *cis*, *cis*-3,5-di(methylcarbonyloxy)cyclohexylacetate **165** (6.5g, 25.19 mmol) was suspended in 0.1 M sodium phosphate buffer (pH 7) (135 ml) and stirred vigorously. To the stirred suspension Porcine Liver Esterase (0.110g, 1760 units) was added and reaction mixture was stirred vigorously at 30°C for 12 hr. pH of the reaction mixture was monitored at every 2 hr and was maintained at pH 7 using 1N NaOH solution. After completion of reaction (12 hr), it was extracted with ethyl acetate (3 x 150 ml). Organic layers were combined and washed with brine, dried on anhydrous sodium sulphate and concentrated under vacuum to yield *cis*, *cis*-3-hydroxy-5-methylcarbonyloxycyclohexylacetate (**166**, yield 4.92g, 90%) as a semisolid.

^{1}H NMR($CDCl_3$): δ 1.43 (qn, 3H), 2.01 (s, 6H), 2.24 (m, 3H), 2.71 (bs, 1H), 3.72 (m, 1H), 4.72 (m, 2H)

^{13}C NMR (CDCl3): δ 20.76, 35.99, 39.59, 64.83, 67.31, 170.04

IR (KBr): cm^{-1} 754.60, 884.15, 1029.29, 1140.34, 1250.00, 1367.64, 1738.92, 2871.17, 2953.14, 3445.47
Mass: Base m/e = 96 other m/e: 156, 138, 114, 73, 67, 67, 60, 55
Elemental analysis: calculated for $C_{10}H_{16}O_5$: C 55.56%, H 7.40% Found C 55.30%, H 7.58%

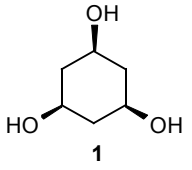
*Preparation of cis-3,5-diacetoxycyclohexanone **(167)***



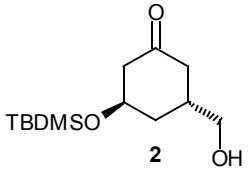
167

Cis, cis-3-hydroxy-5-methylcarbonyloxycyclohexylacetate (**166**, 4.5g, 20.83 mmol) was dissolved in dichloromethane (25 ml). To the solution, anhydrous sodium acetate (0.1g) and pyridinium chlorochromate (6.74g, 31.24 mmol) were added in one portion. Reaction mixture was stirred at RT for 2 hr. It was then diluted with ether (25 ml) and stirred for few minutes. Allowed to settle and supernatant was decanted out. Residue was extracted with ether (3x20 ml). Decanted supernatant and all organic extracts were combined and washed with 1:1 brine + water mixture (3 x 25 ml) followed by brine. Organic layer was dried on anhydrous sodium sulphate and solvent was evaporated under vacuum. Residue was recrystallized from ethyl acetate-pet. ether mixture to afford *cis*-3,5-diacetoxycyclohexanone (**167**, 3.8g, yield 85%) as crystalline needles.
^{1}H NMR ($CDCl_3$): δ 2.08 (s, 6H), 2.18 (q, 2H), 2.50 (dd, 2H), 2.72 (dd, 2H), 5.35 (qn, 2H)
^{13}C NMR (CD_Cl3)δ (20.96, 33.88, 45.66, 68.05, 169.76, 204.10
IR (CH_Cl3): $^{cm-}$1 666.79, 1025.89, 1074.95, 1242.05, 1424.10, 1731.71, 3022.39
Mass: Base m/e = 69, 154 other m/e: 215, 186, 172, 154, 137, 126, 112, 94, 84, 69, 65, 60, 55
Elemental analysis: calculated for $_C1_{0H}1_4$O3: C 56.07%, H 6.54% Found C 56.1%, 6.69%

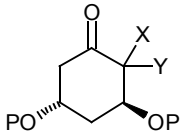
*Attempted desymmetrization of cis-3,5-diacetoxycyclohexanone (**1**67) through enzymatic*



1



2

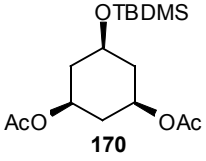


3 X, Y = CH_2
4 X = Y = H

hydrolysi §

*C*is-3,5-diacetoxycyclohexanone (**1**67, 0.10g, 0.467 mmol) was added to 0.1 M phosphate buffer (pH 7-3, 5 ml). To the mixture, enzyme (0.01g) was added and reaction mixture was stirred magnetically. Reaction was monitored periodically by TLC. After few minutes, few spotsere observed on TLC apart from that of starting material. Within a hour, starting material disappeared. Reaction was worked up by extraction with ethyl acetate (3 x 5 ml). Isolated spots mainly consisted of eliminated compounds, viz. phenol and 5-oxo-3-cyclohexenylacetate (**168**). Compound **168** has following ^{1}H NMR data.
^{1}H NMR($CDCl_3$): δ 2.08 (s, 3H), 2.45-2.85 (m, 4H), 5.38 (m, 1H), 6.10 (dm, 1H), 6.89 (m, 1H)

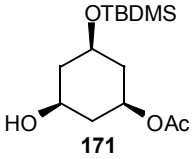
*Preparation of cis, cis-3-(methylcarbonyloxy)-5-(tert-butyldimethylsilyloxy)cyclohexyl acetate **(170)***



170

Cis, *cis*-3-hydroxy-5-methylcarbonyloxycyclohexylacetate (**166**, 2g, 9.26 mmol) and DMAP (0.113g, 0.926 mmol) were placed in 100 ml two-necked round bottom flask equipped with dropping funnel and two-way stopcock. It was evacuated and flushed with argon. To it, dry dichloromethane (10 ml) and dry HMPA (2 ml) was added and stirred to dissolve. The solution was cooled to -10°C with stirring. To it, solution of *tert*-butyldimethylsilyl chloride (1.96g, 13 mmol) in 5 ml dry dichloromethane was added dropwise while maintaining temperature below 0°C. Reaction mixture was stirred for 15 min and to it dry triethylamine (2.02g, 20 mmol) was added dropwise. Reaction mixture was stirred at room temperature for 12 hr. It was then transferred to a separating funnel and washed successively with cold, dil. HCl water, aq. $NaHCO_3$ and then brine. Organic layer was dried on anhydrous sodium sulphate and solvent was removed under vacuum. Residue was purified by flash column chromatography (eluent 2-4% ethyl acetate in petroleum ether) to yield *cis*, *cis*-3-(methylcarbonyloxy)-5-(*tert*-butyldimethylsilyloxy)cyclohexylacetate (**170,** yield 2.85g, 90%) as an oily liquid.
^{1}H NMR ($CDCl_3$): δ 0.06(s, 9H), 0.87 (s, 6H), 1.20-1.45 (m, 3H), 2.04 (s, 6H), 2.2 (m, 3H), 3.69 (m, 1H), 4.73 (m, 2H).
^{13}C NMR ($CDCl_3$): δ -5.07, 17.61, 20.73, 25.40, 36.17, 40.40, 65.43, 67.01, 169.64
IR ($CHCl_3$): cm^{-1} 758.90, 838.05, 1034.91, 1106.32, 1246.82, 1368.91, 1734.01, 2858.81, 2955.07
Mass: Base m/e = 117 other m/e: 273, 213, 171, 159, 129, 117, 97, 79, 75, 57
Elemental analysis: calculated for $C_{16}H_{30}O_5Si$: C 58.18%, H 9.10% Found C 58.19%, H 9.50%

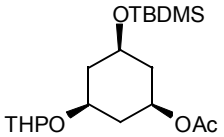
Preparation of 3-hydroxy-5-tert-butyldimethylsilyloxy-(1S,3R,5R)-cyclohexylacetate ***(171)***



171

Cis, *cis*-3-(methylcarbonyloxy)-5-(*tert*-butyldimethylsilyloxy)cyclohexylacetate (**170**, 8g, 242 mmol) was dissolved in *tert*-butanol (32 ml). To the solution, 0.1 M sodium phosphate buffer (368 ml, pH 8) was added and mixture was stirred vigorously. To the stirred emulsion, Pig Liver Esterase (0.30g, 4800 units) was added and the mixture was stirred vigorously at 30°C for 48 hr. During reaction pH was maintained at 8 using 1N sodium hydroxide solution. Reaction mixture was extracted with ethyl acetate (3 x 300 ml). Organic layers were combined and washed with brine. It was then dried on anhydrous sodium sulphate and solvent was removed under vacuum. Oily residue contained 3-hydroxy-5-*tert*-butyldimethylsilyloxy-(1*S*,3*R*,5*R*)-cyclohexylacetate **171** along with unreacted **170** and over hydrolyzed product **172**. These were separated by flash column chromatography. *Cis*, *cis*-3-(methylcarbonyloxy)-5-(*tert*-butyldimethylsilyloxy)cyclohexyl acetate recovered: 2.04g; 3-hydroxy-5-*tert*.butyldimethylsilyloxy-(1*S*,3*R*,5*R*)-cyclohexylacetate as a viscous liquid (**171**, yield 3.7g, 70% based on recovered starting material).
^{1}H NMR ($CDCl_3$): δ 0.06 (s, 9H), 0.87 (s, 6H), 1.35-1.60, (m, 3H), 2.05 (s, 3H), 2.14 (m, 3H), 3.74 (m, 2H), 4.77 (m, 1H)
^{13}C NMR ($CDCl_3$): -4.99, 17.68, 20.89, 25.49, 39.66, 40.11, 43.71, 64.80, 65.96, 67.83, 170.16
IR ($CHCl_3$): cm^{-1} 758.43, 838.93, 1049.42, 1109.15, 1218.09, 1254.01, 1370.09, 1725.03, 2859.8, 2887.95, 2952.33, 3017.48
Mass: Base m/e = 75 other m/e: 231, 171, 129, 117, 105, 97, 79, 75, 67, 59
Elemental analysis: calculated for $C_{14}H_{28}O_4Si$: C 58.33%, H 9.72% Found C 58.15%, H 9.95%
Specific rotation $[\alpha]_D$ = -4.8 (c = 1, $CHCl_3$) e.e. = 96.4% (determined by chiral HPLC of corresponding Mosher ester. Column: Whelk-O1 [4.0 mm Id x 25 cm] AT-256; λ =254 nm, flow rate: 1 ml/min; mobile phase: Hexane:isopropanol 98:02; retention time for Mosher ester of **171** = 4.59, for Mosher ester of **ent-171** = 4.34, see page No. 158 for HPLC chart).

Preparation of 3-tetrahydro-2H-2-pyranyloxy-5-tert-butyldimethylsilyloxy-(1S,3R,5R)-cyclohexylacetate ***(173)***



173

3-Hydroxy-5-*tert*-butyldimethylsilyloxy-(1*S*,3*R*,5*R*)-cyclohexylacetate (**171**, 2.9g, 9.73 mmol) was dissolved in dry dichloromethane (30 ml). The solution was cooled below 0°C in ice-salt bath. To the stirred solution, dihydropyran (1g, 12 mmol) was added and p-toluenesulfonic acid monohydrate (0.1g) was added as catalyst. Reaction mixture was stirred at -10°C for 2 hr. Reaction was quenched by adding aqueous sodium bicarbonate solution. Both the layers were separated. Aqueous layer was extracted with dichloromethane (2 x 10ml). Organic layers were combined and washed with water followed by brine. It was then dried on anhydrous sodium sulphate and solvent was removed under vacuum. Residue was purified by flash column chromatography to yield 3-tetrahydro-2*H*-2-pyranyloxy-5-*tert*-butyldimethylsilyloxy-(1*S*,3*R*,5*R*)-cyclohexylacetate **173** (yield 3.7g, 99.5%) as an oily liquid.

^{1}H NMR ($CDCl_3$): δ 0.05 (s, 6H), 0.87 (s, 9H), 1.25-1.90 (m, 9H), 2.04 (s, 3H), 2.05-2.40 (m, 3H), 3.40-3.75 (m, 3H), 3.86 (m, 1H), 4.55-4.80 (m, 2H)

^{13}C NMR ($CDCl_3$): δ -4.90, 18.00, 19.40, 19.50, 25.20, 25.80, 30.90, 36.80, 40.70, 41.00, 42.80, 62.00, 62.50, 66.00, 68.00, 69.70, 95.00, 96.80, 169.90

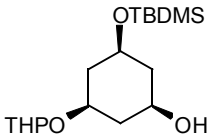
IR ($CHCl_3$): cm^{-1} 752.08, 768.22, 838.35, 1029.76, 1114.56, 1215.63, 1251.94, 1727.19, 2858.86, 2950.80, 3016.74

Mass: Base m/e = 85 other m/e: 231, 211, 171, 159, 129, 117, 105, 101, 85, 79, 75, 67, 55

Elemental analysis: calculated for $C_{19}H_{36}O_5Si$: C 61.29%, H 9.68% Found C 61.37%, H 10.03%

Specific rotation $[\alpha]_D$ = +1.39 (c = 1, $CHCl_3$)

Preparation of 3-tetrahydro-2H-2-pyranyloxy-5-tert-butyldimethylsilyloxy-(1S,3R,5S)-cyclohexan-1-ol ***(174)***



174

3-Tetrahydro-2*H*-2-pyranyloxy-5-*tert*-butyldimethylsilyloxy-(1*S*,3*R*,5*R*)-cyclohexylacetate (**173**, 3.5g, 9.16 mmol) was dissolved in dry methanol (25 ml). To the solution anhydrous potassium carbonate (0.828g, 6 mmol) was added and the mixture was stirred at room temperature for 2 hr. Methanol was removed under vacuum and residue was extracted with dichloromethane (3 x 10ml). Dichloromethane layers were combined and washed with water followed by brine wash. It was dried on anhydrous sodium sulphate. Solvent was removed under vacuum and residue was purified by flash column chromatography to yield 3-tetrahydro-2*H*-2-pyranyloxy-5-*tert*.butyldimethylsilyloxy-(1*S*,3*R*,5*S*)-cyclohexan-1-ol (**174**, yield 3g, 96%) as a viscous liquid.

^{1}H NMR ($CDCl_3$): δ 0.06 (s, 6H), 0.87 (s, 9H), 1.35-1.90 (m, 10H), 2.05-2.35 (m, 3H), 3.40-3.75 (m, 4H), 3.80-3.98 (m, 1H), 4.73 (s, 1H)

^{13}C NMR ($CDCl_3$): δ -4.90, 18.00, 19.50, 25.20, 25.80, 30.90, 40.00, 40.70, 42.00, 42.30, 44.70, 62.00, 62.50, 65.80, 66.90, 69.50, 70.00, 96.40, 96.80

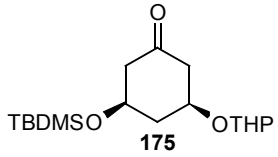
IR ($CHCl_3$): cm^{-1} 753.06, 766.83, 838.54, 867.39, 1020.84, 1048.02, 1114.23, 1215.02, 1253.72, 2858.73, 28884.47, 2947.49, 3013.80, 3418.48

Mass: Base m/e = 75 other m/e: 309, 189, 171, 129, 119, 101, 85, 79, 75, 67, 55

Elemental analysis: calculated for $C_{17}H_{34}O_4Si$: C 61.82%, H 10.30% Found C 61.83%, H 11.00%

Specific rotation $[\alpha]_D$ = +0.93 (c = 1, $CHCl_3$)

Preparation of 3-tetrahydro-2H-2-pyranyloxy-5-tert-butyldimethylsilyloxy-(3S,5R)-cyclohexan-1-one ***(175)***



175

3-Tetrahydro-2*H*-2-pyranyloxy-5-*tert*-butyldimethylsilyloxy-(1*S*,3*R*,5*S*)-cyclohexan-1-ol (**174,** 2.85 g, 8.38 mmol) was dissolved in dry dichloromethane (25 ml) under argon atmosphere. To it, anhydrous sodium acetate (0.2g) and pyridinium chlorochromate (13g, 12.6 mmol) were added in one portion and the mixture was stirred under argon atmosphere for 8 hr. Then reaction mixture was diluted with diethyl ether (30 ml) and stirred well. The solution was decanted and the remaining black tar was extracted with diethyl ether (3 x 15 ml). Organic layers were combined and were filtered through small celite bed. Then, the organic layer was washed with water (3 x 20 ml) followed by brine. It was dried on anhydrous sodium sulphate and solvent was removed under vacuum. Residue was purified by flash column chromatography. Yield of 3-tetrahydro-2*H*-2-pyranyloxy-5-*tert*-butyldimethylsilyloxy-(3*S*,5*R*)-cyclohexan-1-one (**175,** 2.24g, 80%) as an oily liquid.

^{1}H NMR ($CDCl_3$): δ 0.07 (s, 6H), 0.87 (s, 9H), 1.35-1.95 (m, 8H), 2.2-2.8 (m, 4H), 3.50 (m, 1H), 3.85 (m, 3H), 4.60 & 4.75 (2s, 1H)

^{13}C NMR ($CDCl_3$): δ -4.9, 18.0, 19.5, 19.9, 25.8, 26.0, 30.9, 41.0, 42.5, 47.0, 48.5, 51.5, 62.5, 63.0, 69.0, 69.8, 97.5, 206.5, 207.0

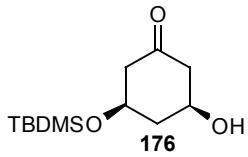
IR ($CHCl_3$): cm^{-1} 858.87, 980.49, 1028.79, 1053.00, 1254.14, 1360.70, 1377.86, 1462.85, 1717.24, 2857.60, 2892.24

Mass: Base m/e = 187, 85 other m/e: 271, 227, 169, 159, 143, 127, 95, 75, 67

Elemental analysis: calculated for $C_{17}H_{32}O_4Si$: C 62.19%, H 9.75% Found C 61.90%, H 10.10%

Specific rotation $[\alpha]_D$ = +3.71 (c = 1, $CHCl_3$)

Preparation of 3-hydroxy-5-tert-butyldimethylsilyloxy-(3S,5R)-cyclohexan-1-one ***(176)***



176

3-Tetrahydro-2*H*-2-pyranyloxy-5-*tert*-butyldimethylsilyloxy-(3*S*,5*R*)-cyclohexan-1-one (**175,** 1g, 2.96 mmol) was placed in 50 ml two-necked round bottom flask equipped with two-way stopcock and rubber septum. Flask was evacuated and flushed with argon. To it, dry ether (10 ml) was added and the resulting solution was stirred vigorously. To the stirred solution magnesium bromide etherate (2.3g, 8.9 mmol) was added and the mixture was stirred for 3 hr. The reaction mixture was cooled in ice-bath and reaction was quenched by adding saturated ammonium chloride solution. Both the layers were separated. Aqueous layer was extracted with ether (3 x 10 ml). Organic layers were combined and washed with brine. Then it was dried on anhydrous sodium sulphate and solvent was removed under vacuum. Residue was purified to yield 3-hydroxy-5-*tert*-butyldimethylsilyloxy-(3*S*,5*R*)-cyclohexan-1-one (**176,** yield 0.69g, 90%) as a viscous liquid.

^{1}H NMR ($CDCl_3$): δ 0.10 (s, 6H), 0.88 (s, 9H), 1.95-2.30 (m, 2H), 2.45-2.78 (m, 4H), 3.95 (d, 1H), 4.36 (m, 1H), 4.56 (m, 1H)

^{13}C NMR ($CDCl_3$): δ -5.50, -5.28, 17.55, 25.34, 38.24, 49.56, 49.89, 68.86, 70.48, 206.78

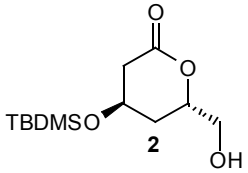
IR ($CHCl_3$): cm^{-1} 777.13, 835.81, 1010.66, 1045.71, 1095.81, 1254.55, 1381.17, 1413.71, 1464.09, 1715.72.2857.70, 2892.64, 2932.26, 2951.84, 3439.28

Mass: Base m/e = 75 other m/e: 187, 169, 145, 129, 101, 95, 75, 69, 59

Elemental analysis: calculated for $C_{12}H_{24}O_3Si$: C 59.01%, H 9.83% Found C 58.85%, H 10.18%

Specific rotation $[\alpha]_D$ = +16.20 (c = 1, $CHCl_3$)

Preparation of 6-hydroxymethyl-4-tert-butyldimethylsilyloxy-(4R,6S)-tetrahydro-2H-2-pyranone ***(2)***



2

3-Hydroxy-5-*tert*-butyldimethylsilyloxy-(3*S*,5*R*)-cyclohexan-1-one (**176**, 0.1g, 0.394 mmol) and 50% 3-chloroperbenzoic acid (0.275g, 0.79 mmol) were mixed and kept in dark for 15 hr. Reaction mixture was dissolved in ethyl acetate (5 ml) and washed successively with cold sodium metabisulfite solution, sodium bicarbonate solution followed by brine wash. It was then dried on anhydrous sodium sulphate and solvent was removed under vacuum. Residue was purified by flash column chromatography to yield white, crystalline 6-hydroxymethyl-4-*tert*-butyldimethylsilyloxy-(4*R*,6*S*)-tetrahydro-2*H*-2-pyranone (**1,** yield 0.048g, 45%, m.p. 91°C). It was recrystallized from pet. ether (2 ml) containing 5% chloroform (yield = 0.036 g, 75%, m.p. 95°C)

^{1}H NMR ($CDCl_3$): δ 0.09, 0.07 (2s, 6H), 0.89 (s, 9H), 1.64 (bs, 1H), 1.74-1.92 (m, 2H), 2.60 (d, 2H, J = 3), 3.65 (dd, 1H, J = 12), 3.88 (dd, 1H, J =12), 4.39 (m, 1H), 4.81 (m, 1H)
(see page no. 147 for N.O.E. data)

^{13}C NMR ($CDCl_3$): δ -4.93, 17.93, 25.68, 32.15, 39.26, 63.61, 64.59, 76.86, 170.10

IR ($CHCl_3$): cm^{-1} 666.31, 898.12, 1021.88, 1061.06, 1086.17, 1118.98, 1390.84, 1463.17, 1729.43, 2857.28, 3418.25

Mass: Base m/e = 75 other m/e: 260, 229, 203, 185, 161, 143, 129, 111, 101, 69, 59

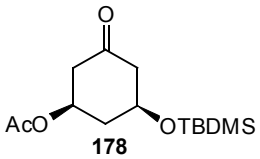
Elemental analysis: calculated for $C_{12}H_{24}O_4Si$: C 55.38%, H 9.20% Found C 55.42%, H 9.03%

Specific rotation $[\alpha]_D$ = +3.0 (c = 1, $CHCl_3$) before recrystallization e.e. 86%

$[\alpha]_D$ = +1.9 (c = 1, $CHCl_3$) after recrystallization e.e. 98%

(e.e. determined by chiral HPLC of corresponding benzoate derivative, column-Whelk-O1 [4.0 mm Id x 25 cm] AT-256; λ =254 nm, flow rate: 1 ml/min; mobile phase: Hexane:isopropanol 92:08; retention time for benzoate of **2** = 13.78, for benzoate of **ent-2** = 15.72, for HPLC chart see page no. 159).

Preparation of 3-oxo-5-tert-butyldimethylsilyloxy-(1R, 5S)-cyclohexylacetate ***(178)***



178

Cis, *cis*-3-hydroxy-5-*tert*-butyldimethylsilyloxy-(1*S*, 3*R*, 5*R*)-cyclohexylacetate (**171,** 1.40g, 2.78 mmol) was dissolved in dichloromethane (5 ml). To the solution sodium acetate (0.1g) and pyridinium chlorochromate (1.57g, 7.3 mmol) were added. The mixture was stirred for 5 hr at RT. Residue was extracted with ether (3x10 ml). Organic extracts were combined, filtered through celite. Filtrate was washed with brine + water (1:1) and finally with brine. Organic layer was dried on anhydrous sodium sulphate and concentrated under vacuum. Residue was filtered through silica gel column to afford 3-oxo-5-*tert*-butyldimethylsilyloxy-(1*R*, 5*S*)-cyclohexylacetate (**178**, 1.30 g, 94%) as an oily liquid.

^{1}H NMR ($CDCl_3$): δ 0.07 (d, 6H), 0.88 (d, 9H), 2.06 (s, 3H), 2.41 (m, 4H), 2.65 (m, 2H), 4.01 (m, 1H), 5.00 (m, 1H)

^{13}C NMR ($CDCl_3$): δ -4.96, 17.80, 20.95, 25.56, 39.32, 45.91, 50.28, 65.96, 67.21, 169.85, 205.25

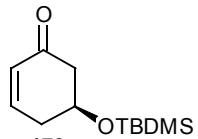
IR ($CHCl_3$): cm^{-1} 442.57, 756.83, 1218.50, 1244.99, 1723.00, 2857.44, 2932.80, 2953.64, 3019.98

Mass: Base m/e = 163 other m/e: 185, 169, 145, 127, 117, 111, 101, 95, 75, 59

Elemental analysis: calculated for $C_{14}H_{26}O_4Si$: C 58.74%, 9.09% Found C 58.60%, H 9.28%

Specific rotation $[\alpha]_D$ = -11.54 (c = 1, $CHCl_3$)

Preparation 5-tert-butyldimethylsilyloxy-(5S)-2-cyclohexenone ***(179)***



179

3-Oxo-5-*tert*-butyldimethylsilyloxy-(1*R*, 5*S*)-cyclohexylacetate (**178**, 0.30g, 1 mmol) was dissolved in dichloromethane (5 ml). To the solution, DBU (0.150g, 1mmol) was added and reaction mixture was stirred for 2 hr. Reaction was quenched by adding cold, ammonium chloride solution. Both the layers were separated. Organic layer was washed with ammonium chloride solution and was dried on anhydrous sodium sulphate. Solvent was removed under vacuum and residue was purified on silica gel column to afford 5-*tert*-butyldimethylsilyloxy-(5*S*)-2-cyclohexanone (**179**, yield 0.224g, 95%) as an oily liquid.

^{1}H NMR ($CDCl_3$): δ 0.07(s, 6H), 0.87 (s, 9H), 2.25-2.75 (m, 4H), 4.23 (m, 1H), 6.05 (dm, 1H), 6.9 (m, 1H)

^{13}C NMR ($CDCl_3$): δ -5.10, 17.62, 25.38, 35.23, 47.69, 67.32, 129.77, 146.27, 197.92

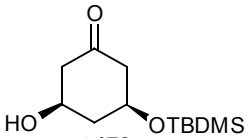
IR ($CHCl_3$): cm^{-1} 757.33, 834.08, 869.03, 1102.56, 1217.89, 1254.46, 1385.92, 1678.44, 2857.57, 2892.50, 2934.06, 2953.54, 3015

Mass: Base m/e = 74 other m/e: 211, 169, 151, 127, 110, 94, 66, 58

Elemental analysis: calculated for $C_{12}H_{22}O_2Si$: C 63.72%, H 9.73% Found C 63.65%, H 9.91%

Specific rotation $[\alpha]_D$ = +9.41 (c = 1, $CHCl_3$) Lit.[35a] $[\alpha]_D$ = +9.8 (c 1, $CHCl_3$)

*Preparation of 3-hydroxy-5-tert-butyldimethylsilyloxy-(3R, 5S)-cyclohexan-1-one (****ent-176****)*



ent-176

3-Oxo-5-*tert*-butyldimethylsilyloxy-(1*R*, 5*S*)-cyclohexylacetate (**178**, 1.2g, 4.2 mmol) was dissolved in DMSO (10 ml) and 0.1 M phosphate buffer (pH 6.5, 90 ml). To the mixture, PLE (0.1 g) was added and the reaction mixture was shaked for 48 hr. It was filtered through celite and filtrate was extracted with ethyl acetate (3 x 100 ml). Organic extracts were combined and washed with brine. Organic layer was dried on anhydrous sodium sulphate and concentrated under vacuum. Residue was chromatographed on silica gel to afford 3-hydroxy-5-*tert*-butyldimethylsilyloxy-(3*R*,5*S*)-cyclohexan-1-one (**ent-176**, 0.77 g, 75%) as viscous liquid.

^{1}H NMR ($CDCl_3$): δ 0.10 (s, 6H), 0.87 (s, 9H), 1.95-2.4 (m, 2H), 2.45-2.78 (m, 4H), 3.98 (bs, 1H), 4.39 (m, 1H), 4.58 (m, 1H)

^{13}C NMR ($CDCl_3$): δ -5.50, -5.28, 17.55, 25.34, 38.24, 49.56, 49.89, 68.86, 70.48, 206.78

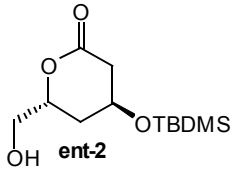
IR ($CHCl_3$): cm^{-1} 668.15, 759.18, 837.70, 1216.14, 1255.02, 1676.48, 1712.47, 2930.89, 2955.00, 3018.43, 3387.52, 3406.91

Mass: Base m/e = 75 other m/e: 187, 169, 145, 129, 101, 95, 75, 69, 59

Elemental analysis: calculated for $C_{12}H_{24}O_3Si$: C 59.01%, H 9.83% Found C 59.1%, H 9.98%

Specific rotation $[\alpha]_D$ = -22.06 (c = 1, $CHCl_3$)

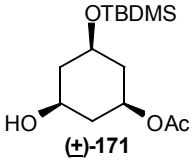
*Preparation of 6-hydroxymethyl-4-tert-butyldimethylsilyloxy-(4S,6R)-tetrahydro-2H-2-pyranone (****ent-2****)*



ent-2

3-Hydroxy-5-*tert*-butyldimethylsilyloxy-(3*R*,5*S*)-cyclohexan-1-one (**ent-176**, 0.1g, 0.394 mmol) and 50% 3-chloroperbenzoic acid (0.275g, 0.79 mmol) were mixed and kept in dark for 15 hr. Reaction mixture was dissolved in ethyl acetate (5 ml) and washed successively with cold sodium metabisulfite solution, sodium bicarbonate solution followed by brine wash. It was then dried on anhydrous sodium sulphate and solvent was removed under vacuum. Residue was purified by flash column chromatography to yield white, crystalline 6-hydroxymethyl-4-*tert*-butyldimethylsilyloxy-(4*S*,6*R*)-tetrahydro-2*H*-2-pyranone (**ent-2**, yield 0.047g, 45%). M.p. 95°C
^{1}H NMR ($CDCl_3$): δ 0.08, 0.09 (2s, 6H), 0.89 (s, 9H), 1.55-2.00(m + bs, 3H), 2.60 (d, 2H, J = 3), 3.66 (dd, 1H, J = 12), 3.90 (dd, 1H, J = 12), 4.38 (m, 1H), 4.80 (m, 1H)
^{13}C NMR ($CDCl_3$): δ -5.17, 17.66, 25.41, 31.59, 38.90, 63.24, 64.19, 76.73, 170.31
IR ($CHCl_3$): cm^{-1} 666.31, 898.12, 1021.88, 1061.06, 1086.17, 1118.98, 1390.84, 1463.17, 1729.43, 2857.28, 3418.25
Mass: Base m/e = 101 other m/e: 260, 229, 203, 185, 161, 143, 129, 111, 75, 68, 59
Elemental analysis: calculated for $C_{12}H_{24}O_4Si$: C 55.38%, H 9.20% Found C 55.40%, H 9.30%
Specific rotation $[\alpha]_D$ = -1.96 (c 1, $CHCl_3$) e.e. 99% (determined by chiral HPLC of corresponding benzoate derivative, column-Whelk-O1 [4.0 mm Id x 25 cm] AT-256; λ =254 nm, flow rate: 1 ml/min; mobile phase: Hexane:isopropanol 92:08; retention time for benzoate of **2** = 17.96, for benzoate of **ent-2** = 19.97, for HPLC chart see page no. 160).

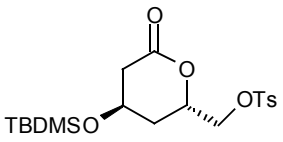
Preparation of racemic *3-hydroxy-5-tert-butyldimethylsilyloxycyclohexylacetate [(±)-171]*



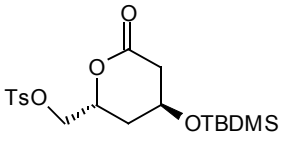
(±)-171

Cis, *cis*-3-(methylcarbonyloxy)-5-(*tert*-butyldimethylsilyloxy)cyclohexyl acetate (**170**, 1.0g mmol) was dissolved in THF (30 ml). To the solution, methanol (5 ml) and K_2CO_3 (0.250 g) was added and reaction mixture was stirred at RT for 3 hr. Reaction mixture was concentrated under vacuum. Residue was extracted with dichloromethane (3 x 5 ml). Organic extracts were combined and washed with brine and dried over anhydrous sodium sulphate. It was concentrated under vacuum. Residue was chromatographed on silica gel column to afford racemic 3-hydroxy-5-*tert*-butyldimethylsilyloxycyclohexylacetate [**(±)-171**, yield 0.65g, 84.4% based on recovered starting material] along with unreacted starting material **170** (0.25g). Spectral data for racemic **171** was same as reported for (-)-**171**.

Preparation of 6-p-toluenesulfonylmethyl-4-tert-butyldimethylsilyloxy-(4R,6S)-tetrahydro-2H-2-pyranone and its enantiomer



Tosyl of **2**



Tosyl of **ent-2**

6-hydroxymethyl-4-*tert*-butyldimethylsilyloxy-(4*R*,6*S*)-tetrahydro-2*H*-2-pyranone (**2**, 0.02g, 0.077 mmol) was dissolved in dry pyridine (0.2 ml). Solution was cooled to 0°C in ice-salt mixture and to the cold solution *p*-toluenesulfonylchloride (0.025g, 0.13 mmol) was added and reaction mixture was stirred at RT for 12 hr. Reaction mixture was diluted with ether (5 ml). Ether layer was washed with dilute hydrochloric acid followed by washing with by brine + water, aqueous. $NaHCO_3$ and finally with brine. Organic layer was dried on anhydrous sodium sulphate and

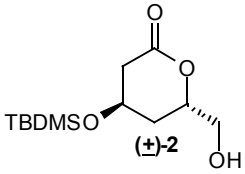
solvent was removed under vacuum. Residue was purified on silica gel column (20% ethyl acetate in pet. ether) to afford tosyl of lactone **2** (yield 0.27g). Same procedure was followed for preparation of tosyl of **ent-2**. ^{1}H NMR data for both the tosyl derivatives was identical and is as follows

^{1}H NMR ($CDCl_3$): δ 0.06, 0.07 (2s, 6H), 0.86 (s, 9H), 1.76-2 (m, 2H), 2.46 (s, 3H), 2.54 (d, 2H, J = 3), 4.18 (m, 2H), 4.36 (m, 1H), 7.36 (d, 2H, J = 12), 7.80 (d, 2H, J = 12)

Specific rotation for tosyl of **2**: $[\alpha]_D$ = +6.85 (c = 1.94, $CHCl_3$) Lit.[32] $[\alpha]_D$ = +5 (c = 0.82, $CHCl_3$)

Specific rotation for tosyl of **ent-2** $[\alpha]_D$ = -6.61 (c = 1.87, $CHCl_3$)

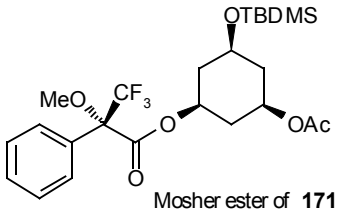
*Preparation of racemic 6-hydroxymethyl-4-tert-butyldimethylsilyloxy-tetrahydro-2H-2-pyranone **[(±)-2]***



(±)-2

Racemic 3-hydroxy-5-*tert*-butyldimethylsilyloxycyclohexylacetate**, (±)-171** was processed as mentioned earlier for the preparation of (**+**)-**2** to afford racemic sample of 6-hydroxymethyl-4-*tert*-butyldimethylsilyloxy-tetrahydro-2*H*-2-pyranone **[(±)-2]**.

