Università Degli Studi di Salerno

Facoltà di Scienze Matematiche Fisiche e Naturali
-
Dipartimento di Chimica e Biologia

DOTTORATO DI RICERCA IN CHIMICA

X Ciclo-Nuova Serie

Total synthesis of terpenoidic unsatured dialdehydes and evaluation of their activity towards TRP receptors

Tutor

Prof. Aldo Spinella

Coordinatore
Prof. Gaetano Guerra

PhD Candidate

Mariantonietta D'Acunto

Anno 2012

ALLA MIA CARA FAMIGLIA

Ci sono momenti in cui la vita regala attimi di bellezza inattesa. Smetti di fare una cosa e ti accorgi che attorno a te tutto è perfetto, il dono di un Dio meno distratto del solito. Tutto sembra sincero. La nascita di una nuova vita, l'alba di un cambiamento, qualcosa di profondo o semplicemente la conferma di un affetto tenuto nascosto, di un sentimento segreto, custodito in silenzio dentro di noi con pudore. O anche la fine di qualcosa, la fine di un momento, di un periodo difficile sempre più faticoso da sostenere. Quando terminano i respiri corti, lasciando spazio a uno lungo profondo che riempie e svuota il petto.
In quei momenti non mi manca nulla.

QUESTA TESI è NOSTRA.,

NOI, CHE SIAMO STATI PIU' FORTI DI TUTTO E TUTTI.

Index

CHAPTER 6

LIST OF ABBREVIATION

Cl$_2$Pd(dppp)   1,3-bis(diphenylphosphine)propane

Super Hydride   Lithium triethylborohydride

Ac$_2$O   acetic anhydride

DBU   1,8-diazabiciclo[5.4.0]undec-7-ene

DCC   diciclohexylcarbodiimmide

DMAD   dimethyl acetilendicarboxylate

DMAP   4-N,N-dimethylamminopiridine

DMSO   dimethyl sulfoxide

e.e.   enantiomeric excess

MCPBA   m-chloroperbenzoic acid

MTPA   methoxy(trifluoromethyl)phenilacetic acid

PPTS   piridinium p-toluensulfonate

TBAF   tetrabutyilammonium fluoride

TBAI   tetrabutyl ammonium iodide

TBDMSCl   tert-butyldimethylsylil chloride

TBDPDCl   tert-butyldiphenilsylil chloride

THF   tetrahydrofurane

p-TsOH   p-toluensolfonic acid

p-TsCl   p-tosil Chloride

MsCl   Methanesulfonyl chloride

ABSTRACT

The aim of this PhD project has been to develop new synthetic strategies in enantioselective preparation of natural products.

In particular my attention has been focused on preparation of some natural metabolite, containing an α,β-unsaturated dialdehyde in a polycyclic backbone, and their synthetic analogue, in order to better understand structure activity relationship towards TRP receptors ion channels.

The recent discover of a new thermoreceptor TRPA1 and given that these natural metabolites show also a widespread of bioactivities, such as antiproliferative and cytotoxic activity, has increased our interest towards these target ever more.

Our purpose is to assay the bioactivity of synthesized products both as TRP receptor agonists and as antiproliferative compounds .

The first chapter of this work is an introduction to these terpenoidic molecules, with a wide range of described natural occurring metabolite and their classification in drimane, isocopalane, and scalarane dialdehydes.

Thus, the structure of TRP receptor is described with a brief history of these ion channels, starting from the first cloned receptor ,the TRPV1vanilloid.

In the chapter 2 total syntheses of polygodial derivatives, both C-1 and C-3 functionalised, are described.

Polygodial and C-1 functionalised drimanes have been prepared with an approach whose key step is a Diels Alder reaction; Drimane C-3 functionalised have been prepared with a radical chemistry approach.

Polygodial   C1-hydroxy derivative   C3 acethoxydroxy derivative
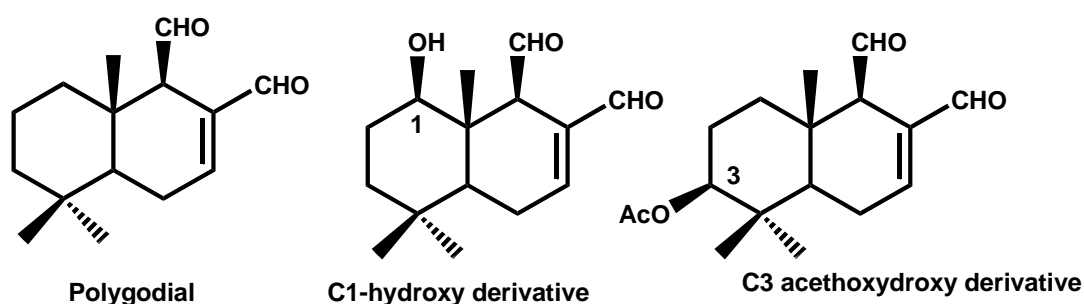
In the chapter three an approach to the enantioselectve syntheses of both diterpenoidic and seserterpenoidic unsaturated dialdehydes, structural analogues of occurring natural product such as ent-isocopalendial, scalaradial and deacethoxyscalaradial, is described.

Isocopalendial　　　**Scalaradial**　　　**Deacethoxyscalaradial**
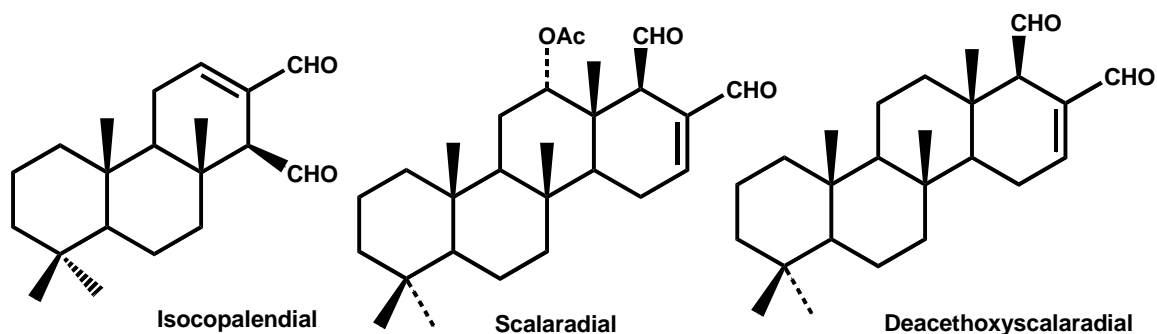
Chapter four is dedicated to discussion of some bioactivity assays, the receptorial one have been in collaboration with Dott. Luciano De Petrocellis and Dott.Vinenzo Di Marzo, Istituto di Chimica Biomolecolare del CNR (Pozzuoli, Na).The antiproliferative assays have been made in collaboration with Prof. Giuseppina Autore, Dott Giuseppe Bianco,Dipartimento di Farmacia, Unversità degli Studi di Salerno,

During my PhD studies I have spent six months at The University of York, working in Professor Richard Taylor Research Group.

During these months my research has been focused on developing of a new methodology in synthesis of α –alkylidene γ butyrolactones.

In the chapter five some natural occurring α –alkylidene γ butyrolactones. and relevant synthetic routes to prepare these molecules are described, in the chapter six is described a new methodology in synthesis of α –alkylidene γ butyrolactones motif involving Rh(II) chemistry

CHAPTER 1

INTRODUCTION

1.1 <u>BIOACTIVE TERPENOIDIC DIALDEHYDES: AN OVERVIEW</u>

Natural products afford a window of opportunity to study important biological skills. This is the reason why many interesting metabolites have been isolated and characterized.

These secondary metabolites play an important role in many organisms; their production is connected with several external factors.

Often they play a prominent role in the coexistence and co-evolution of species having a wide range of function in several areas of chemical control:

- Sexual attractants
- Development, metamorhphosis, growth suppressors
- Social behavior, territorial claims, track indicators
- Defence allomones and alarm pheromones

Plants and other organisms need to protect themselves against predators and several strategies have been developed during their evolution.

One of these implies the use of chemical "weapons" and this is often done by producing repellents, *antifeedant* and toxins.[1]

Repellency implies a role in the natural defense system of their host organisms. In some cases this strategy is so efficient that the predator do not even have to chew the plant. For instance, aphids detect the *antifeedants* from the plant with sensilla located on their antennal tips.

A large variety of plant-derived natural products and other chemical agents evoke sensory responses with an infinite shade of perceptual qualities. The perception of chemical stimuli by sensory means is referred to as chemosensation or chemoreception. The presence of many irritant chemicals in plants is due to their action as deterrents against animals searching for food.

In humans, the olfactory and the gustatory systems are the principal chemosensory systems.

---

[1] Jansen, B. J. M.; de Groot, A. *Nat. Prod. Rep.* **2004**, 21, 449 and references therein.

Many of these compounds have an α–β unsaturated 1,4-dialdehyde moiety in their framework. These allomones often have a terpenoidic backbone and can be classified into: sesquiterpenoids, diterpenoids and sesterterpenoids and few of these are monoterpenes.

Some example are: polygodial (**1.1**) isolated from *Polygonum hydropiper*[2] and from mollusks belonging to the subclass of *Opistobranchia* (these mollusks have lost, during the evolution, the usual mechanical defensive due to their shell and so they have developed a chemical defence), warburganal (**1.2**)[3] from *Warburgia ugadensis*, miogadial (**1.3**) isolated from *Zingiber mioga,*[4] isovelleral (**1.4**) from fungus *Lactarius vellereus*,[5] ent-isocopalendial (**1.5**) from *Spongia officinalis,*[6] scalaradial (**1.6**)[7] and deacetoxyscalaradial (**1.7**) from *Cacospongia mollior*[8] (**Figure 1.1**).

[2] Brown, C.S; Loder, J. *Aust. J. Chem.* **1962**, *15,* 322.

[3] Kubo, I.; Lee, Y.; Pettei, M.; Pilkiewicz, F.; Nakanishi, K. *J. Chem. Soc., Chem. Comm.* **1976**, 1013.

[4] Itokawa, H.; Morita, H.; Mihashi, S, *Chem Pharm Bull* **1986**, 28, 3452.

[5] Magnusson, G.; Thoren, s.; Wickberg, B. *Tetrahedron Lett* **1972**, 1105.

[6] Zubia, E.; Gavagnin, M.; Scognamiglio, G.; Cimino, G. *J. Nat Prod* **1994**, 57, 725.

[7] Cimino,G.; De Stefano, S.; Minale, L. *Experientia* **1974**, 30, 846.

[8] De Rosa, S.; Puliti, R.; Crispino, A.; De Giulio, A. *J Nat. Prod,* **1994**, 57, 256.
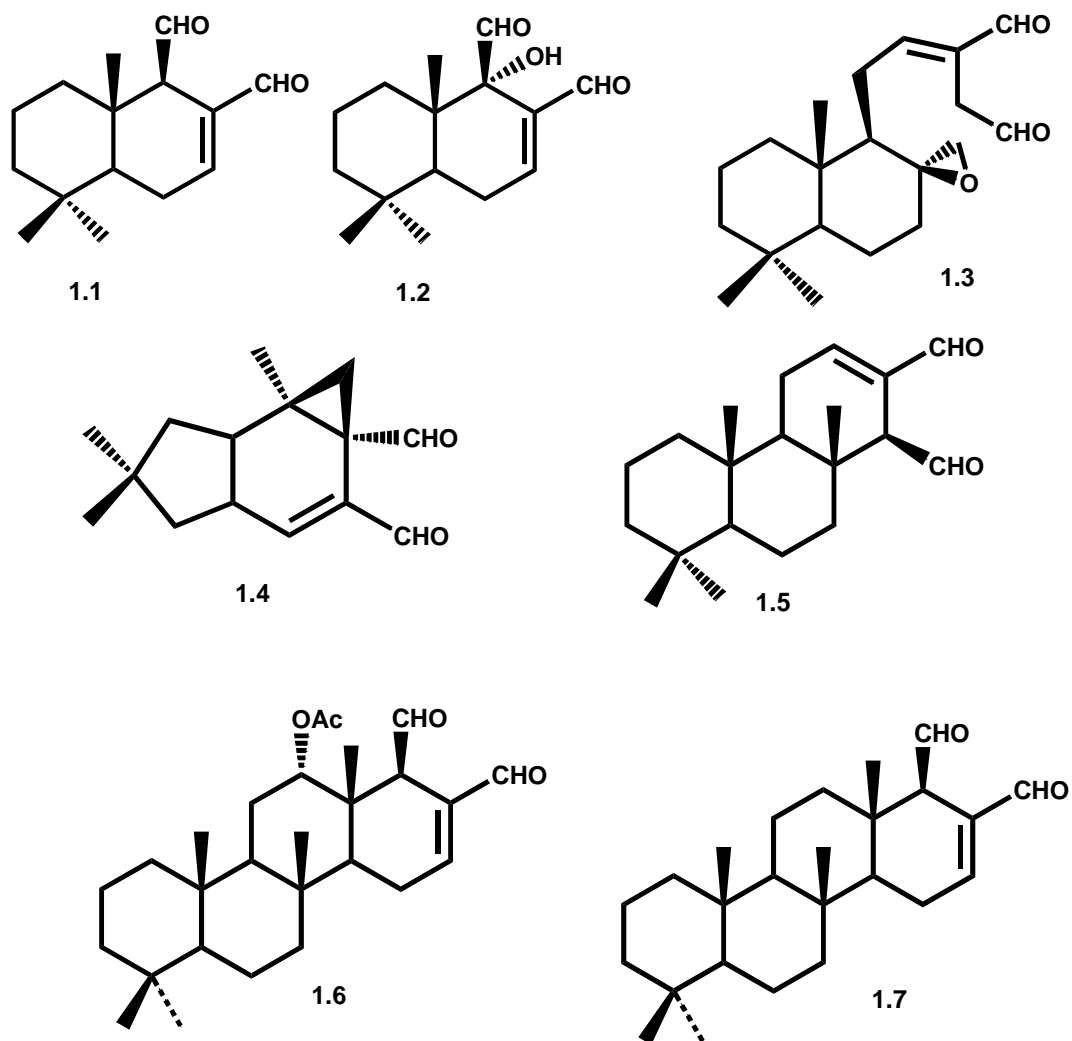
FIGURE 1.1- Some Natural occurring terpenoidic dialdehydes.

We have focused our attention to those compounds having an α,β unsaturated 1,4-dialdehyde moiety in a polycyclic backbone. These compounds may grouped on the base of the skeleton backbone. The most common terpenoid skeletons found for these compounds are the bicyclic backbone of driman **1.1a**, the tricyclic backbone of isocopalane **1.5a** and the tetracyclic scalarane **1.6a** skeleton. (**Figure 1.2**)

1.1 a   **1.5 a**   **1.6 a**
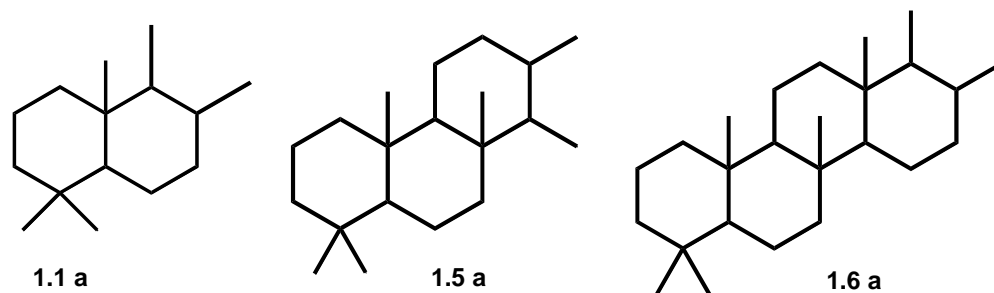
<u>**FIGURE 1.2-**</u> Driman,isocopalane and scalarane backbones

In the following section natural sources and bioactivity of these terpenoid dialdehyde groups are described.

1.2 SESQUITERPENOIDIC DIALDEHYDES

Several sesquiterpenoidic unsaturated dialdehydes with drimane skeleton have been isolated from many natural sources both marine and terrestrial.

The plant *Polygonum hydropiper* (**Figure 1.3**) is also known as marsh pepper because of its habitat and a hot taste experienced on chewing the leaves.

The plant contains the unsaturated dialdehydes polygodial (**1.1**), warburganal (**1.2**) and isotadeonal (**1.8**)[9] (**Figure 1.4**). The presence of polygodial is probably a key factor which makes this plant species resistant to the predators attack.[10]

---

[9] Ying, B.P.; Peiser, G.; Mathias, J.Y.; Tutko, D.; Hwang, Y.S.; *Phytochem* **1995**, 38, 909-915.