Preparation of Mosher esters of racemic and optically enriched 3-hydroxy-5-tert-butyldimethylsilyloxycyclohexylacetate

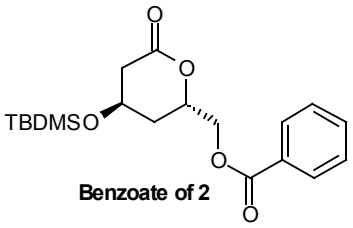


Mosher ester of **171**

3-hydroxy-5-*tert*-butyldimethylsilyloxycyclohexylacetate (**171**, 0.020g, 0.0694 mmol) was dissolved in dry dichloromethane (1 ml). To the solution pyridine (0.01g, 0.126 mmol) was added and the solution was cooled to 0^{o}C in ice-salt bath. To the cold solution, a solution of (*S*)-Mosher acid chloride (0.023g, 0.09 mmol) in dry dichloromethane (0.5 ml) was added. Reaction mixture was stirred at RT for 5 hr. Reaction was quenched by adding cold, dilute hydrochloric acid. Organic layer was separated and washed with dilute hydrochloric acid followed washing with by brine + water, aqueous. $NaHCO_3$ and finally with brine. Organic layer was dried on anhydrous sodium sulphate and solvent was removed under vacuum to afford Mosher ester of **171** (0.025 g).

^{1}H NMR ($CDCl_3$): δ 0.09 (s, 6H), 0.88 (s, 9H), 1.43 (m, 3H), 2.05, 2.07 (2s, 3H), 2.25 (m, 3H), 3.54, 3.55 (2s, 3H), 3.73 (m, 1H), 4.77 (m, 1H), 5.00 (m, 1H), 7.45 (m, 5H)

*Preparation of benzoates of lactones **2**, **ent-2**, and racemic **2**.*



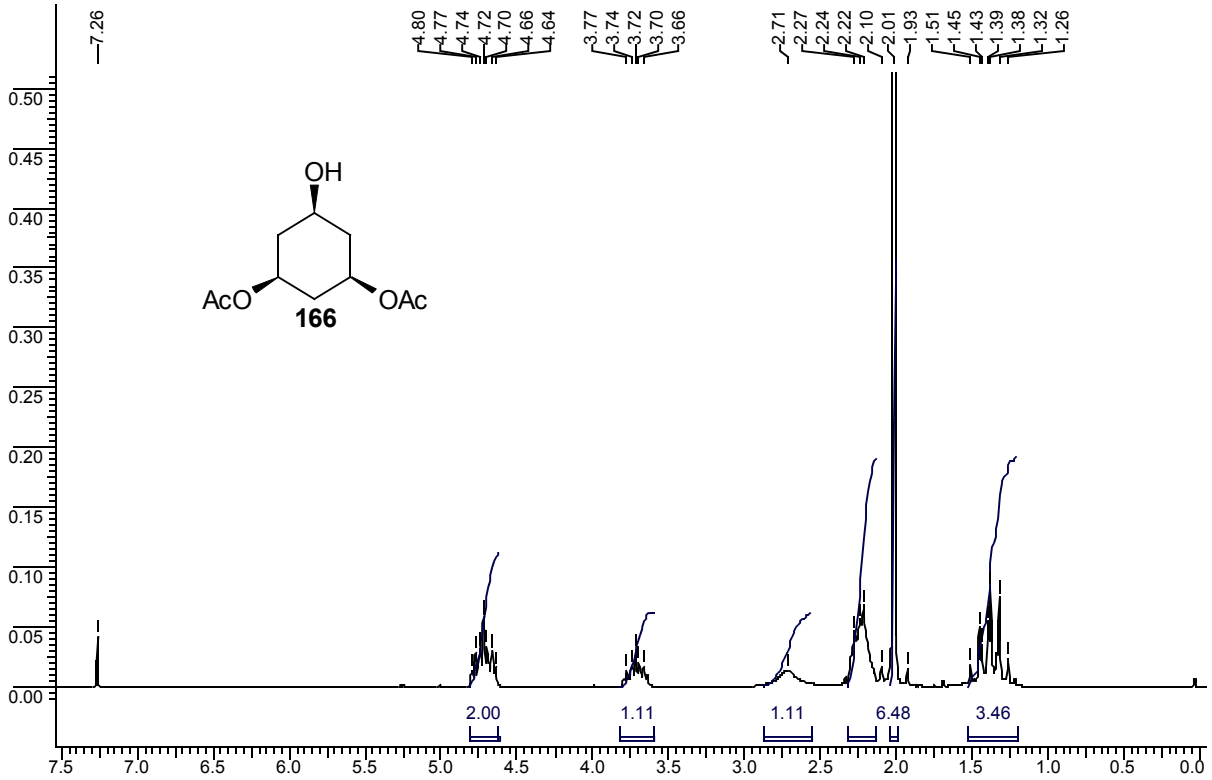
Benzoate of 2

Lactone **2** (0.01g, 0.038 mmol) was dissolved in dichloromethane (1 ml). To the solution triethylamine (0.014g, 0.02 ml, 0.138 mmol) was added and solution was cooled to 0°C in ice-salt bath. To the cold reaction mixture, solution of benzoyl chloride (0.008g, 0.057 mmol) in dry dichloromethane (0.5 ml) was added. Reaction mixture was stirred at RT for 10 hr. Reaction was quenched by adding cold, dilute hydrochloric acid. Organic layer was separated and washed with dilute hydrochloric acid followed washing with by brine + water, aqueous. $NaHCO_3$ and finally with brine. Organic layer was dried on anhydrous sodium sulphate and solvent was removed under vacuum to afford benzoate of lactone **2** (yield 0.01g). Same procedure was followed for other two lactones. ^{1}H NMR data for benzoate was as follows:

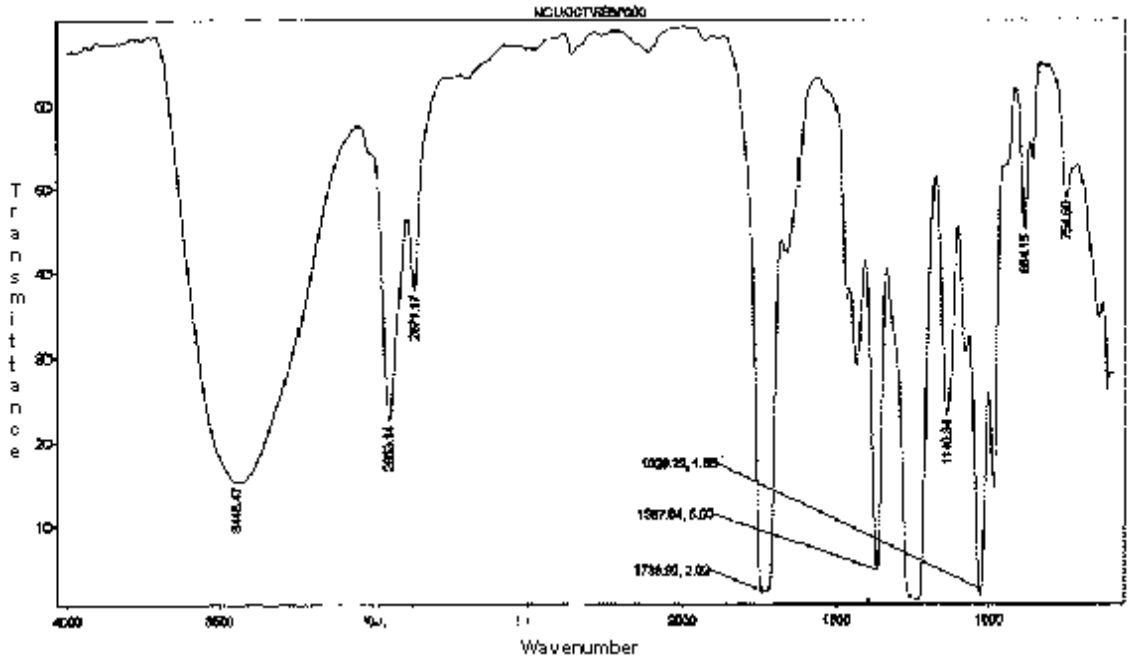
^{1}H NMR ($CDCl_3$): δ 0.07 (s, 6H), 0.90 (s, 9H), 1.93 (m, 2H), 2.64 (d, 2H), 4.40 (m, 1H), 4.53 (d, 2H), 5.00 (m, 1H), 7.45 (t, 2H), 7.59 (t, 1H), 8.06 (d, 2H)