[10] Brown, C.S; Loder, J. *Aust. J. Chem.* **1962**, *15,* 322.

FIGURE 1.3- *Polygonum hydropiper*
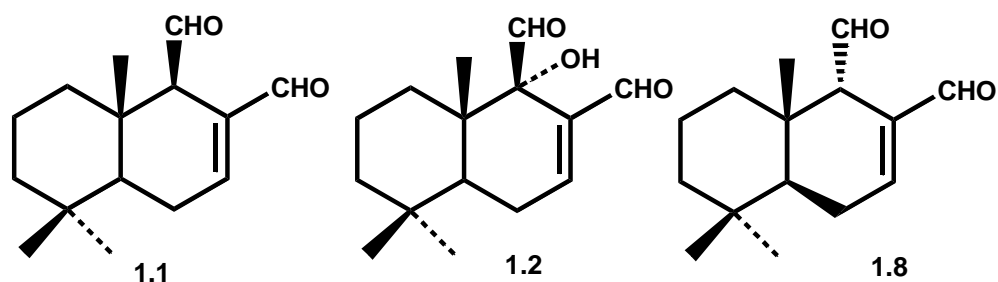
FIGURE 1.4- Some metabolites from *Polygonum hydropiper*

There is no doubt that plants are good sources of biologically active natural products. Drug discovery have therefore often been based on ethnobotanical information. Hence, it is not surprising that several pungent unsatured dialdehydes have been isolated from plants used in folk medicine and as spices in food. The folk medicinal plant waterpepper, *Polygonum hydropiper*, contains the intense pungent substances polygodial **1.1** and warburganal **1.2** in leaf and seed.

Waterpepper has sometimes been used as a substitute for pepper in Europe and as hot tasting spice in China and Japan. It has been used as a folk medicine against tumors, uterine fibromas, and malignant ulcers.

On the other side it is known to be toxic to fishes, pigs and sheeps. Some metabolites in the barks of *Canellaceae and Warbugia* are widely used in folk medicine to alleviate toothache, rheumatisms, general pains, malaria, and also widely used as spices in food.

Polygodial (1.**1**) has been also isolated from the marine mollusk *Dendrodoris limbata* (**Figure 1.5**)

FIGURE 1.5- *Dendrodoris limbata*

Due to their defensive role these compounds must be easily available by the producer organisms, however their toxicity requires a careful storage.

To this purpose at least two strategies are used: the first and most straightforward is to store the dialdehydes in special cavities or cells. For example unsaturated dialdehydes have been found in leaves cavities of the plant *Polygonum hydropiper*. When an herbivore bites and chews the plant the cavity walls are crushed and the pungent dialdehydeis distributed.

Other organisms have elaborated more sophisticated systems for the storage of these metabolites, transforming them in a masked form. For instance, the nudibranch species *Dendrodoris limbata* stores olepupuane (**1.9**), at the end of the mantle. The precursor olepupuane is transformed to polygodial (**1.1**), by the contact with predators. (**Scheme 1.1**)
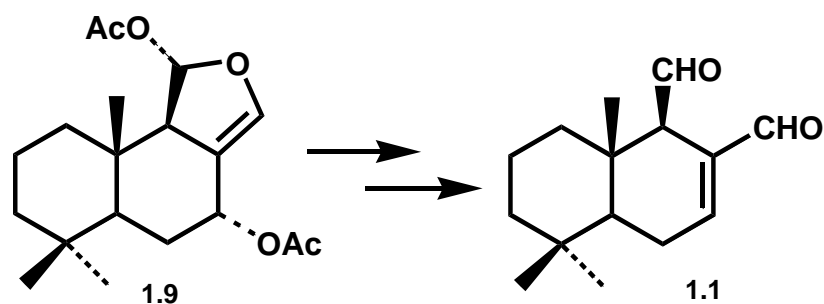
SCHEME 1.1

Some metabolites related to poygodial but C-1 functionalized have been isolated from plants of the Winteracee family, typically growing up in South America: *Drimis winterii* and *Drimis brasiliensis* .

Drymis winterii is a plant used in popular medicine in the treatment of inflammatory diseases.[11] (**Figure 1.6**)

From its bark, drimanial (**1.10**) and 1-β-(*p*-metossicinnamoil)polygodial (**1.11**) metabolites, with high antinociceptive activity, were isolated.[12] (**Figure 1.7**)

FIGURE 1.6- *Drymis winterii*

---

[11] Malheiros A.; Filho, V.C.; Schmitt, C. B.; Santos, A. R. S.; Scheidt, C.; Calixto, J. B.; Delle Monache, F.; Yunes, R. *Phytochemistry* **2001**, *57*, 103.

[12] Filho, V. C.; Schlemper, V.; Santos, A. R. S.; Pinheiro, T. R.; Yunes, R.; Mendes, G. L.; Calixto, J. B.; Delle Monache, F. *Journal of Ethnopharmacology* **1998**, *62*, 223.
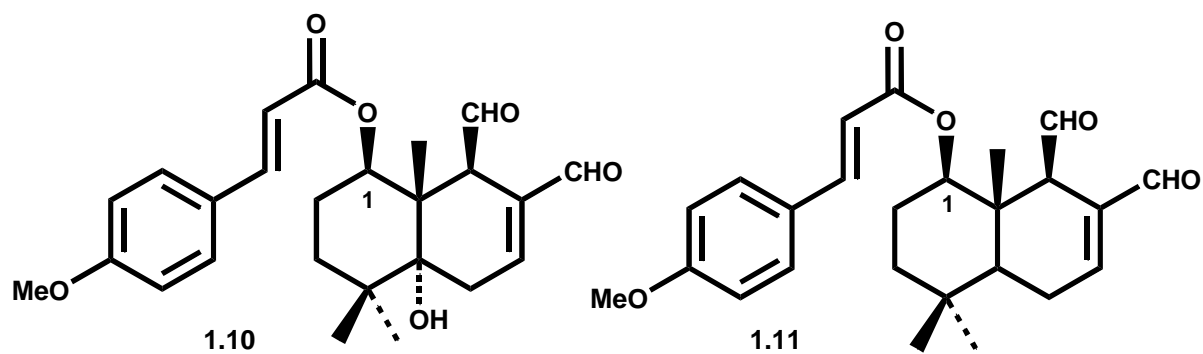
FIGURE 1.7- Metabolites from *Drymis winterii*

1-β-*p*-Cumaroilossipolygodial (**1.12**) was isolated from *Drymis brasiliensis*. The bioactivity of this compound it is still not known.[13] (**Figure 1.8-1.9**)

FIGURE 1.8 *Drymis brasiliensis*

---

13 Vichnewsky, W.; Palaniappan, K.; Herz, W. *Phytochemistry* **1986,** *6*, 1476.

1.12
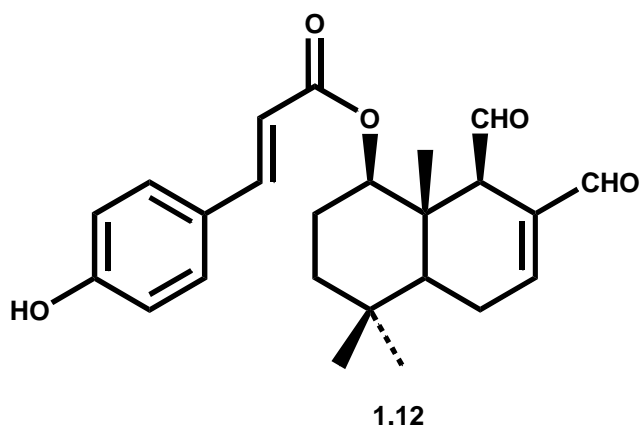
FIGURE 1.9- 1-β-*p*-Cumaroilossipolygodial from *Drymis brasiliensis*

Other drimanic compounds related to polygodial (**1.1**) have been isolated from *Canellaceae*, a small family of plants consisting of nine species, grouped in four genera. Of these, *Winteriana* are endemic of south Africa and *Warbugia* from Madagascar.

Five unsaturated dialdehydes have been isolated from the bark of *Canella winteriana* (**Figure 1.10**), the major ones are muzigadial **(1.13)**, 3-β-acetoxypolygodial **(1.14)** and its C-3 epimer **(1.15)**.[14] (Figure **1.11**)
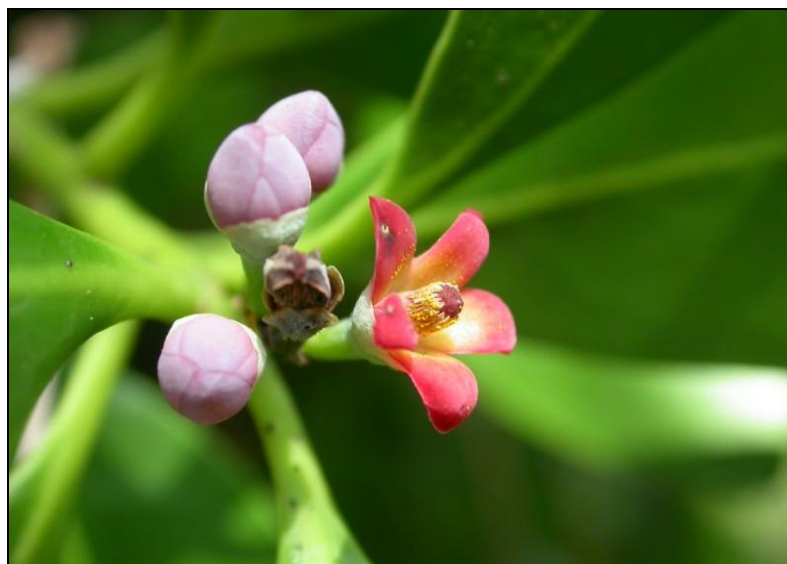
FIGURE 1.10- *Canella winteriana*

---

[14] Ying; B.P; Peiser, G; Yuan; J.Y., Mathias,K; Tutko, D. *Phytochem.* **1995**, 38, 900.
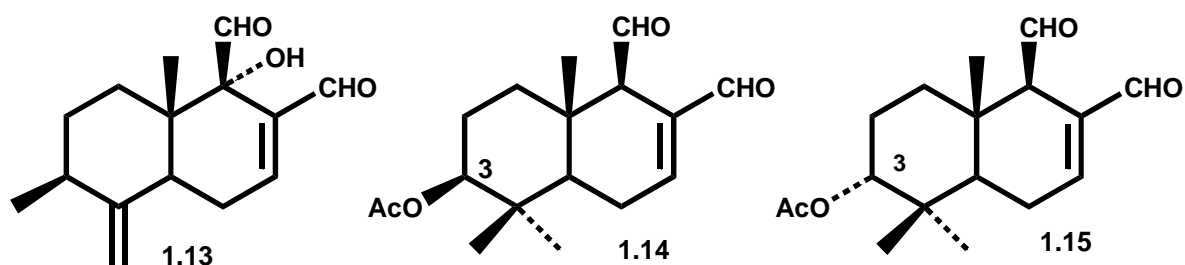
FIGURE 1.11-Some metabolites from *Canella winteriana*

For these compounds some studies toward structure activity relationship have been reported.

It has been demonstrated that the dialdehyde is an essential moiety, no phytotoxic activity was shown by other metabolites with just one aldehyde group or protected as acetals or cyclised to form a lactone ring.

In addition, the β configuration of 9-CHO is important for the activity, in fact the C-9 epimer of **1.14** is much less active than **1.14** itself because of different stereochemistry at C-9 carbon.

It seems that the β orientation of the acetyl group is important for bioactivity: in fact compound **1.15,** the epimer of **1.14** with a different stereochemistry at C-3 is not active.[15, 16]

Some sesquiterpenoidic dialdehydes have been isolated also from *fungi.*

Isovelleral (**1.4**) and velleral (**1.16**) have been isolated from fungus *Lactarius vellereus*, piperdial (**1.17**) from *Lactarius piperatus* and *epi*-piperdial (**1.18**) from *Lactarius necator*. (**Figure 1.12**)

---

[15] Ying; B.P; Peiser, G; Yuan; J.Y., Mathias,K; Tutko, D. *Phitochem.* **1995** 38, 900.

[16] Ying, B.P.; Peiser, G.D.;Yuan, Y, J.;Mathias, Karesina, F. *J. Agric. Food. Chem.***1995**, 43,826.

1.4

1.16

1.17

1.18
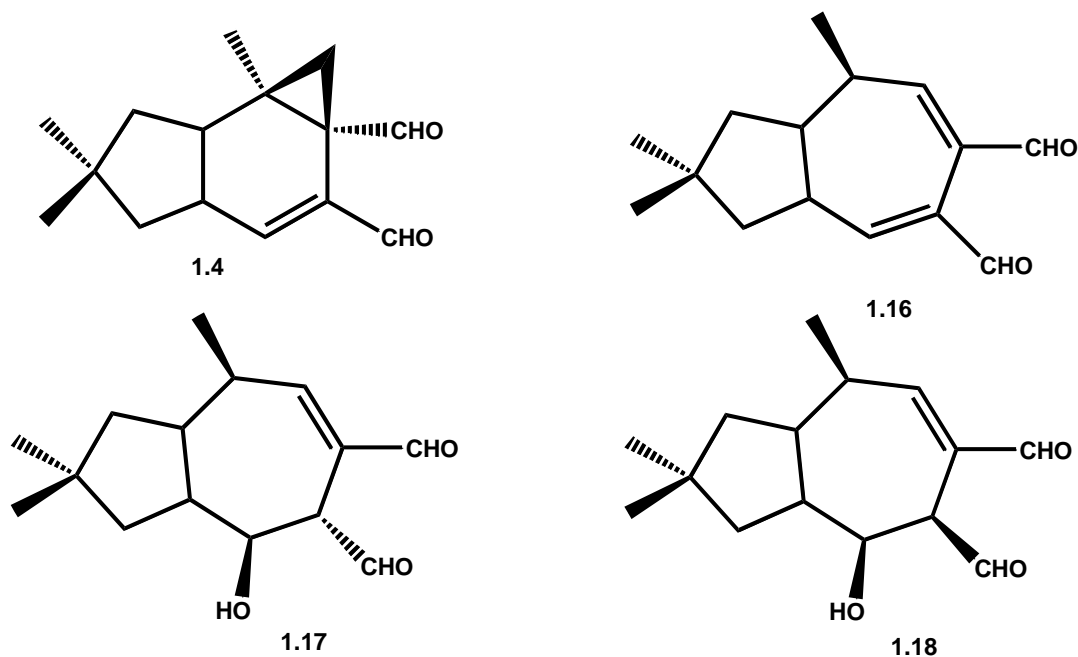
FIGURE 1.12-Some metabolites from fungi

1.3 SPONGIANE AND ENT-ISOCOPALANE DIALDEHYDES.

Spongiane and ent-isocopalane diterpenoids are widely present in marine sponges. The Mediterranean *Spongia zimocca*[17] (**Figure 1.13**) and *Spongia officinalis L.*[18] yielded the first spongiane diterpenoid, isoagatholactone **1.19,** and also some related metabolites possessing either spongiane or ent-isocopalane skeletons (**1.20-1.22**). (**Figure 1.14**)

---

[17] Zubia, E.; Gavagnin, M.; Scognamiglio, G., Cimino, G.; *J.Nat.Prod* **1994**, 57, 725.

[18] Puliti, R.; Mattia,C. *Acta Cryst.* **1999**, C 55, 2160.