4.2.6. Spectra

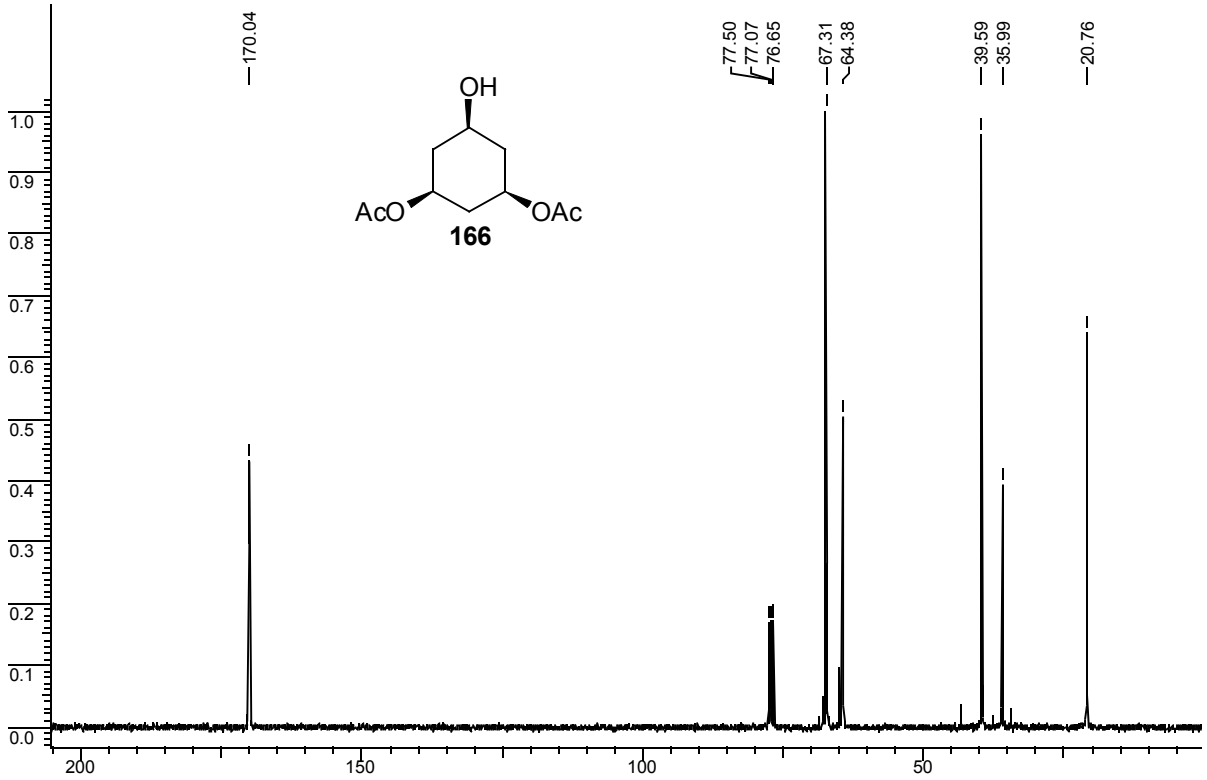
*^{1}H NMR spectrum of **166***

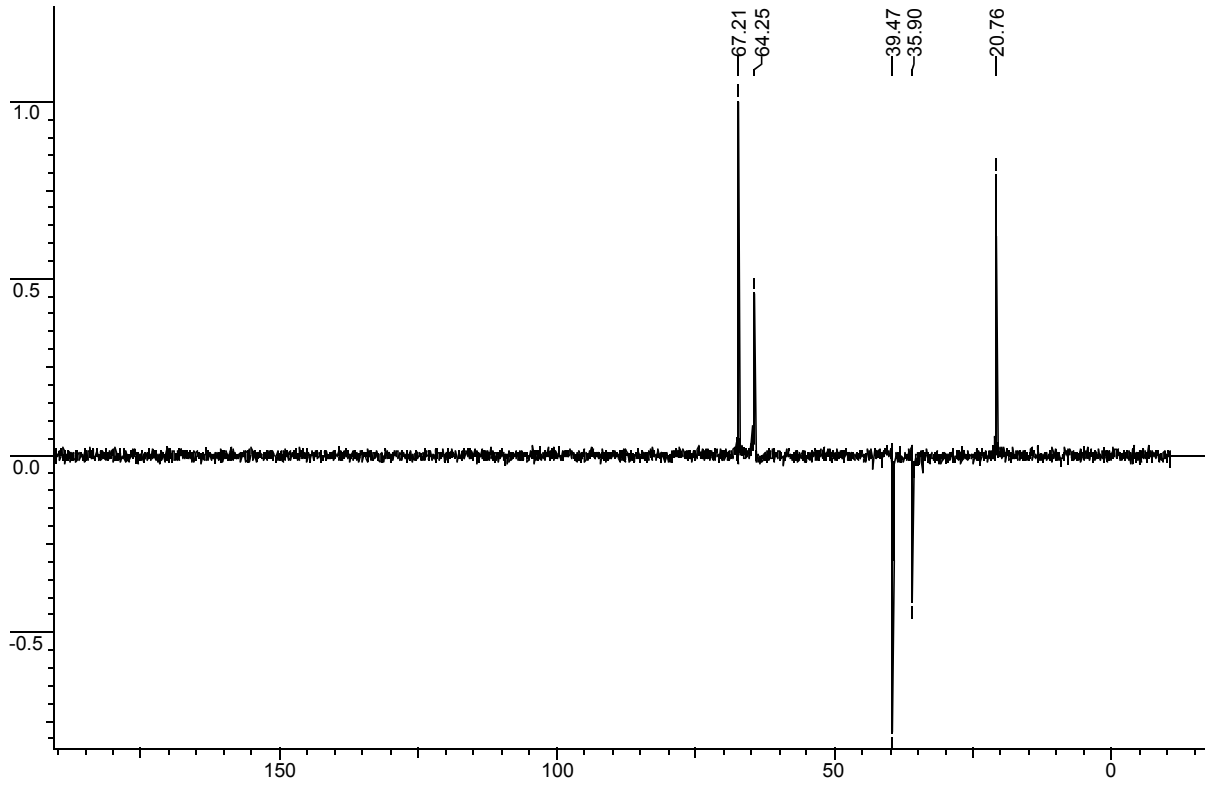


*IR spectrum of **166***



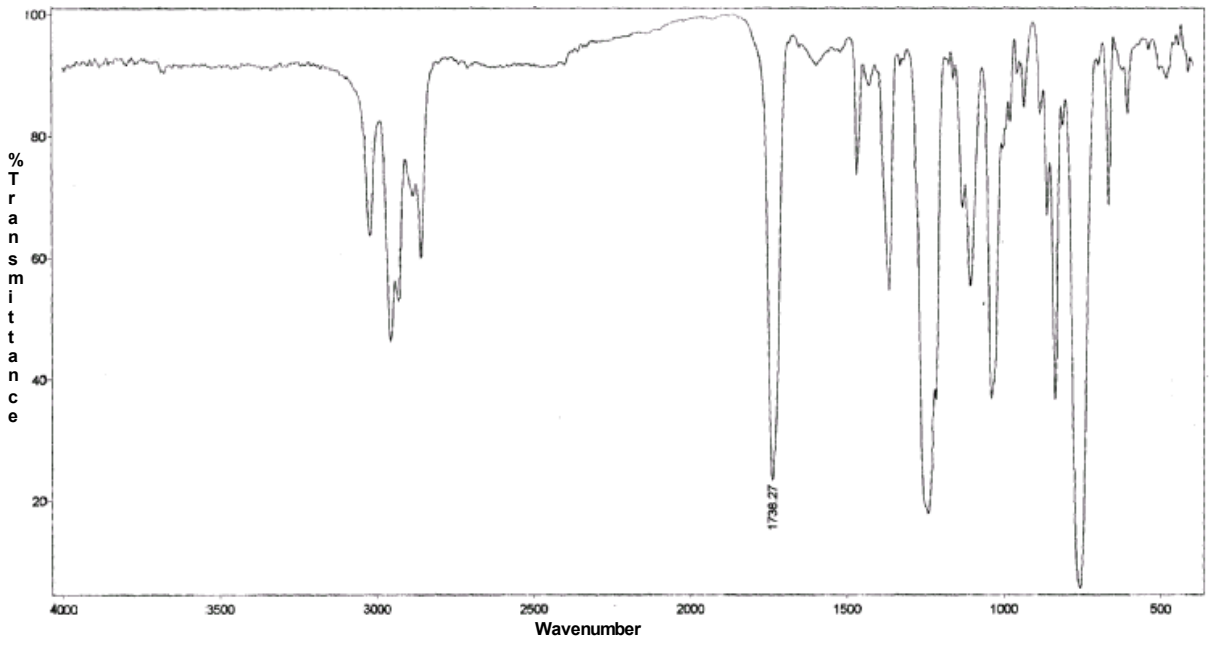
*^{13}C NMR Spectra of **166***



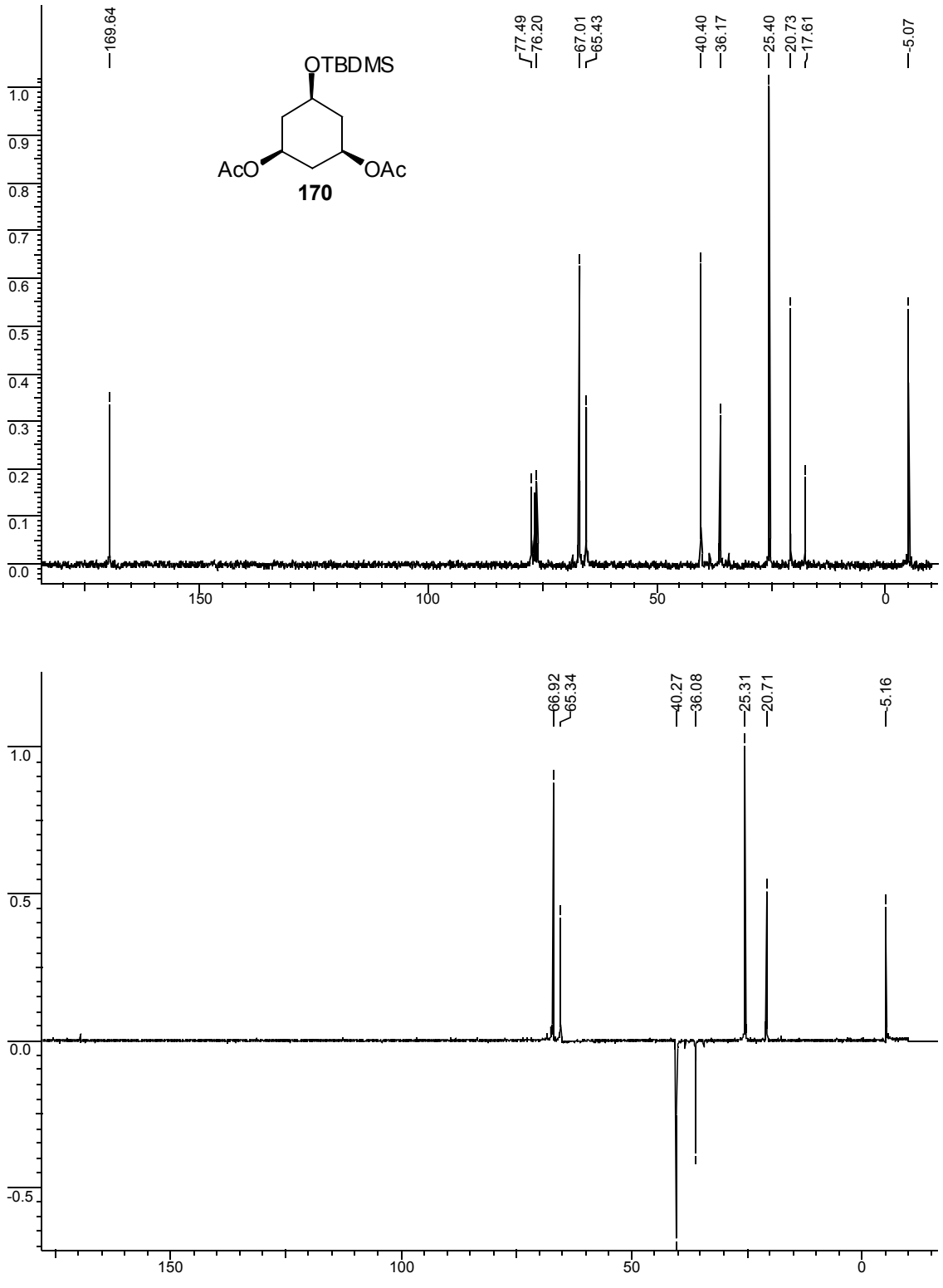


*^{1}H NMR spectrum of **170***

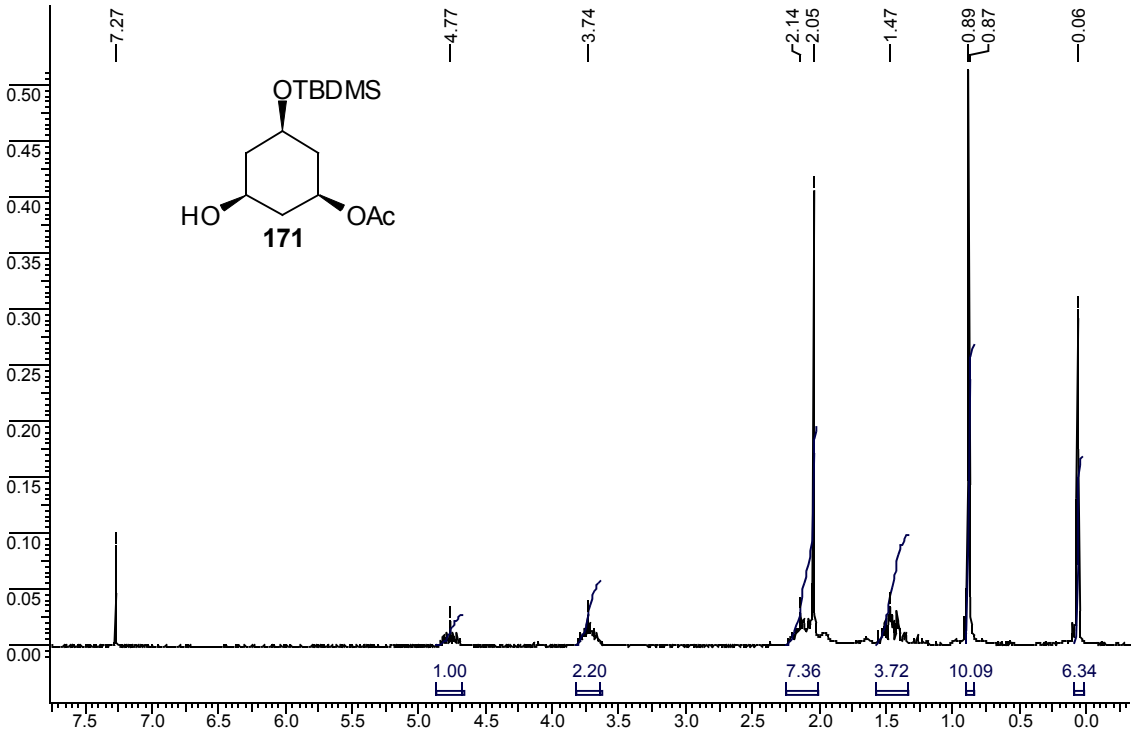
*IR spectrum of **170***



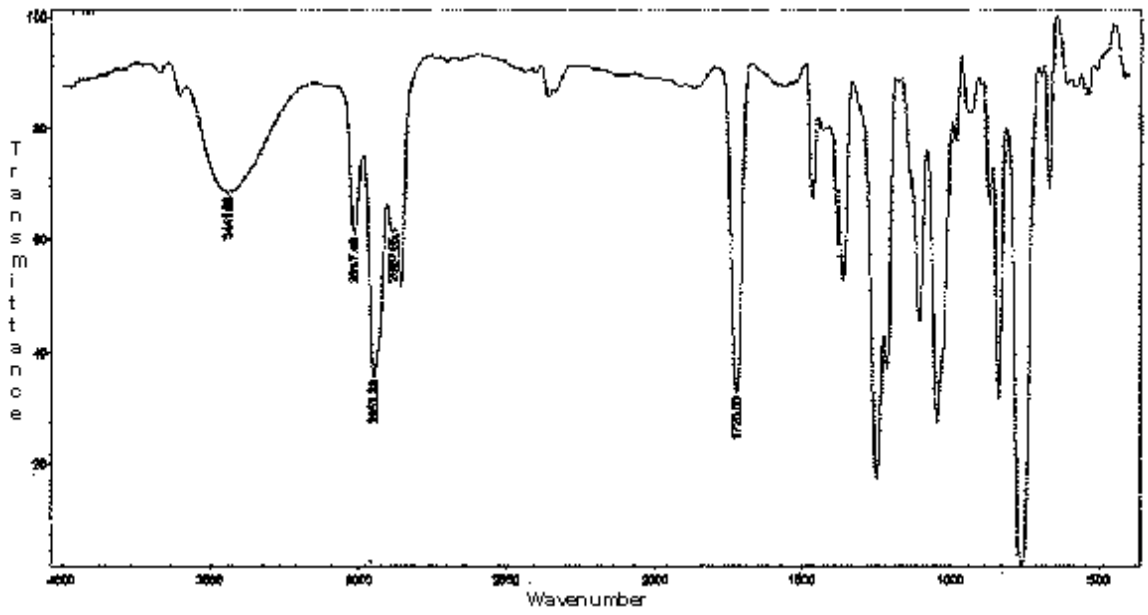
*^{13}C NMR spectra of **170***



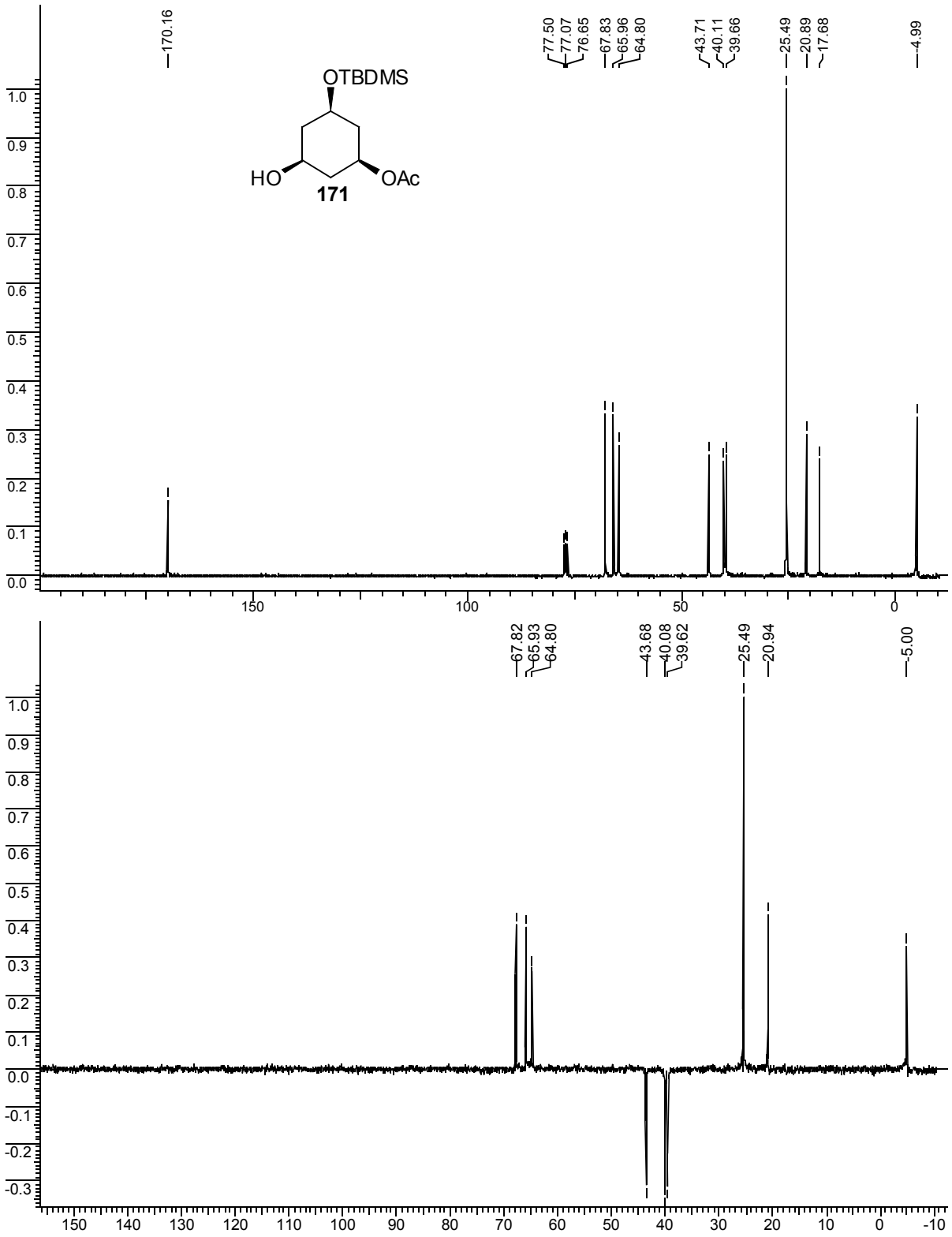
*[1]H NMR spectrum of **171***



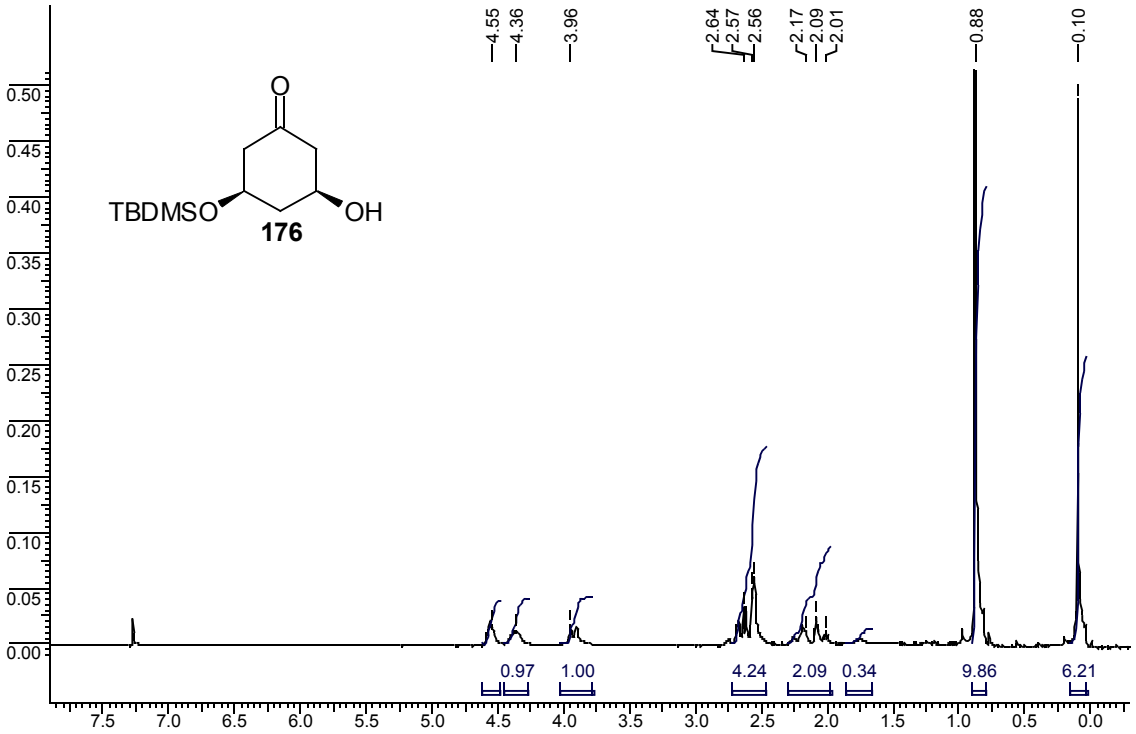
*IR spectrum of **171***



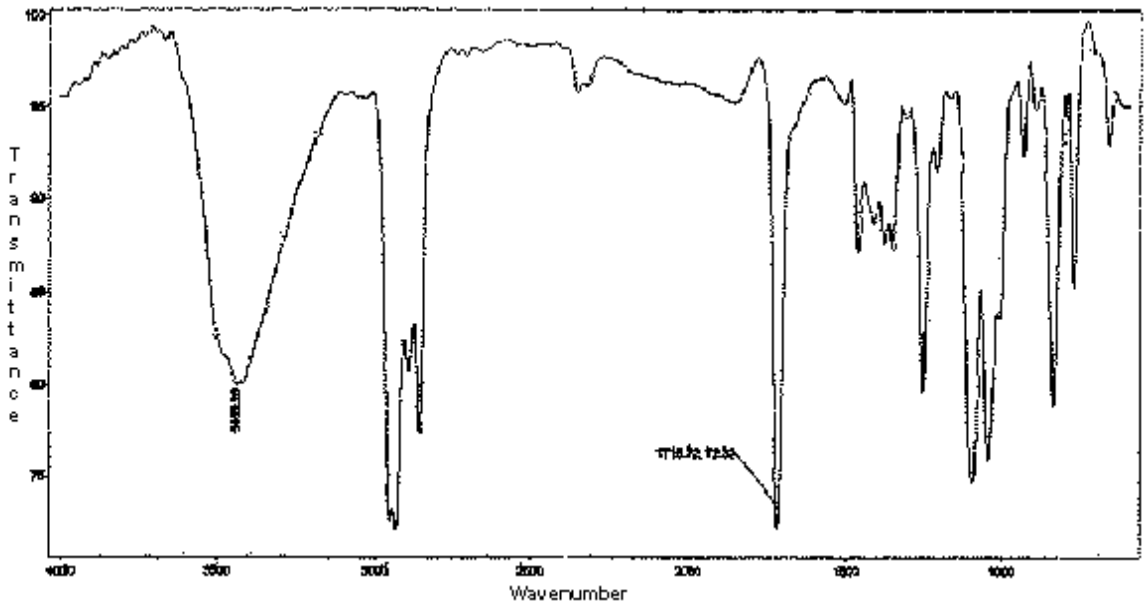
*^{13}C NMR spectra of **171***



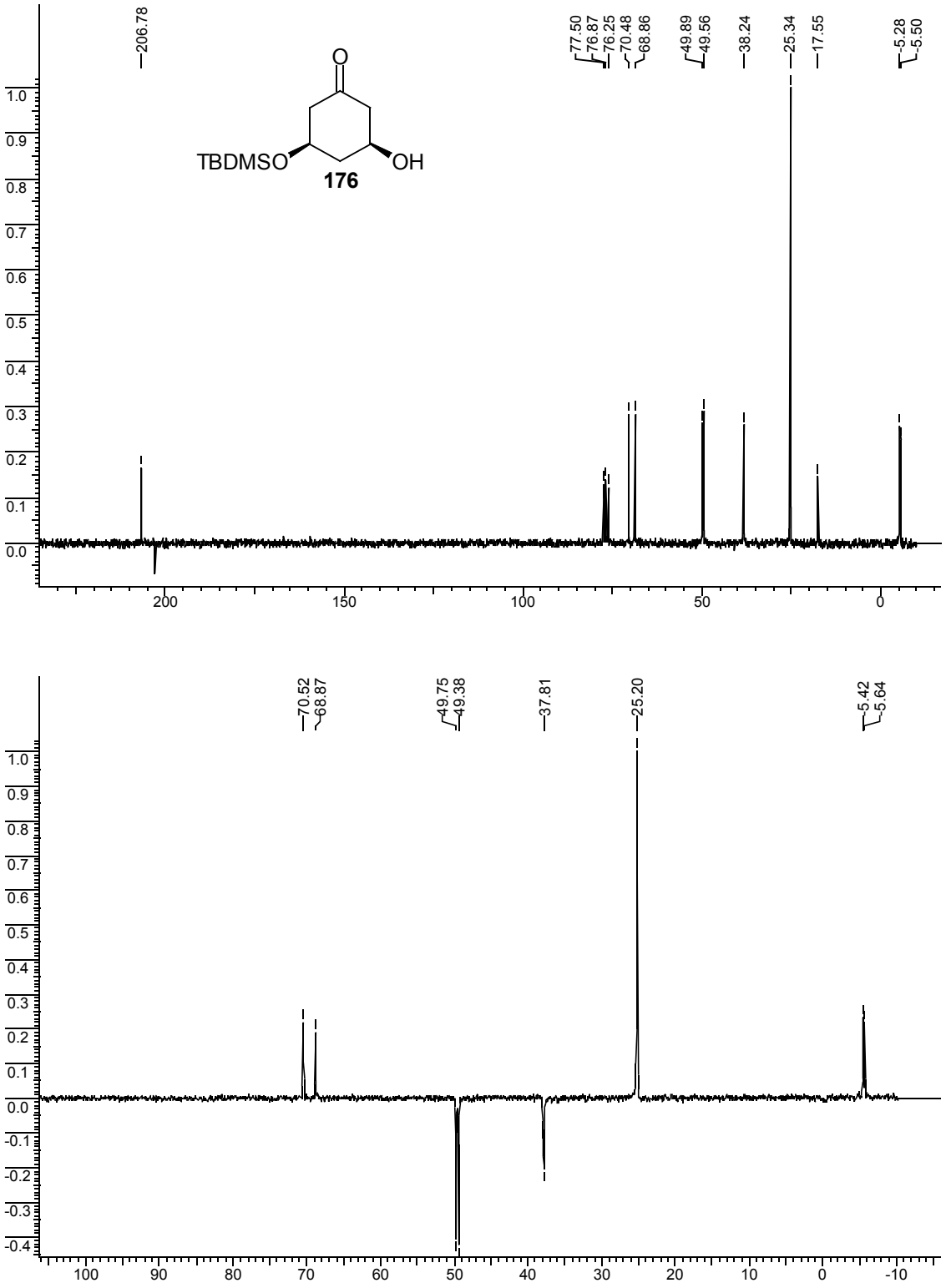
*[1]H NMR spectrum of **176***



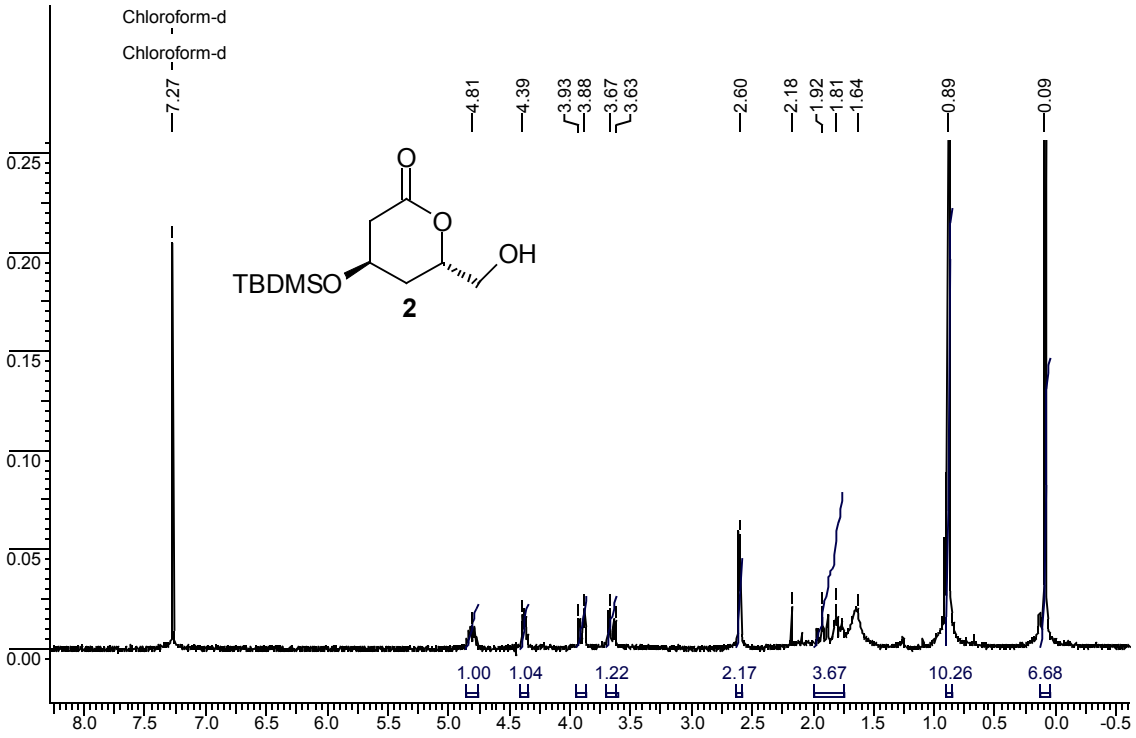
*IR spectrum of **176***



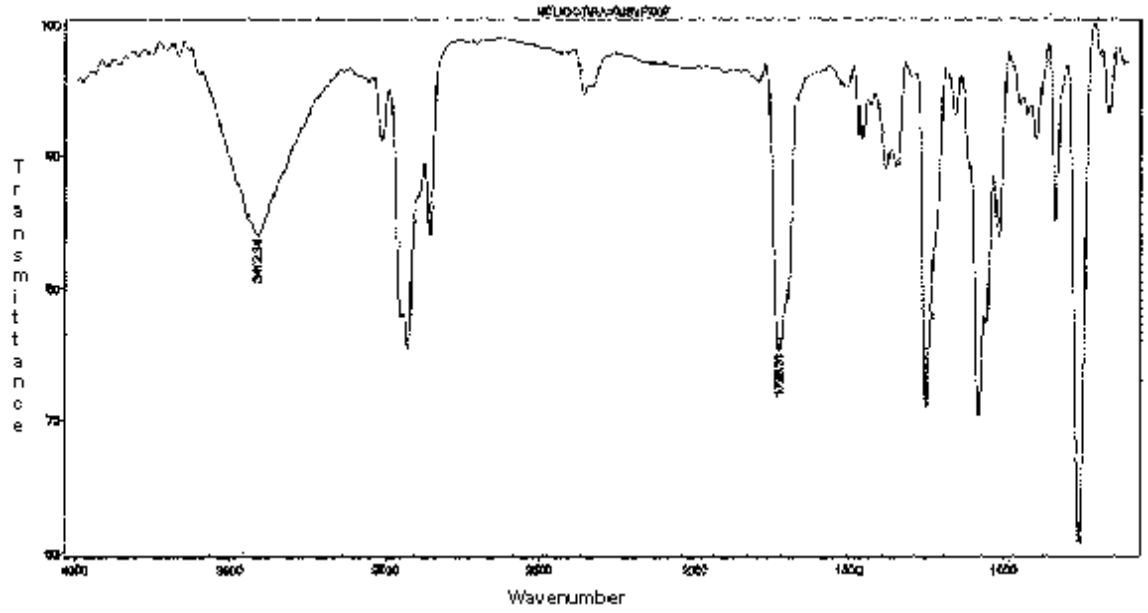
*^{13}C NMR spectra of **176***



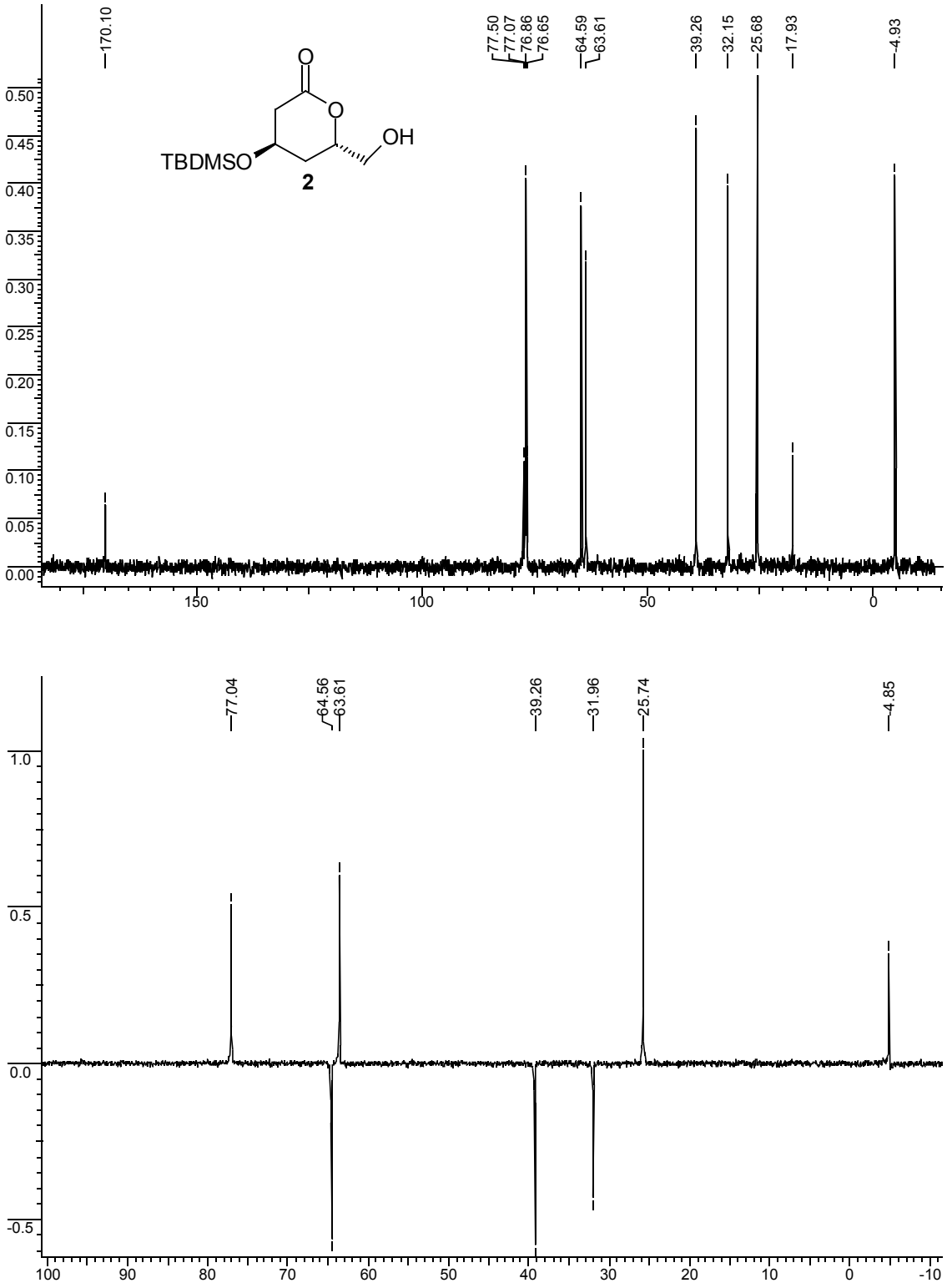
*[1]H NMR spectrum of **2***



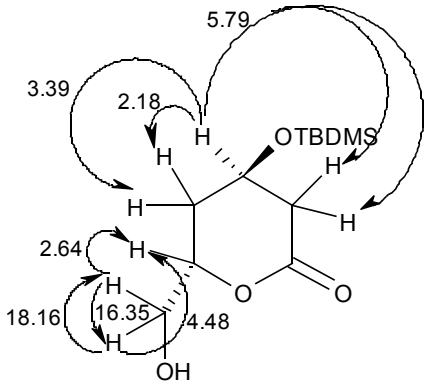
*IR spectrum of **2***

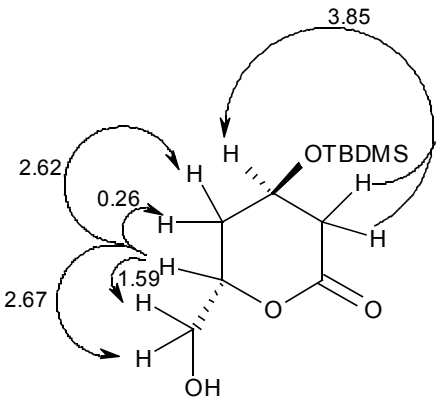


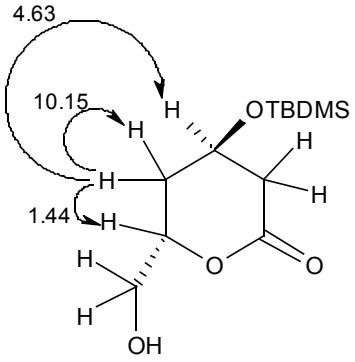
^{13}C NMR spectra of **2**

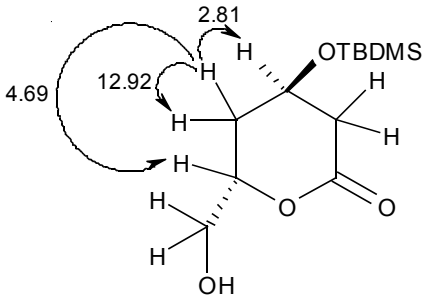


*N. O. E. difference for lactone **1**, 400 MHz ($CDCl_3$)*

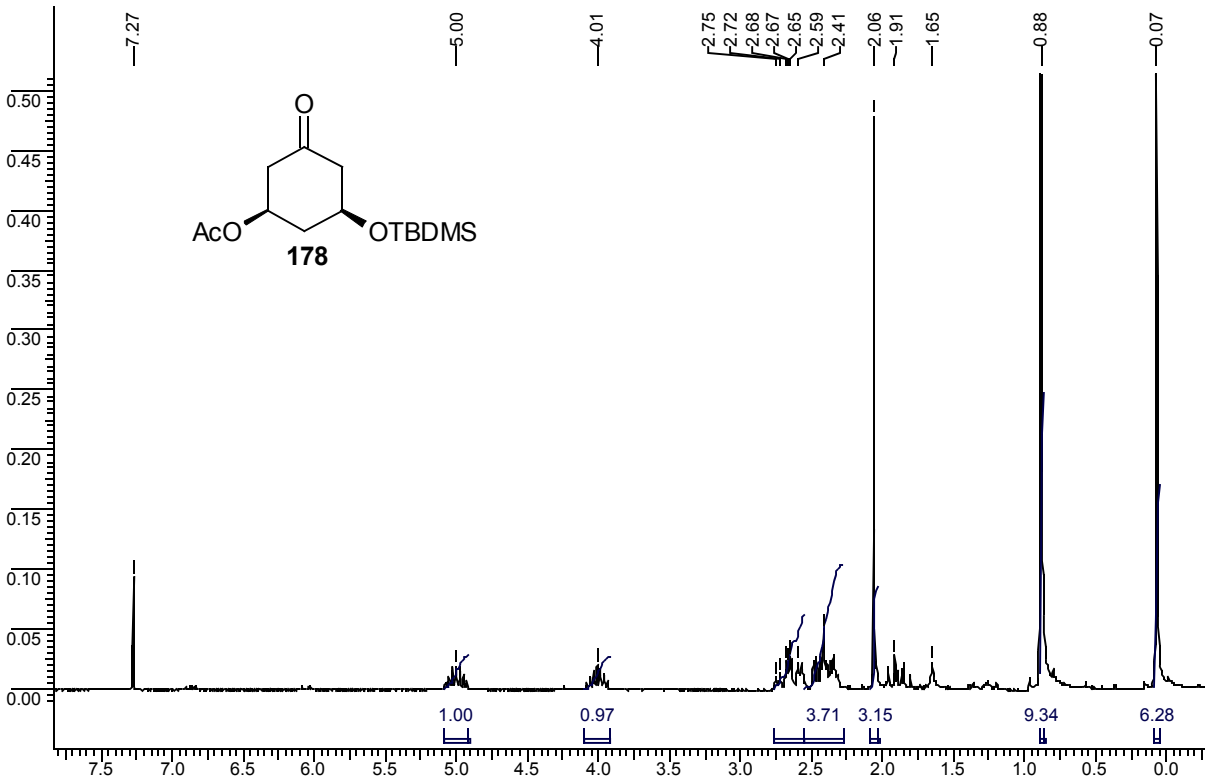




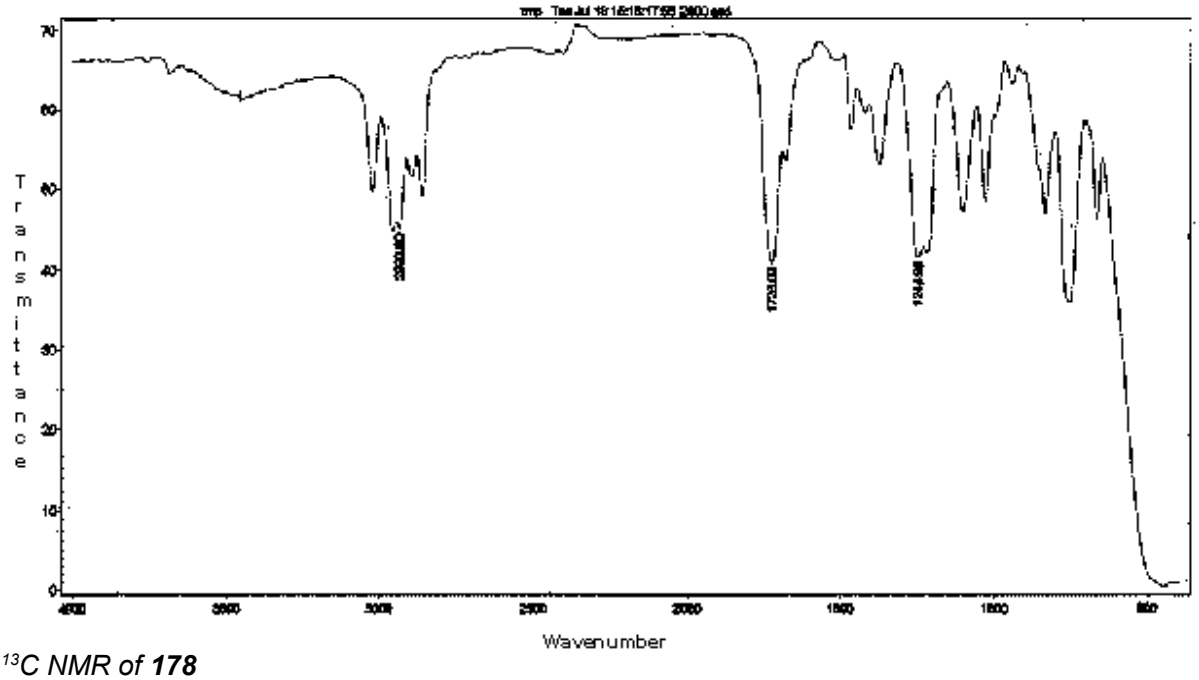




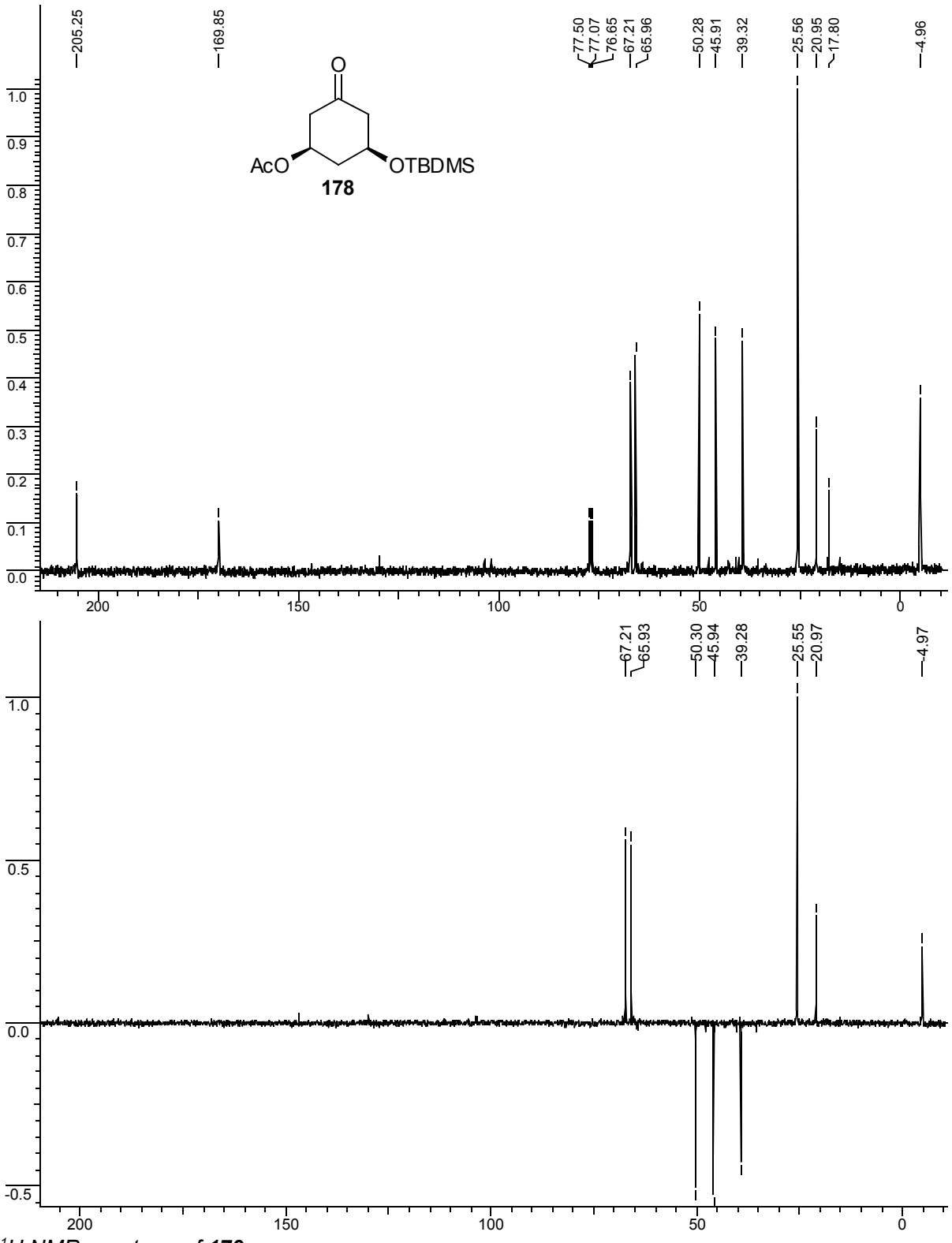
*^{1}H NMR spectrum of **178***



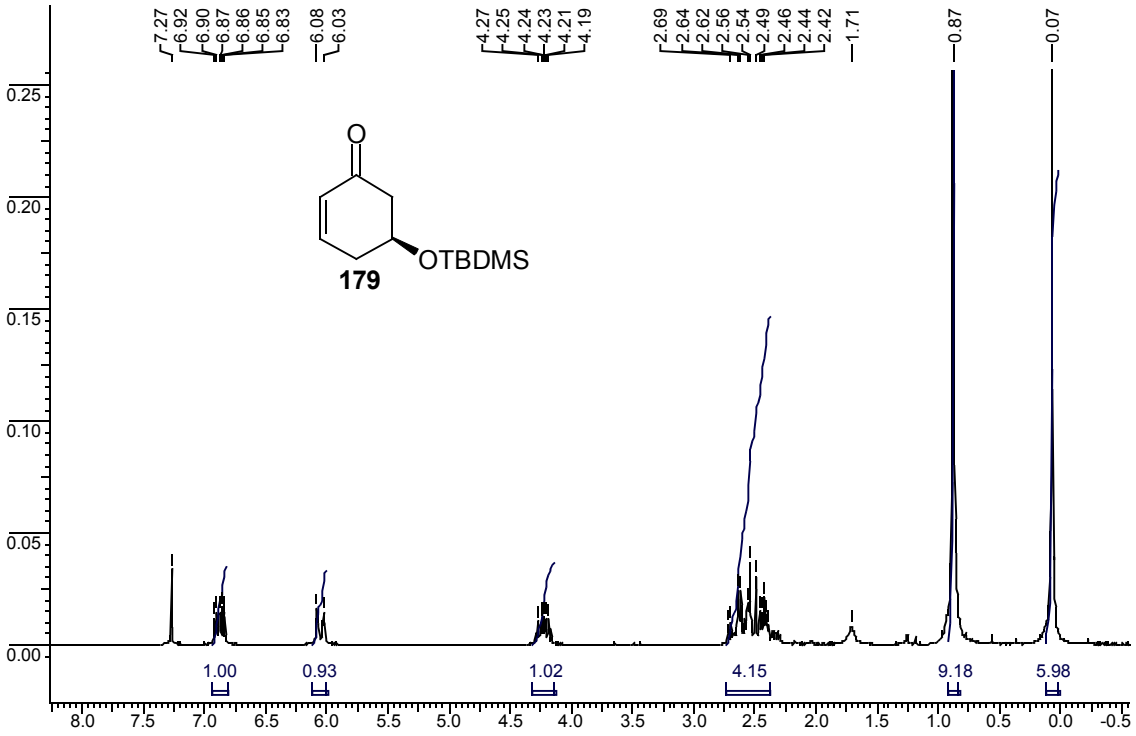
*IR spectrum of **178***



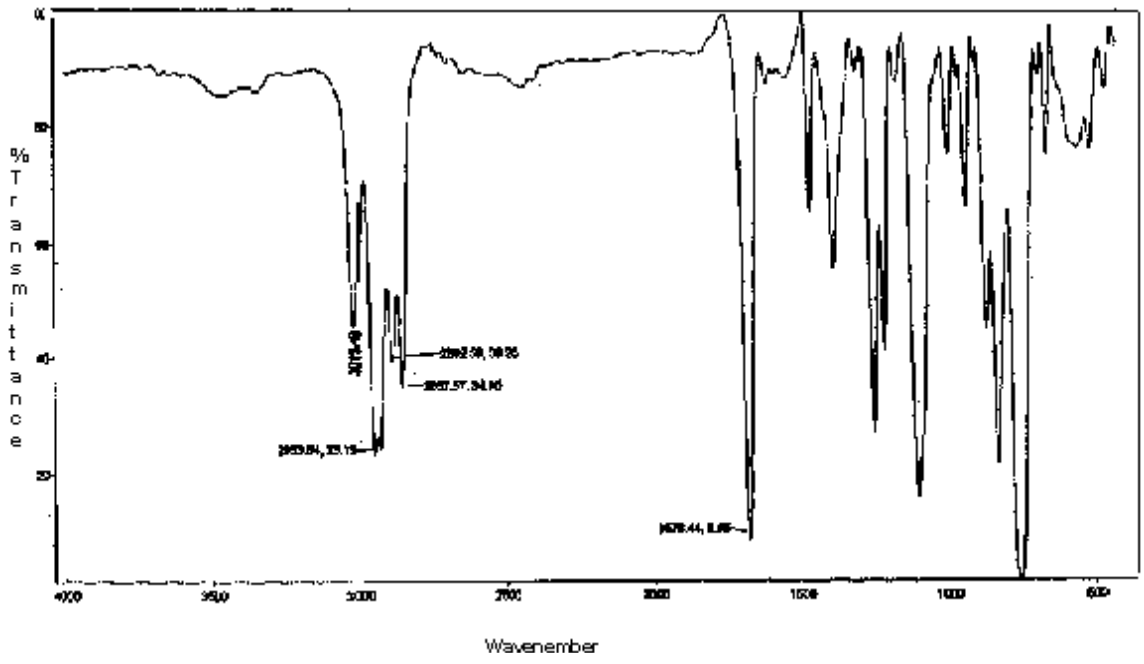
*^{13}C NMR of **178***



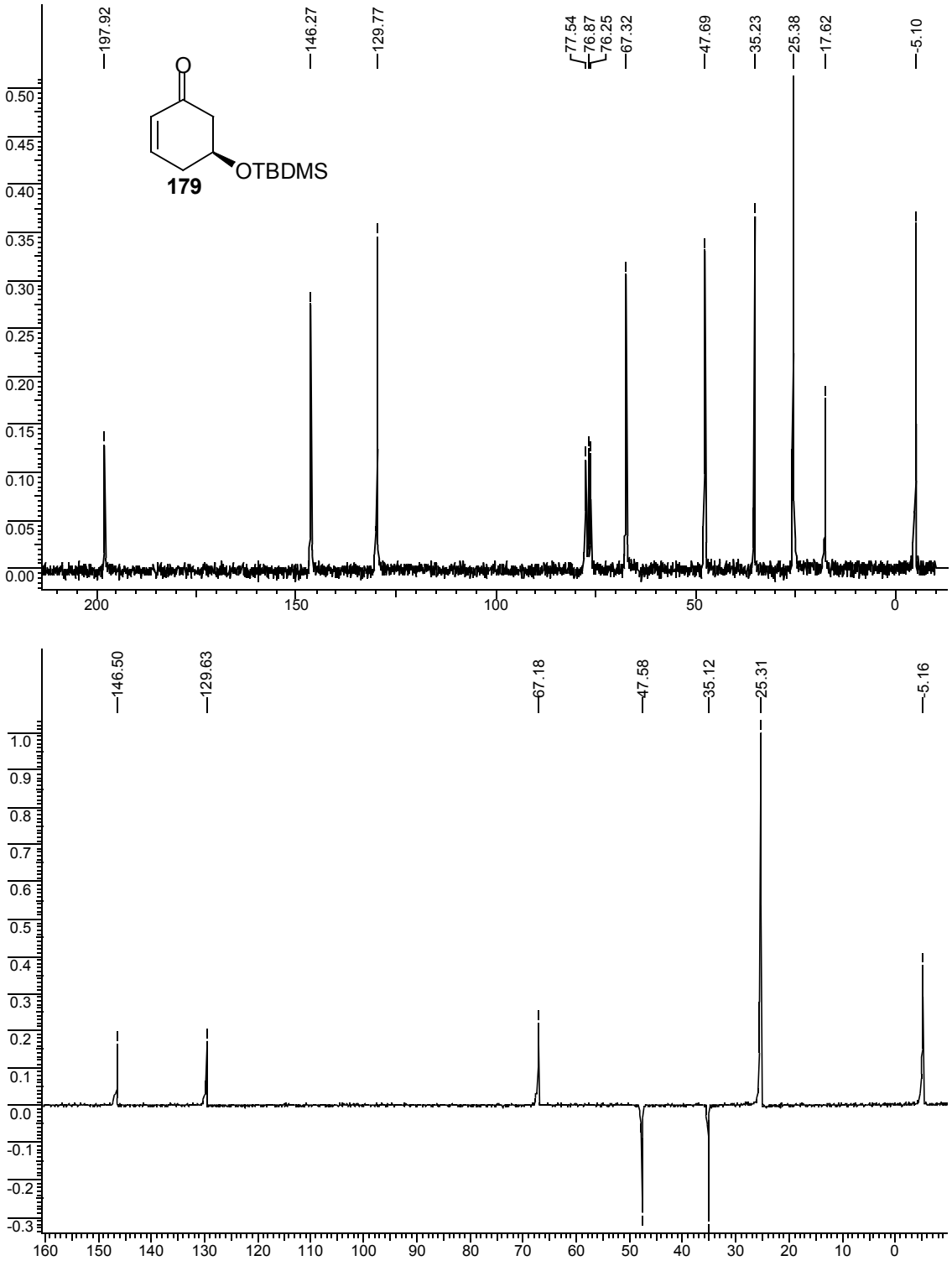
*^{1}H NMR spectrum of **179***



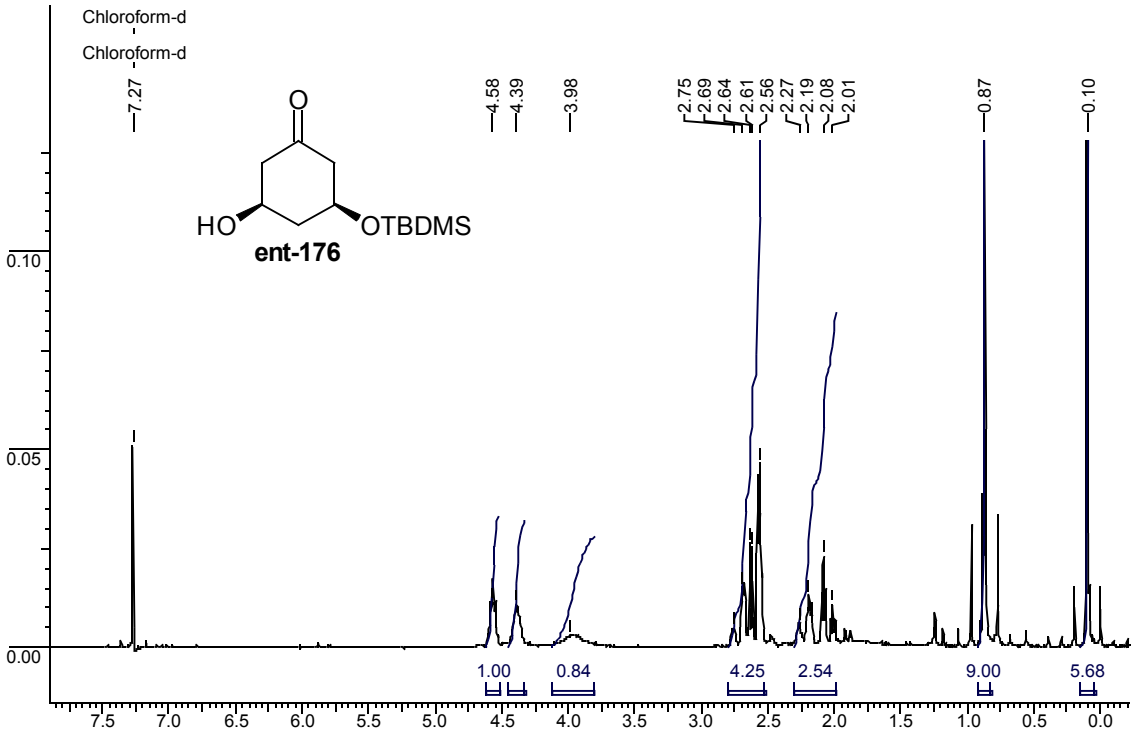
*IR Spectrum of **179***



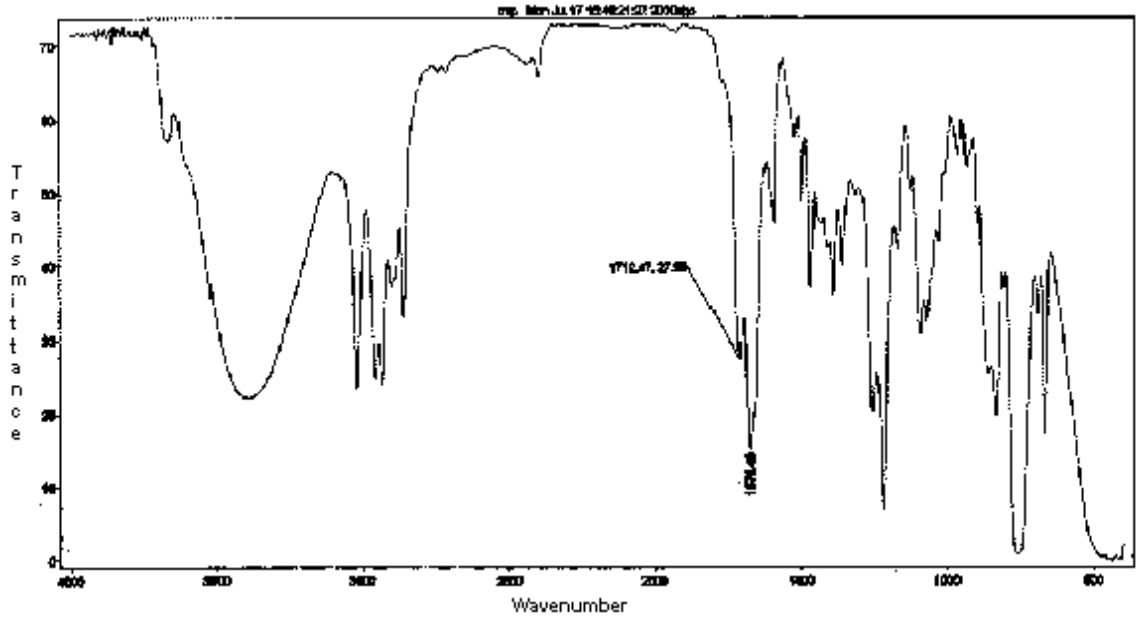
*^{13}C NMR spectra of **179***



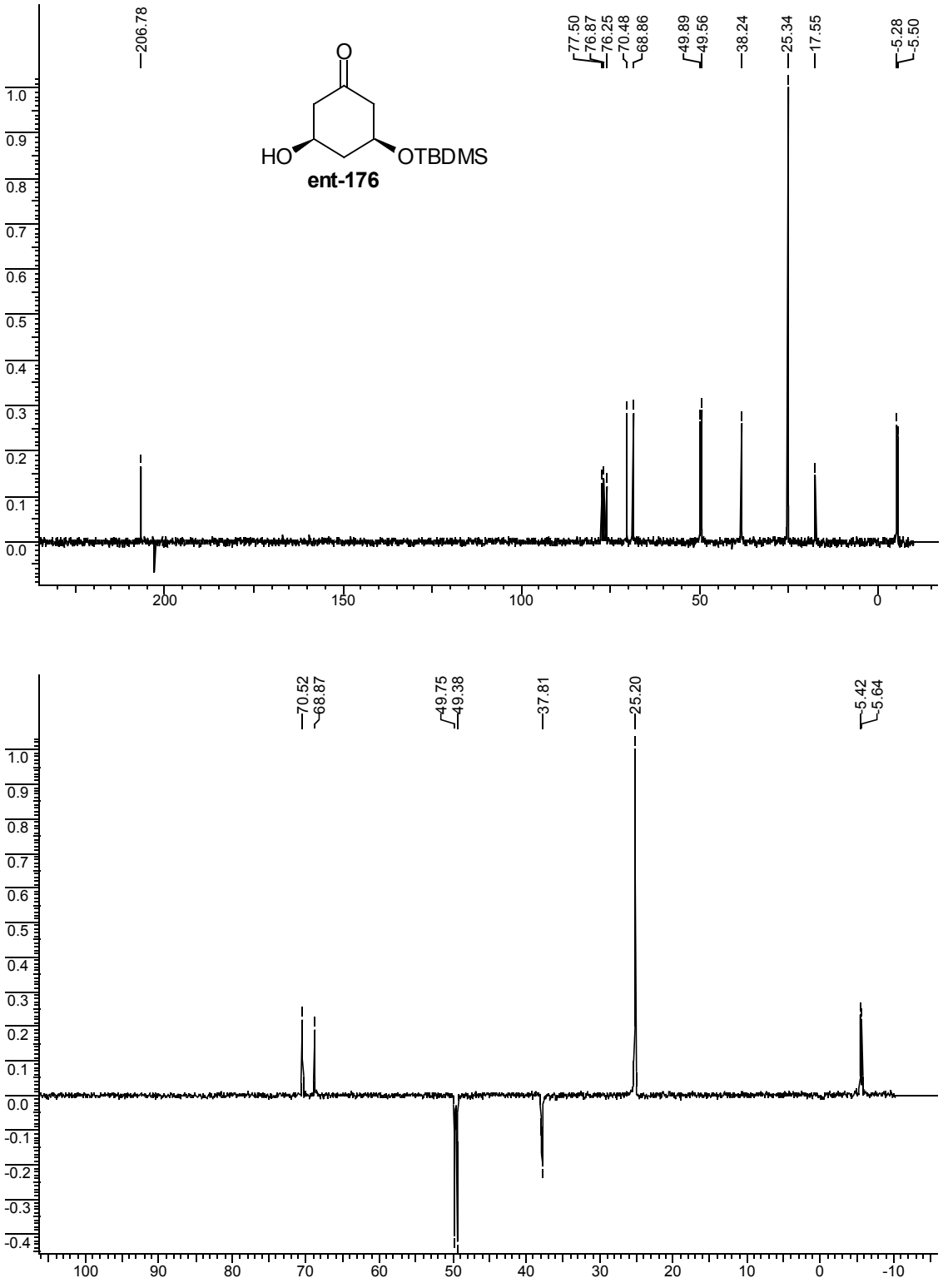
*^{1}H NMR spectrum of **ent-176***



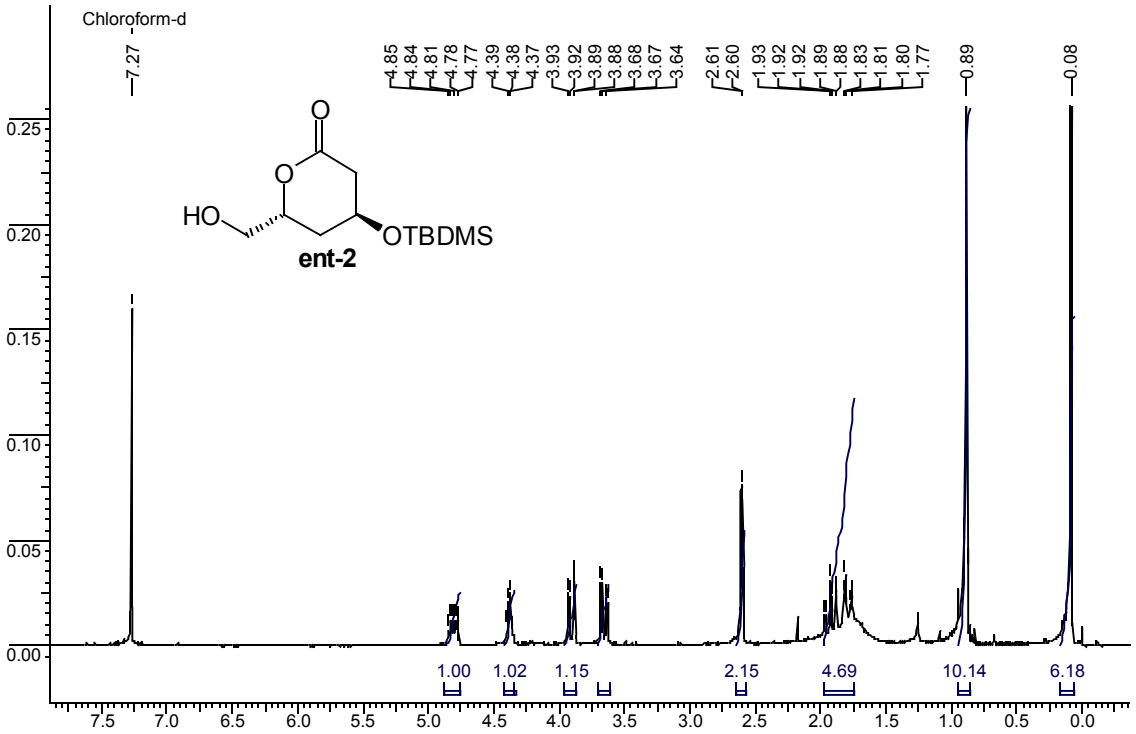
*IR spectrum of **ent-176***



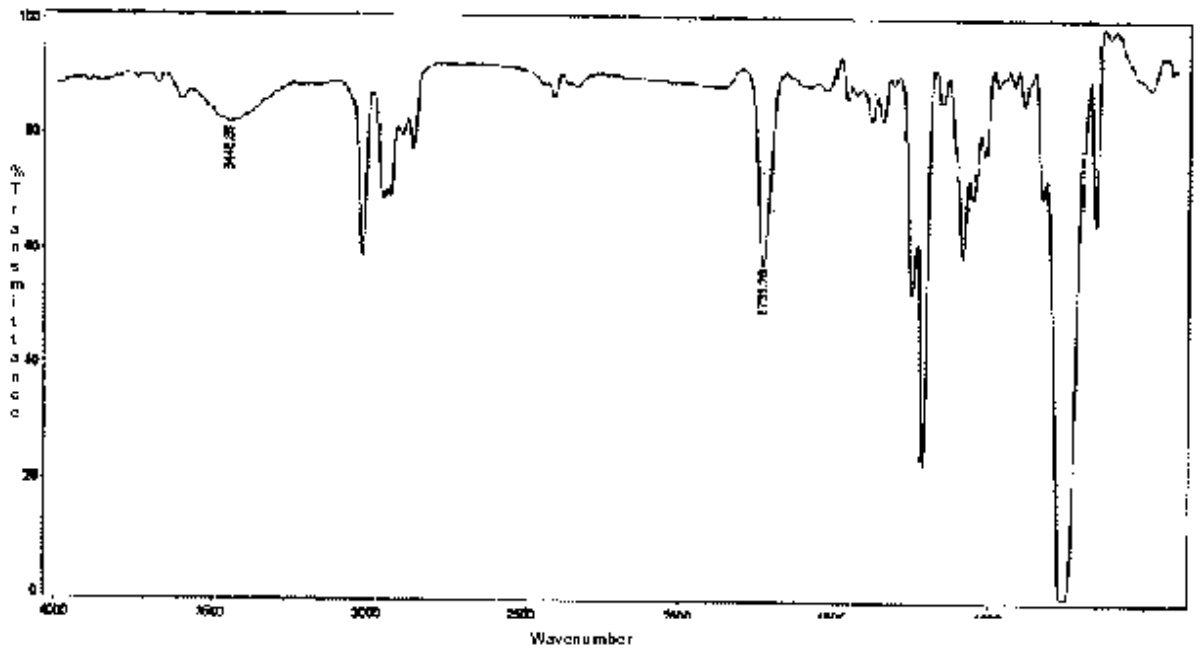
*^{13}C NMR of **ent-176***



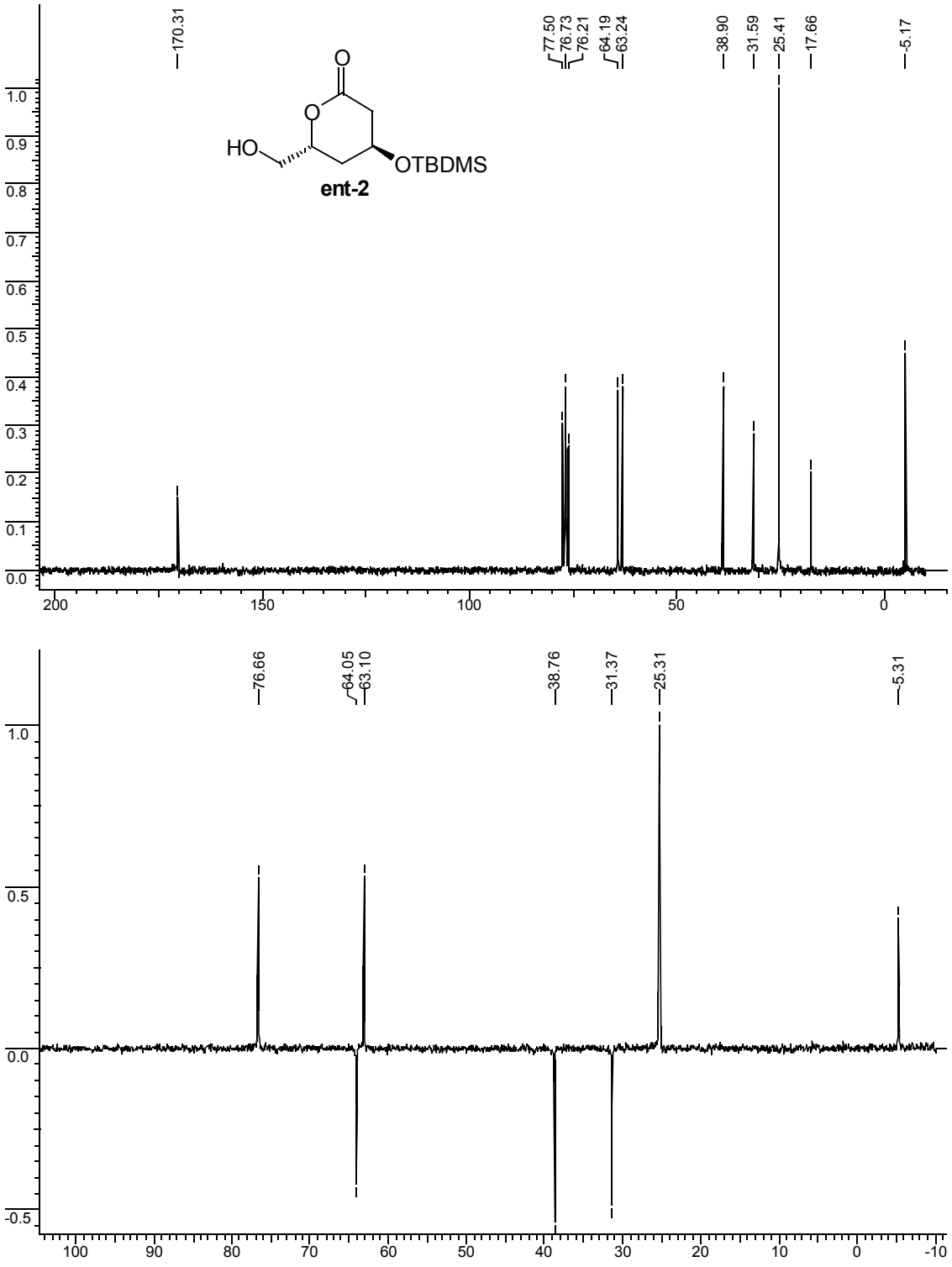
*[1]H NMR spectrum of **ent-2***



IR spectrum of ***ent-2***



*^{13}C NMR spectra of **ent-2***



Single Crystal X-ray analysis of ***ent-2***

Single crystal of **ent-2** was grown in ethyl acetate by slow evaporation in a chamber saturated with pet. ether. The compound **ent-2** having formula $(C_{12}H_{24}O_4Si)_2$ (two molecules in unit cell) belong to Monoclinic space group $P2_1$ with unit cell dimensions as given below

a = 6.687 (5),	b = 6.748 (1)	c = 34.303 (11) (Å)
β = 90.15 (4)°,	V = 1547.8 (12) Å^3	

Data was collected on *Enrat Nonius CAD-4 (MACH-3) diffractometer* at IIT, Powai, Mumbai using MoKα radiations (λ = 0.7107 Å)

The structure was solved by direct methods using SHELEX-97. From the analysis it can be seen that there are two molecules with similar conformation in the asymmetric unit. The figure 4.2 shows the ORTEP diagram of one of the molecules. The torsion angles (degree) are given in Table 4.3.

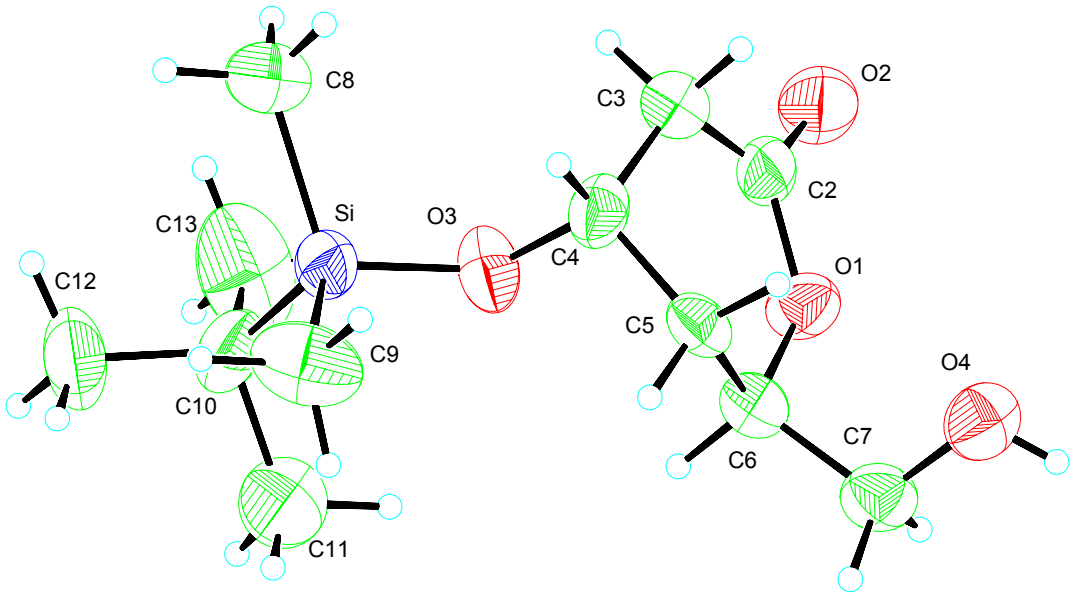


Figure 4.2.

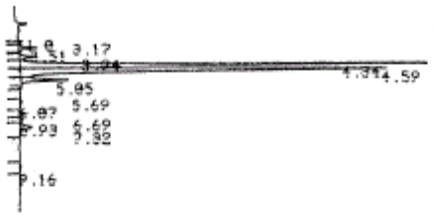
Table 4.3. Torsion angles for ring structure of **ent-2**

Entry No.BondTorsion angle
(degree)1.C(10)-Si-O(3)-C(4)178.4 (8)2.C(8)-Si-O(3)-C(4)58. 1 (9)3.C(9)-Si-O(3)-C(4)-61. 8 (9)4.C(6)-O(1)-C(2)-C(3)179.0 (9)5.C(6)-O(1)-C(2)-O(2)3.8 (16)6.O(2)-C(2)-C(3)-C(4)168.5 (10)7.O(1)-C(2)-C(3)-C-(4)-16.6 (16)8.Si-O(3)-C(4)-C(5)116.3 (9)9.Si-O(3)-C(4)-C(3)-124.1 (9)10.C(2)-C(3)-C(4)-O(3)-75.0 (12)11.C(2)-C(3)-C(4)-C(5)45.5 (13)12.O(3)-C(4)-C(5)-C(6)56.7 (12)13.C(3)-C(4)-C(5)-C(6)-62.3 (12)14.C(2)-O(1)-C(6)-C(7)-143.6 (10)15.C(2)-O(1)-C(6)-C(5)-20.1 (14)16.C(4)-C(5)-C(6)-O(1)49.0 (12)17.C(4)-C(5)-C(6)-C(7)166.3 (8)18.O(1)-C(6)-C(7)-O(4)71.9 (11)19.C(5)-C(6)-C(7)-O(4)-49.3 (12)20.O(3)-Si-C(10)-C(13)-61.8 (12)21.C(8)-Si-C(10)-C(13)57.4 (13)22.C(9)-Si-C(10)-C(13)179.7 (12)23.O(3)-Si-C(10)-C(12)178.7 (8)24.C(8)-Si-C(10)-C(12)-62.0 (10)25.C(9)-Si-C(10)-C(12)60.3 (10)26.O(3)-Si-C(10)-C(11)59.6 (10)27.C(8)-Si-C(10)-C(11)178.9 (10)28.C(9)-Si-C(10)-C(11)-58.9 (12)

Bond length of C2O2 – 1.205 (14) Å ⇒ Double bond
Bond length of C7O4 – 1.415 (14) Å ⇒ Single bond

*Chiral HPLC charts for Mosher ester of **171***

Racemic Mosher ester Optically enriched Mosher ester

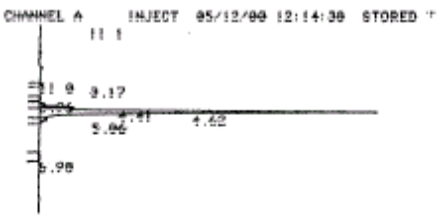


DATA SAVED TO BIN # 1

SANDEEP.CHORPADE 05/12/00 12:00:45

FILE 1. METHOD 0. RUN 1 INDEX 1

PEAK#	AREA%	RT	AREA BC
1	0.274	3.17	13417 01
2	1.479	3.51	72386 01
3	1.546	3.94	75655 02
4	43.549	4.34	2131211 02
5	44.357	4.59	2170720 02
6	5.065	5.85	247873 03
7	0.149	5.69	7295 02
8	0.466	6.87	22795 02
9	0.521	6.69	25518 02
10	0.483	6.93	23661 02
11	1.949	7.32	95389 03
12	0.16	9.16	7845 01
TOTAL	100.		4893273



DATA SAVED TO BIN # 2

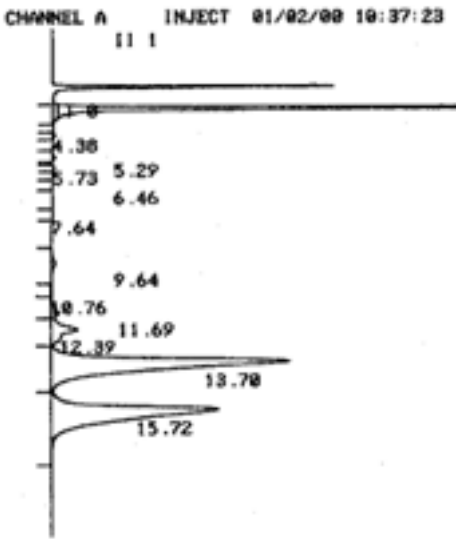
SANDEEP.CHORPADE 05/12/00 12:14:30

FILE 1. METHOD 0. RUN 2 INDEX

PEAK#	AREA%	RT	AREA BC
1	0.395	3.17	9588 01
2	0.227	3.96	5464 01
3	7.209	4.41	173269 02
4	90.231	4.62	2169111 02
5	1.731	5.86	41614 03
6	0.209	6.98	5088 01
TOTAL	100.		2403966

*Chiral HPLC charts for Benzoates of Lactone **2***

Racemic Before crystallization After crystallization



DATA SAVED TO BIN # 2

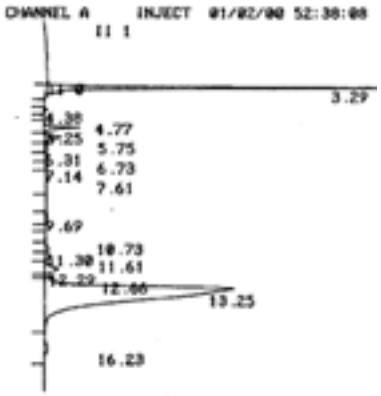
S.R.GHORPADE 01/02/00 1

FILE 1. METHOD 0. RUN 2

PEAK#	AREAX	RT	AREA	BC
1	22.526	3.27	4790425	01
2	0.066	4.38	14039	01
3	0.108	5.29	22911	01
4	0.046	5.73	9811	01
5	0.03	6.46	6287	01
6	0.023	7.64	4795	01
7	0.475	9.64	100918	02
8	0.051	10.76	10756	02
9	1.02	11.69	216820	02
10	3.937	12.39	837266	02
11	39.092	13.7	8313453	02
12	32.629	15.72	6939007	03

TOTAL 100. 21266488

Read Error



DATA SAVED TO BIN # 1

S.R.GHORPADE 01/02/00 52

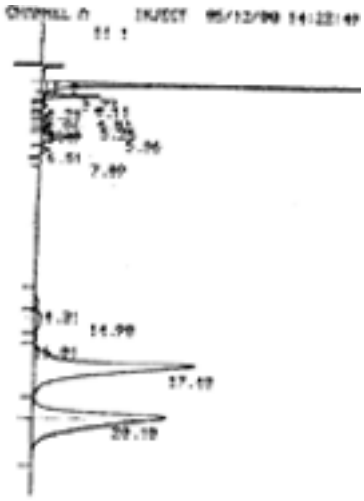
FILE 1. METHOD 0. RUN 1

PEAK#	AREAX	RT	AREA	BC
1	17.525	3.29	5791684	01
2	0.172	4.38	56854	01
3	0.018	4.77	6002	02
4	2.427	5.25	802163	02
5	1.578	5.75	521585	02
6	0.523	6.31	172829	02
7	0.426	6.73	140983	02
8	0.29	7.14	95985	02
9	0.283	7.61	93457	03
10	0.185	9.69	61188	01
11	0.097	10.73	32188	02
12	0.526	11.3	173839	02
13	0.613	11.61	202718	02
14	2.033	12.29	671812	02
15	0.468	12.66	154653	02
16	72.098	13.25	23827300	02
17	0.736	16.23	243097	03

TOTAL 100. 33048329

Chiral HPLC charts for Lactone ***ent-2***

Racemic Optically enriched



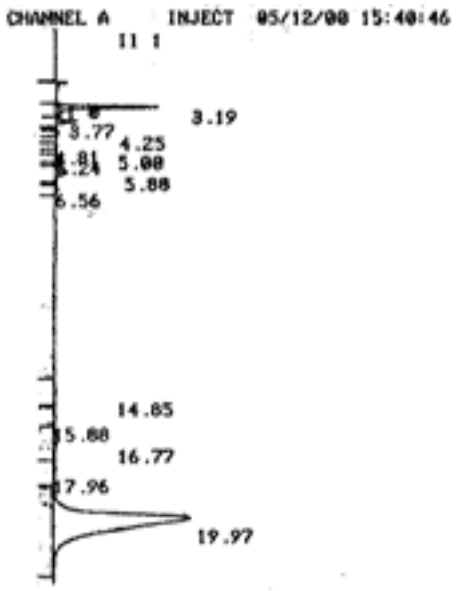
DATA SAVED TO BIN # 9

INPUT OVERRANGE AT RT= 2.10

SANDEEP.CHORPADE 05/12/00 1

FILE 1. METHOD 0. RUN 9

PEAK#	AREAX	RT	AREA %C
1	16.513	3.38	2003387 99
2	1.782	3.77	216732 95
3	0.035	4.11	4243 96
4	0.289	4.39	35061 97
5	0.261	4.81	31629 02
6	0.243	4.96	29530 02
7	0.513	5.25	62231 02
8	0.272	5.67	32956 02
9	2.599	5.96	314974 02
10	0.111	6.31	13516 01
11	0.109	7.09	13231 01
12	0.572	14.31	69277 02
13	2.097	14.99	253995 02
14	0.259	16.91	31369 02
15	39.233	17.19	4139977 02
16	32.01	20.1	4248799 99
TOTAL	100		12133613



DATA SAVED TO BIN # 6

SANDEEP.CHORPADE 05/12/00 1

FILE 1. METHOD 0. RUN 6

PEAK#	AREAX	RT	AREA %C
1	~~9.878~~	~~3.19~~	~~396900 88~~
2	1.749	3.77	70259 05
3	0.233	4.25	9362 01
4	0.162	4.81	6492 02
5	0.175	5.	7015 02
6	0.468	5.24	18799 03
7	1.922	5.88	77222 01
8	0.284	6.56	11403 01
9	0.407	14.85	16358 01
10	0.28	15.88	11266 02
11	1.628	16.77	65408 02
12	0.467	12.96	18755 03
13	82.349	19.97	3308998 01
TOTAL	100		4018287

4.3. Development of Chemoenzymatic Route for the intermediates of the phosphine oxide A ring synthon for 1α, 25-dihydroxyvitamin D_3

4.3.1. Introduction

In recent years, vitamin D research from the viewpoint of its chemistry and pharmacology has expanded enormously with the discovery that 1α, 25-dihydroxyvitamin D_3 (**180**, Figure 4.3, also known as 1α, 25-dihydroxycholecalciferol or calcitriol), the hormonally active metabolite of vitamin D, exhibits a much broader spectrum of biological activities than originally thought, well beyond its classical functions in regulating calcium and phosphorus metabolism.[38] The steroid hormone 1,25-D_3 and its analogues have been used or have high potential for application as drugs in treating a diverse range of human disease such as rickets,[39] renal osteodystrophy,[40] osteoporosis,[41] psoriasis,[42] leukemia,[43] breast cancer,[44] prostate cancer,[45] AIDS,[46] and Alzheimer's disese.[47]

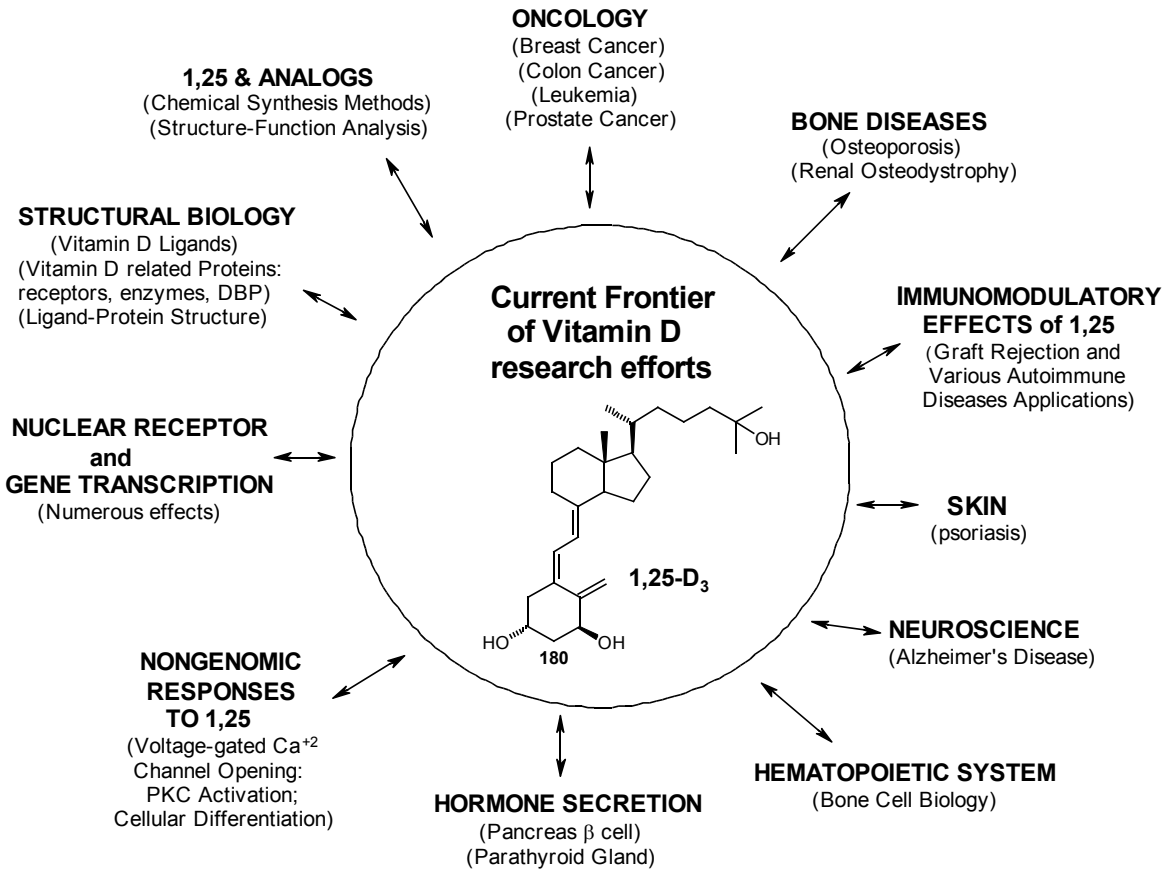


Figure 4.3.