FIGURE 1.13 - *Spongia zimocca*

Isogatholactone 1.19   Spongiane Skeleton (1.20)   *ent* isocopalane skeleton

1-21;R= α CHO
1.22;R= β CHO
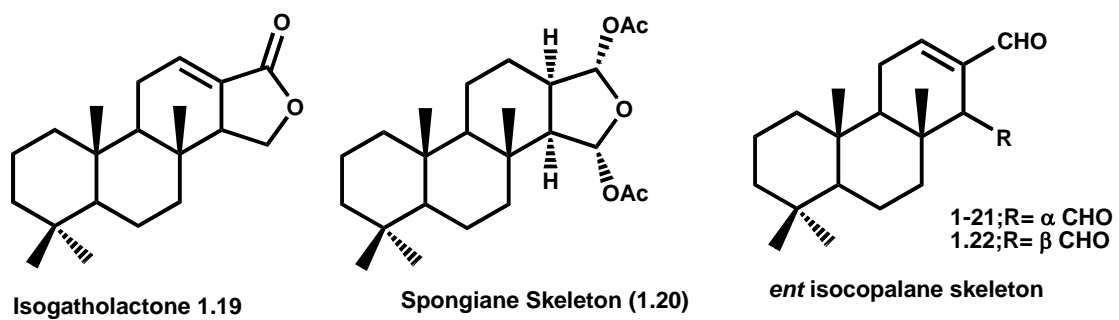
FIGURE 1.14 -Some natural occurring spongiane and *ent*-isocopalane compounds

From *Spongia officinalis* (**Figure 1.15)** in the 1999 has been isolated *ent*-Isocopal- 12-ene- 15,16-dialdehyde **1.5**, a C-20 diterpenoid presents a trans-fused tricyclic system with a 1,4-dialdehyde function, adjacent to the double bond of the cyclohexene ring.[19] (**Figure 1.16**)

FIGURE 1.15 - *Spongia officinalis*

1.5
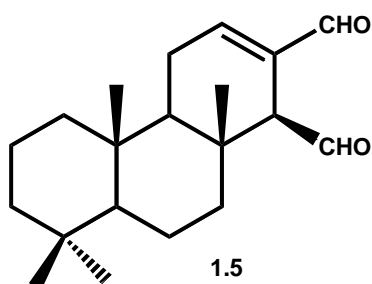
FIGURE 1.16 - *ent*-Isocopal- 12-ene- 15,16-dialdehyde from *Spongia officinalis*

1.4 SCALARANE DIALDEHYDES

Scalarane–type sesterterpenoids (**Figure 1.17**) were isolated from many natural sources and are a very interesting group of natural products typically isolated from both marine sponges and mollusks.

These molecules show diverse pharmacological properties including cytotoxicity, antimicrobial and anti-inflammatory activity, platelet aggregation inhibition.
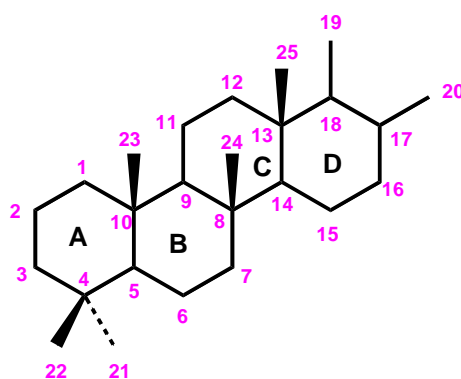
FIGURE 1.17 Scalaranic backbone

An involvement of these sesterterpenoids in the defensive mechanisms of sponges and molluscs has also been demonstrated. The structural diversity in the scalarane family mainly arises from the different arrangement of the oxidized carbons C-19 and C-20, which can be involved in a cyclization process to form a five-membered ring with higher or lower oxidation [i.e., scalarin (**1.23)**] The majority of natural scalaranes are characterized by the presence of an oxygenated functional group at C-12, such as scalaradial **(1.6)** and related compounds. However, a number of natural scalaranes display additional oxygenated groups at different positions of the tetracyclic framework [i.e., heteronemin (**1.24**), 6 3-keto-deoxoscalarin (**1.25**), 6-keto-deoxoscalarin (**1-26**)]. (**Figure 1.18**)

The B-ring functionalization has also been reported for a series of scalaranes (i.e., **1.27**) isolated from ferns, the only report of scalarane occurrence in plants. Scalaradial **(1.6)** the first sesterterpenoidic molecule with a scalarane skeleton was isolated in 1974 from *Cacospongia mollior* (Figure **1.19**) by Cimino and co-workers and shows a relevant anti-inflammatory profile in both *in vitro* and *in vivo* models.

Scalaradial (1.6)   Scalarin (1.23)   Heteronemin (1.24)

3-keto-deoxoscalarin (1.25)   6-keto-deoxoscalarin (1.26)   1.27
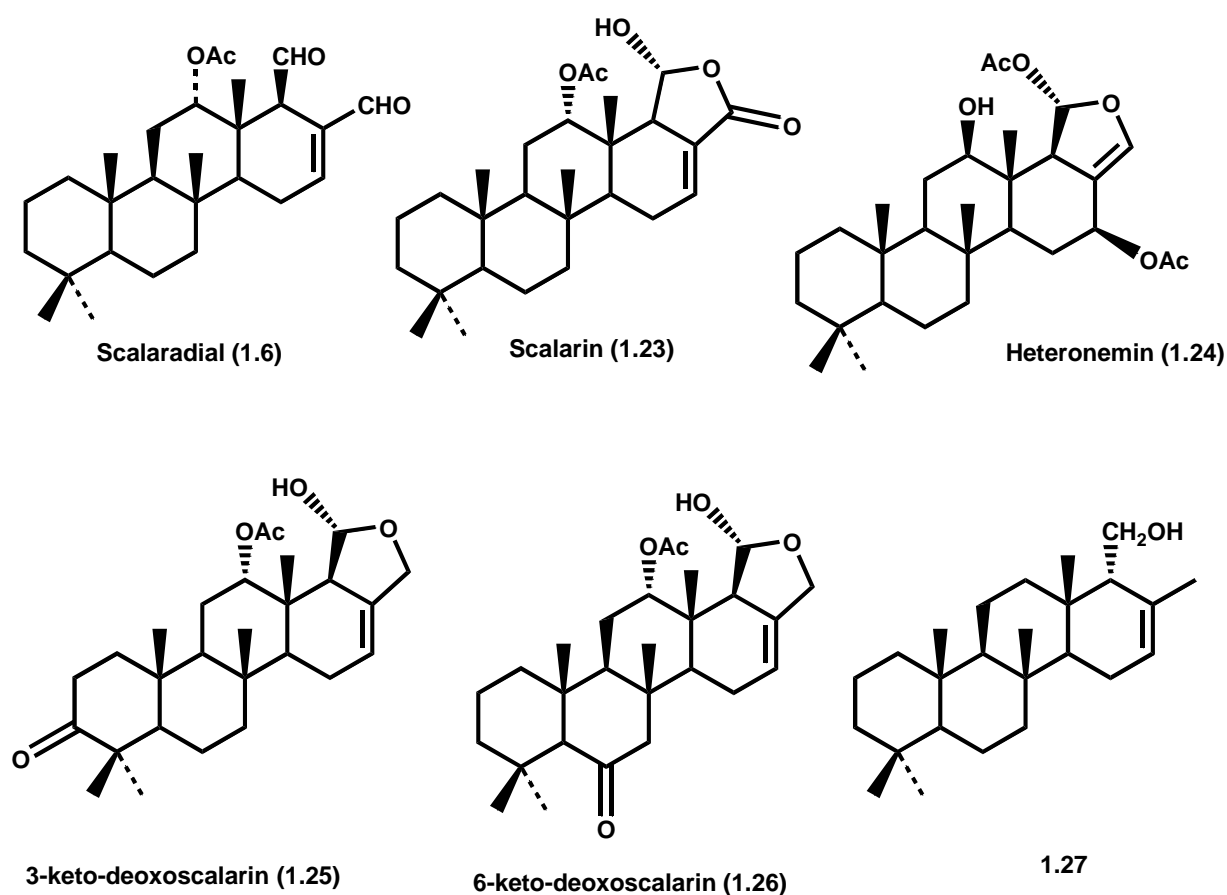
FIGURE 1.18- Some natural occurring scalarane

FIGURE 1.19 *Cacospongia mollior*

Cimino et al.[19] suggested that the marine mollusk *Hypselodoris orsini* converts the dialdehyde compounds scalaradial (**1.6**) to deoxoscalarin (**1.28**) and further to 6-keto- deoxoscalarin (**1.26**) which is specifically accumulated in the glands of the mantle. (**Figure 1.20**)

Scalaradial (1.6)   **Deoxoscalarin (1.28)**   **6-keto-deoxoscalarin**
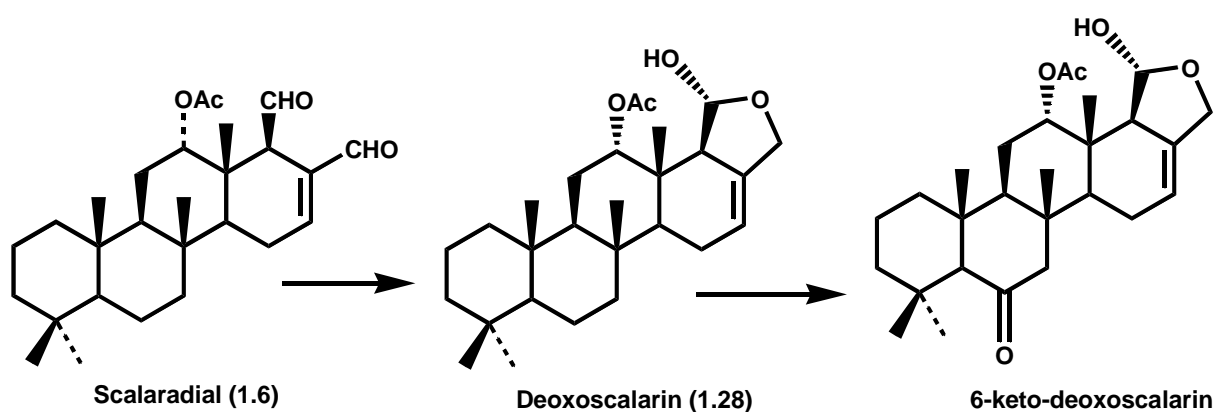
FIGURE 1.20- Storage of scalaradial

---

19 Kulcit¸ V.; Ungur, N.; Gavagnin, M.; Castelluccio, F.; and Cimino G. *Tetrahedron* **2007**, 63, 7617.

It has been found that some sponges produce dialdehyde scalaradial or its epimer **1.29** without any further transformation while other sponges produce heteronemin (**1.24**). This metabolite is very similar to olepupuane (**1.9**). (Figure **1.21**)

It has been shown that 12 epi-scalaradial has an important effect on the inhibition of epidermal growth factor receptor (EGFR). [20]

12-*Epi*-Scalaradial (1.29)   **Heteronemin (1.24)**
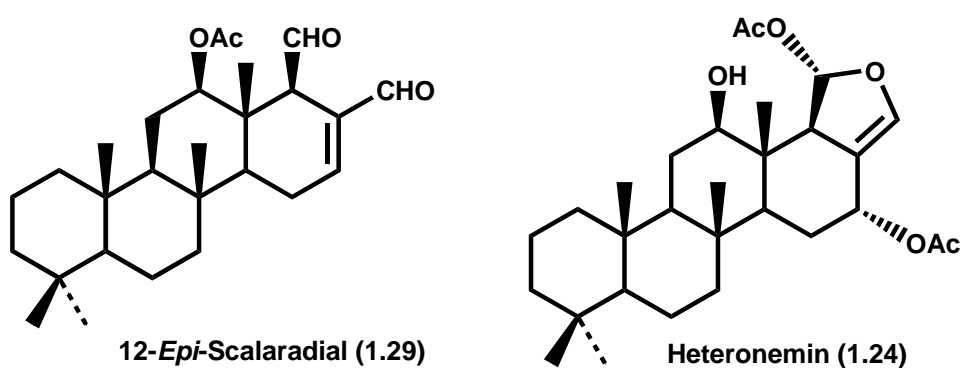
FIGURE 1.21

[20] Xie,Y.; Liu, L.; Huang, X .; Guo, Y.; Lou,L. J. *Pharmacol. and Exp. ther.* **2005**, 314, 1210.

1.5 BIOACTIVITY: STRUCTURAL REQUIREMENTS AND TRP RECEPTORS INTERACTION

Most unsaturated dialdehydes possess a wide variety of biological activities: antibacterical, antifungal, anti-inflammatory, both antitumor and tumor promoting, cytotoxic, enzyme inhibiting, phytotoxic, pungent, and so on.

The biological activities have in many cases been shown to be strongly linked to the unsaturated dialdehyde functionality. For instance both the enal moiety and the aldehyde group were essential for inhibition of tumor promotion on mouse skin as well as for mutagenicity of isovelleral **4** in Ames Salmonella[21] assay. Stereoisomerisation and other structural changes may affect the biological activity drammmatically.

While polygodial **1.1** is one of the most potent natural unsaturated dialdehydes, its epimer isotadeonal **1.8** is not active,[22] the isovelleral isomer **1.30** has less antibiotic cytotoxic and phytotoxic potentials when compared to isovelleral itself,[23] and the mutagenic activity of merulidial (**1.31**) is lost after isomerisation to **1.32** or by acetylation of its secondary alcohol.[24] (Figure **1.22**)

---

[21] Sterner, O.; Carter, R.E.; Nilsson, L.M. *Mutat. Res.,* **1987**, 188,169.

[22] Kubo, I.; Lee, Y.; Nakanishi, K. *J. Chem. Soc. Chem Comm.* **1976**, 1013.

[23] Anke, H.; Sterner, O. *Planta Med* **1991**,57, 344.

[24] Anke, H.; Sterner O.,Steglich,W. *J.Antibiot* **1989***,* 42, 738.

1.1   **1.8**

1.4   **1.30**

1.31   **1.32**

FIGURE 1.22 Different structural features can affect bioactivity: *polygodial **1.1** is a potent natural occurring dialdehyde, its epimer isotadeonal **1.8** is not active, the isovelleral isomer **1.30** is less active if compared to isovelleral itself, and the mutag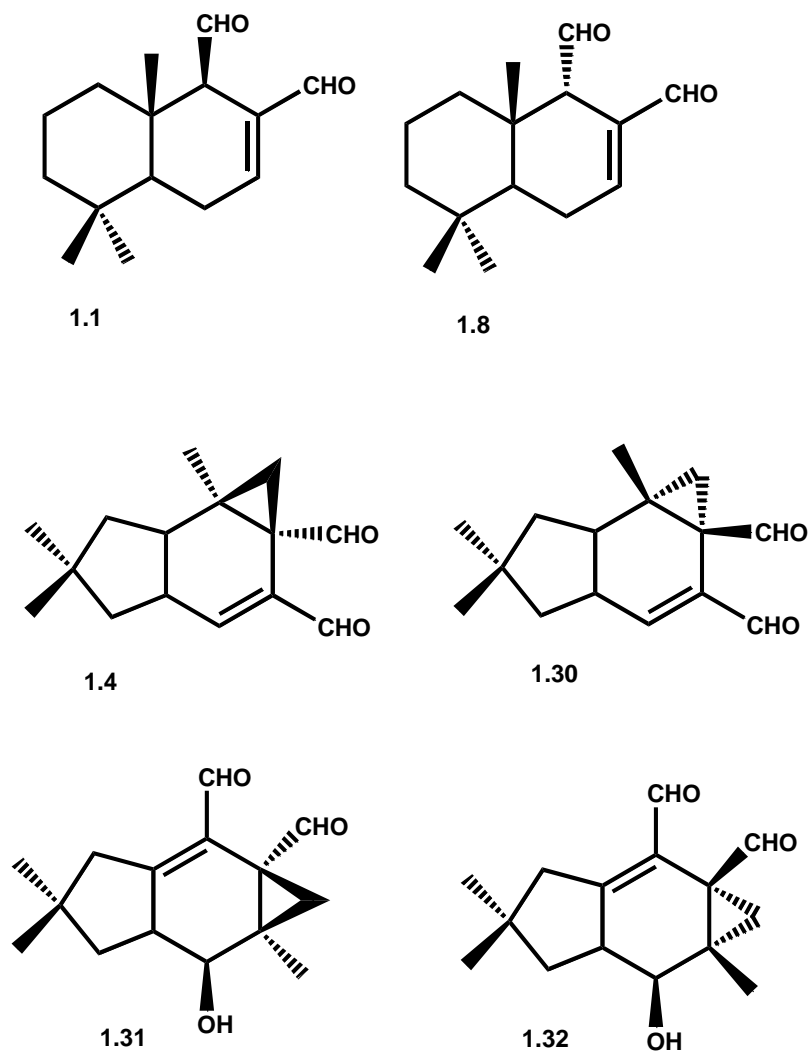enic activity of merulidial (**1.31**) is lost after isomerisation to **1.32** or by acetylation of its secondary alcohol.*

1.5.1 EARLY INVESTIGATIONS ON THE MECHANISM OF ACTION OF BIOACTIVE DIALDEHYDES

Many unsaturated dialdehydes, terpenoidic and none, are known to be pungent on human tongue and it has been shown that all *antifeedant* dialdehydes taste hot and spicy to the human tongue. Whereas nonantifeedant derivatives are devoid of hot taste.[25]

It has been supposed that both the enal moiety and the other aldehyde group should be essential for pungency, as well as many other bioactivities.