In view of this extraordinary flexibility current research is aimed at the synthesis of analogues of **180** with superagonistic potency. During the last decade a large number of analogues have been synthesized and tested biologically; comprising modifications in the A-ring, in the CD ring fragment and especially in the side chain.[48] Among the A-ring modifications, deletion of the 19-exomethylene function has been shown to induce interesting biological activities. 19-Nor-1α, 25-dihydroxyvitamin D_3 (**181**) displays reduced calcemic effect (< 10%) while retaining good cell differentiating properties.[49]

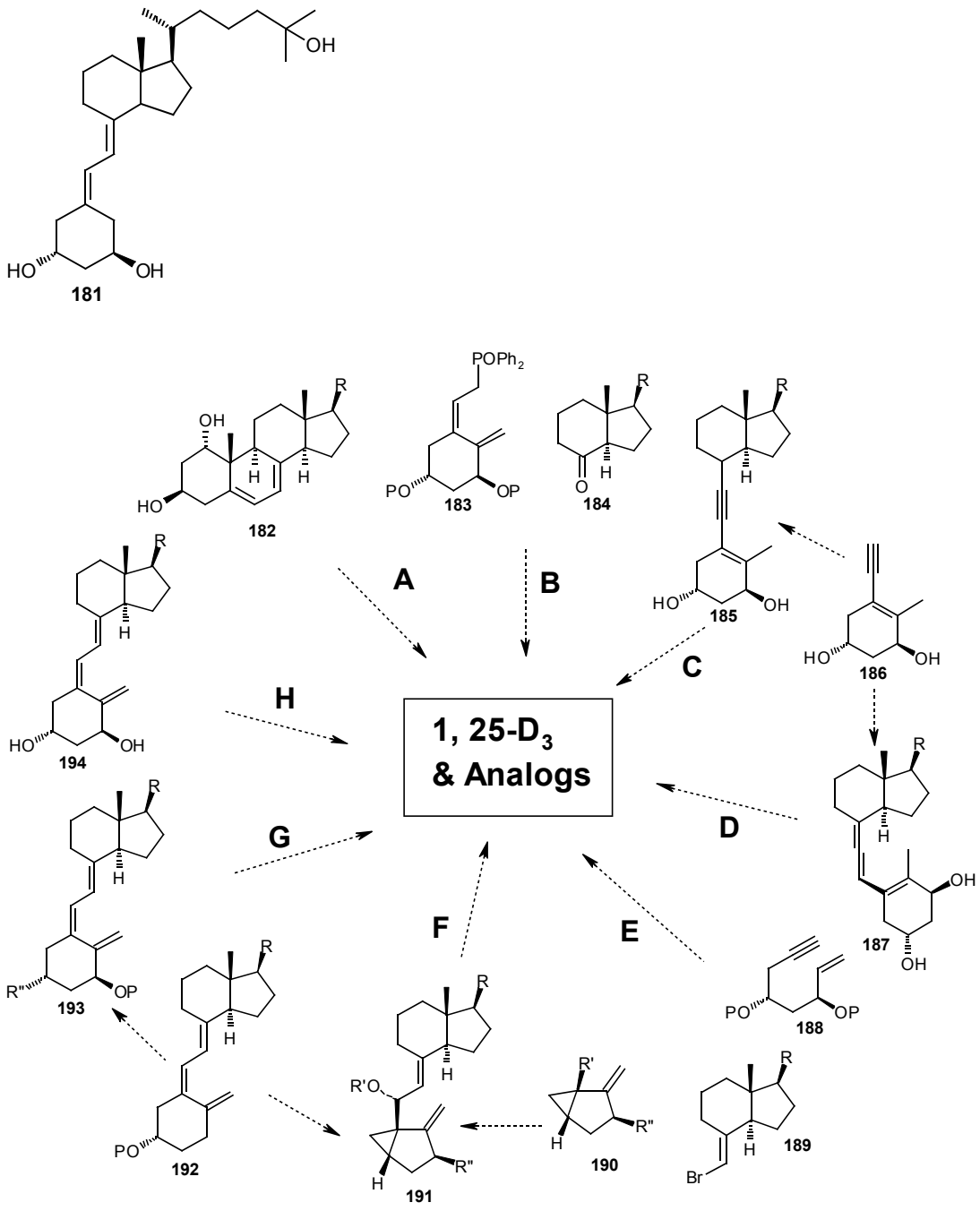
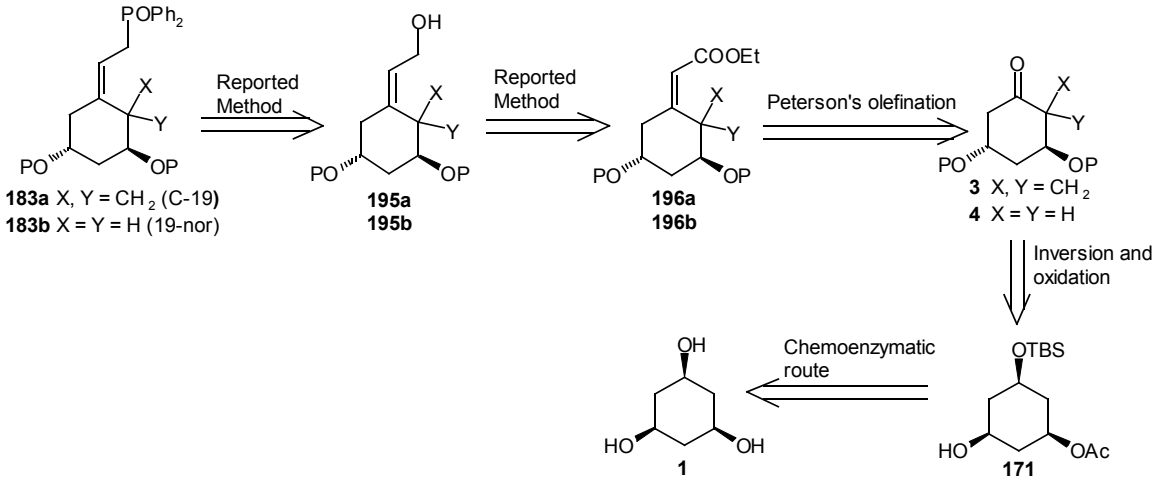


Figure 4.4.

Several synthetic approaches for the synthesis of the hormone 1α,25-D_3 and its various analogues have been developed. Some of the major synthetic variations and methods utilized in recent years are A to H depicted in Figure 4.4.

Among all these approaches, the phosphine oxide coupling approach B is probably the most useful method for producing side-chain and other analogues. In this method first developed by Lythoge,[50] the phosphine oxide **183** is directly coupled to a Grundmann's ketone derivative of type **184**, producing the 1α,25-D_3 skeleton. The shortcoming of this route is that the synthesis of the A-ring fragment **183** is somewhat tedious. Even though, some of the laboratories have come up

with good syntheses, more simple and efficient syntheses are desirable. We decided to develop simple chemoenzymatic route to **183** starting from phloroglucitol. Following retrosynthetic approach can be outlined (Scheme 4.28).

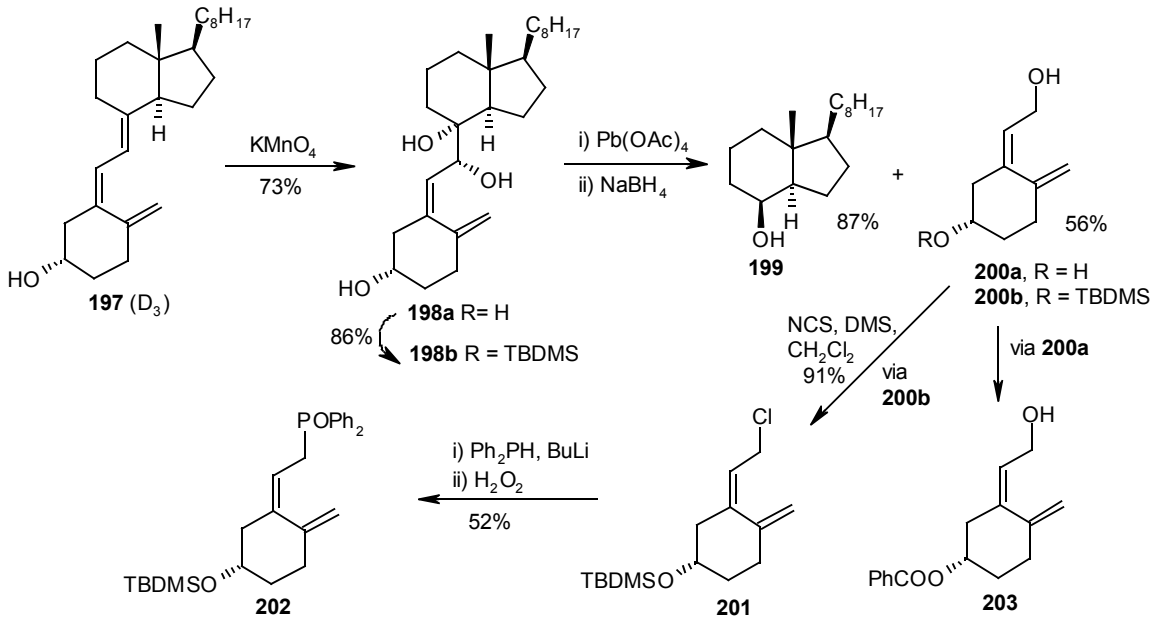


Scheme 4.28.

Synthesis of phosphine oxide fragments **183a** and **183b** are reported from cyclohexylidene esters **196a** and **196b** resp.[50c,f, 51] These cyclohexylidene esters are obtained from 3,5-*trans*-disubstituted cyclohexanones **3** and **4** resp. through Peterson's olefination in good yields.[51] We decided to develop syntheses of these two chiral ketones from the chiral intermediate **171** obtained in chemoenzymatic synthesis of lactone **2** starting from phloroglucitol **1** (Section 4.2.3.). Before presenting our work, a brief review of various methods for the synthesis of phosphine oxide fragment **183** is presented below.

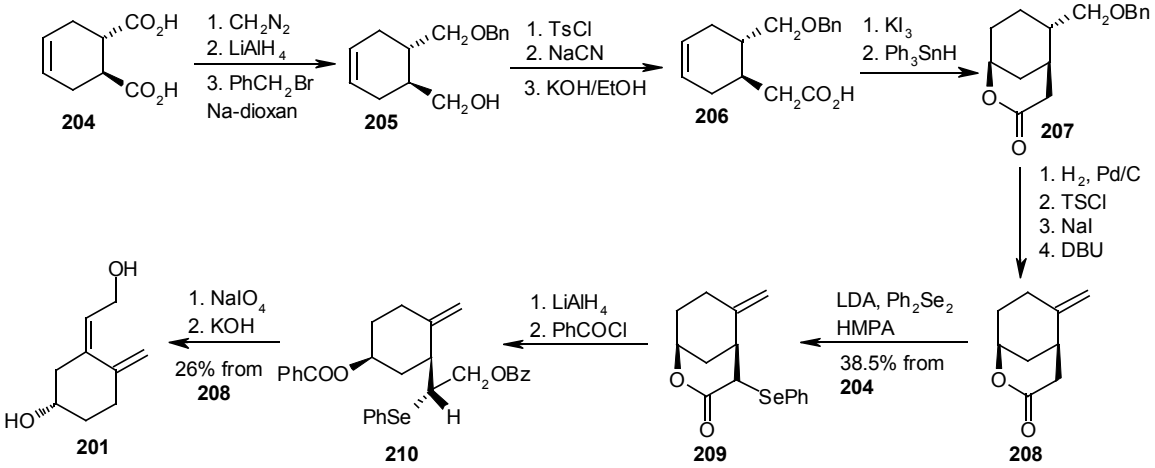
4.3.2. Reported methods of A-ring Phosphine oxides

The A-ring phosphine oxide **202** is most easily obtained through degradation of Vitamin D_3 (**197**) as shown in Scheme 4.29.[50f] The initial selective $\Delta^{7\alpha,\,8\alpha}$ -bishydroxylation of D_3 (**197**) is carried out using $KMnO_4$. The C-C bond of 7α, 8α-glycol **198** is easily cleaved via a lead tetracetate oxidation–$NaBH_4$ sequence. The phosphine oxide **202** is obtained from **200** by reaction of the corresponding TBDMS protected chloride **201** with lithium diphenylphosphide, followed by hydrogen peroxide treatment.[50c,f] Lythoge's procedure via the intermediate **203** was reported still earlier.[50f]



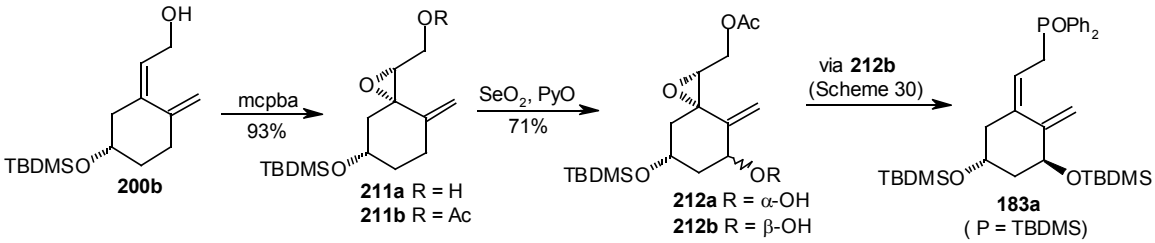
Scheme 4.29.

The total synthesis of the intermediate diol **200a** or a related C-3 derivative presents two stereochemical problems. In Lythoge's synthesis (Scheme 4.30)[50b,e] the first problem of securing the proper stereogenicity at the secondary hydroxyl center was solved by using the readily available (*S*)-cyclohex-4-en-1-*trans*-2-dicarboxylic acid (**204**) as starting material. When this acid was converted into the lactone **208**, chirality was transferred to provide the required one at the secondary hydroxyl center in the end product. The second problem, the stereospecific formation of the *Z* geometry of the trisubstituted double bond was solved by selective thermal syn elimination of the selenoxide **209**.



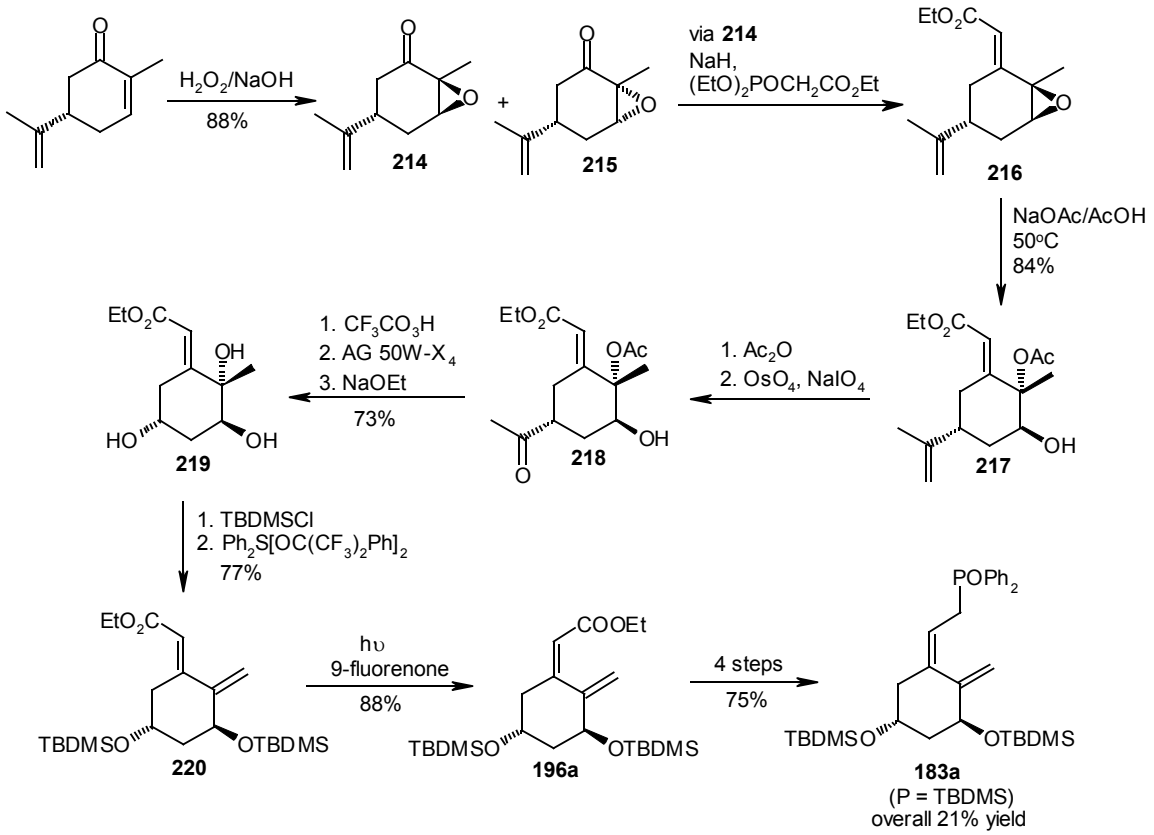
Scheme 4.30.

The Roche investigation of the chemical conversion of vitamin D_3 to its 1α,25-dihydroxy metabolite makes it possible to convert **200b** directly to the 1α-hydroxylated phosphine oxide **183a** (Scheme 4.31).[52] The more reactive Δ5,6-double bond of **200b** was protected as the corresponding epoxide using mcpba, affording exclusively α-epoxide **211a**. Oxidation of acetate **211b** with SeO_2/pyridine selectively afforded C-1 hydroxylated product **212** as 78:22 mixture of 1α and 1β-hydroxy isomers **212a** and **212b** respectively. Compound **212b** was further converted to phosphine oxide **183a** (P = -TBDMS)



Scheme 4.31.

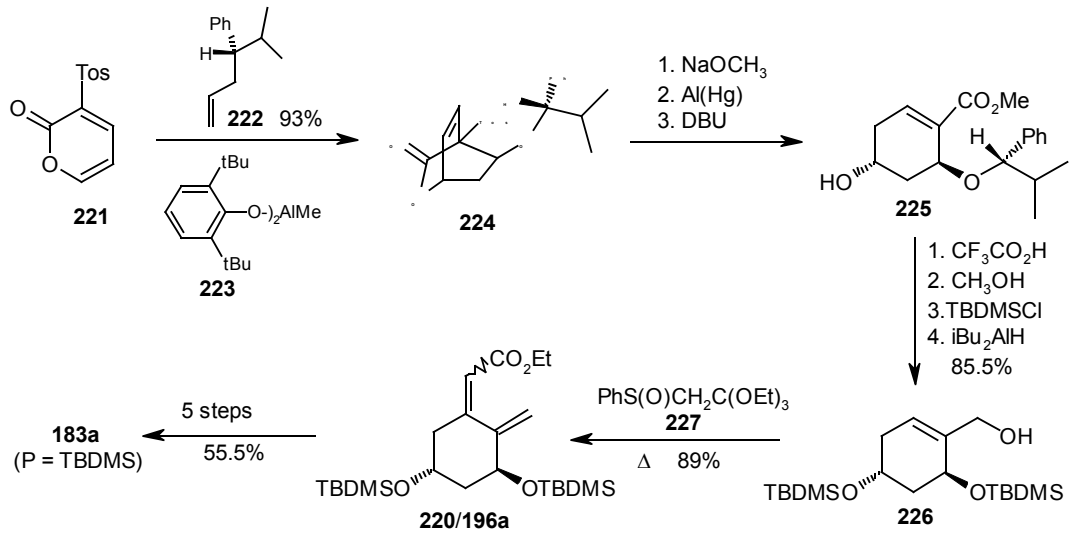
Carvone approach: The 14-step procedure of the Hoffmann-La Roche group (Scheme 4.32)[53] remains one of the most efficient total syntheses of the 1α-hydroxy A-ring phosphine oxide **183a** (P = TBDMS). Horner-Emmons reaction of (*S*)-carvone epoxide **214** gave ester epoxide **216**, which was cleaved with sodium acetate in acetic acid and esterified to produce **217**. The latter on oxidative cleavage gave methyl ketone **218**. Baeyer-Villiger oxidation and hydrolysis gave triol ester **219**. The two secondary alcohols were protected and the exomethylene group introduced by treatment with dialkoxydiaryl sulfurane reagent to induce β-elimination. The resulting E-ester **220** was isomerized to the *Z*-ester **196a** (P = TBDMS) via triplet-sensitized photoisomerization. Compound **196a** was converted to **183a** in four steps.



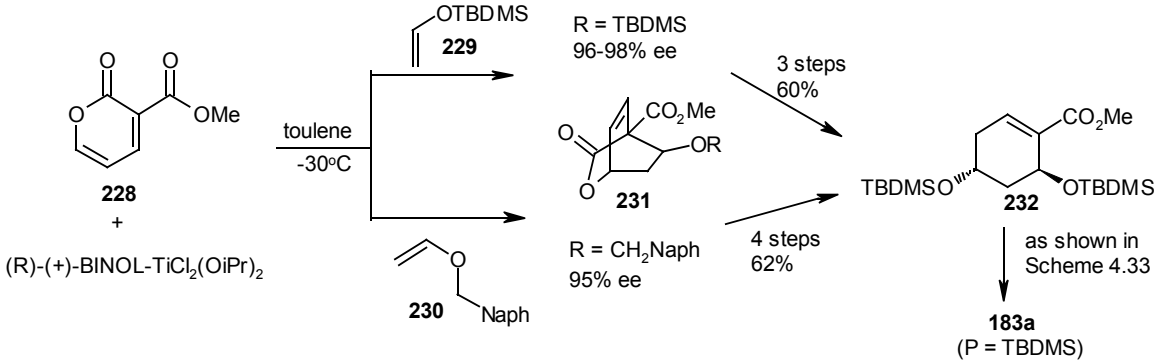
Scheme 4.32.

Diels-alder approach: Utilizing a Lewis acid-catalyzed [4+2] cycloaddition process, Posner[54] reported a 14-step synthesis of **183a** (P = TBDMS) in 34.6% overall yield (Scheme 4.33). The reaction of 3-pyrone sulfone **221** with enatiomerically pure vinyl ether **222** in the presence Yamamoto's "MAD" Lewis acid **223** gave bicyclic lactone **224** with 98:2 endo to exo diastereoselectivity. Methanolysis of lactone ring in **224** followed by reductive desulfonylation with Al/Hg produced a mixture of α,β- and β,γ-unsturated ester, which on double bond isomerization with DBU afforded, conjugated enoate ester **225**. Chiral auxiliary was sacrificed via trifluoroacetolysis. The tandem Claisen rearrangement-sulfoxide thermolysis of **226** using novel sulfonylorthoacetate **227** produced the known dienoate ester **220**/**196a** as 4:1 mixture in 89% yield. The mixture was transformed to the A-ring phosphine oxide **183a** by the Roche procedure shown in Scheme 4.32.

Investigations by the Posner laboratory on the Lewis acid promoted [4+2] cycloaddition scheme[55] revealed that the reaction of commercially available vinyl ethers **229** or **230** with methyl 12-pyrone-3-carboxylate (**228**), in the presence of chiral Lewis acidic titanium species produces adduct **231** exhibiting high enantiopurity (Scheme 4.34). The adducts are easily transformed in 3-4 steps to the intermediate **232** which can be converted to **183a** as shown in Scheme 4.33.



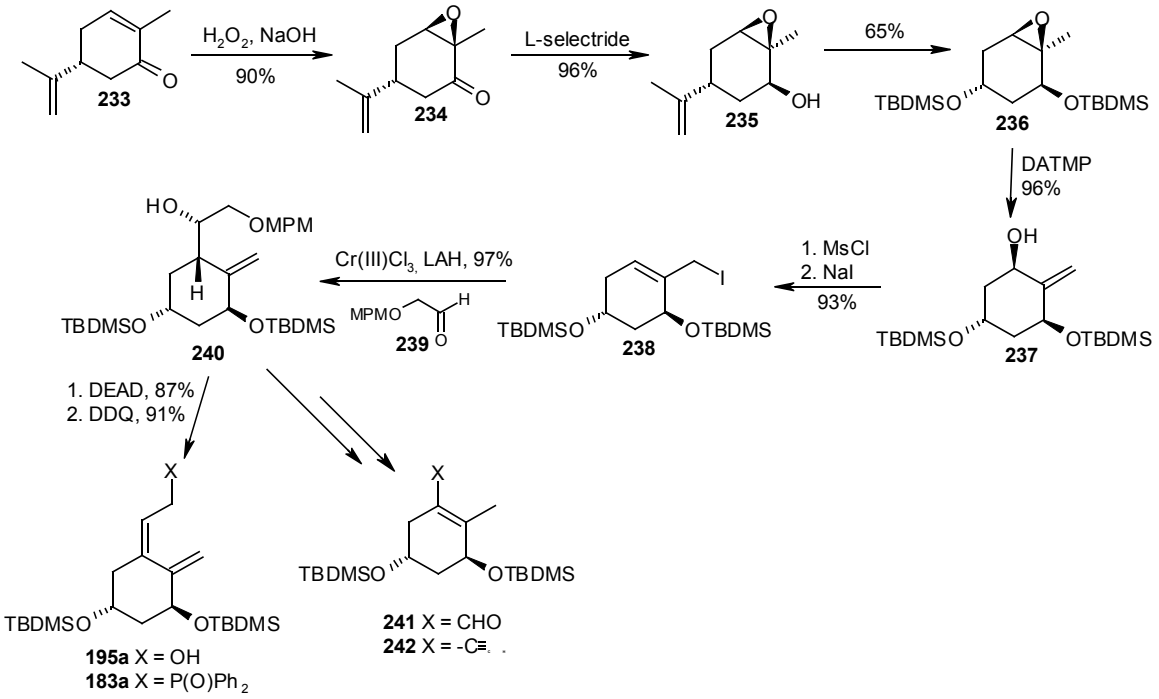
Scheme 4.33.



Scheme 4.34.

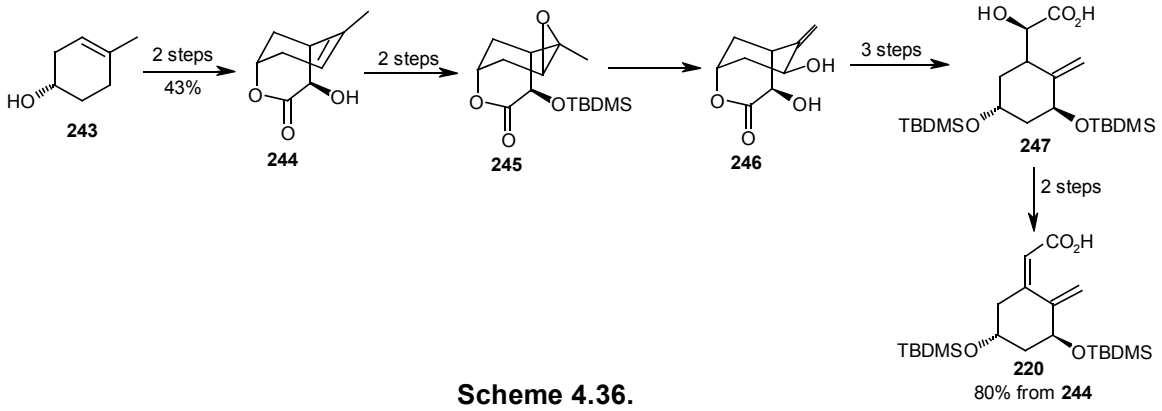
Other syntheses based on Diels-Alder approach are also reported by De Clerq[56] and Posner[57].

Cr(II)-mediated reactions: In the approach of Htakeyama et al,[58] as shown in Scheme 4.35, the intermediate **239** for synthesis of A-ring phosphine oxide **183a** was synthesized starting from (*R*)-(-)-carvone (**233**), using a diastereoselective chromium (II)-mediated addition of an allylic halide to aldehyde as a key step. The epoxide **234** obtained from **233** was stereoselectively reduced with lithium tri-sec-butylborohydride to give 13:1 mixture of **235** and its epimer. Oxidative degradation of the isopropenyl group, TBDMS protection and the epoxide rearrangement with diethylaluminium 2,2,6,6-tetramethylpiperidine (DATMP) led to regioselective formation of allyl alcohol **237**. The latter was then converted to iodide **238**, which was utilized in the crucial chromium (II)-mediated reaction with aldehyde **239** to afford alcohol **240** with excellent diastereolselectivity (~100% yield of single diastereomer). Dehydration of the alcohol (Mitsunobu reagent) afforded, after oxidative deprotection, the desired alcohol **195a**, which could be converted to the A-ring phosphine oxide **183a** by a procedure outlined earlier. This 15 step procedure from (*R*)-carvone, 25% overall yield, is comparable to that of the Hoffmann-La Roche group (Scheme 4.32) and is of additional use since other A-ring precursors **241** and **242** are also available from the common intermediate **240**.

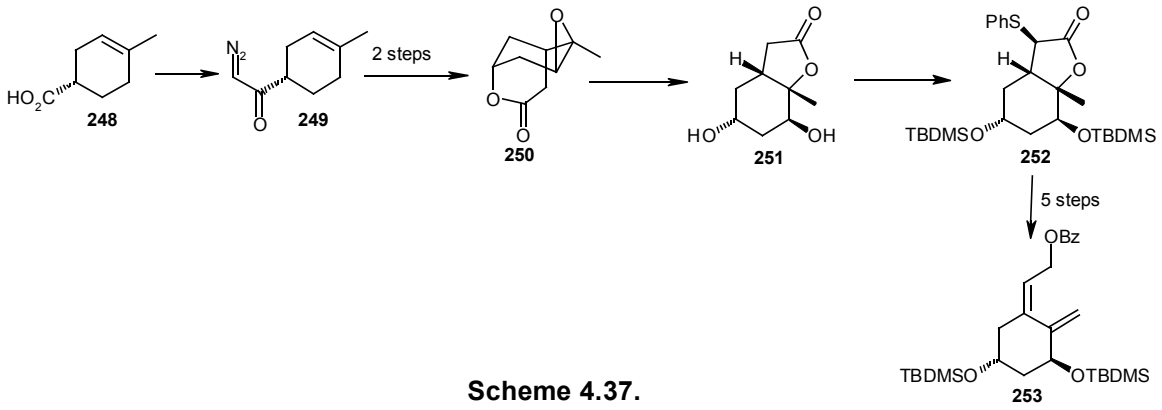


Scheme 4.35.

Ene reaction: In both procedures of Stork (Scheme 4.36 and 4.37),[59] an intermolecular chirality transfer was used for two-carbon homologation at C-5 and the 1β-hydroxyl was introduced through epoxidation-ring opening of the resulting double bond introduced in the first step. The two routes are conceptually related but the first one (Scheme 4.36) is starting with the difficultly accessible 4-methyl-3-cyclohexenol (**243**) having high optical purity. The advantage of the second route is that the starting material is the readily available optically pure 4-methyl-3-cyclohexenecarboxylic acid (**248**, Scheme 4.37). The two routes lead to the attractive intermediate **220** and **253** respectively. Similarly Uskokovic and coworkers have also developed synthesis of **183a** based on stereoselective intermolecular ene reaction.[60]

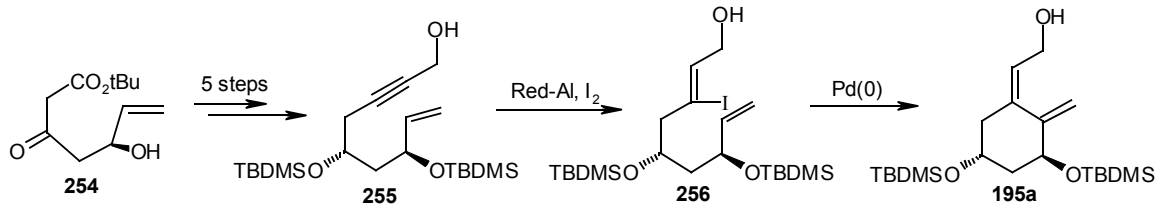


Scheme 4.36.



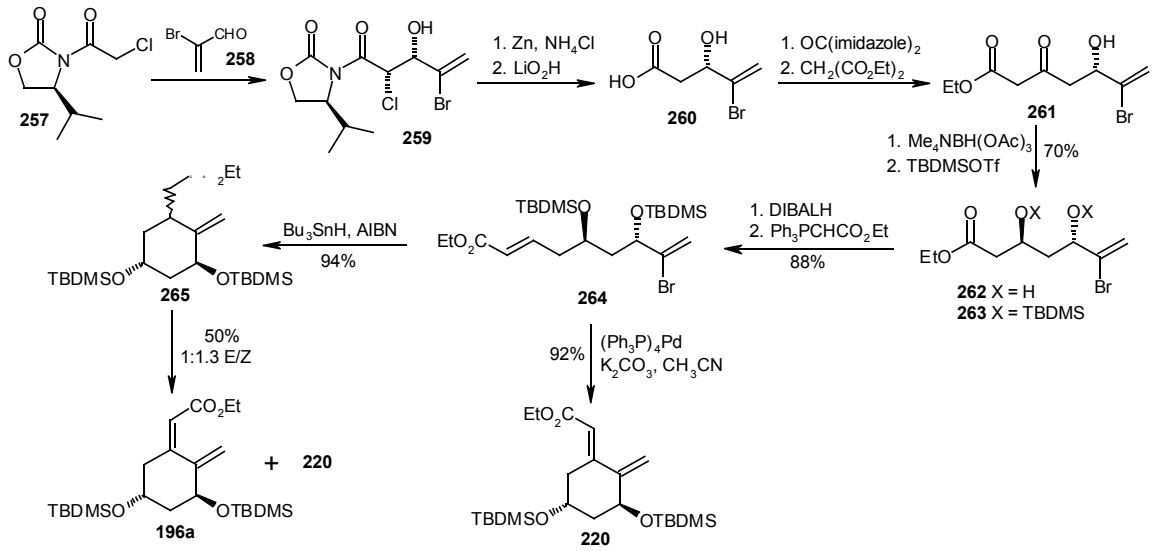
Scheme 4.37.

Pd-catalyzed cyclization: A short and versatile approach for regio- and stereoselective synthesis of bis-exocyclic conjugated dienes was developed in Mourino's laboratory in Spain (Scheme 4.38).[61] The requisite (*Z*)-vinyl iodide **256** for cyclization was prepared by Corey's reductive iodination method or Denmark's modification. Reflux of the vinyl iodide **256** with 5 mol% $Pd(PPh_3)_4$ and Et_3N gave exclusively desired cyclization product **195a** (22% overall yield). Few other synthesis based on Pd-catalyzed cyclization are reported by Shimizu,[62,63] Ogasawara.[64]



Scheme 4.38.

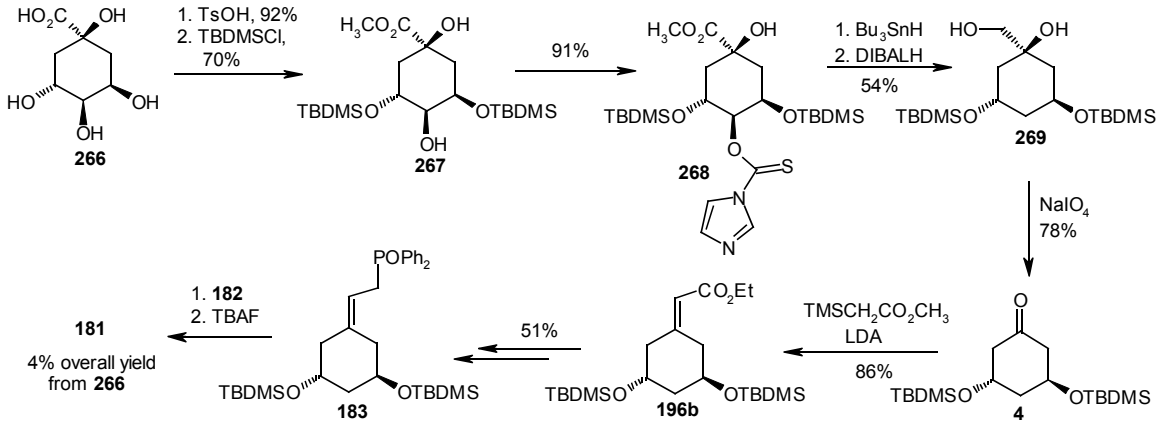
Radical cyclization: Radical cyclization of acyclic vinyl selenide or an aryl radical cyclization leading to the synthesis of A-ring synthons have been studied.[65,66] The improved version of this strategy (nine step to **220**, 26% overall yield) is outlined in Scheme 4.39 starting from α-bromoacrolein (**258**).[67] Evans type syn-selective aldol reaction of bromoacrolein **258** with the bornenolate of 3-(chloroacetyl)-4-(*S*)-isopropyloxazolidinone (**257**) gave a single diastereomer **259**. Reductive removal of the unwanted chlorine atom followed by saponification with lithium hydroperoxide (LiO_2H) afforded **260**. The two-carbon homologated β-hydroxyketone **261** was reduced by the Evans protocol (tetramethylammonium triacetoxyborohydride), affording the diol **262** as a 13:1 anti/syn mixture (87%). After further transformation, radical cyclization of the resulting **264** (Bu_3SnH, AIBN) generated **265** (94%). The latter was dehydrogenated to mixture of **220** and **196a**. However the bromide **264** could be efficiently converted to the single E-isomer **220** by Pd-catalyzed cyclization [$(Ph_3P)_4Pd$, K_2CO_3, CH_3CN, 92%].