In earlier investigations Kubo supposed that cysteine was involved, in a Michael reaction-type addition, with unsaturated dialdehydes, even if no adduct has ever been isolated.[26]

This expectation was reasonable, since it is known that cysteine reacts preferably in 1,4 additions with α, β-unsaturated dialdehydes. Saturated aldehydes, however, readily give thiazolidine adducts with cysteine.

Taniguchi et al [27]reported that several physiological effects due to polygodial, e.g inhibition of growth, alcohol fermentation etc appeared to result from its irreversible reaction with sulfhydryl groups.

However, based on kinetic data, Sodano and co-workers [28]proposed that the biological activity of the unsaturated dialdehyde is primarly related to their ability to form adducts with amino groups rather than sulfhydryl group on the receptor.

They proposed that the biological mechanisms, investigated under biomimetic conditions by reaction of terpenoidic dialdehydes with methylamine, occurs with formation of azomethinic intermediate that give a pyrrol-type compound.[29] (**Scheme 1.2**)

---

[25] Caprioli, V.; Cimino, G.; Colle, R.; Gavagnin, M.; Sodano, G.; Spinella,A. *J.Nat.Prod* **1987**, 50, 146.

[26] Kubo, I. , Ganjian, I. *Experientia* **1981,** *37,* 1063.

[27] Taniguchi, M.; Adachi, T.; Haraguchi, H.; Oi, S.; Kubo, I.*J.Biochem*, **1983**, 94, 149.

[28] D'Ischia, M.; Prota, G.; Sodano, G. *Tetrahedron Lett.* **1982**, 23, 4139.

[29] a) Cimino, G.Sodano, G.; Spinella, A.*Tetrahedron* **1987**, 43, 5401. b) Cimino, G.; Spinella, A.; G.Sodano. *Tetrahedron Lett.* **1984**, 25, 4151.

This is what could be happen when a dialdehyde is approaching to the receptor.
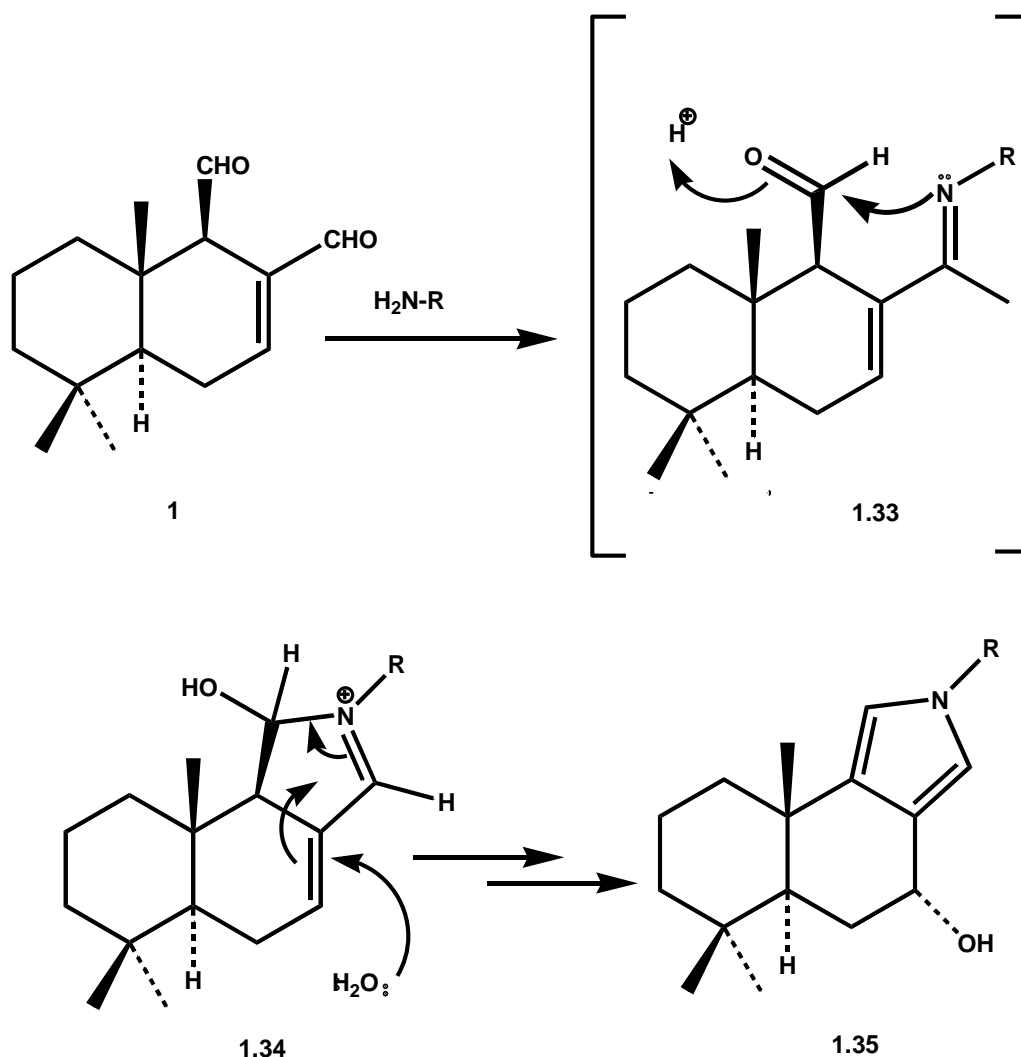
Scheme 1.2

However both the aldehyde functionalities could play a role in the interaction with the receptor. The first example of a reaction between a bi-functional nucleophile and a unsaturated dialdehyde was a reaction between cysteine methyl ester and muzigadial, that gives a tetracyclic adduct in this reaction and polygodial as well can give other possible adducts by reaction with peptides.

Another mechanism proposed for reaction between unsaturated dialdehydes and peptides was suggested by Fritz[30] this mechanism included both a pyrrole formation and a Michael-addition. (**Scheme 1.3**)
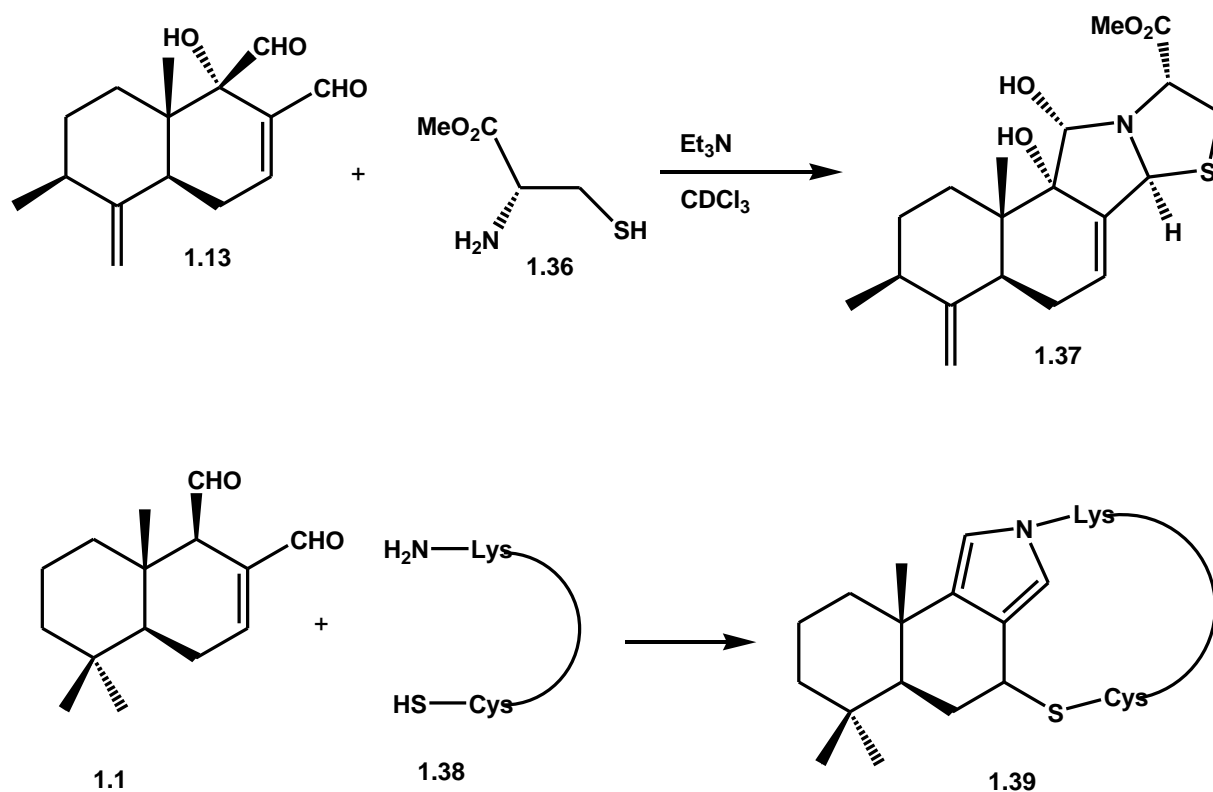
SCHEME 1.3

The high reactivity of unsaturated dialdehydes toward primary amino groups is often proposed to explain their antibiotic activity and their enzyme inhibiting properties. In fact their reactivity towards primary amines *in vivo* may produce some of natural pyrrols (molliorins **1.41**) which are co-isolated with the corresponding aldehyde scalaradial. This compound is formed *in vivo* in the sponge *Cacospongia mollior*, most probably by reaction with scalaradial **1.6** and ornitin **1.40**. (**Scheme 1.4**)

---

[30] Fritz, G.L; Mills, G.D.; Warthen, J.D.; Waters, R.M. *J.Chem.Ecol*. **1989**, 15, 2607

1.6

1.40

1.41
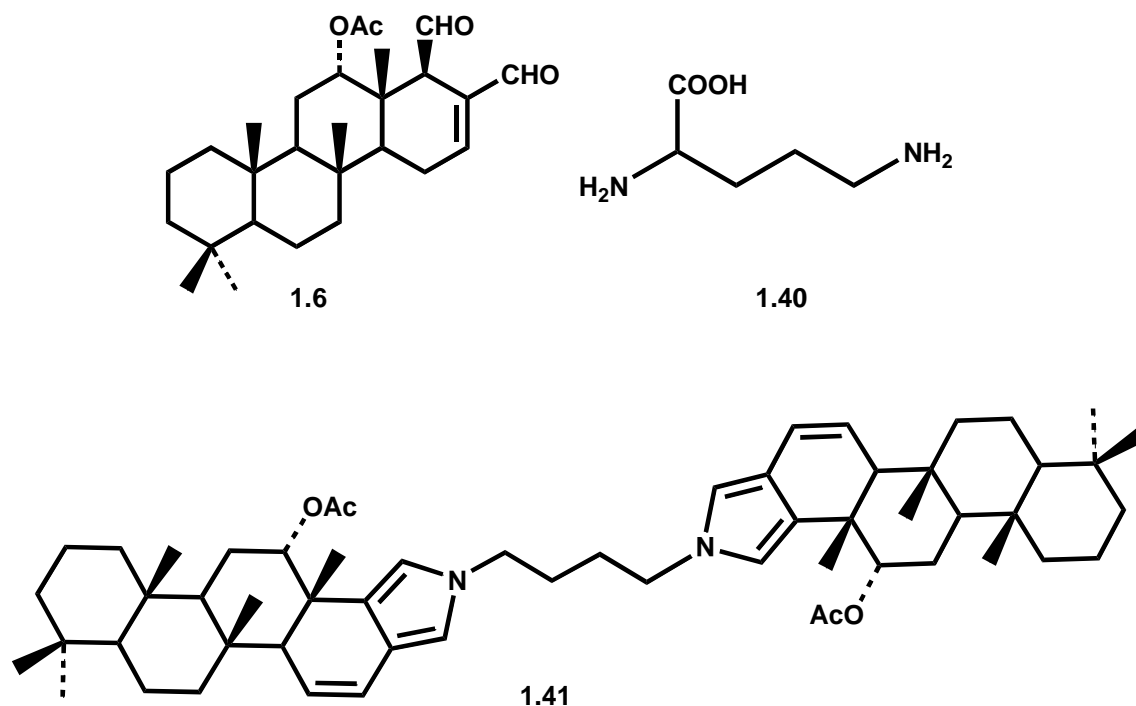
SCHEME 1.4

In conclusion the molecular mechanisms by which these type of compounds exert their bioactivities is still not unknown and many questions concerning the unsaturated dialdehyde and their activity remain. However it seems that the mechanism proposed by Sodano and co-workers has been confirmed from some evidences.

In fact, when the dialdehyde moiety has a specific orientation with C-9 CHO β oriented (instead of C-9 CHO α) the pyrrole compound is easily formed, because this orientation gives an optimal distance between the two groups, and allows easy reaction with bionucleophiles.

This hypothesis could explain why polygodial is pungent to human tongue while is C-9 isomer is inactive. (**Figure 1.23**)

.

Polygodial
hot tastig

Isotadeonal
not active
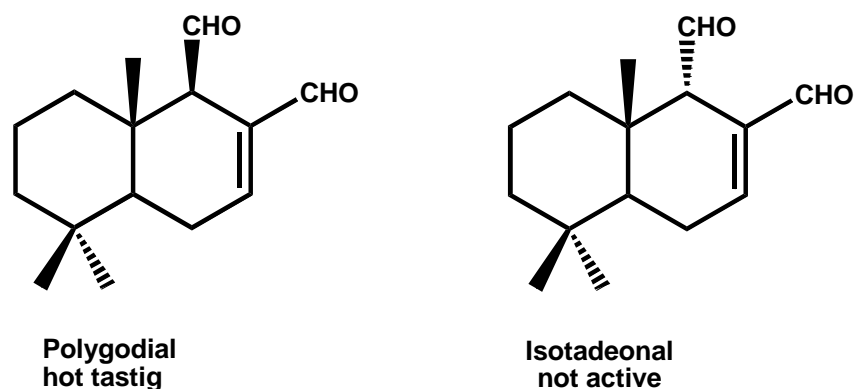
FIGURE 1.23

Anyway this structural feature is not enough to explain why other molecules like scalaradial, having an α,β-unsaturated dialdehyde moiety with the correct stereochemistry, are not active. The lack of activity is probably related to the size of the molecule: too big to interact with the receptors.

The isolation from *Cacospongia mollior*, of desacethyscalaradial .resulted hot tasting, pointed out that a presence of a free hydroxyl group in the framework can increase the activity toward receptors.

Furthermore, in other cases, it has been shown that presence of a polar group can decrease dramatically the activity, such as in the case of both isovelleraloids , merulidial and its analogues.

In fact, some studies carried out to evaluate pungency amount of some unsaturated dialdehydes on the human tongue and their affinity for specific [³H]-resiniferatoxin binding sites in rat spinal cord, showed that the hydroxyl group (see derivatives **1.43**-**1.44a,b**) in isovelleral **1.4** and merulidial **1.31** decreases highly the affinity of these compounds for [³H]-resiniferatoxin **1.45** binding sites. (**Figure 1.24**)

Scalaradial (1.6)
not active

Desacethylscalaradial (1.42)
Hot tasting

1.4

1.43

1.31

1.44 a;R=OAc
1.44 b;R=OH

1.44

1.45
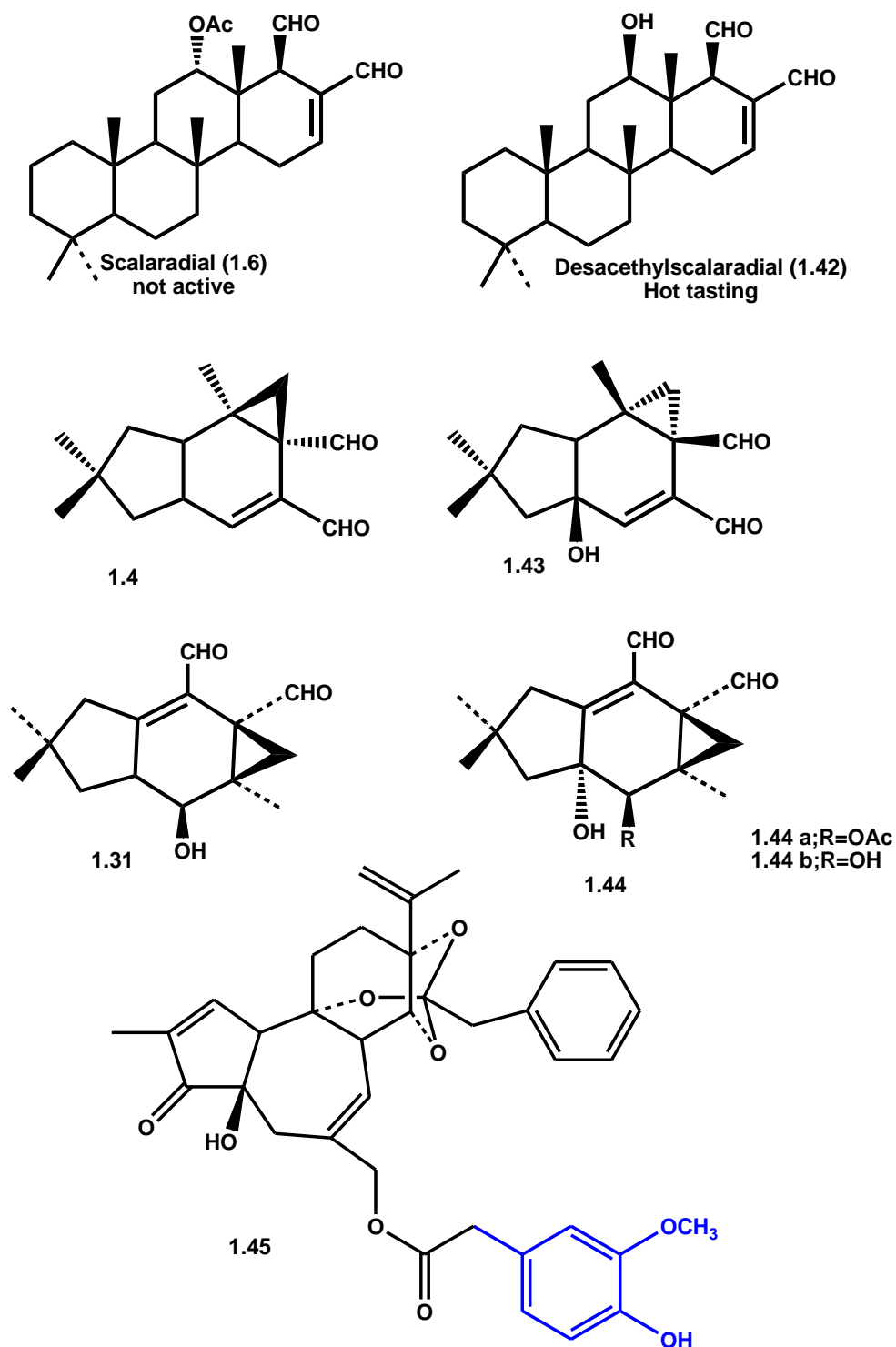
FIGURE 1.24 *Pungency of some natural occurring dialdehydes related to the presence of polar group in their framework. In* **1.43** *the presence of a polar group increases the bioactivity if compared with* **1.6***; surprisingly, the presence of a polar group in* **1.43** *and* **1.44** *decreases the bioactivity if compared with* **1.4** *and* **1.31** *respectively.*

Finally some unsaturated dialdehydes have been related to capsaicin **1.46**, isolated from *Capsicum spp*,[31] the responsible of the burning sensation of the hot peppers in the human tongue.

Pungency of capsaicin has been largely investigated and early studies showed an interaction with a specific receptor and this was demonstrated by the specific binding of [^{3}H]-resiniferatoxin (RTX) **1.45,** a naturally occurring ultrapotent capsaicine analogue, isolated in 1975 from the latex of *Euphorbia resinifera.*[32] (**Figure 1.25**)

1.46

1.47

FIGURE 1.25

---

[31] Nelson EK *J Am Chem Soc* **1919**, 41,1115.

[32] Szallasi A ,Blumberg PM. *LifeSci* **1990**, 47,1399.

It has been supposed that some natural occurring terpenoidic dialdehydes, that are hot taste to human tongue, could activate this chemoreceptor.

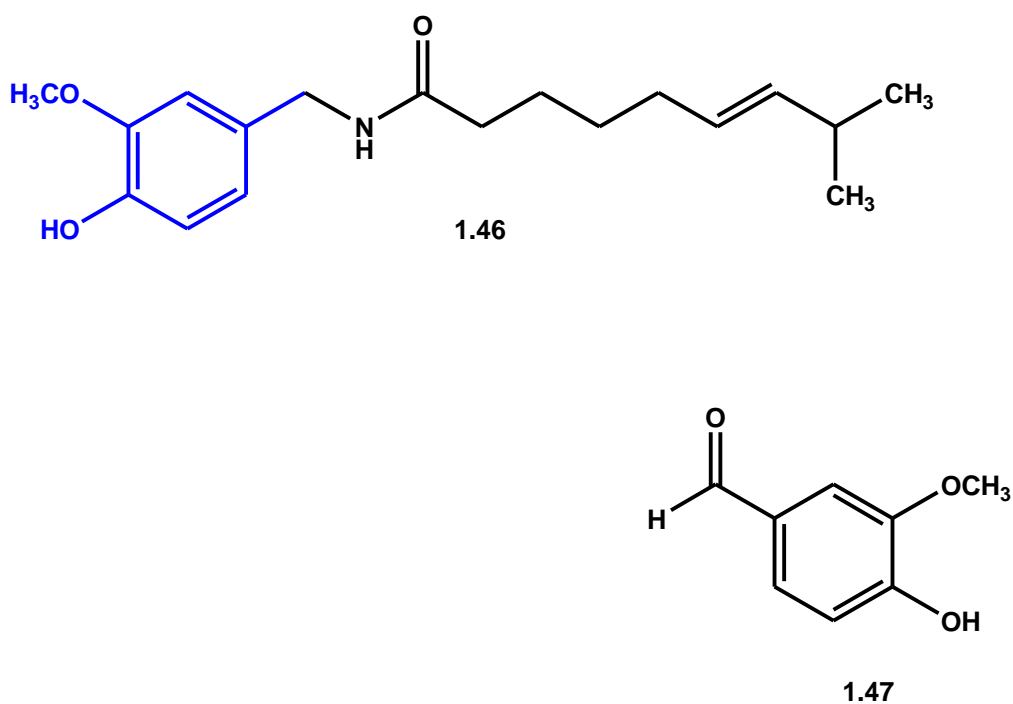This is the reason why these compound are important targets for structure-activity relationship investigation.

The receptor has been described as the vanilloid receptors because RTX and capsaicin have the homo vanilloid moiety (**1.47**) in common. This vanilloid receptor, called TRPV1[33] is a member of a big family of receptors involved in the chemo and thermo reception, the TRP family. These receptors deserve a special comment not only because of their interaction with agonists is the cause of burning sensation which is the base of defensive response but also because their activation Is involved in inflammatory processes.

1.5.2 <u>TRP RECEPTORS ION CHANNELS</u>

The ability to detect both ambient and body temperature is vital for animal survival. Thermosensation seems in most cases to be carried out by the direct activation of thermally-gated ion channels in the surface membranes of sensory neurones, rather than by any more indirect mechanism such as modulation by temperature of the activity of intracellular signalling pathways. To date all such temperature-sensitive ion channels are members of the extensive TRP family. (**Figure 1.26**)

---

[33] Szallasi A , Blumberg PM Pharmacol Rev.1999, 51, 159.

Figure 1

Temperature (°C)

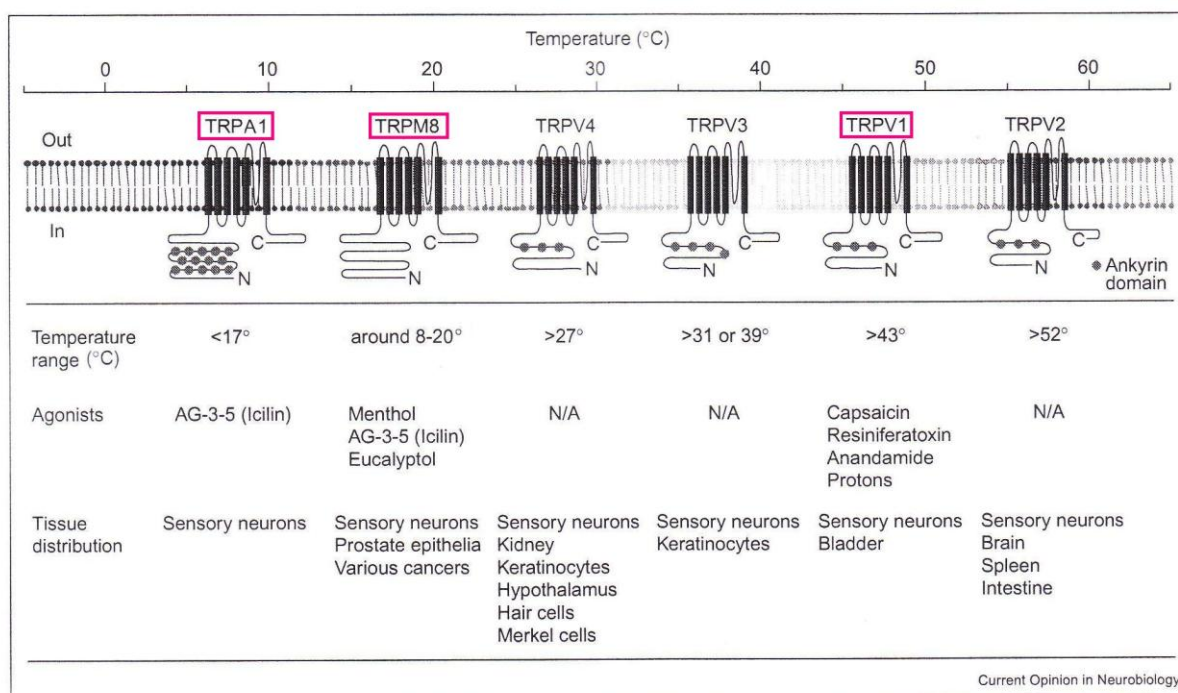|	TRPA1	TRPM8	TRPV4	TRPV3	TRPV1	TRPV2
Temperature range (°C)	<17°	around 8-20°	>27°	>31 or 39°	>43°	>52°
Agonists	AG-3-5 (Icilin)	Menthol AG-3-5 (Icilin) Eucalyptol	N/A	N/A	Capsaicin Resiniferatoxin Anandamide Protons	N/A
Tissue distribution	Sensory neurons	Sensory neurons Prostate epithelia Various cancers	Sensory neurons Kidney Keratinocytes Hypothalamus Hair cells Merkel cells	Sensory neurons Keratinocytes	Sensory neurons Bladder	Sensory neurons Brain Spleen Intestine

Current Opinion in Neurobiology

FIGURE 1.26- *TRP Receptors Family: Temperature-Range Of Activation, Agonists And Tissue Distibution*

It has been shown that TRP channels are involved in trigeminal chemosensory. Trigeminal somatosensory system play a key role in chemosensation, Sensory ending of trigeminal nerves are located all around the face, or in mucous membrane of nasal and oral cavities and are activated by physical stimuli (such as temperature) or chemical stimuli and evoked sensation of touch or pain.

Protective responses evoked by trigeminal stimulation include salivation, tearing, coughing, respiratory depression, and sneezing .Moreover, pungent irritation or burning pain is a very common side effect of drugs applied topically to the skin.

Chemical signaling of taste and smell involves the following mechanism; binding of the chemical agent to the receptor activates a protein that initiates a transduction cascade. In contrast, activation of somatosensory afferents by chemicals involves the direct gating of an ion channel by the chemical stimulus and is called ionotropic transduction. In either case, the chemical signal leads to

38

a change in ionic permeability and the depolarization of the sensory receptor. The sensations evoked by chemical agents applied to the skin or mucous membranes vary in their quality (e.g., burning, stinging, itch, tingling, cold), temporal profile, and intensity.[34]
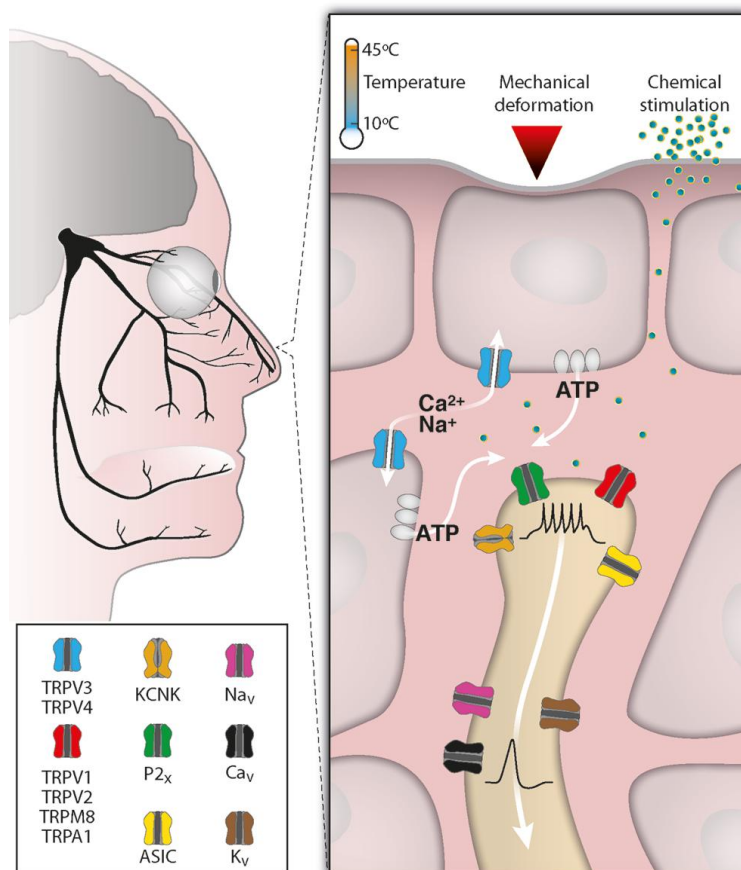

FIGURE 1.27 *Molecular determinants of chemosensation in trigeminal nerve terminals. Branches of the human trigeminal nerve innervating the face, the eye, and the nasal and oral cavities. Temperature and many chemical agents can stimulate chemosensitive channels directly. These channels are expressed in sensory nerve terminals and mucosal epithelial cells. The opening of cationic channels (TRP) generates a graded transduction current depolarization with subsequent propagation of the nerve impulse to the brain system.*

---

[34] Felix Viana ACS Chem. Neurosci. **2011**, 2, 38–50.

The origin of the discovery of the TRP (*Transient receptor potential*) channels can be traced back to the 1960s, when a *Drosophila* mutant was found to show a transient response to prolonged bright light . The *trp* gene was cloned in 1989, and was shown to encode a light-activated Ca2+ channel (TRP) in *Drosophila.* Later studies provided evidence for the existence of many different TRP homologues. The mammalian TRP channel family contains ~30 members, which can be divided into at least seven subfamilies (TRPA, TRPC, TRPM, TRPML, TRPN, TRPP and TRPV1-4). Thermo-TRPs currently comprise nine members from the TRPV (TRPV1–4), TRPM (M2 to M8) and TRPA (TRPA1) subfamilies.