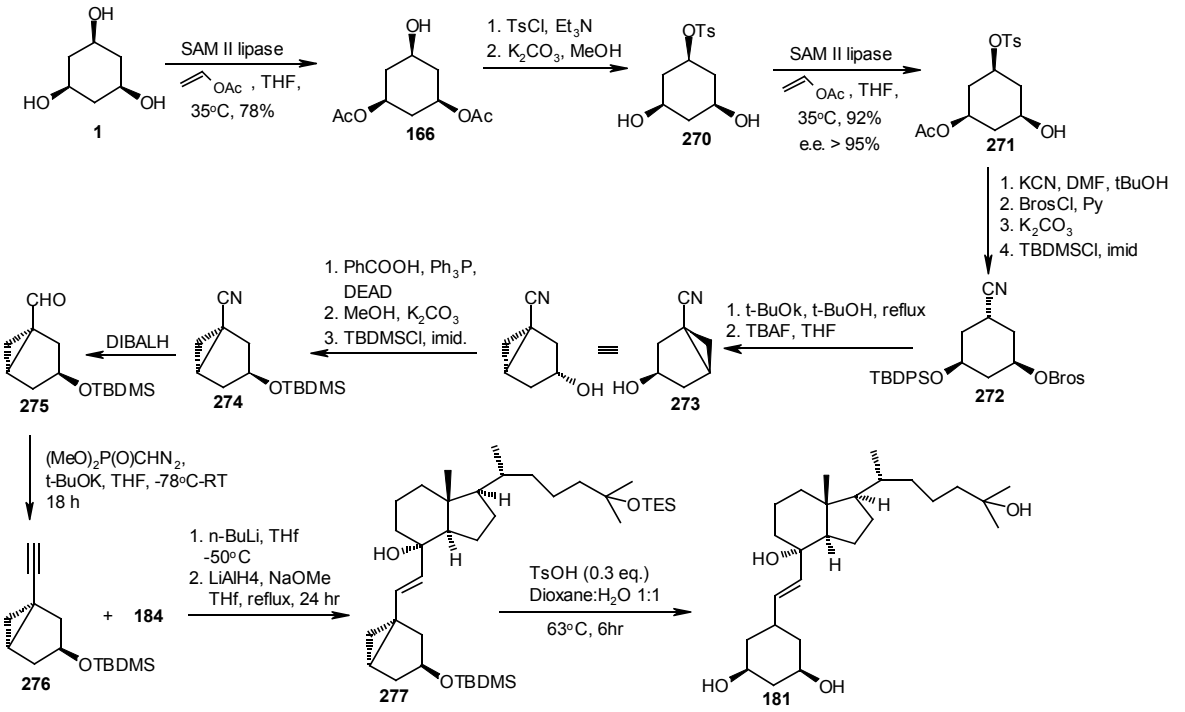
Scheme 4.39.

De Luca reported the synthesis of 19-nor-A-ring phosphine oxide **183b** via elaboration of (-)-quinic acid (**266**) as shown in Scheme 4.40.[51] Esterification of the latter followed by silylation gave ester **267** in which the secondary alcohol was removed via Barton's procedure and then the ester was reduced to alcohol **269** with DIBALH. Oxidation with $NaIO_4$ followed by Peterson's olefination [ethyl(trimethylsilyl)acetate/LDA, -78°C) gave the cyclohexylidene ester **196b**. The latter was converted to the 19-nor-A-ring phosphine oxide **183b** and then Horner-Wittig condensation with CD-ring fragment **184** yielded the 19-noranalogue **181** (4% overall yield from **266**).

Vandewalle reported chemoenzymatic synthesis of A-ring synthon starting from phloroglucitol **1**.[33] Chiral intermediate **271** was prepared chemoenzymatically as shown in Scheme 4.41. Tosylate **271** was converted to cyanide **272**, which after protecting group interconversion, intramolecular alkylation and deprotection afforded **273**. This alcohol after Mitsunobu inversion, followed by methanolysis of benzoate, TBDMS protection and DIBALH reduction of cyanide afforded aldehyde **275** which was converted to alkyne **276** via Seyferth's method using dimethyldiazomethylphosphonate. Reaction of lithiated alkyne **276** with ketone **184** gave the propargylic alcohol, which was subsequently reduced to (E)-allylic alcohol **277**. Acid-catalyzed solvolysis of **277** with concomitant deprotection afforded 19-nor-1α, 25-dihydroxyvitamin D_3 (**181**).



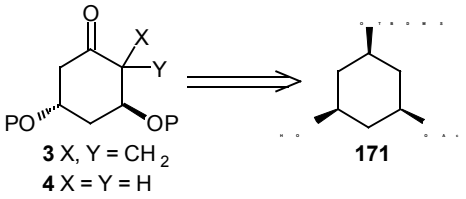
Scheme 4.40.



Scheme 4.41.

4.3.3. Present work and Discussion

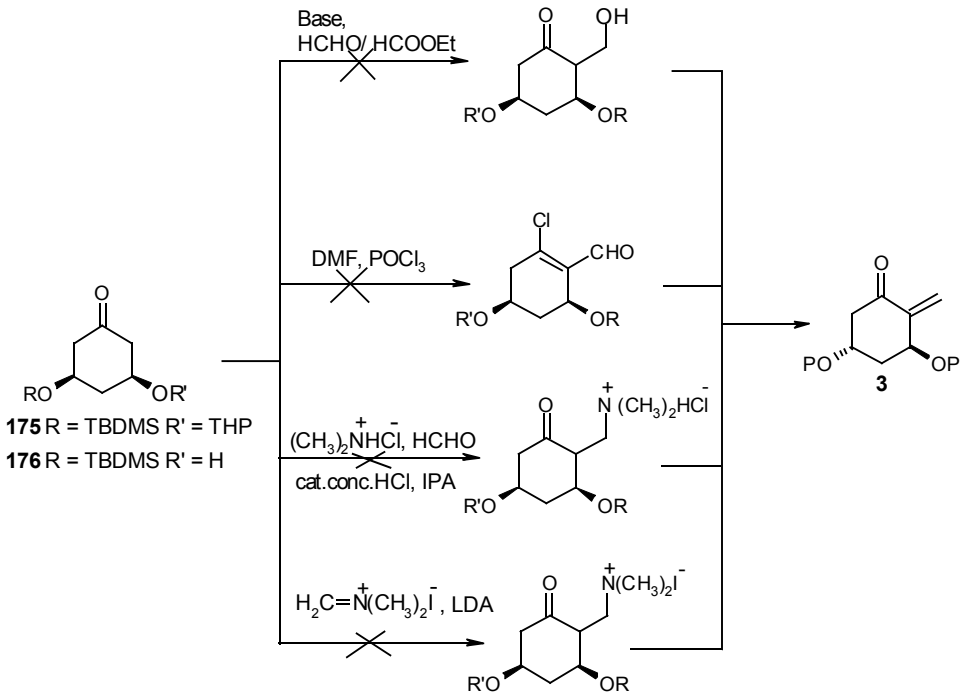
We planned to utilize optically active intermediate **171** for the syntheses of 4,5-*trans*-substituted ketones **3** and **4** (Scheme 4.42). The major tasks involved for the synthesis of these ketones would be (i) inversion at one of the protected hydroxy groups to get desired *trans*-geometry (ii) Introduction of exomethylene group on the cyclohexane ring to get **3**.



Scheme 4.42.

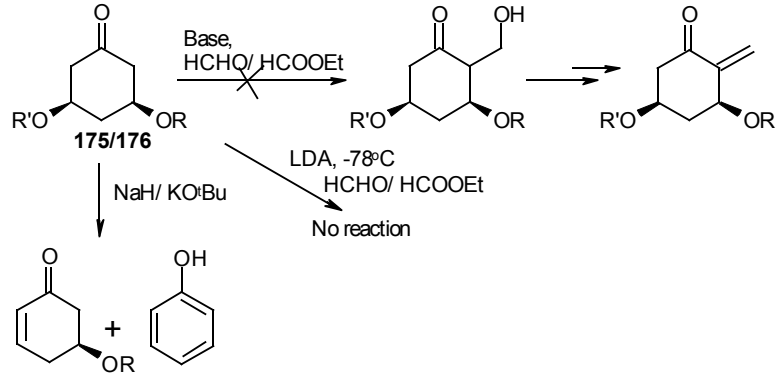
4.3.3.1. Attempted synthesis of diprotected 3,5-dihydroxy-2-methylene-(3*S*, 5*S*)-cyclohexan-1-one (3)

We planned to utilize ketones **175** and **176** (Scheme 4.26, Route A). Various attempts were made for the introduction of exomethylene group on these ketones as shown in Scheme 4.43.



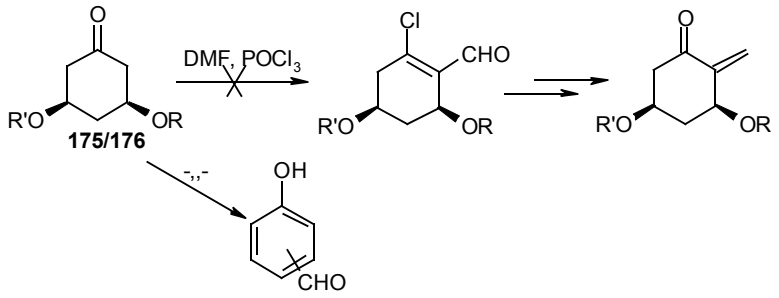
Scheme 4.43.

Introduction of exomethylene was planned through hydroxymethylation[68] followed by elimination. Ketones **175** and **176** were treated with bases as NaH, KO^{t}Bu and LDA at low temperature followed by treatment with formaldehyde or ethyl formate. But when NaH and KO^{t}Bu were used only elimination products were recovered whereas when LDA was used as starting materials could be recovered (Scheme 4.44).



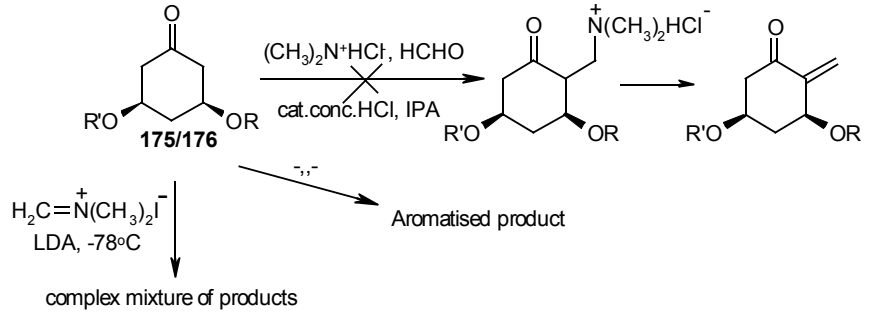
Scheme 4.44.

Next we planned to carry out Vilsmeier formylation[69] on ketones **175** and **176** to afford chloroaldehyde, which can further be converted to desired exomethylene intermediate. But when **175** and **176** were treated with DMF-$POCl_3$ at low temperature only formylated aromatized compounds i.e. salicylaldehyde could be isolated (Scheme 4.45).



Scheme 4.45.

Further we attempted acid catalyzed Mannich reaction on **175** and **176** to afford Mannich base, which can be further eliminated, to furnish exomethylene group. But under Mannich conditions also aromatized compounds were obtained (Scheme 4.46). Therefore, we attempted LDA catalyzed Mannich reaction at –78°C using dimethylmethyleneammonium iodide,[70] but only a complex mixture of products could be obtained (Scheme 4.46).

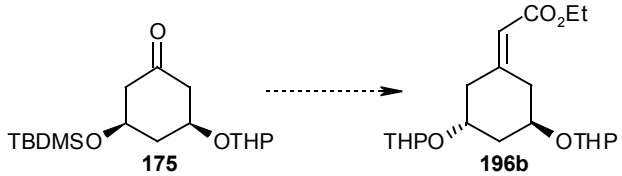


Scheme 4.46.

Thus our attempts for the synthesis of intermediate **3** with exomethylene were unsuccessful. Therefore we decided to focus on intermediate **196b** and **4** required for 19-norvitamin D_3.

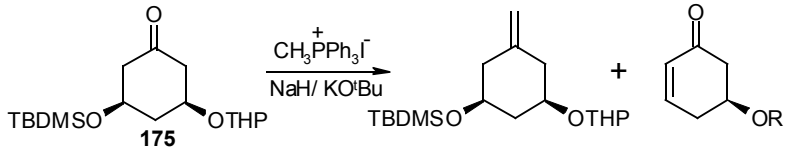
4.3.3.2. Attempted synthesis of ethyl-2-[3,5-di(tetrahydro-2*H*-2-pyranyloxy)-(3*R*,5*R*)-cyclohexylidene] acetate (196b) from ketone 175

Synthesis of **196b** from ketone **175** would require Wittig reaction on carbonyl and inversion at one of the protected hydroxy center (Scheme 4.47).



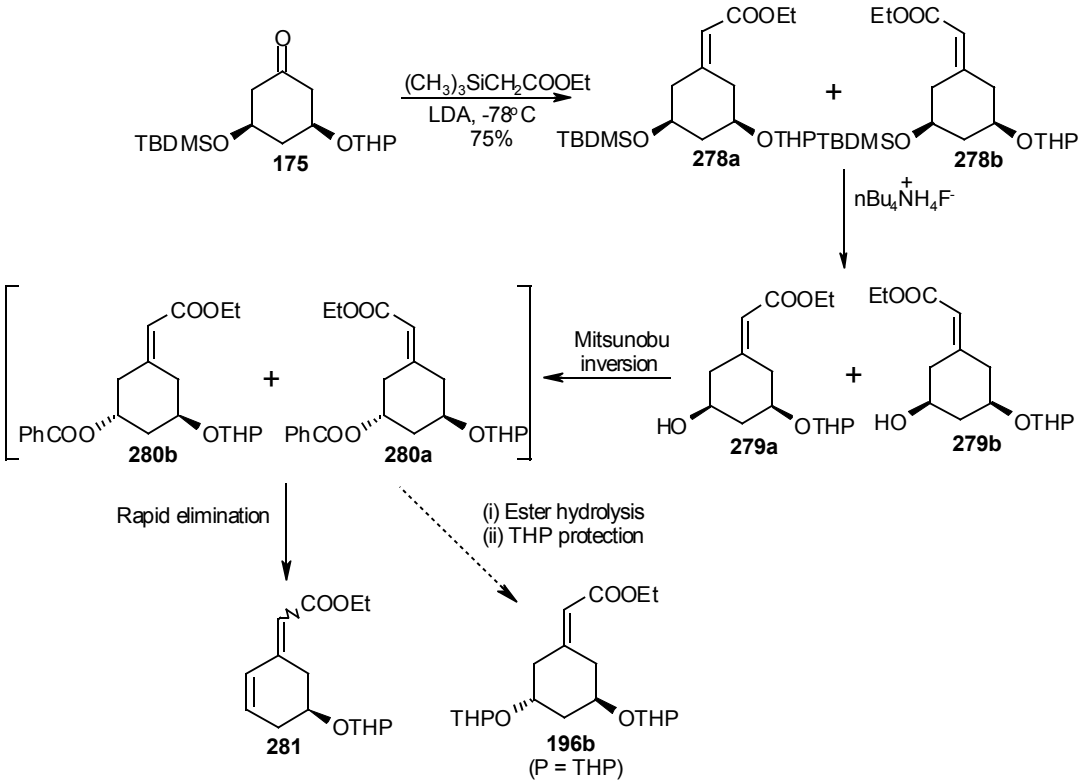
Scheme 4.47.

When a model Wittig reaction on **175** with $CH_3P^+Ph_3I^-$ was carried out using NaH and KO^{t}Bu as bases, a Wittig compound along with large amount of eliminated products was obtained (Scheme 4.48). Thus, ketone **175** is reactive enough to give Wittig reaction, but is sensitive for bases as NaH, KO^{t}Bu.



Scheme 4.48.

As per our earlier observation, this compound is stable with LDA at low temperatures. Therefore we decided to carry out Wittig reaction using LDA at –78°C. Peterson's olefination using ethyl(trimethylsilyl)acetate and LDA at –78°C is reported to give exocarboethoxymethylene on cyclohexanone derivatives.[51,71] When we treated ketone **175** with LDA and ethyl(trimethylsilyl)acetate at –78°C, we could isolate mixture of cyclohexylidene esters **278a** and **278b** from reaction mixture in 75% yield (Scheme 4.49). Here we planned to exploit symmetric nature of 3,5-disubstituted cyclohexane ring to obtain required stereochemistry in A ring synthon. Thus, deprotection of –OTBDMS would afford mixture of **279a** and **279b**. This mixture on Mitsunobu inversion would afford mixture of benzoates **280a** and **280b**, which on further ester hydrolysis and protection of inverted hydroxy group, would furnish **196b** (P = THP). We could successfully obtain mixture of **279a** and **279b** by treating **278** with TBAF in aqueous THF in 85% yield. Mixture was further reacted under Mitsunobu conditions[72] using DEAD and benzoic acid to obtain benzoates with inverted configuration, but corresponding benzoates were found to be very prone to elimination and only a mixture of eliminated compounds **281** could be isolated.

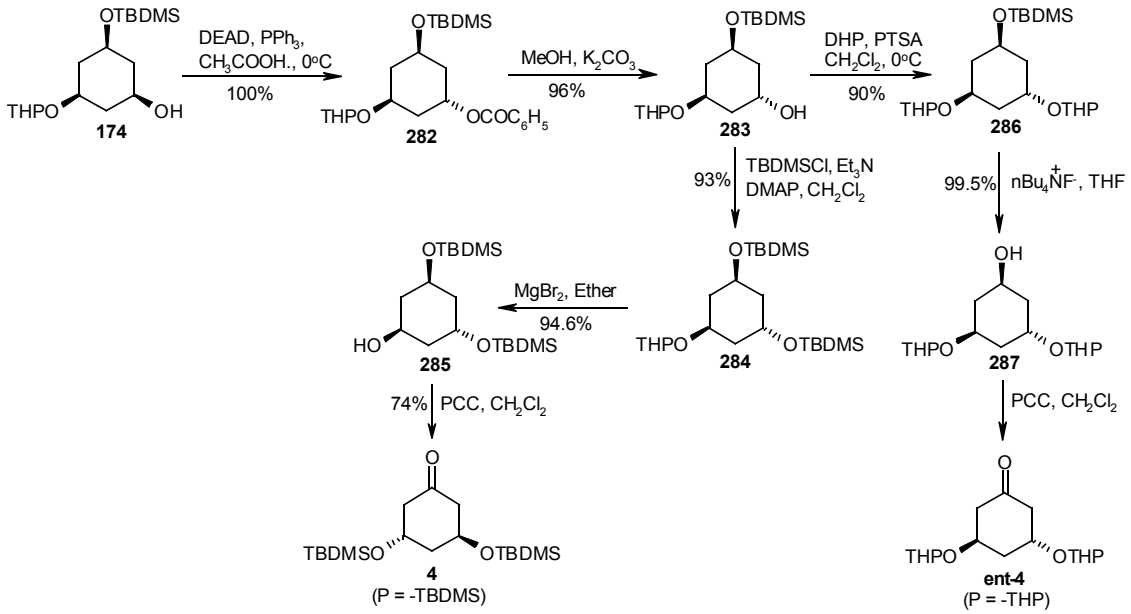


Scheme 4.49.

Thus, the scheme was not successful due to elimination problem. Therefore we decided to postpone introduction of exo-carboethoxymethylene after Mitsunobu inversion. We planned synthesis of ketone **195b** from optically active intermediate **174**.

4.3.3.3. Synthesis of enantiomers of diprotected *trans* 3,5-dihydroxy cyclohexanone (4 and ent-4) from 174.

For the synthesis of ketone **4** from **174** would involve Mitsunobu inversion and protecting group interchange as critical steps. Compound **174** was subjected to Mitsunobu inversion at 0°C using DEAD and benzoic acid (Scheme 4.50). Reaction was neat and fast and we could isolate required inverted benzoate **282** in almost quantitative yield. Methanolysis of **282** with MeOH/K_2CO_3 afforded cyclohexanol **283** with required *trans* substitution pattern in 96% yield. Further, TBDMS protection (TBDMSCl, Et_3N, DMAP in dichloromethane, 90% yield), THP deprotection using $MgBr_2$ (95% yield) and PCC oxidation (75% yield) afforded the required ketone **4** (P = TBDMS) in 62% overall yield from **174** and in 33% overall yield from **1**. We also made **ent-4** (P = THP) from **283**. In this case, free –OH in **283** was protected as THP ether **286** (DHP, PTSA, 0°C, 98% yield) followed by TBDMS deprotection (TBAF, THF, 90% yield) and PCC oxidation (90% yield) to obtain **ent-4** (P = THP) in 76% overall yield from **174** and 42% overall yield from **1**. Enantiomeric excess of **ent-4** (P =THP) was determined to be 94% by HPLC analysis on chiral column Lichrocart (*R*, *R*) Whelk- O 1 (5μm) column (Page 194). Enantiomeric excess of the ketone **4** could not be determined directly as its enantiomers did not separate on chiral columns available with us. Since all the reaction were carried out under nonracemizing conditions, e.e. of **4** was also assumed to be near 95% similar to that of **ent-4** (P = THP).



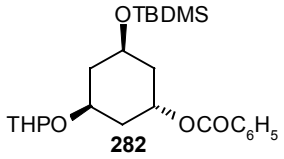
Scheme 4.50.

4.3.4. Conclusion

*Thus, even though our attempts to synthesize ketone **3** with exomethylne double bond were not successful, we have developed a simple and efficient synthesis of both enantiomers of ketone **4**, a useful intermediate of phosphine oxide A-ring synthon **183b** for 19-nor D_3 (**181**), to which it can be converted as shown before (Scheme 4.40).*

4.3.5. Experimental

*Preparation of 1-phenylcarbonyloxy-3-tetrahydro-2H-2-pyranyloxy-5-tert-butyldimethylsilyl oxy-(1R, 3R, 5R)-cyclohexane (**282**)*



282

3-Tetrahydro-2*H*-2-pyranyloxy-5-*tert*-butyldimethylsilyloxy-(1*S*,*3R*,5*S*)-cyclohexan-1-ol **(174**, 1.2g, 3.64 mmol), benzoic acid (0.61g, 5 mmol), triphenylphosphine (1.31g, 5 mmol) were placed in 50 ml two-necked round-bottomed flask equipped with two-way stopcock and sidearm addition funnel. Assembly was evacuated and flushed with argon. Anhydrous THF (10 ml) was added and the solution was cooled to -10°C in ice-salt mixture with magnetic stirring. To the cold, stirred solution, diethylazodicarboxylate (0.87g, 5 mmol) in anhydrous THF (5 ml) was added dropwise over the period of 30 min. while maintaining the temperature well below 0°C. After the completion of addition, reaction mixture was further stirred at 0°C for 3 hr. Then it was filtered through bed of silica gel. Organic layer was separated and THF was evaporated under vacuum. Aqueous layer was extracted with ethyl acetate (2x10ml). Residue from THF layer was dissolved in combined ethyl acetate and washed with aqueous sodium bicarbonate solution, followed by brine wash. It was dried on anhydrous sodium sulphate and solvent was evaporated under vacuum. Residue was purified on silica gel column (8% ethyl acetate in pet.ether) to afford 1-phenylcarbonyloxy-3-tetrahydro-2*H*-2-pyranyloxy-5-*tert*-butyldimethylsilyloxy-(1*R*,3*R*,5*R*)-cyclohexane (**282** yield 1.57g, 100%) as a viscous liquid.

^{1}H NMR ($CDCl_3$): δ 0.06 (s, 6H), 0.88 (s, 9H), 1.20-1.95 (m, 9H), 2.05-2.60 (m, 3H), 3.51 (m, 1H), 4.00 (m, 2H), 4.69, 4.80 (2m, 1H), 5.52 (m,1H), 7.40-7.65 (m, 3H), 8.00 (md, 2H)

^{13}C NMR ($CDCl_3$): δ -4.50, 18.18, 19.57, 19.79, 25.93, 31.23, 35.64, 37.00, 39.46, 41.70, 43.58, 60.34, 62.32, 66.18, 69.71, 70.45, 96.54, 97.61, 128.49, 129.59, 130.10, 130.80, 132.93, 165.58

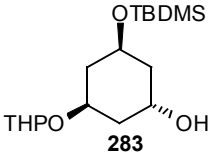
IR ($CHCl_3$): cm^{-1} 418.47, 440.12, 469.92, 668.20, 748.14, 770.55, 1026.85, 1097.70, 1215.23, 1275.35, 1712.12, 2857.35, 1712.12, 2857.25, 2951.01, 3017.6

Mass: Base m/e = 105 other m/e: 293, 179, 171, 159, 135, 129, 122, 105, 85, 67, 55

Elemental analysis: calculated for $C_{24}H_{38}O_5Si$: C 66.36%, H 8.76% Found C 66.24%, H 9.06%

Specific rotation $[\alpha]_D$ = +3.71 (c = 1.3, $CHCl_3$)

Preparation of 3-tetrahydro-2H-2-pyranyloxy-5-tert-butyldimethylsilyloxy-(1R,3R,5S)-cyclohexan-1-ol (283**)**

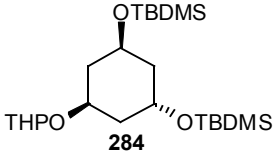


283

1-Phenylcarbonyloxy-3-tetrahydro-2*H*-2-pyranyloxy-5-(*tert*-butyldimethylsilyloxy)-(1*R*,3*R*,5*R*)-cyclohexane (**282**,1.7g, 3.92 mmol) was dissolved in dry methanol (15 ml). To the solution, anhydrous potassium carbonate (0.5g, 3.6 mmol) was added and the reaction mixture was stirred at RT for 12 hr. Methanol was removed under vacuum and residue was extracted with ethyl acetate (3x10 ml). Organic extracts were combined, washed with brine and dried on anhydrous sodium sulphate. Solvent was removed under vacuum and residue was purified on silica gel column (20% ethyl acetate in pet. ether) to afford 3-tetrahydro-2*H*-2-pyranyloxy-5-*tert*.butyldimethylsilyloxy-(1*R*,3*R*,5*S*)-cyclohexan-1-ol (**283**, yield = 1.24 g (96%) as an oily liquid.

^{1}H NMR ($CDCl_3$): δ 0.07 (s, 6H), 0.84, 0.86 (2s, 9H), 1.20-2.35 (m, 13H), 3.49 (m, 1H), 3.90 (m, 1H), 4.00(m, 2H), 4.28 (m, 1H), 4.71 (m, 1H)
^{13}C NMR ($CDCl_3$): δ -4.66, 16.00, 19.70, 25.40, 25.80, 31.14, 37.82, 39.78, 41.73, 41.91, 43.53, 62.55, 62.67, 65.75, 66.36, 69.41, 70.02, 96.82, 97.34
IR ($CHCl_3$): cm^{-1} 434.79, 471.18, 667.93, 750.97, 769.50, 834.92, 1024.76, 1099.22, 1123.63, 1214.00, 1252.49, 2856.97, 2892.10, 2947.86, 3013.26, 3450.00
Mass: Base m/e = 85 other m/e309, 229, 213, 187, 171, 159, 145, 119, 97, 79, 75, 69, 55
Elemental analysis: calculated for $C_{17}H_{34}O_4Si$: C 61.82%, H 10.30% Found C 61.76%, H 10.76%
Specific rotation $[\alpha]_D$ = +1.59 (c = 1, $CHCl_3$)

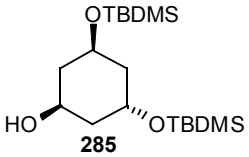
*Preparation of 2-[3,5-di(tert-butyldimethylsilyloxy)cyclohexyloxy]tetrhydro-2H-pyran (**284**)*



284

3-Tetrahydro-2*H*-2-pyranyloxy-5-*tert*-butyldimethylsilyloxy-(1*R*,3*R*,5*S*)-cyclo-hexan-1-ol (**283,** 0.5g, 1.5 mmol) was dissolved in dry dichloromethane (5 ml) and dry HMPA (0.5 ml) under the atmosphere of argon. Solution was cooled to 0^{o}C in ice-salt mixture. To the cold, stirred solution, *tert*.butyldimethylsilyl chloride (0.35g, 2.3 mmol) and DMAP (0.02g, 0.16 mmol) dissolved in dry dichloromethane (2 ml) was added dropwise while maintaining temperature below 0^{o}C. Reaction mixture was stirred for 5 min. To the cold reaction mixture, triethylamine (0.3g, 2.97 mmol, 0.41 ml) was added dropwise while maintaining temperature below 0^{o}C. After the addition was over, reaction mixture was stirred at RT for 12 hr. The reaction was quenched by adding ice-cold, dilute hydrochloric acid. Organic layer was separated. Aqueous layer was extracted with dichloromethane. Organic layers were combined and washed with dilute hydrochloric acid, brine + water, 10% sodium bicarbonate solution and finally with brine + water. The organic layer was dried on anhydrous sodium sulphate and solvent was evaporated under vacuum. Residue was purified on silica gel column (2% ethyl acetate in pet. ether) to afford pure 2-[3,5-di(*tert*-butyldimethylsilyloxy)cyclohexyloxy]tetrhydro-2*H*-pyran (**284**, yield 0.625g, 93%) as an oily liquid.
^{1}H NMR ($CDCl_3$): δ 0.05, 0.06 (2s, 12H), 0.88 (s, 18H), 1.20-2.35 (m, 12H), 3.51 (m, 1H), 4.01 (m, 3H), 4.19(m, 1H), 4.26 (m, 1H), 4.65,4.73 (2m, 1H)
^{13}C NMR ($CDCl_3$): δ -5.02, -4.66, 17.96, 18.20, 19.82, 20.06, 25.53, 25.71, 25.92, 31.11, 31.29, 38.74, 40.33, 41.94, 42.80, 43.65, 62.36, 62.79, 66.06, 67.12, 67.25, 69.81, 70.24, 96.85, 97.37
IR ($CHCl_3$): cm^{-1} 418.47, 450.49, 475.90, 668.55, 754.95, 766.85, 834.90, 1026.26, 1214.41, 2856.33, 2890.66, 2949.72, 3017.62
Mass: Base m/e = 85 other m/e: 303, 285, 211, 185, 171, 159, 145, 129, 115, 101, 75
Elemental analysis: calculated for $C_{23}H_{48}O_4Si_2$: C 62.16%, H 10.81% Found C 62.51%, H 11.01%
Specific rotation $[\alpha]_D$ = +3.98 (c = 1, $CHCl_3$)

*Preparation of 3,5-di(tert-butyldimethylsilyloxy)cyclohexan-1-ol (**285**)*



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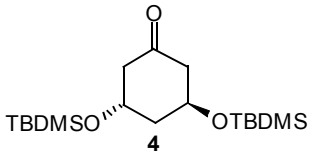
2-[3,5-Di(*tert*-butyldimethylsilyloxy)cyclohexyloxy]tetrahydro-2*H*-pyran (**284**, 0.3g, 676 mmol) was dissolved in dry ether under argon atmosphere. To the solution, magnesium bromide etherate (0.524g, 2.03 mmol) was added and reaction mixture was stirred for 3 hr at RT. Reaction was quenched by adding cold, saturated ammonium chloride solution. Ether layer was separated and aqueous layer was extracted with ether (ether 3 x 5ml). Organic extracts were combined, washed with brine and dried on anhydrous sodium sulphate. Solvent was evaporated under vacuum and residue was purified on silica gel column (10% ethyl acetate in pet. ether) to afford 3,5-di(*tert*-butyldimethylsilyloxy)cyclohexan-1-ol (**285**, yield 0.23 g, 95%) as a viscous liquid.