Each thermo-TRP is activated over a specific temperature range, and when working together in vivo they cover a wide cumulative temperature range from noxious heat (>52 C°) to noxious cold (<10 C°). Activation of the thermo-TRPs seems to be the main mechanism for peripheral thermosensation. In addition to temperature, thermo-TRP channels respond to a variety of chemical and other physical stimuli, allowing them to work as polymodal sensors.[35] (**Figure 1.28**)

---

[35] a) Huang, J.; Zhang, X.; McNaughton, P.A. *Seminars in Cell & Developmental Biology* **2006** 17638, b) Venkatachalam, K.; Montell, M. *Annu. Rev. Biochem*. **2007**. 76, 387.
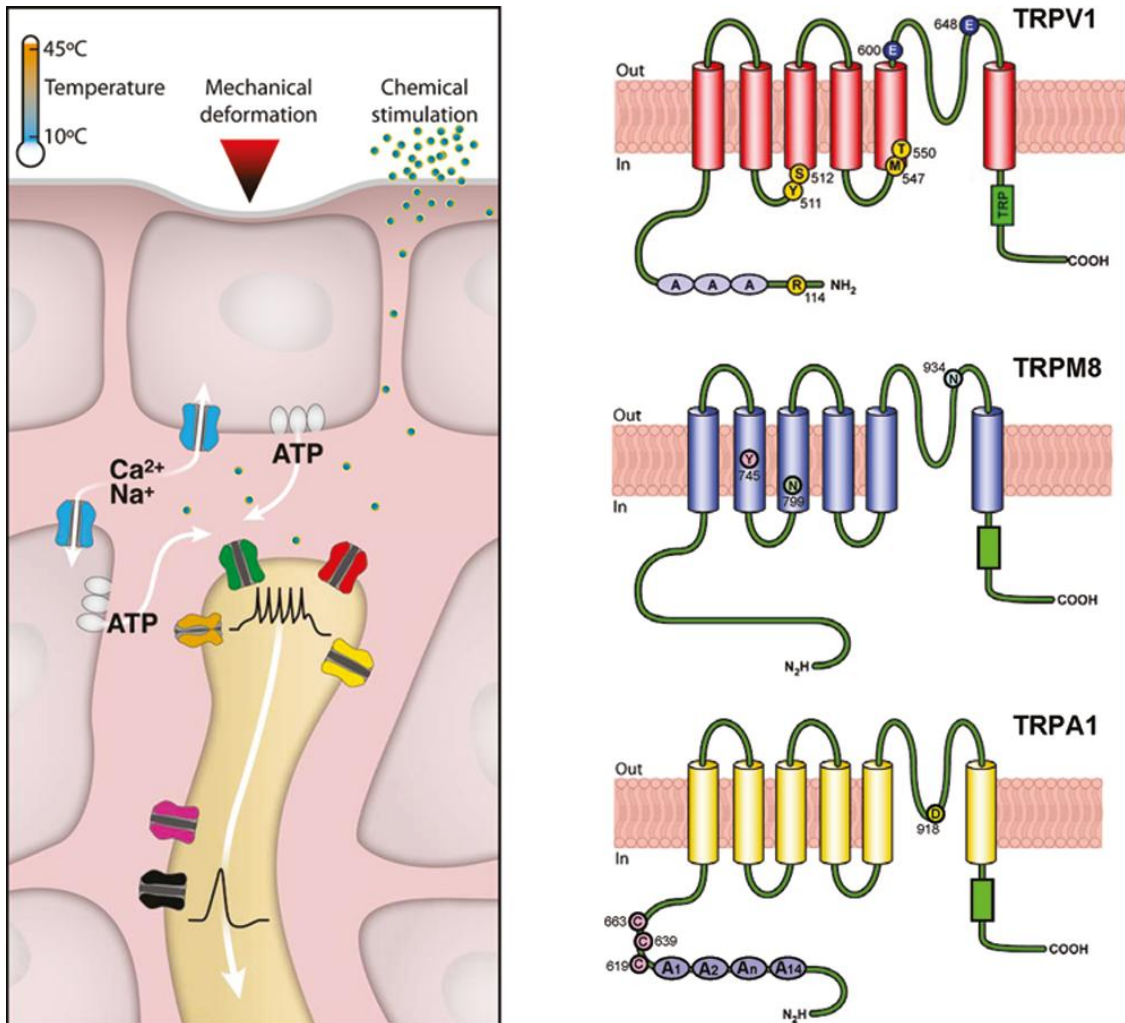
FIGURE 1.28 *Activation of TRP receptors by both chemical anf physical stimuli*

In common with other members of the TRP family, the thermo-TRPs are homo- or heterotetramers of four single subunits. Each subunit has a predicted structure of six transmembrane (TM) domains with a pore loop between domain five (TM5) and six (TM6). Both the amino- and carboxy-terminal domains of thermo-TRPs are proposed to be localized intracellularly. The N-terminal domain of the thermo-TRPs contains several ankyrin repeat elements (except TRPM8 in which ankyrin motifs have not been identified). The ankyrin repeat, a 33-residue sequence motif, has been found to mediate protein–protein interaction in a number of biologically important proteins.

In the C-terminal region, the TRP domain, a highly conserved region of 25 amino acids, is common amongst all TRPV, TRPC and TRPM channels

The functional role of the TRP domain in these channels is unclear. Some evidence suggests that in TRPV1, the TRP domain serves as an association domain for tetramerization and is therefore critical for assembly of TRPV1 subunits. In TRPM ion channels, however, it has been proposed to play a role in activation and desensitization [35a](**Figure 1.29**)
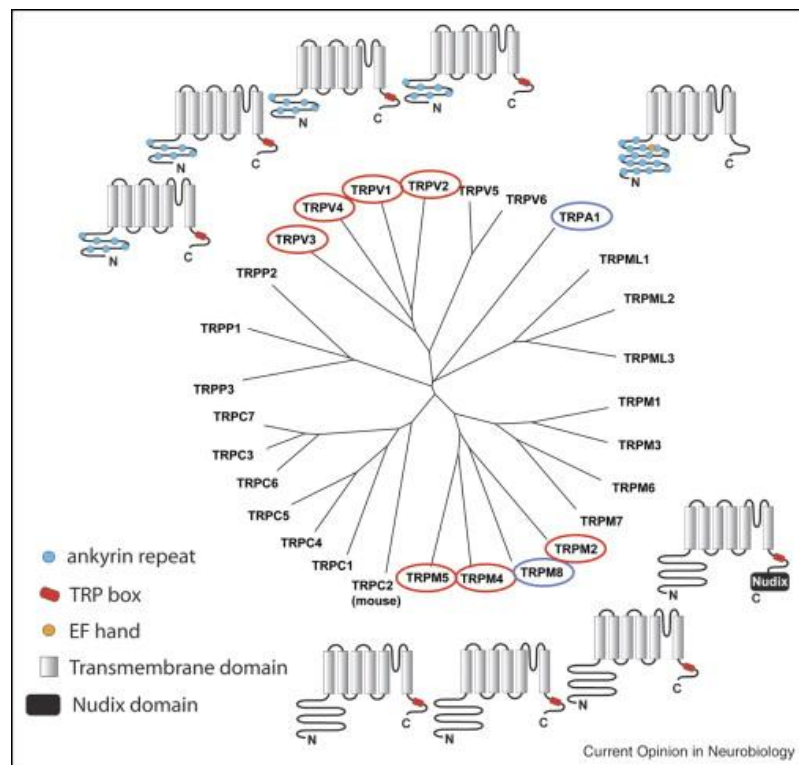

FIGURE 1.29 *Topology of TRP receptors family*

Our research has been focused in particular on two receptorial targets: TRPV1 and TRPA1.
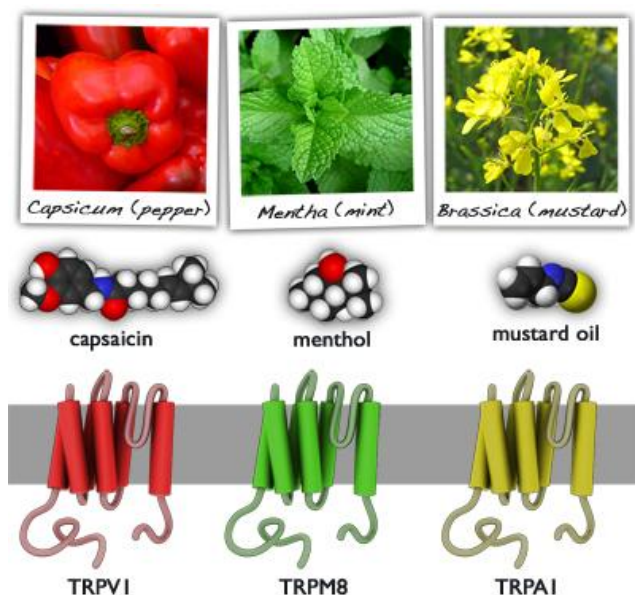

FIGURE 1.30 SOME NATURAL OCCURRING AGONIST

1.5.3   FROM HOT CHILI PEPPER TO THERMORECEPTION: TRPV1 VANILLOID RECEPTOR

The first mammalian TRPV was identified by expression cloning in a search for channels activated by the inflammatory vanilloid compound capsaicin, which gives spicy foods their characteristic hot taste and was the first identified in dorsal root ganglion and trigeminal ganglion neurons, and is also highly expressed in spinal and peripheral nerve terminals and in the pancreas.

TRPV1 is broadly involved in nociception and analysis of vanilloid receptor gene confirmed that the channel contributes to detection and integration of painful chemical and thermal stimuli. TRPV1 is selective for Ca^{2+} and Mg^{2+} ions and is activated by vanilloid compounds such as capsaicin **1.46** and resiniferatoxin **1.45**, as well as by heat (<43 C°), low pH (<5) and many other compound including endocannabinoids, anandamide **1.48** and allicin **1.49**, a metabolite from garlic.[36] (**Figure 1.31**)

---

[36] Macpherson, L.; Geierstanger, B.; Bandell,M.; Patapoutian, A. *Current Biol*. *2005*, 15, 929.

1.48

1.49

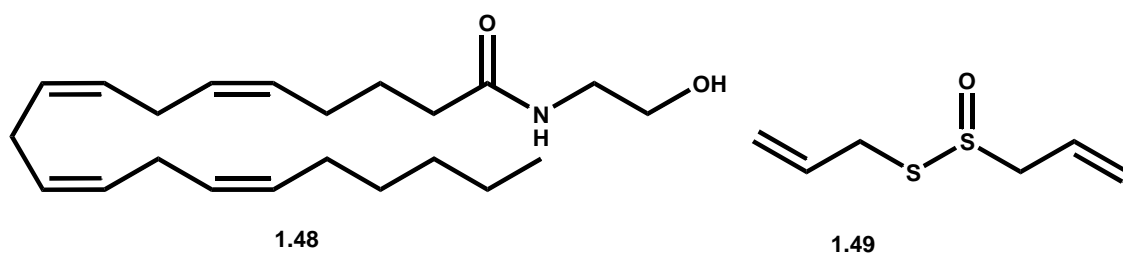**FIGURE 1.31**

TRPV1-mediated cation influx initiated by the application of noxious chemicals or heat is further enhanced by low pH, In fact, pH $\leq$ 5.9, characteristic of proalgesic tissue acidosis, induces a shift in the thermal activation threshold of TRPV1 so that it can be activated at room temperature .

While initially painful, capsaicin and other compound (eg polygodial **1.1**) acts as an analgesic through desensitization of sensory neurons: the initial TRPV1 activation, however often leads to pain and extreme discomfort.

The first studies on chemoreception were carried out on capsaicin, the primary chemical irritant in hot peppers, because its burning sensation in the human tongue. These assays were carried out on rat dorsal root ganglion neurones, so in a non selective medium.

Capsaicin **1.46** has long been thought to interact at a specific receptor whose existence has been demonstrated by the specific binding of [³H]-resiniferatoxin **1.45** (RTX), a naturally occurring ultrapotent capsaicin analogue, and by the development of a synthetic, competitive capsaicin antagonist, capsazepine (**1.50**) [37](Figure **1.32**)

As capsaicin and RTX differ dramatically in the rest of the molecule, but share a (homo)vanillyl substituent essential for biological activity, the receptor appears to be best described as the vanilloid receptor VR.[38]

---

[37] Walpole,C.; Bevan,S.; Bovermann,G.; Boelsterli, J.; Breckenridge, R.;Davies,J.; Hughes,G.; James, I.; Oberer,L. *J. Med. Chem.*, **1994**, 37, 1942.
[38] Szallasi ,A.; Blumberg,M. *Gen Pharmacol* **1994**, 25,223.
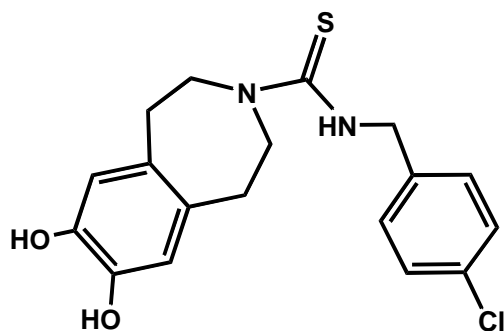
Capsazepine (1.50)

FIGURE 1.32

As hot tasting compounds, also some natural unsaturated dialdehydes such as isovelleral **1.4**, have been assayed, like capsaicin **1.46**, on rat dorsal root ganglion neurons and it was found that isovelleral inhibited specific binding of [^{3}H]-resiniferatoxin (RTX), to rat trigeminal ganglion or spinal cord. An investigation on 14 sesquiterpenoids with an unsaturated 1,4-dialdehyde, furnished positive results in similar assays on the rat spinal cord. The results suggested that isovelleral-like compounds produce their irritant effect by interacting with receptors on capsaicin-sensitive sensory neurones. Since these pungent diterpenes are structurally distinct from the known classes of vanilloids, these data provided new insights into structure-activity relationship and afforded new opportunities for the development of drugs targeting capsaicin-sensitive pathways.[39]

Another investigation on vanilloid activity of natural dialdehydes was conducted on a wider group of compounds. In these assays isovelleral **1.4** and polygodial **1.1** were active toward receptors. However surprisingly in these assays also tastless dialdehydes such as scalaradial resulted active. [40]

The TRPV1 receptor was cloned in the 1997 by Julius *et al.*[41] starting from the evidence that capsaicin, the main pungent ingredient in hot chili peppers elicits a sensation of burning pain, thinking that it happen by activation of sensory

[39] A. Szallasi, M. Jonassohn, G. Acs, T. Biro, P. Acs, P.M. Blumberg, 0. *Sterner British Journal of Pharmacol*, **I996**, 119, 283.

[40] Szallasi, A.; Bìrò, T.; Modarres, S.; Garlaschelli, L.; Petersen, M.; Klusch, A.; Vidari, G.; Jonassohn, M.; De Rosa, S.; Sterner, O.; Blumberg, P. M.; Krause, J. E. *Eur. J. of Pharmacol.* **1998**, *356,* 81.

[41] Caterina, M. J.; Schumacherk,M.; Tominaga,M.; Rosen, T.;Levine,J.; Julius, D. *Nature* **1997**, 389, 816

neurons that convey information about noxious stimuli to the central nervous system. They made an expression cloning strategy based on calcium influx to isolate a functional cDNA encoding a capsaicin receptor from sensory neurons.

HEK type cells were transfected with pools of clones from a rodent dorsal root ganglion cDNA and subjected to microscopy fluorescent calcium imaging before and during treatment with capsaicin. Cells transfected with vector alone exhibited no response to capsaicin. Cells transfected with pool exhibited marked increases in cytoplasmatic calcium. This pool was iteratively subdivided and reassayed until a single clone (VR1) was isolated.

It has been found that this receptor is a non selective cation channel that is structurally related to members of the TRP family of ion channels. The cloned capsaicin receptor is also activated by increase in the temperature in the noxious range, suggesting that it functions as a transducer of painful thermal stimuli in vivo.

Once cloned TRPV receptor it has been made specific assays toward binding of both vanilloid compounds capsaicin and resiniferatoxin and plant derived sesquiterpenes like isovelleral.

Assays carried out on the recombinant rat vanilloid VR1 receptor expressed in HEK 293 cells has been shown that Ca^{2+} response were induced by resiniferatoxin and capsaicin but isovelleral was inactive.[42]

Further studies were carried out on this receptor by Geppetti *et al* to better understand the biological effects induced by the plant derived sesquiterpene polygodial and drimanial, in term of how they can displace of [^{3}H]-resiniferatoxin (RTX). The assay was carried out both on rat spinal cord membranes and rat trigeminal ganglion neurons.

In the first medium it has been demonstred that capsaicin polygodial and drimanial can displace the specific binding sites of of [^{3}H]-resiniferatoxin and, likewise, they promoted an increase of Ca^{2+} uptake.

In addition the assays were carried out using specific inhibitor as well, such as capsazepine and ruthenium red and in these cases the contraction induced by vanilloids compounds were totally inhibited.

---

[42] Ravelic, V.; Jerman, J.C.; Brough, S.J.; Davis, J.B.; Egerton, J.; Smart, D. *Biochem Pharmacol.* **2003,** *65,* 143.

When the assays were carried out on cultured rat trigeminal neurons polygodial **1.1** drimanial **1.10** and capsaicin **1.46** were able to significantly increase intracellular Ca $^{2+}$ uptake effects that was significantly prevented by capsazepine **1.50**.

Together the present results strongly suggest that pharmacological actions of plants derived sesquiterpenes seems to be partially mediated by activation of TRPV1.[43]

1.5.4 <u>THE DISCOVER OF TRPA1 RECEPTOR</u>

TRPA1 channel was identified in 2003[44] as a receptor activated by noxious cold. The ankyrin transient receptor potential channel TRPA1 is a non-selective cationic channel that is expressed by sensory neurons, where it can be activated by pungent chemicals.

Macpherson *et al* have discovered a key role of TRPA1 channels in chemical damage and pain sensing, responding to a wide range of chemical compounds produced during tissue damage.[45]

The activation mode of TRPA by its agonist resemble the behavior of other thermo TRP are activated: initially, pain is followed by desensitization of sensory neurons.

More recently some assays carried out on cultured murine root ganglion neurons have demonstrated that sesquiterpenoids such as polygodial **1.1** and isovelleral **1.2** can activate TRPA1 receptor with a great increase of Ca^{2+} influx through gated receptor.

Recent studies have demonstrated that TRPA1 is a receptor for a wide range of noxious electrophilic agents, activating the channel through covalent modification of Cysteine and Lysine in TRPA protein.

These modifications come out from interaction of agonists containing an $\alpha, \beta-$ unsaturated dialdehydes moiety capable of forming both Michael adduct and Shiff base with reactive amino acid residues.

[43] Andrè, E.; Campi, B.; Trevisani, M.; Ferreira, J.; Mahleiros, A.; Yunes, R.A.; Calixto, J.B.; Geppetti, P. *Biochem Pharmacol.* **2006,** 71, 1248.

[44] Story, G.M., Peier,A.M., Reeve, A.J., Eid, S.R.,Mosbacher, J., Hricik, T.R.,Earley, T.J., Hergarden, A.C., Andersson, D.A., Hwang, S.W., McIntyre,P., Jegla, T., Bevan, S., Patapoutian, A. *Cell* **2003,**112, 819.

[45] Chao Tai; Shanshan Zhu and Ning Zhou *The journal of Neuroscience* **2008**, 28, 1019.

TRPA1 is rendered insensitive to these agonists when specific covalent acceptor sites are substituted with non reactive residues.

In fact both polygodial and isovelleral have been tested on these TRPA1 mutants and these mutants were totally insensitive to progressive addition of agonists.

Seems that C-terminus is a critical modulatory domain for TRPA1 activation, and this end-tail is rich in Lys residue,[46] that means might be possible the hypothesis of Sodano and co workers that in chemoreception could be involved a reaction between a Lys and the α, β-unsaturated dialdehydic moiety.

Recently it has been shown that oleocanthal (**Figure 1.28**) a phenolic compound isolated by extra virgin oil[47] having an unsaturated dialdehydic moiety, is able to activate TRPA1 receptor.(**Figure 1.33**)

1.51
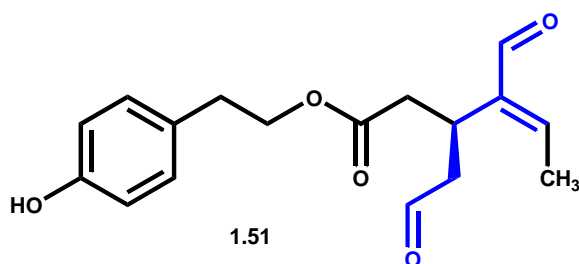
FIGURE 1.33

This compound, synthesised by Amos Smith III and co-workers,[48] has been implicated in the bitterness, pungency, and astringency of extra virgin olive oil and shows inhibitory activities COX-1 and COX-2 , similar in potency to the NSAID ibuprofen.[49]

The irritant effect of oleocanthal is especially in the throat and this is in contrast with other oral irritants such as cynnamaldehyde, capsaicin which irritate mucus membranes throughtout the oral cavity.

---

[46] Abdul Samad, Lucie Sura, Jan Benedikt, Rudiger Ettrich, Babak Minofar, Jan Teisinger And Viktorie Vlachova Biochem. J. (**2011**) 433, 197–204.

[47] Andrewes P, Busch JL, de Joode T, Groenewegen A, Alexandre H. *J Agric Food Chem* **2003**, 51,1415–1420.

[48] Amos B. Smith, III,* Jeffrey B. Sperry, and Qiang Han *J. Org. Chem.* **2007**, *72*, 6891-6900

[49] Beauchamp, G.; Keast, R.; Morel, D.; Liu, J.; Pika, J.; Han, Q.; Lee, C.; Smith, A. B., III; Breslin, P. *Nature* **2005**, *437*, 45-46.

It has been shown that this rare irritation pattern is a consequence of both the specificity of oleocanthal for a single sensory receptor and the anatomical restriction of this sensory receptor in the oral cavity.

In vitro assays have demonstrated that oleocanthal selectively activates the TRPA1 channel in HEK 293 cells.[50]

---

[50] Peyrot des Gachons et al. *J. Neurosci.* **2011** , 31(*3*), 999 −1009.

CHAPTER 2

TOTAL SYNTHESIS OF SESQUITERPENOIDIC DIALDEHYDES

2.1 OUTLINE

In this chapter the total syntheses of polygodial derivatives, both C-1 and C-3 functionalised, are described.

Our purpose is to assay the bioactivity of synthesized natural products and analogues both as TRP receptor agonists and as antiproliferative compounds .

Because of their different structural features, two different synthetic strategies have been developed to prepare these C-1 and C-3 drimane:

- Polygodial and C-1 functionalised drimanes have been prepared with an approach whose key step is a Diels Alder reaction; derivatives **2.3- 2.5** have been prepared following a synthetic protocol previously developed in our group for preparation of compounds **2.1, 2.2**, **1.11** .[51] (Figure **2.1- 2.2**)
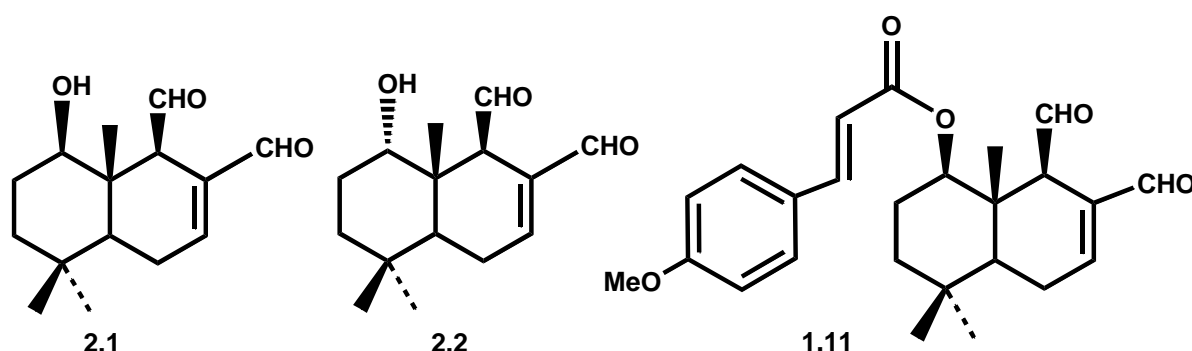
FIGURE 2.1 compounds already synthesized in our research group

[51] a) Della Monica, C.; Della Sala, G.; Izzo, I.; De Petrocellis, L.; Di Marzo, V.; Spinella, A. *Tetrahedron* **2007**, *63*, 6866. b) Della Monica, C.; De Petrocellis, L.; Di Marzo, V.; Landi, R.; Irene, I.; Spinella, A. *Bioorg. Med. Chem. Lett.*, **2007**, 6444.

2.3

2.4

2.5
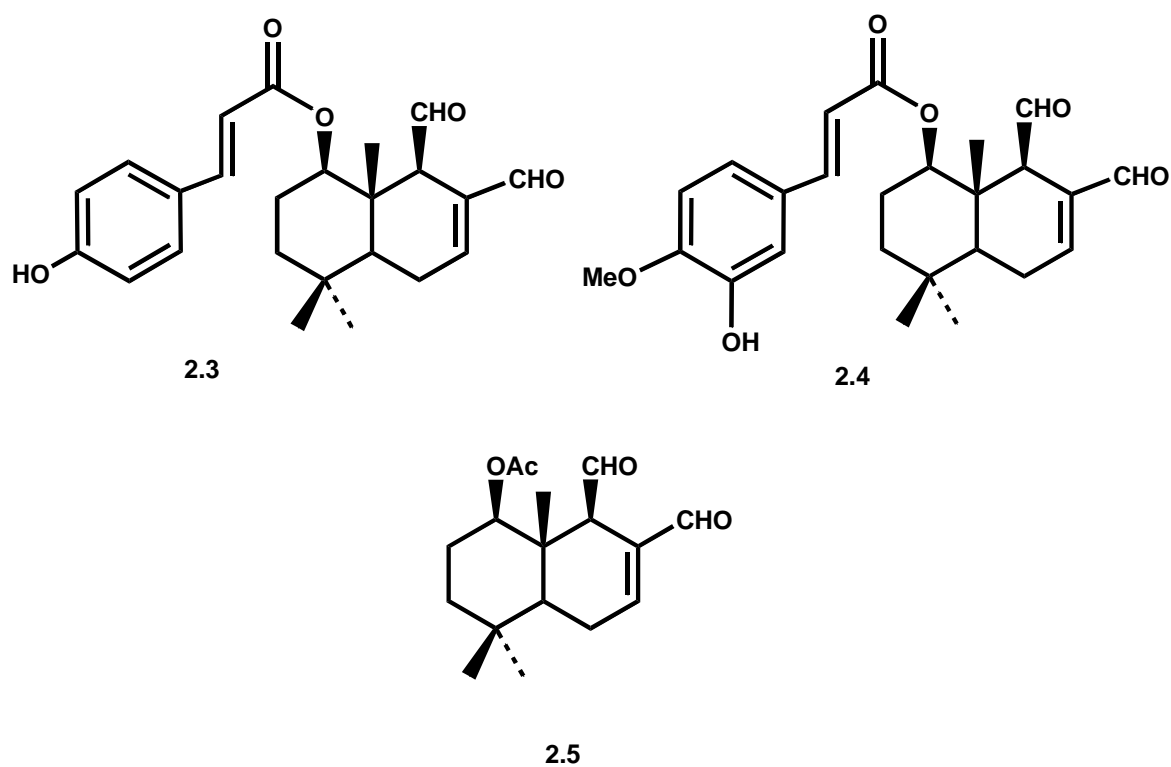
FIGURE 2.2 New synthetic targets

- Drimane C-3 functionalised have been prepared with a radical chemistry approach starting from an easily prepared chiral epoxypolyene.[52] (**Figure 2.3**).
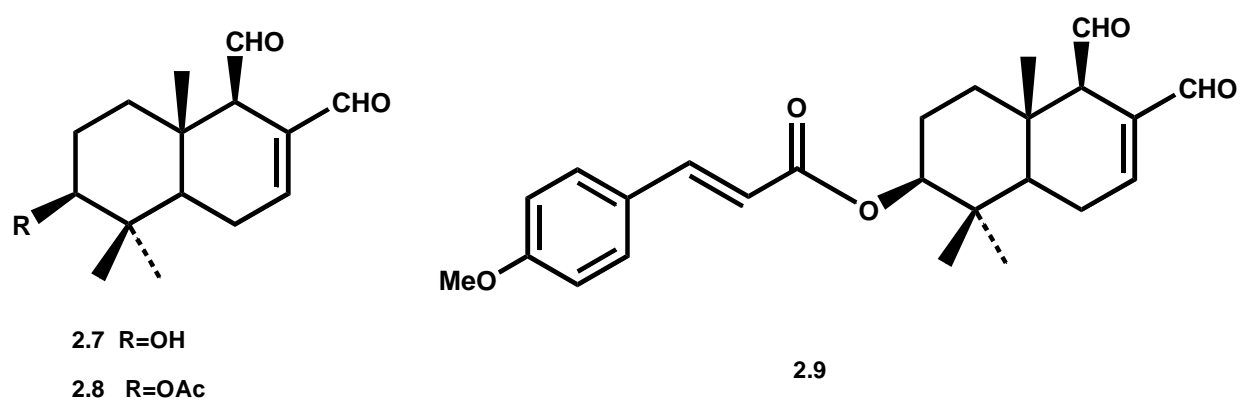

2.7 R=OH

2.8 R=OAc

2.9

FIGURE 2.3

---

[52] D'Acunto, M; Della Monica,M; Izzo,I; De Petrocellis, L; Di Marzo,V; Spinella, A. *Tetrahedron* **2010**, 66,9785.

**2.2.ENANTIOSELECTIVE TOTAL SYNTHESIS OF POLYGODIAL C1 DERIVATIVES**

In this section enantioselective total synthesis of polygodial C-1 derivatives is described, and Diels–Alder reaction plays the key role in the construction of the bicyclic moiety.

2.2.1 SYNTHESIS OF POLYGODIAL C-1 ANALOGUES: RETROSYNTHETIC ANALYSIS

The preparation of 1-(R)- hydroxypolygodial **2.1** has been achieved following a previously described strategy based on the enantioselective construction of the drimane skeleton *via* Diels–Alder reaction using a chiral hydroxydiene.

In fact, even though different synthetic strategies to natural products with this skeleton have been suggested, the use of an intermolecular Diels–Alder reaction of an appropriate 1,3-diene with dimethyl acetylene dicarboxylate (DMAD) to construct the drimane skeleton was particularly attractive.

The hydroxydiene **2.10** was prepared through a stereocontrolled Corey–Bakshi–Shibata[53] reduction of ketone **2.15**. (**Scheme 2.1**)
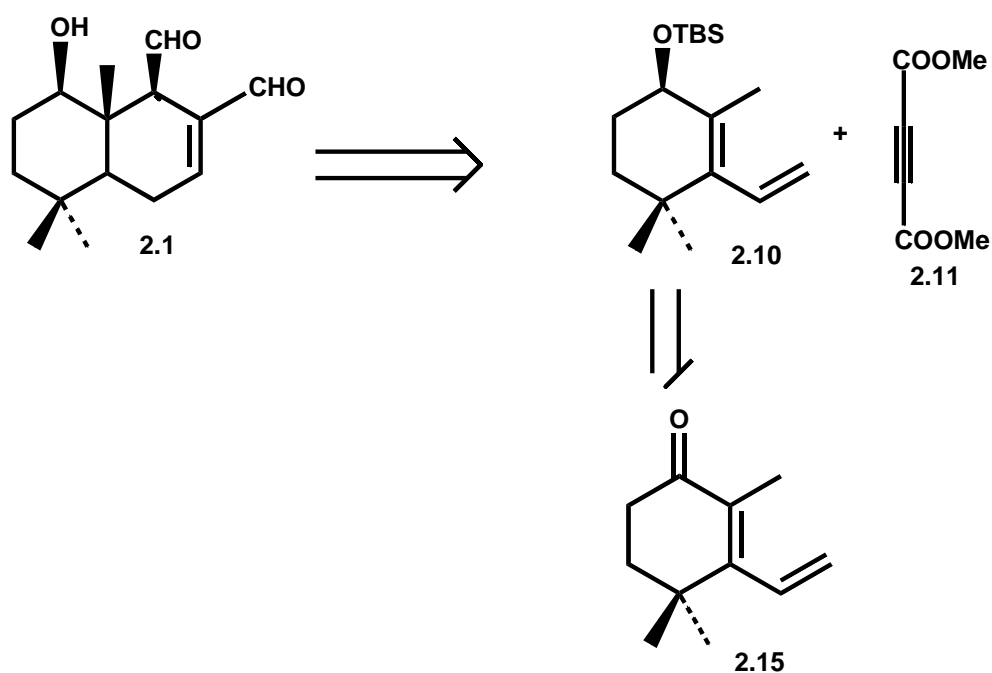
SCHEME 2.1

[53] Corey, E. J.; Bakshi, R. K.; Shibata, S. *J. Am. Chem. Soc.* **1987**, 109, 5551.