^{1}H NMR ($CDCl_3$): δ 0.09 (s, 12H), 0.91(s, 18H), 1.48-2.05 (m, 7H), 4.10(m, 1H), 4.27 (m, 2H)
^{13}C NMR ($CDCl_3$): δ -4.93, -4.75, 17.96, 18.08, 25.77, 42.68, 64.50, 67.55, 68.89
IR ($CHCl_3$): cm^{-1} 436.48, 456.97, 753.18, 769.02, 834.27, 1038.84, 1059.71. 1092.27, 1117.36, 1215.26, 1253.91, 2856.84, 2891.84, 2934.32, 2950.00, 3013.66, 3481.73
Mass: Base m/e = 145 other m/e: 303, 227, 211, 185, 171, 133, 129, 115, 101, 79, 72, 59
Elemental analysis: calculated for $C_{18}H_{40}O_3Si_2$: C 60.00%, H 11.11% Found C 60.12%, H 11.54%
Specific rotation $[\alpha]_D$ = +2.98 (c = 1.17, $CHCl_3$)

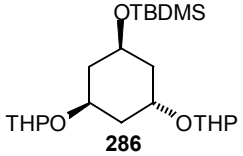
*Preparation of 3,5-di(tert-butyldimethylsilyloxy)-(3S,5S)-cyclohexan-1-one (**4**)*



4

3,5-Di(*tert*-butyldimethylsilyloxy)cyclohexan-1-ol (**285**, 0.15g, 0.412 mmol) was dissolved in dichloromethane (3 ml). To the solution, pyridinium chlorochromate (0.153g, 0.708 mmol) and anhydrous sodium acetate (0.02g) was added and reaction was stirred for 1 hr. Reaction mixture was diluted with ether (5 ml) and solvent was decanted. Sticky residue was extracted with ether (3 x 5ml). Ether extracts were combined, washed successively with brine + water and brine. The organic layer was dried on anhydrous sodium sulphate and solvent was removed under vacuum. Residue was purified on silica gel column (1% ethyl acetate in pet. ether) to afford 3,5-di(*tert*-butyldimethylsilyloxy)-(3*R*,5*S*)-cyclohexan-1-one (**4**, yield 0.10g, 70%) as an oily liquid.
^{1}H NMR ($CDCl_3$): δ 0.06 (s, 12H), 0.87 (s, 18H), 1.95 (t, 2H, J = 9), 2.38 (dd, 2H, J = 12), 2.55 (dd, 2H, J = 6), 4.35 (qn, 2H, J = 6)
^{13}C NMR ($CDCl_3$): δ -4.94, -4.88, 17.99, 25.71, 42.13, 50.25, 66.83, 207.84
IR ($CHCl_3$): cm^{-1} 411.34, 433.90, 452.41, 460.86, 477.78, 766.81, 805.90, 837.57, 868.49, 902.03, 1035.53, 1063.88, 1100.46, 1216.07, 1255.24, 1719.21, 2858.22, 2954.96, 3019.42
Mass: Base m/e = 143 other m/e: 301, 101
Elemental analysis: calculated for $C_{18}H_{38}O_3Si_2$: C 60.33%, H 10.61% Found C 60.21%, H 11.02%
Specific rotation $[\alpha]_D$ = -14.62 (c = 1.1, $CHCl_3$)

*Preparation of 2-[3-tetrahydro-2H-2-pyranyloxy-5-(tert-butyldimethylsilyloxy)cyclohexyl oxy]tetrahydro-2H-pyran (**286**)*

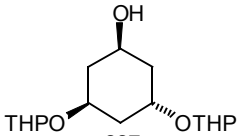


286

3-Tetrahydro-2*H*-2-pyranyloxy-5-*tert*-butyldimethylsilyloxy-(1*R*,3*R*,5*S*)-cyclo-hexan-1-ol (**283**, 0.30g, 9.09 mmol) was dissolved in dry dichloromethane (5 ml) under nitrogen atmosphere. To the solution, 3,4-dihydropyran (0.115g, 1.36 mmol) was added. The solution was cooled below 0°C in ice-salt mixture. To the cold stirred solution PTSA (0.01g) was added and the mixture was stirred at 0°C for 2 hr. Reaction was quenched by adding aqueous sodium bicarbonate solution. Organic layer was separated and aqueous layer was extracted with dichloromethane (2 x 3ml). Combined organic extracts were washed with brine + water, dried on anhydrous sodium sulphate. Solvent was evaporated under vacuum and residue was purified on silica gel column (5% ethyl acetate in pet. ether) to afford 2-[3-tetrahydro-2*H*-2-pyranyloxy-5-(*tert*-butyldimethylsilyloxy) cyclohexyloxy]tetrahydro-2*H*-pyran (**286**, yield 0.34g, 90%) as an oily liquid.
^{1}H NMR ($CDCl_3$): δ 0.06, 0.07 (2s, 6H), 0.89 (s, 9H), 1.15-2.38 (m, 18H), 3.5 (m, 2H), 3.8 (m, 4H), 4.14 (m, 1H), 4.72 (m, 2H)

^{13}C NMR ($CDCl_3$): δ -4.84, 17.88, 19.31, 19.53, 25.30, 25.63, 30.93, 34.49, 35.82, 36.81, 38.00, 38.43, 40.52, 41.44, 43.31, 43.50, 62.10, 62.39, 65.70, 65.96, 69.45, 70.29, 70.70, 96.50, 97.05
IR ($CHCl_3$): cm^{-1} 437.51, 477.66, 746.74, 771.39, 1024.42, 1076.27, 1121.29, 1213.07, 1250.47, 2856.57, 2944.37, 3011.75
Mass: Base m/e = 84 other m/e: 272, 255, 210, 171, 128
Elemental analysis: calculated for $C_{22}H_{42}O_5Si$: C 63.77%, H 10.14% Found C 63.71%, H 10.36%
Specific rotation $[\alpha]_D$ = -0.55 (c = 1.11, $CHCl_3$)

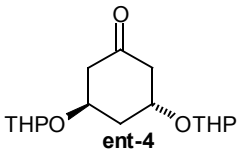
*Preparation 3,5-di(tetrahydro-2H-2-pyraynyloxy)-1-cyclohexanol (**287**)*



287

2-[3-Tetrahydro-2*H*-2-pyranyloxy-5-(*tert*-butyldimethylsilyloxy)cyclohexyloxy]tetrahydro-2H-pyran (**286,** 0.25g, 0.60 mmol) was dissolved in THF (3 ml). To the solution, 1M tetrabutylammonium fluoride solution (0.9ml, 0.235g, 0.9 mmol) was added and reaction was stirred for 1 hr. Solvent was evaporated under vacuum and residue was purified on silica gel column (20% ethyl acetate in pet. ether) to afford compound 3,5-di(tetrahydro-2H-2-pyraynyloxy)-1-cyclohexanol (**287**, yield 0.18g, 99.5%) as a viscous liquid.
^{1}H NMR ($CDCl_3$): δ 1.4-2.15 (m, 19H), 3.52 (m, 2H), 3.90 (m, 2H), 4.13 (m, 3H), 4.72 (m, 2H)
^{13}C NMR ($CDCl_3$): δ 19.23, 19.33, 19.50, 25.10, 30.76, 34.41, 35.98, 36.42, 38.04, 38.79, 40.02, 40.18, 62.06, 62.20, 62.32, 62.41, 65.51, 65.78, 69.18, 69.34, 69.60, 70.66, 71.01, 71.21, 96.51, 96.65, 96.80, 96.96
IR ($CHCl_3$): cm^{-1} 667.15, 754.52, 1022.52, 1067.96, 1119.94, 121.29, 1350.93, 1447.80, 2857.82, 2943.27, 3009.42, 3452.65
Mass: Base m/e = 85 other m/e: 263, 215, 199, 185, 175, 115, 97
Elemental analysis: calculated for $C_{16}H_{28}O_5$: C 64.00%, H 9.33% Found C 64.07%, H 9.41%
Specific rotation $[\alpha]_D$ = +2.58 (c = 1.18, $CHCl_3$)

*Preparation of 3,5-di(tetrahydro-2H-2-pyraynyloxy)-(3R, 5R)-cyclohexan-1-one (**ent-4**)*

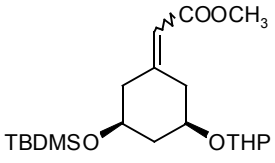


ent-4

3,5-Di(tetrahydro-2*H*-2-pyraynyloxy)-1-cyclohexanol (**287**, 0.1g, 0.33 mmol)) was dissolved in dichloromethane (2 ml). To the solution, sodium acetate (0.02g) and pyridinium chlorochromate (0.108g, 0.5 mmol) was added. Reaction mixture was stirred for 1 hr. reaction mixture was diluted with ether (2 ml). Solvent was decanted and residue was extracted with ether (3 x 2 ml). Organic extracts were combined and washed with 1:1 brine + water mixture followed by brine. The organic layer was dried on anhydrous sodium sulphate and solvent was evaporated under vacuum. Residue was purified on silica gel column (10% ethyl acetate in pet. ether) to afford 3,5-di(tetrahydro-2*H*-2-pyraynyloxy)-(3*R*, 5*R*)-cyclohexan-1-one (**ent-4** P = THP yield 0.080g, 80%) as an oily liquid.
^{1}H NMR ($CDCl_3$): δ 1.35-1.95 (m, 12H), 2.00-2.33 (m, 2H), 2.38-2.85 (m, 4H), 3.50 (m, 2H), 3.85 (m, 2H), 4.30 (m, 2H), 4.68 (m, 2H)
^{13}C NMR ($CDCl_3$): δ 18.86, 19.12, 19.26, 25.04, 30.51, 34.33, 36.06, 37.72, 45.80, 46.06, 47.94, 48.16, 61.79, 61.94, 62.12, 62.42, 69.70, 70.03, 70.28, 70.61, 96.46, 96.64, 96.97, 159.46, 206.95, 207.10, 207.32

IR ($CHCl_3$): cm^{-1} 436.38, 667.70, 756.59, 1214.37, 2946.90, 3018.45
Mass: Base m/e = 85 other m/e: 213, 197, 113, 101, 95, 67, 55
Elemental analysis: calculated for $C_{16}H_{26}O_5$: C 64.43%, H 8.72% Found C 64.48%, H 9.01%
Specific rotation $[\alpha]_D$ = +16.36 (c 1.12, $CHCl_3$)

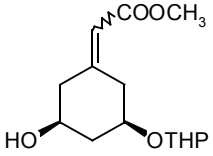
Preparation of ethyl-2-[3-tetrahydro-2H-2-pyranyloxy-5-tert-butyldimethylsilyloxy-(3R,5S)-cyclohexylidene]acetate (278a/278b**)**



278a/278b

In a flame dried two-necked round bottom flask fitted with rubber septum and two-way stopcock, anhydrous THF (2 ml) was injected under argon atmosphere. It was cooled to –78ºC in dry ice-acetone bath. To it, 10% n-butyllithium in pet.ether (0.43 ml, 0.43g, 0.667 mmol) and anhydrous diisopropylamine (0.0673g, 0.093 ml, 0.667 mmol) were added and mixture was stirred for 15 min. To this LDA solution, trimethylsilylacetoacetate (0.107 g, 0.667 mmol) in anhydrous THF (2 ml) was added and mixture was stirred for 15 min. To the reaction mixture, solution of 3-tetrahydro-2*H*-2-pyranyloxy-5-*tert*-butyldimethylsilyloxy-(3*S*, 5*R*)-cyclohexan-1-one (**175**, 0.075 g, 0.222 mmol) in anhydrous THF (2 ml) was added slowly. Reaction mixture was stirred for 4 hr at –78ºC. Reaction was quenched by adding cold, saturated ammonium chloride solution. THF layer was separated and solvent was evaporated under vacuum. Aqueous layer was extracted with ethyl acetate (2x5ml). Organic extracts were combined and residue from THF layer was dissolved in it. It was then washed with brine and was dried on anhydrous sodium sulphate. Solvent was evaporated under vacuum and residue was purified on silica gel column (2% ethyl acetate in pet. ether) to afford ethyl-2-[3-tetrahydro-2*H*-2-pyranyloxy-5-*tert*-butyldimethylsilyloxy-(3*R*,5*S*)-cyclohexylidene]acetate (**278a**/**278b**, yield 0.065g, 72.2%))
^{1}H NMR ($CDCl_3$): δ 0.09 (m, 6H), 0.90 (d, 9H), 1.28 (t, 3H), 1.40-1.95 (m, 8H), 2.00-2.70 (m, 4H), 3.56 (m, 2H), 3.89 (m, 2H), 4.18 (q, 2H), 4.72 (m, 1H), 5.73 (m, 1H)

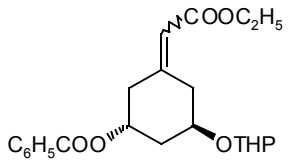
*Preparation of Ethyl-2-[3-hydroxy-5-tetrahydro-2H-2-pyranyloxy-(3S,5R)-cyclohexylidene] acetate (**279a/279b**)*



279a/279b

Ethyl-2-[3-tetrahydro-2*H*-2-pyranyloxy-5-*t*-butyldimethylsilyloxy-(3*R*,5*S*)-cyclohexylidene]acetate (**278a/278b**, 0.40g, 0.0983 mmol) was dissolved in THF(2 ml) containing 1% water. To the solution, 1M tetrabutyl ammonium fluoride solution (0.1 ml, 0.026g, 0.0983 mmol) was added and reaction was stirred for 1 hr. THF was removed under vacuum and residue was chromatographed on silica gel column (10% ethyl acetate in pet. ether) to afford of ethyl-2-[3-hydroxy-5-tetrahydro-2*H*-2-pyranyloxy-(3*S*,5*R*)-cyclohexylidene]acetate (**279a/279b**, 0.024g, 83%).
^{1}H NMR ($CDCl_3$): δ 1.26 (t, 3H), 1.40-1.85 (m, 6H), 1.89-2.17 (m, 3H), 2.30-2.55 (m, 3H), 2.90-3.20 (mdd, 1H), 3.51 (m, 1H), 3.9 (m, 2H), 4.12 (m, 3H), 4.78 (dm, 1H), 5.81 (dm, 1H)

*Attempted synthesis of 3-[1-ethyloxycarbonyl-(E/Z)-methylidene]-1-phenylcarbonyloxy-5-tetrahydro-2H-2-pyranyloxy-(1R, 5R)-cyclohexane (**280a/280b**)*



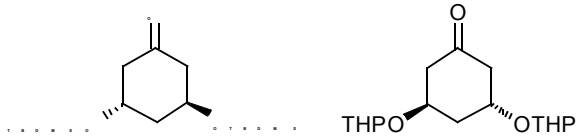
280a/280b

Ethyl-2-[3-hydroxy-5-tetrahydro-2*H*-2-pyranyloxy-(3*S*,5*R*)-cyclohexylidene]acetate (**279a/279b**, 0.045g, 0.154 mmol), benzoic acid (0.026g, 0.215 mmol), triphenylphosphine (0.056g, 0.215 mmol) were placed in flame-dried two-necked round bottom flask equipped with pressure-equalizing dropping funnel and two-way stopcock. Assembly was evacuated and flushed with argon. Dry THF (2 ml) was injected into the reaction flask and solution was cooled in ice-salt bath. To the cold, stirred solution, diethylazodicarboxylate (0.056g, 0.215 mmol) in dry THF (2 ml) was added dropwise. Reaction mixture was stirred for an hour. Reaction was quenched by addition of ammonium chloride solution. Organic layer was separated and THF was evaporated under vacuum. Aqueous layer was extracted with ethyl acetate (2x10ml). Residue from THF layer was washed with aqueous sodium bicarbonate solution, followed by brine wash. It was dried on anhydrous sodium sulphate and solvent was evaporated under vacuum. Oily residue was obtained which had following ^{1}H NMR values

^{1}H NMR ($CDCl_3$): δ 1.26 (t, 3H), 1.40-1.95 (m, 6H), 2.10-2.80 (m, 4H), 3.52 (m, 2H), 3.59 (m, 2H), 4.18 9q, 2H), 4.74 (m, 1H), 6.11 (m, 2H)

Above data indicates presence of eliminated compounds.

*Preparation of racemic samples for ketone **4** (P = TBDMS) and **ent-4** (P = THP)*



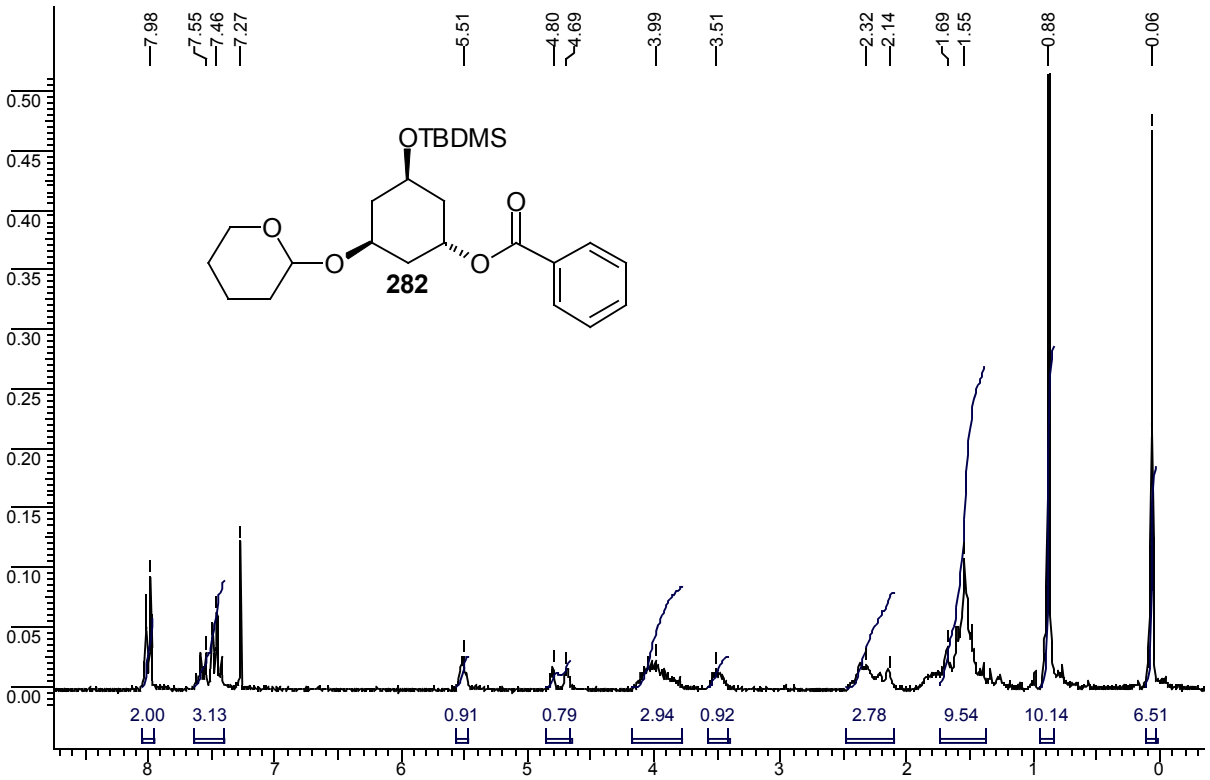
racemic **4** (P = TBDMS)

racemic **ent-4** (P = THP)

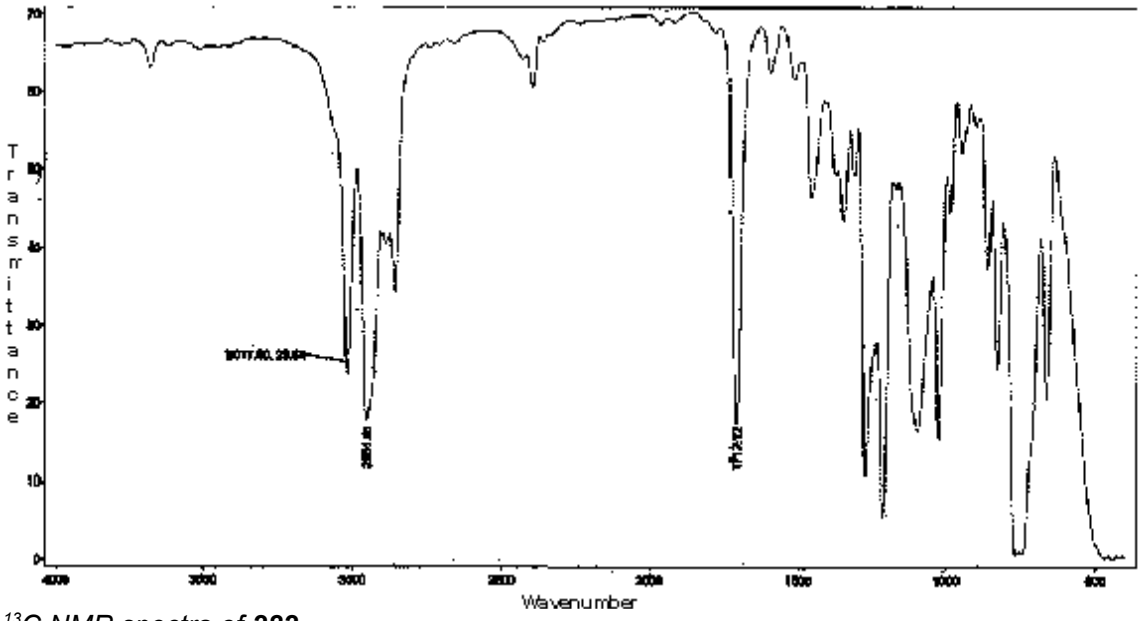
Racemic ketones **4** (P = TBDMS) and **ent-4** (P = THP) were prepared from racemic **171** following the same procedure as described for optically pure samples. Racemic **171** was prepared as described earlier in section 4.2.5. (page no. 134).

4.3.6. Spectra

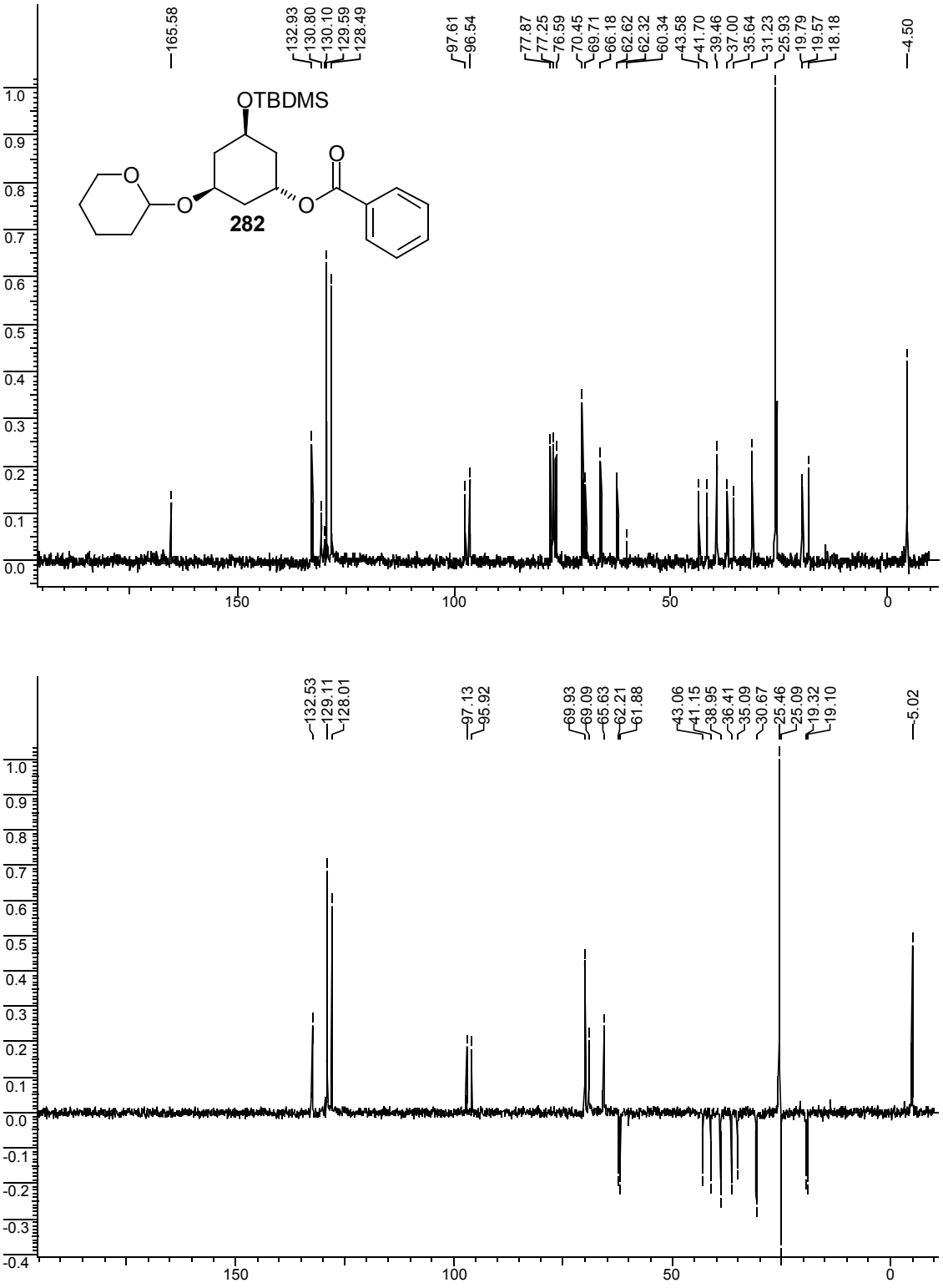
*^{1}H NMR spectrum of **282***



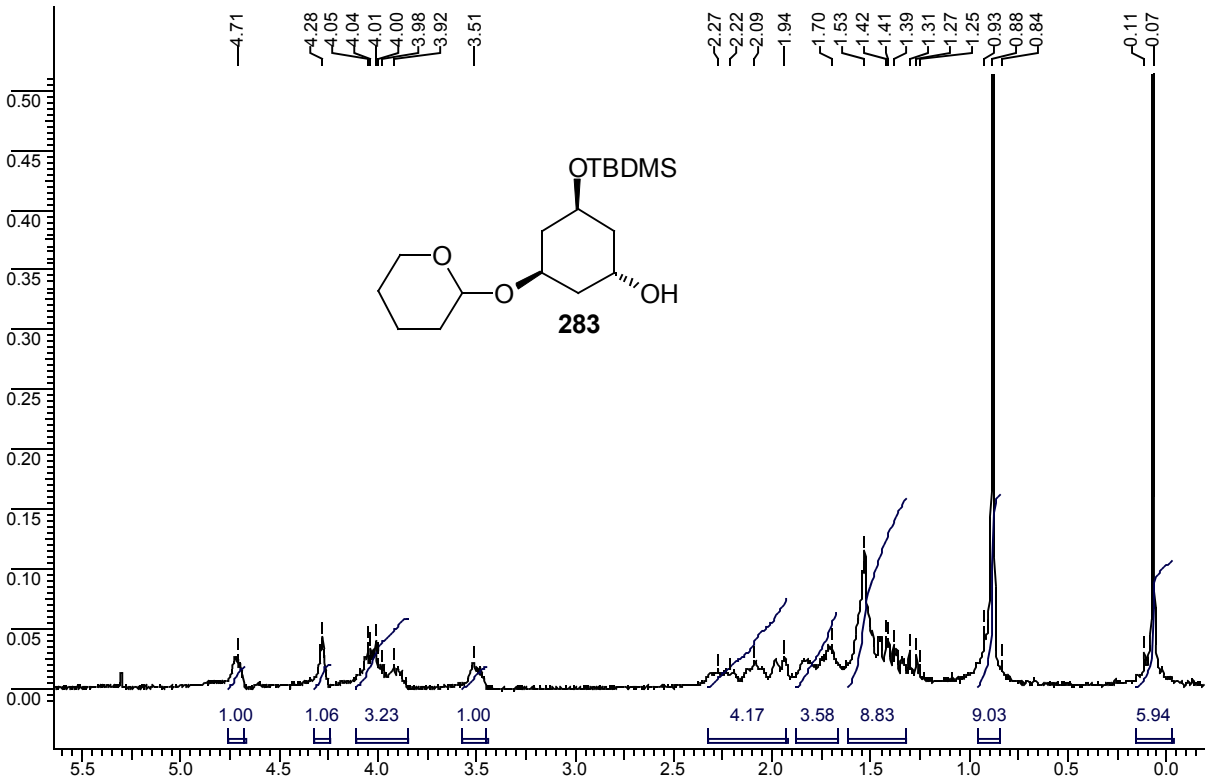
*IR spectrum of **282***



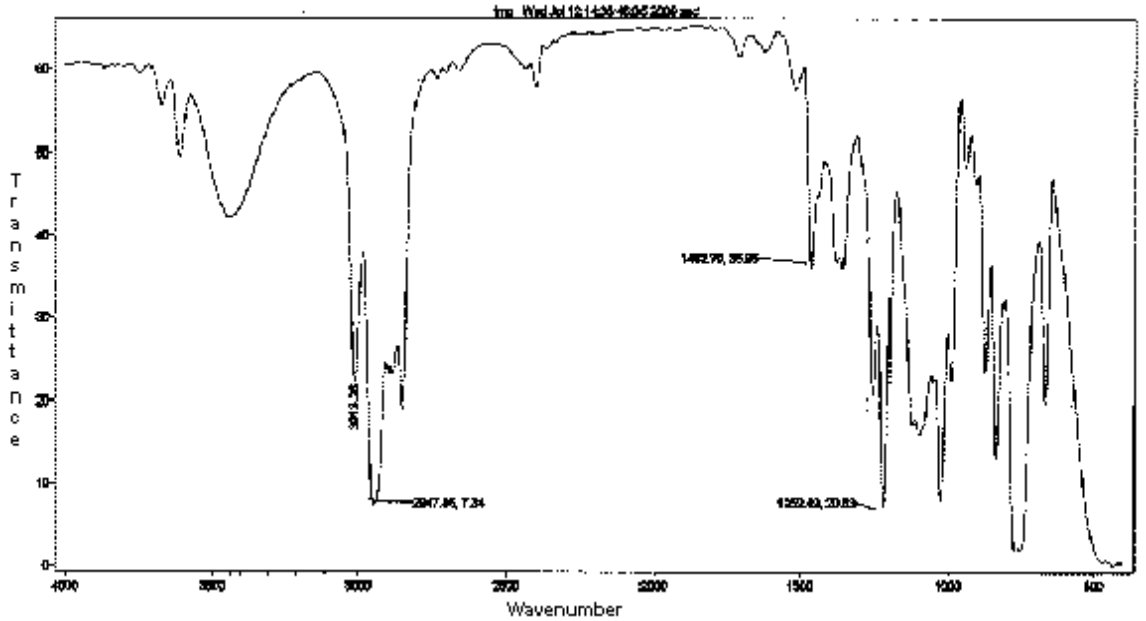
*^{13}C NMR spectra of **282***



*^{1}H NMR spectrum of **283***



*IR spectrum of **283***



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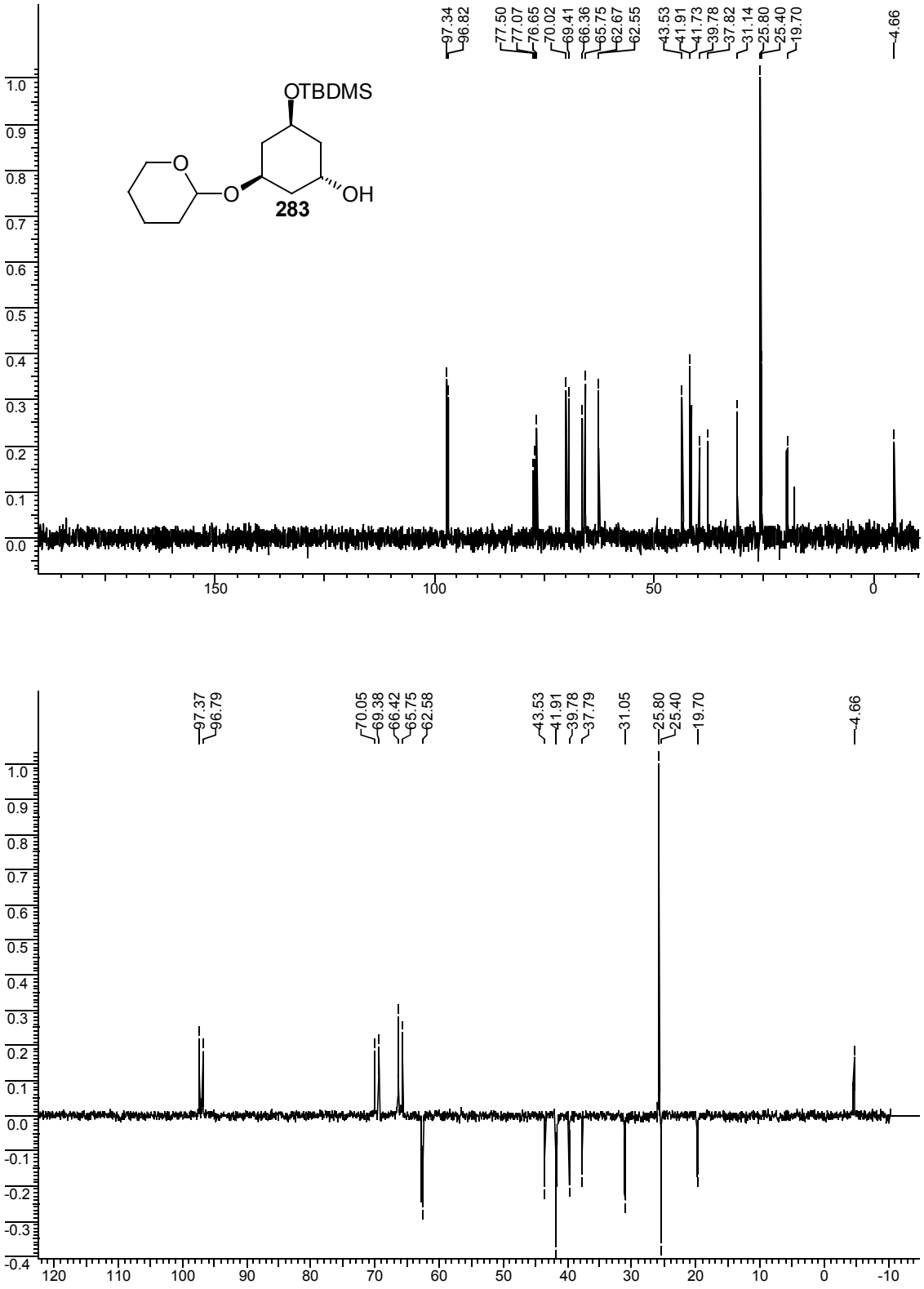

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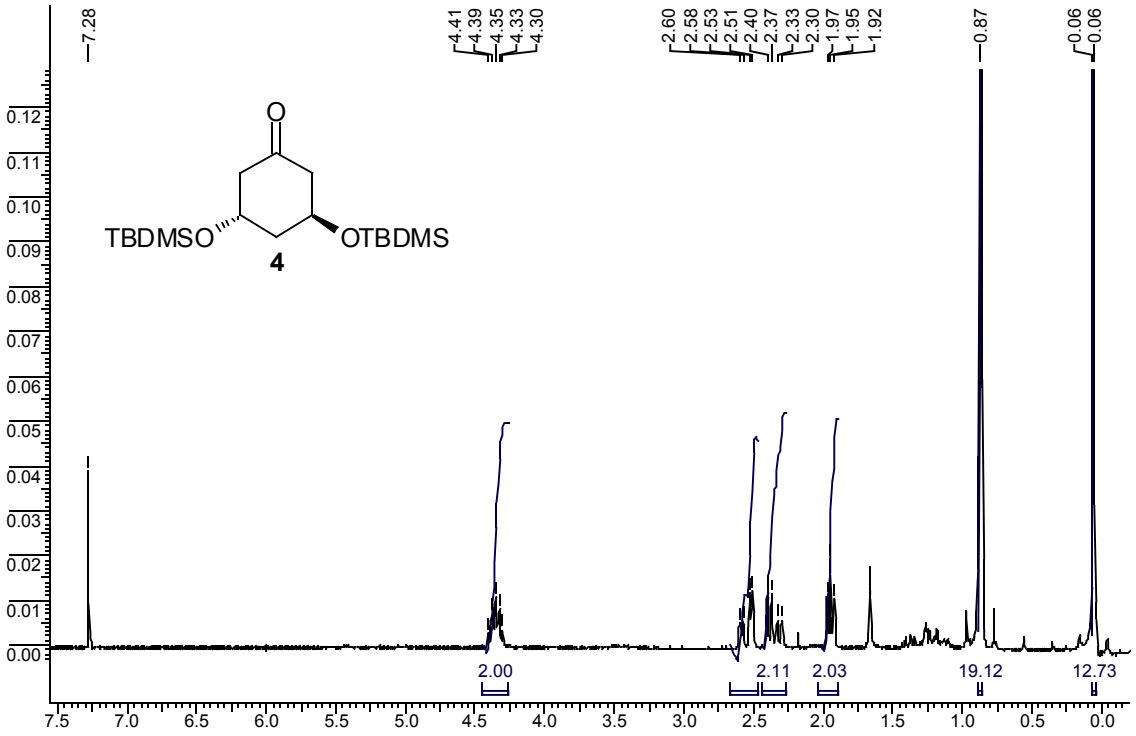

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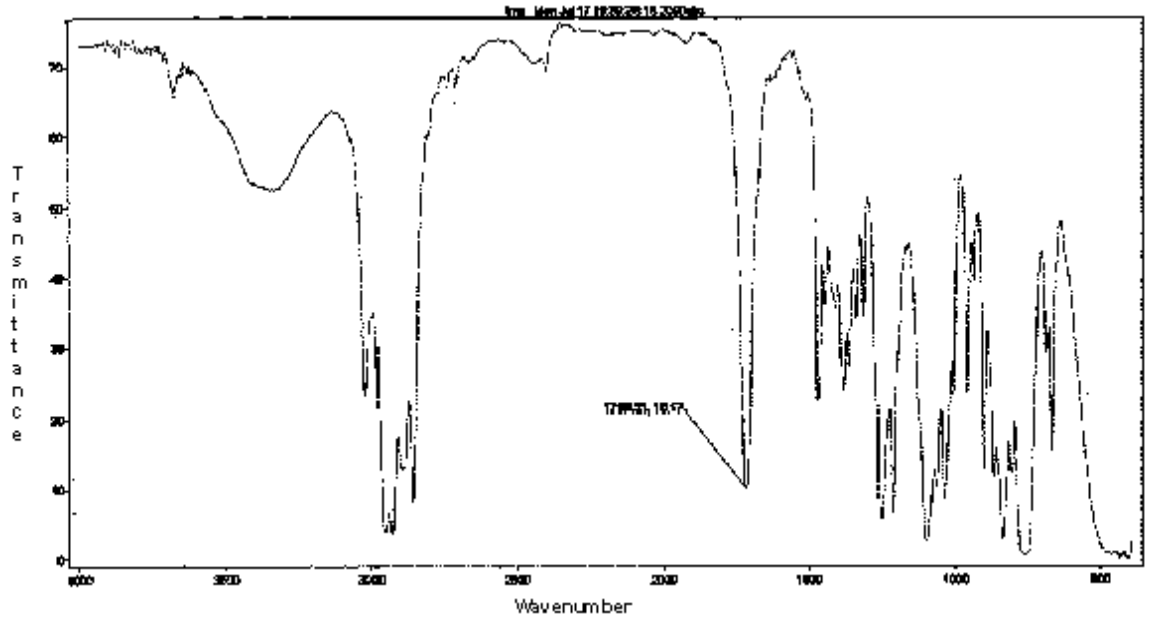
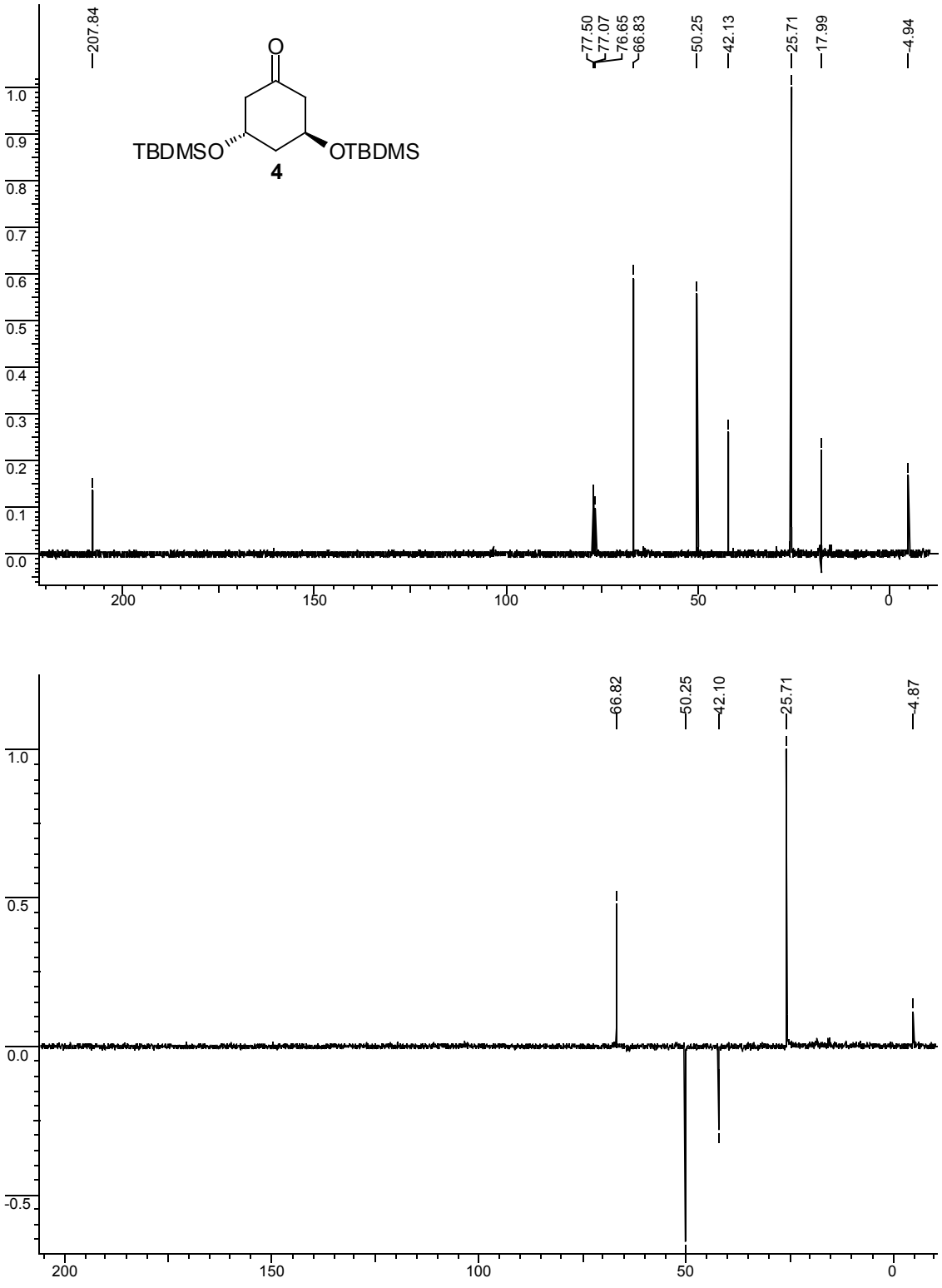


*^{13}C NMR spectra of **4***



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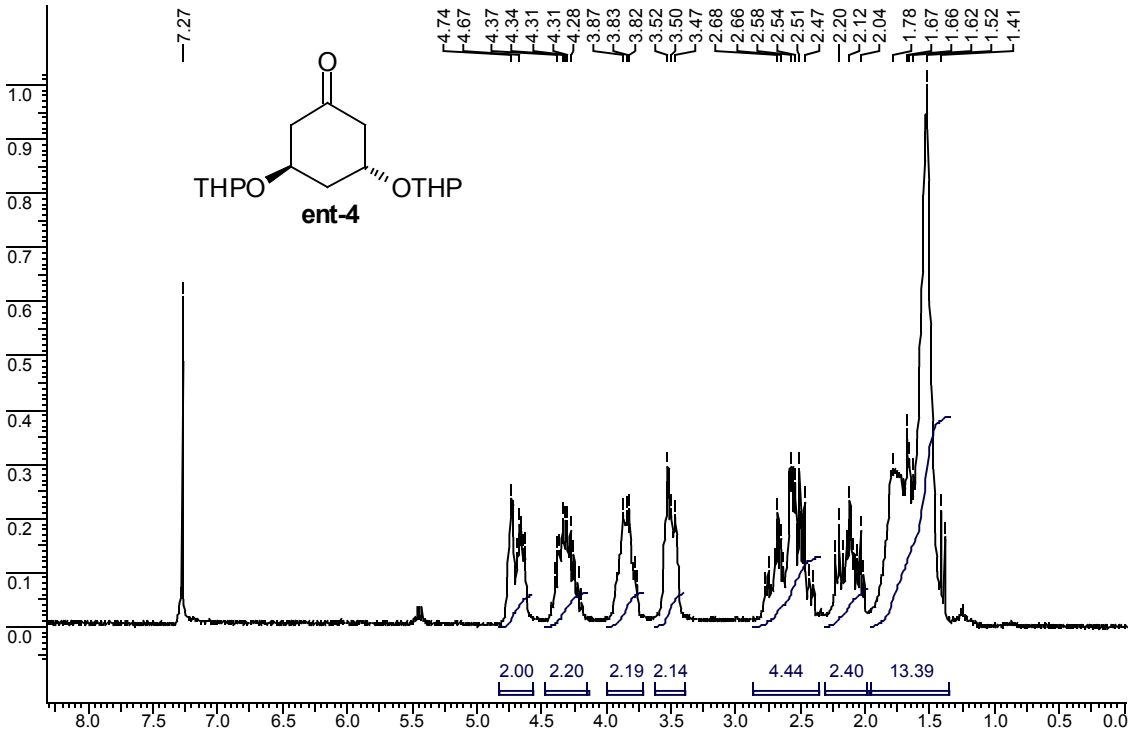

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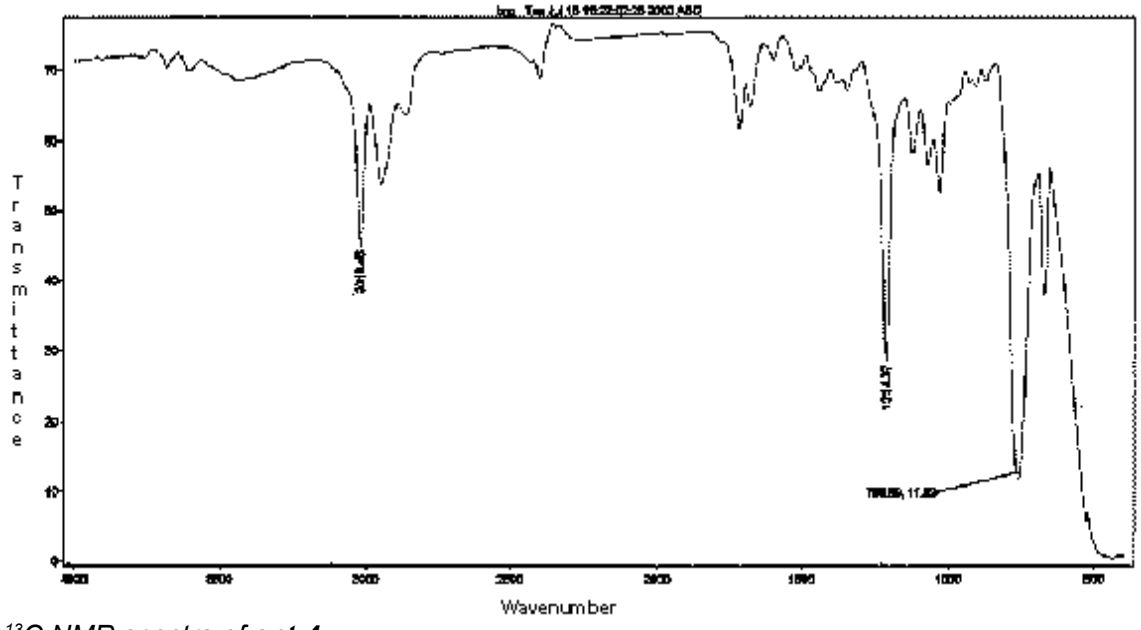
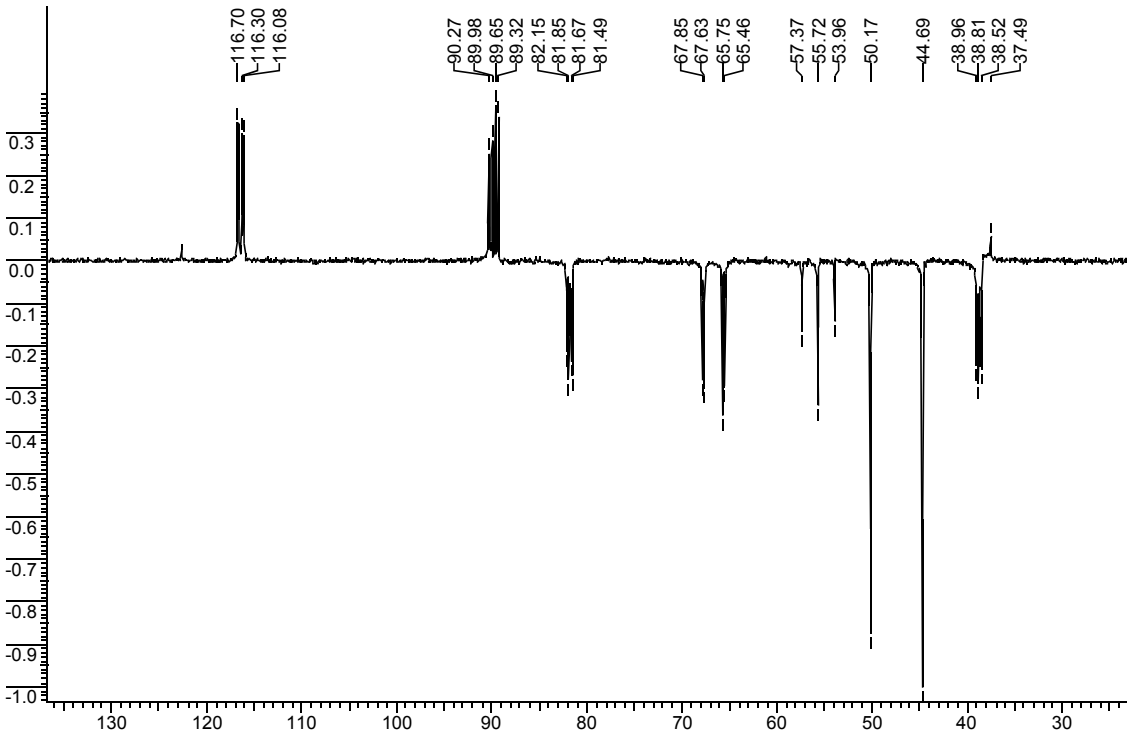


*^{13}C NMR spectra of **ent-4***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65.46
57.37
55.72
53.96
50.17
44.69
38.96
38.81
38.52
37.49
0.3
0.2
0.1
0.0
-0.1
-0.2
-0.3
-0.4
-0.5
-0.6
-0.7
-0.8
-0.9
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120
110
100
90
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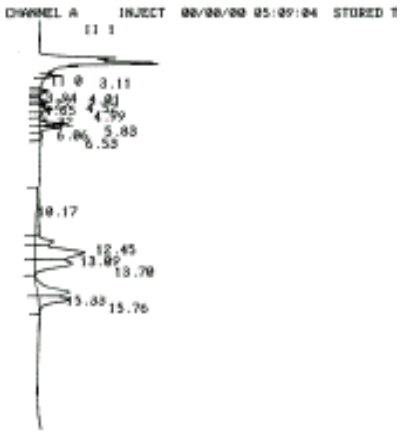
70
60
50
40
30

*Chiral HPLC chart for **ent-4***

Racemic ent-4



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3	0.63	4.01	29551 02
4	0.064	4.27	3019 03
5	0.37	4.52	17382 02
6	0.821	4.65	38537 02
7	2.212	4.99	103818 02
8	0.617	5.32	28946 02
9	3.822	5.83	179336 02
10	3.676	6.06	172481 00
11	0.273	6.58	12818 05
12	5.465	10.17	256400 02
13	6.761	12.45	317251 02
14	28.353	13.09	1330363 02
15	17.854	13.7	837704 02
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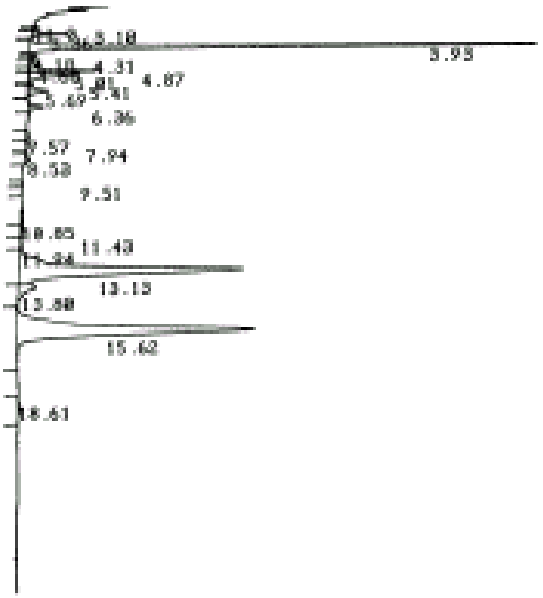
Optically active ent-4



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4	0.013	4.19	1323 06
5	0.191	4.31	19483 06
6	0.522	4.66	52999 06
7	2.941	4.87	298787 06
8	2.873	5.01	291798 06
9	0.142	5.41	14397 06
10	1.171	5.69	118986 07
11	0.776	6.36	78787 01
12	0.178	7.57	18053 02
13	0.138	7.94	14050 03
14	0.294	8.53	29826 01
15	0.031	9.51	3162 01
16	0.554	10.05	56322 02
17	0.379	11.43	38517 02
18	0.429	11.94	43569 02
19	30.899	13.13	3138794 02
20	2.865	13.8	289755 02
21	35.281	15.62	3575798 03
22	0.331	18.61	33617 01
TOTAL	100.		10158277

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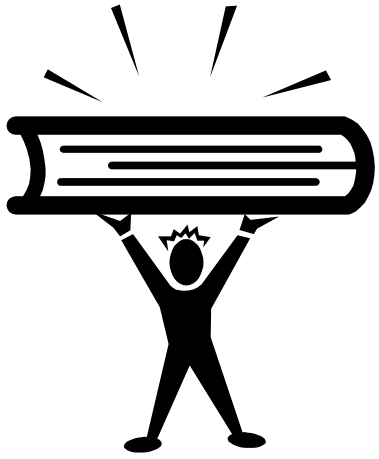
Summary

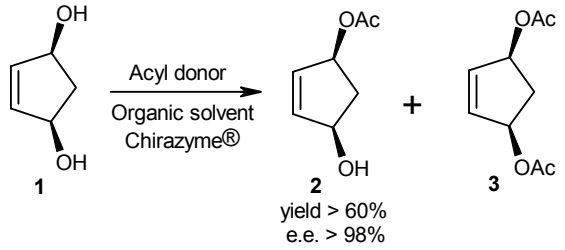


SUMMARY

Contents

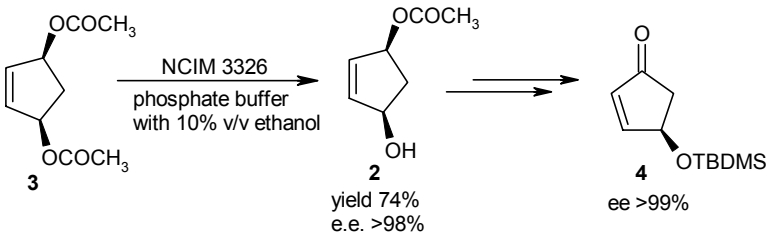
5.0. Summary

☞ Desymmetrization of *meso*-Cyclopent-2-en-1,4-diol (**1**) to 4-(*R*)-hydroxycyclopent-2-en-1-(*S*)-acetate (**2**) was successfully carried out by irreversible transesterification using an immobilized lipase from *Mucor meihei* i.e. Lipozyme®/Chirazyme® through parameter optimization study (Scheme 5.1). This commercial enzyme, available in bulk scale, was previously reported as inefficient for the conversion (<5% yield). The enzyme was studied for the transesterification of **1** in various organic solvents by varying reaction parameters as nature of acyl donor, temperature, enzyme quantity etc. to afford optically active 4-(*R*)-hydroxycyclopent-2-en-1-(*S*)-acetate (**2**) of >98% optical purity in >60% yield.



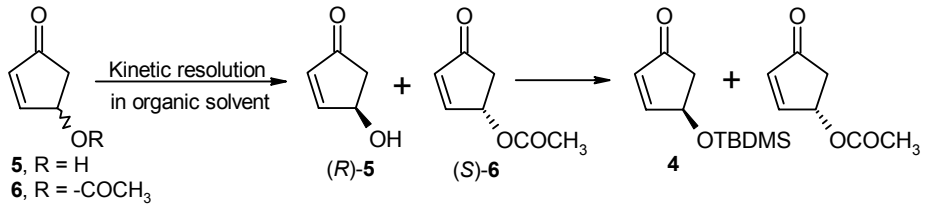
Scheme 5.1.

☞ A practical and scalable process for 4-(*R*)-hydroxycyclopent-2-en-1-(*S*)-acetate (**2**) was developed through desymmetrization of *meso*-Cyclopent-2-en-1,4-diacetate (**3**) catalyzed by *Trichosporon beigelii* (NCIM 3326). Various yeast and fungal cultures from NCIM, NCL, Pune, India were screened for the hydrolysis of **3** to **2** to provide a cheaper and more effective alternative to Porcine Liver Esterase (PLE), which is currently being used for the conversion. Yeast cultures of *Trichosporon* species were identified as having pro-*R* preference in the hydrolysis of **3**; but enantioselectivity was poor. Hence detailed medium-engineering investigations were made for the hydrolysis of **3** to **2** using culture of *Trichosporon beigelii* (NCIM 3326) as catalyst. Addition of 10% v/v ethanol was found to enhance the enantioselectivity of the enzyme, affording **2** of 85% optical purity in 83% yield. Further study and exploration on 'inherent consecutive kinetic resolution to desymmetrization afforded **4** of >98% e.e. in 74% chemical yield (Scheme 5.2). Process was scaled up to 100g scale and compound 2 of >98% e.e. was converted to optically pure component C (**4**) for prostaglandins using reported procedures.



Scheme 5.2.

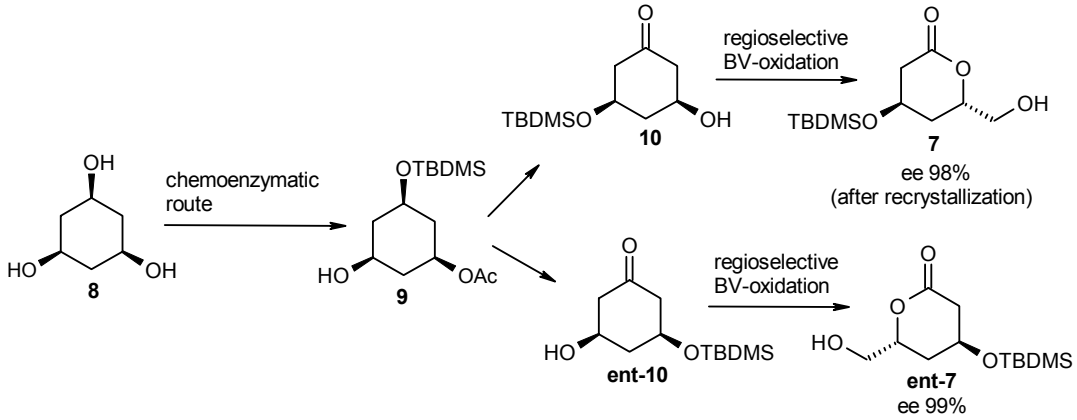
☞ Enzymatic kinetic resolution studies of (±)-4-hydroxycyclopent-2-en-1-one (**5**) were conducted as an alternative to the desymmetrization of *meso*-cyclopentenediol to provide faster and economic access to optically pure 4-(*R*)-*tert*-butyldimethylsilyloxycyclopent-2-en-1-one (**4**).



Scheme 5.3.

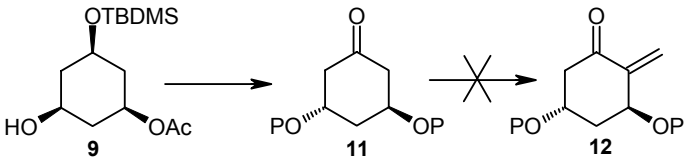
Initially resolution studies were conducted in aqueous buffer media. But, due to the problems of tedious isolation of **5** from aqueous media and possible racemization in aqueous media, resolution studies were taken up in organic solvents by transesterification of **5** with vinyl acetate and alcoholysis of its acetate **6** (Scheme 5.3). Parameters were screened using Lipozyme IM® as catalyst. Although enantioselectivity observed was moderate (E = 24, by alcoholysis of **6** with 2-butanol), trends in the effect of solvent, water content and alcohol structure showed useful general directions and ground rules for screening of enzymes for optimization to useful levels of efficiency.

☞ An efficient chemoenzymatic route for optically pure lactone 6-hydroxymethyl-4-(*tert*-butyldimethylsilyloxy)-(4*R*, 6*S*)-tetrahydro-2*H*-2-pyranone (**7**) and its enantiomer was developed starting from *cis*-phloroglucitol (**8**) (Scheme 5.4). Phloroglucitol **8** was chemoenzymaticlly converted to 3-hydroxy-5-(*tert*.butyldimethylsilyloxy)-(1*S*,3*R*,5*R*)-cyclohexylacetate (**9**, e.e. >95%) through desymmetrization approach which was further explored to yield both the enantiomers of lactone **7** having >98% ee. Hydroxy directed regioselective Baeyer-Villiger oxidation was the crucial step wherein desired lactone was obtained as a single isomer.

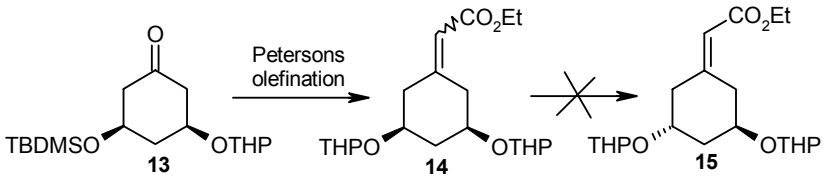


Scheme 5.4.

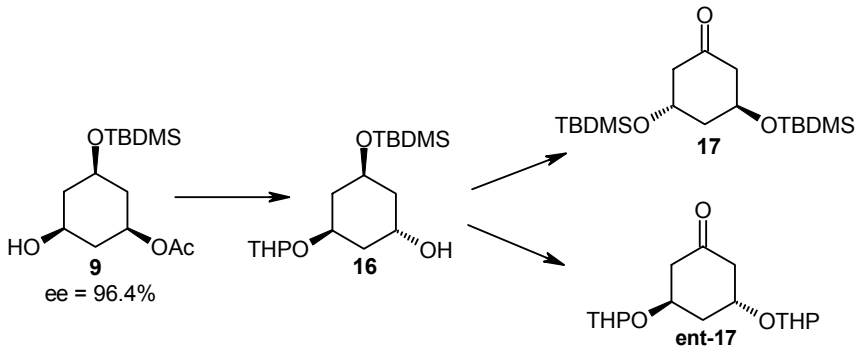
☞ Optically active intermediate obtained in above scheme were also utilized for the synthesis of optically active intermediate for phosphine oxide A-ring synthon of 1α, 25-dihydroxyvitamin D_3 and its 19-nor analogue. Initially attempts were made to obtain ketone **12** with exomethylene functionality (Scheme 5.5). But these were unsuccessful due to unstability/unreactivity of the substrates under the attempted reaction conditions. Synthesis of intermediate **15** for 19-nor-analogue was attempted through Petersons olefination on ketone **13** followed by Mitsunobu inversion (Scheme 5.6). But the scheme was not successful due to rapid elimination of inverted benzoates. Further a scheme was developed for the synthesis of both the enantiomers of diprotected trans-3,5-dihydroxycyclohexanone (**17** and **ent-17**) from intermediate **9**. The key steps include efficient Mitsunobu inversion, followed by protection-deprotection and PCC oxidation (Scheme 5.7).



Scheme 5.5.



Scheme 5.6.



Scheme 5.7.

In conclusion, the study presented in this thesis demonstrates efficient utilization of hydrolytic enzymes for development of elegant routes for few important, high cost optically pure drug intermediates. A cost effective large-scale synthesis of component C, **4** for prostaglandins has

been invented through enzymatic desymmetrization approach. Also two alternative enzymatic approaches were developed for the same purpose. A potentially industrially viable route for optically pure lactone **7**, which is important intermediate for ‘statin’ drugs, has been developed. Even though, synthesis of intermediate **12** with exomethylene functionality for vitamin D_3 was not successful, a simple, elegant route for intermediate **17** for 19-nor D_3 has been developed.

Appendix

APPENDIX

Contents

6.1. Abbreviations

ADP	Adenosine diphosphate
AIBN	2,2'-Azobisisobutyronitrile
AMP	Adenosine monophosphate
ATP	Adenisine triphosphate
$CDCl_3$	Deuteriated chloroform
CLAP	Chicken liver acetone powder
CLEC	Cross linked enzyme crystals
CRL	*Candida rugosa* lipase
d.e.	Diastereomeric excess
DBU	1,8-Diazabicyclo[5.4.0]undec-7-ene
DEAD	Diethylazidodicarboxylate
DHP	3,4-dihydro-2*H*-pyran
DIBALH	Diisobutylaluminium hydride
DIPE	Diisopropyl ether
DMF	Dimethylformamide
e.e.	Enantiomeric excess
GC	Gas chromatography
HMG CoA	Hydroxymethylglutaryl Coenzyme A
HMPA	Hexamethylenephosphoric triamide
HPLC	High performance liquid chromatography
IR	Infrared spectroscopy
mcpba	metachloroperbenzoic acid
NCIM	National Collection of Industrial Microorganisms, NCL, Pune
NMR	Nuclear magnetic resonance spectroscopy
PCC	Pyridinium chlorochromate
PFL	*Pseudomonas fluorescens* lipase
PLAP	Pig liver acetone powder
PLE	Pig liver esterase
PPL	Porcine pancreatic lipase
TBAF	Tetrabutylammonium fluoride
TBME	*Tert*-butyldimethyl ether
THF	Tetrahydrofuran
TLC	Thin layer chromatography

6.2. List of Publications and Patents

☞ *Publications*

Desymmetrization of *meso*-cyclopenten-cis-1, 4-diol to 4-(*R*)-hydroxycyclopent-2-en-1-(*S*)-acetate by irreversible transesterification using Chirazyme®

S. R. Ghorpade, R. K. Kharul, R. R. Joshi, U. R. Kalkote and T. Ravindranathan

Tetrahedron*: Asymmetry* **1999**, *10*, 891-899

Enzymatic kinetic resolution studies of racemic 4-hydroxycyclopent-2-en-1-one

S. R. Ghorpade, K. B. Bastawde, D. V. Gokhale, P. D. Shinde, V. A. Mahajan, U. R. Kalkote and T. Ravindranathan

Tetrahedron: *Asymmetry* **1999**,*10,* 4115-4122

A Practical and Scalable process for 4-(*R*)-Hydroxycyclopent-2-en-1-(*S*)-acetate by desymmetrization of *meso*-Cyclopent-2-en-1,4-diacetate catalyzed by *Trichosporon beigelii* (NCIM 3326), a cheap Biocatalyst.

U. R. Kalkote, **S. R. Ghorpade**, R. R. Joshi, T. Ravindranathan, K. B. Bastawde, D. V. Gokhale

Tetrahedron: *Asymmetry* **2000**, *11,* 2965-2970.

Chemoenzymatic Syntheses of 6-Hydroxymethyl-4-(*tert*.-butyldimethylsilyloxy) -(4*R*, 6*S*)-tetrahydro-2*H*-pyranone and its enantiomer: Hydroxy directed Regioselective Baeyer-Villiger oxidation.

S. R. Ghorpade, U. R. Kalkote, S. P. Chavan, S. R. Bhide, T. Ravindranathan

Communicated to *The Journal of Organic Chemistry*

Simple Chemoenzymatic synthesis of enantiomeric ketone intermediates for 19-nor-1, 25-dihydroxyvitamin D_3.

S. R. Ghorpade, U. R. Kalkote, S. P. Chavan, T. Ravindrnathan

Communicated to *Tetrahedron: Asymmetry*

☞ *Patents*

1. A process for the preparation of 4-(*R*)-hydroxycyclopent-2-en-1-(*S*)-acetate
   U. R. Kalkote, **S. R. Ghorpade**, R. R. Joshi, T. Ravindranathan, K. B. Bastawde, D. V. Gokhale
   Indian patent: File no. NF 359/98, also applied for US and EP patent

2. Chemoenzymatic process for the preparation of 6-Hydroxymethyl-4-(*tert*.-butyldimethylsilyloxy)-(4*R*, 6*S*)-tetrahydro-2*H*-pyranone
   S. R. Ghorpade, U. R. Kalkote, S. P. Chavan, S. R. Bhide, T. Ravindranathan
   Applied for US patent.

3. Chemoenzymatic process for the preparation of 6-Hydroxymethyl-4-(*tert*.-butyldimethylsilyloxy)-(4*S*, 6*R*)-tetrahydro-2*H*-pyranone
   S. R. Ghorpade, U. R. Kalkote, S. R. Bhide, S. P. Chavan, T. Ravindranathan
   Applied for US patent

4. A process for the preparation of a novel cis, cis-3-hydroxy-5-(methylcarbonyloxy) cyclohexyl acetate useful as an intermediate for 6-Hydroxymethyl-4-(*tert*.-butyldimethylsilyloxy)-(4*R*, 6*S*)-tetrahydro-2*H*-pyranone
   S. R. Ghorpade, U. R. Kalkote, S. R. Bhide, S. P. Chavan, T. Ravindranathan
   Indian patent application no. 025NF2001

5. A process for the preparation of a novel cis, cis-3-(methylcarbonyloxy)-5-*tert*-butyldimethylsilyloxycyclohexylacetate useful as an intermediate for 6-Hydroxymethyl-4-(*tert*.-butyldimethylsilyloxy)-(4*R*, 6*S*)-tetrahydro-2*H*-pyranone
   S. R. Ghorpade, U. R. Kalkote, S. R. Bhide, S. P. Chavan, T. Ravindranathan
   Indian patent application no. 026NF2001

6. A process for the preparation of a novel 3-hydroxy-5-*tert*-butyldimethylsilyloxy-(1*S*, 3*R*, 5*R*)-cyclohexylacetate useful as an intermediate for 6-Hydroxymethyl-4-(*tert*.-butyldimethylsilyloxy)-(4*R*, 6*S*)-tetrahydro-2*H*-pyranone
   S. R. Ghorpade, U. R. Kalkote, S. R. Bhide, S. P. Chavan, T. Ravindranathan
   Indian patent application no. 027NF2001

7. A process for the preparation of a novel 3-tetrahydro-2H-2-pyranyloxy--5-*tert*-

butyldimethylsilyloxy-(1*S*, 3*R*, 5*R*)-cyclohexylacetate useful as an intermediate for 6-Hydroxymethyl-4-(*tert*.-butyldimethylsilyloxy)-(4*R*, 6*S*)-tetrahydro-2*H*-pyranone
S. R. Ghorpade, U. R. Kalkote, S. R. Bhide, S. P. Chavan, T. Ravindranathan
Indian patent application no. 028NF2001

8. A process for the preparation of a novel 3-tetrahydro-2H-2-pyranyloxy-5-*tert*-butyldimethylsilyloxy-(1*S*, 3*R*, 5*S*)-cyclohexan-1-ol useful as an intermediate for 6-Hydroxymethyl-4-(*tert*.-butyldimethylsilyloxy)-(4*R*, 6*S*)-tetrahydro-2*H*-pyranone
S. R. Ghorpade, U. R. Kalkote, S. R. Bhide, S. P. Chavan, T. Ravindranathan
Indian patent application no. 029NF2001

9. A process for the preparation of a novel 3-tetrahydro-2*H*-2-pyranyloxy--5-*tert*-butyldimethylsilyloxy-(3S, 5*R*)-cyclohexan-1-one useful as an intermediate for 6-Hydroxymethyl-4-(*tert*.-butyldimethylsilyloxy)-(4*R*, 6*S*)-tetrahydro-2*H*-pyranone
S. R. Ghorpade, U. R. Kalkote, S. R. Bhide, S. P. Chavan, T. Ravindranathan
Indian patent application no. 030NF2001

10. A process for the preparation of a novel 3-hydroxy-5-*tert*-butyldimethylsilyloxy-(3S, 5*R*)-cyclohexan-1-one useful as an intermediate for 6-Hydroxymethyl-4-(*tert*.-butyldimethylsilyloxy)-(4*R*, 6*S*)-tetrahydro-2*H*-pyranone
S. R. Ghorpade, U. R. Kalkote, S. R. Bhide, S. P. Chavan, T. Ravindranathan
Indian patent application no. 031NF2001

11. An improved process for the preparation of 6-Hydroxymethyl-4-(*tert*.-butyldimethylsilyloxy)-(4*R*, 6*S*)-tetrahydro-2*H*-pyranone
S. R. Ghorpade, U. R. Kalkote, S. R. Bhide, S. P. Chavan, T. Ravindranathan
Indian patent application no. 032NF2001

12. A process for the preparation of a novel 3-oxo-5-*tert*-butyldimethylsilyloxy-(1*R*, 5*S*)-cyclohexylacetate useful as an intermediate for 6-Hydroxymethyl-4-(*tert*.-butyldimethylsilyloxy)-(4*S*, 6*R*)-tetrahydro-2*H*-pyranone
S. R. Ghorpade, U. R. Kalkote, S. R. Bhide, S. P. Chavan, T. Ravindranathan
Indian patent application no. 033NF2001

13. A process for the preparation of a novel 3-hydroxy-5-*tert*-butyldimethylsilyloxy-(3*R*, 5*S*)-cyclohexan-1-one useful as an intermediate for 6-Hydroxymethyl-4-(*tert*.-butyldimethylsilyloxy)-(4*S*, 6*R*)-tetrahydro-2*H*-pyranone

S. R. Ghorpade, U. R. Kalkote, S.R. Bhide, S. P. Chavan, T. Ravindranathan
Indian patent application no. 034NF2001

14. An improved process for the preparation of 6-Hydroxymethyl-4-(*tert*.-butyldimethylsilyloxy)-(4*S*, 6*R*)-tetrahydro-2*H*-pyranone
S. R. Ghorpade, U. R. Kalkote, S.R. Bhide, S. P. Chavan, T. Ravindranathan
Indian patent application no. 035NF2001

6.3. Reprints of Publications