2.2.2 SYNTHESIS OF 1-(R)-HYDROXYPOLYGODIAL

The first step of the synthesis (**Scheme 2.2**) is the preparation of epoxide **2.13** from α-ionone **2.12**. Ozonolysis of **2.13** followed by reductive work-up, afforded an epoxyaldehyde which proved to be quite sensible to purification conditions; therefore, the following eliminative ring opening of the epoxide was performed on the crude extract obtained after ozonolysis leading to compound (76% yield from **2.12**). The synthesis of ketone **2.15** was completed by Wittig reaction of aldehyde with CH_2PPh_3, followed by PDC oxidation (79%, two-step yield).
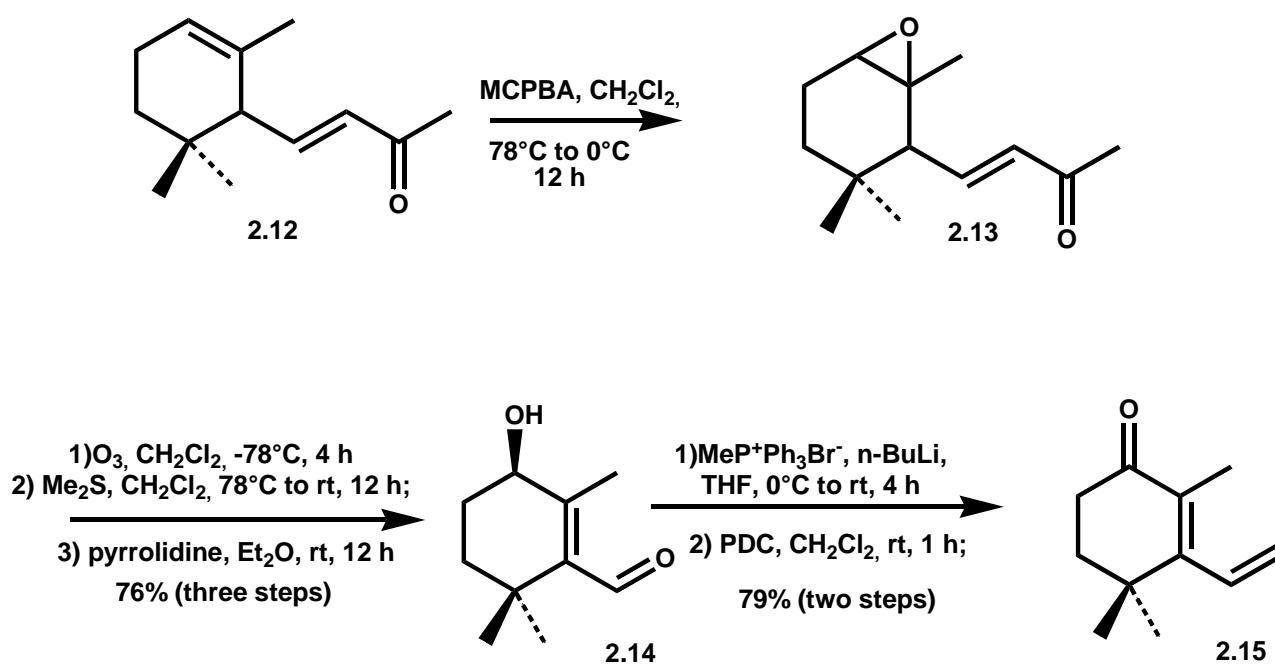
SCHEME 2.2

(R)-2,4,4-Trimethyl-3-vinyl-2-cyclohexene-1-o was prepared by enantioselective reduction of dienone **2.15** with (S)-Me-Corey–Bakshi–Shibata [(S)-MeCBS] oxazaborolidine–borane reagent, giving dienol **2.16** (88% yield and 93% enantiomeric excess) which was then converted into silyl derivative **2.10**. (**Scheme 2.3**)
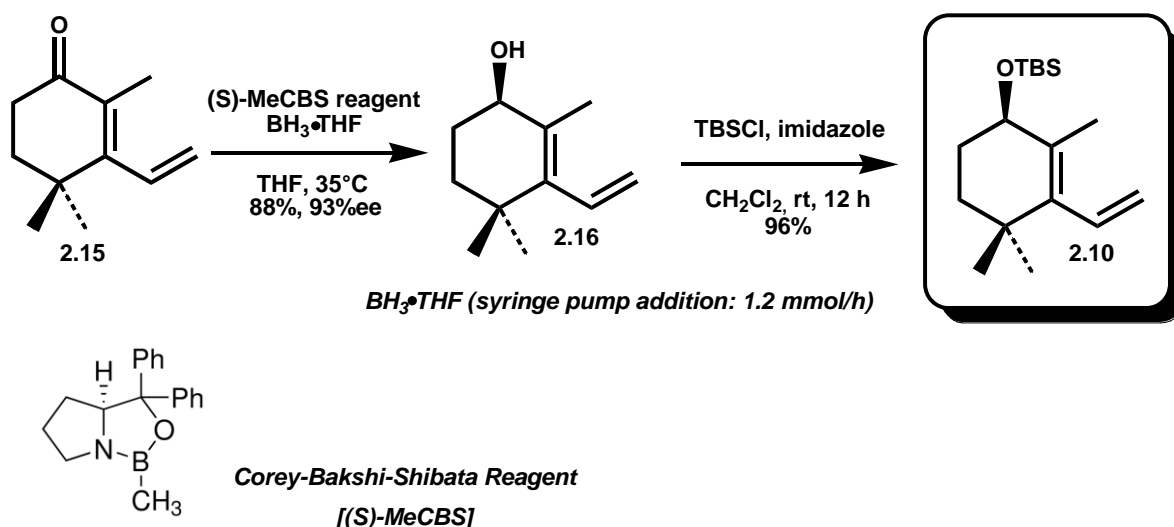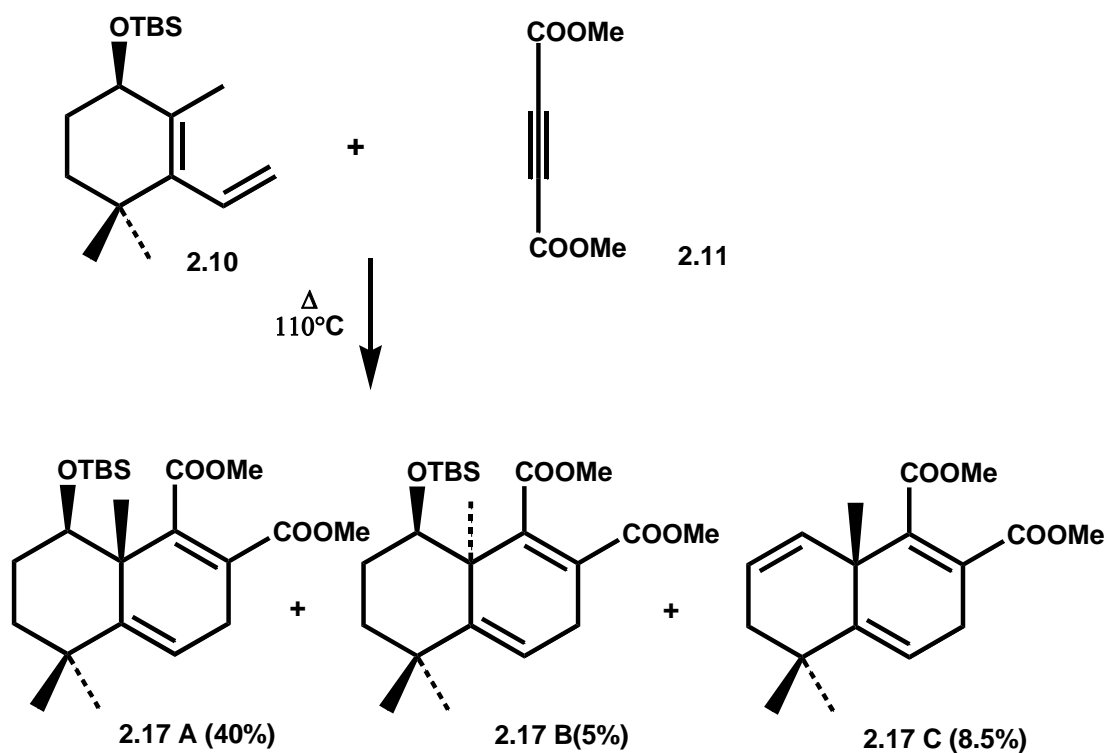
SCHEME 2.3

Once obtained the diene **2.10** it has been carried out the Diels–Alder reaction of with dimethylacetylenedicarboxylate (DMAD). (**Scheme 2.4**)

This reaction proceeded quite slowly (neat, 110°C, 48 h) affording compound **2.17 A** as a major product (40%) together with small amounts of its diastereomer **2.17 B** (5%) and triene **2.17 C** (8.5%).The reaction was stopped after 48 h, although unreacted diene **2.10** was still present, because longer reaction time favoured the increase of triene **2.17 C**. (**Scheme 2.4**)

The stereoselectivity observed can be rationalized by an approach of the dienophile to the diene *anti* to the allylic substituent.

SCHEME 2.4

DBU catalyzed isomerisation of **2.17 A** afforded mainly the conjugated diene **2.18** (90% yield), which was subjected to hydrogenation (H_2, Pd–C and MeOH) affording compound **2.20** (85% yield). (**Scheme 2.5**)
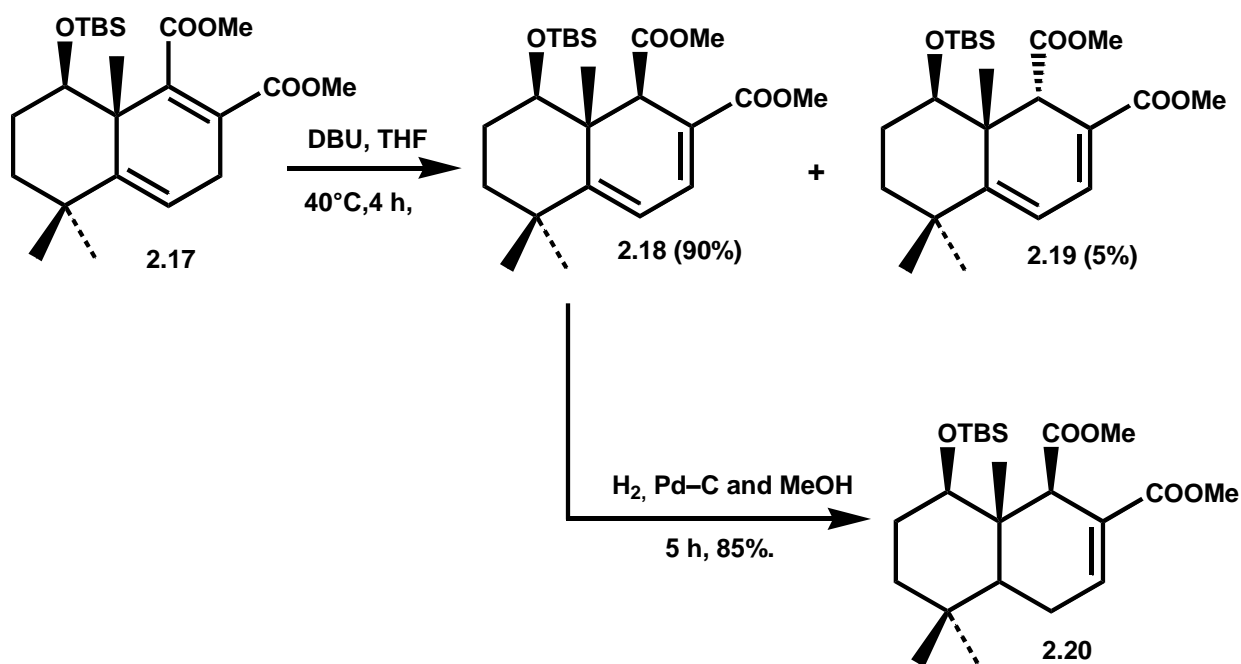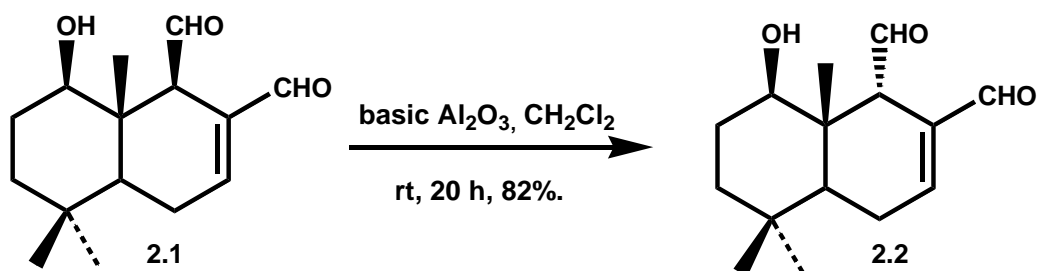

SCHEME 2.5

Reduction of the ester functionalities produced diol **2.21**, which was then oxidized using Swern conditions -(COCl)$_2$, DMSO and NEt$_3$ - to give dialdehyde **2.22** (87%). Finally, 1(R)-hydroxypolygodial **2.1** was produced by deprotection of the TBS ether using HF aq 48% (**Scheme 2.6**).
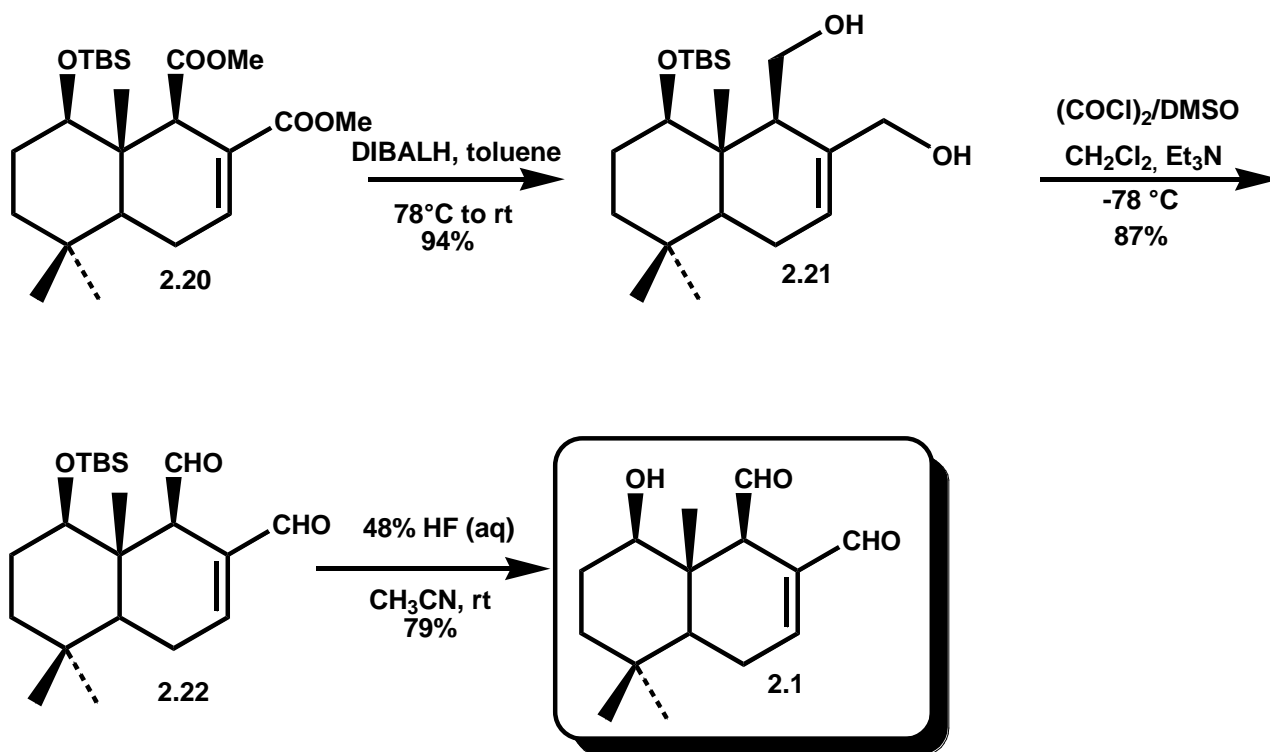
SCHEME 2.6

Easy epimerization of hydroxypolygodial **2.1** was obtained after exposition to basic conditions (basic Al$_2$O$_3$, CH$_2$Cl$_2$, 20 h), which yielded 82% of the epimer 1-(R)-hydroxyisotadeonal **2.2** (**Scheme 2.7**).

SCHEME 2.7

2.2.3 <u>SYNTHESIS OF 1-β-(METHOXYCINNAMOYL)POLYGODIAL AND PREPARATION OF OTHER C-1 ACYL DERIVATIVES.</u>

Once *obtained* 1-(R)-hyrdroxypolygodial **2.1**, other C-1 functionalised dialdehydes with drimanic skeleton were prepared.

1-β-(p-methoxycinnamoyl)polygodial **2.3**, an antinociceptive metabolite from *Drimis winterii* barks, was the first target.

The synthesis of **1.11** was first investigated starting from 1(R)-hydroxypolygodial (**2.1**). However, in this case it is not possible the installation of the cinnamoyl moiety at C1-position directly starting from 1(R)-hydroxypolygodial. In fact, a preliminary study on direct acylation showed that 1-hydroxypolygodial **2.1**, under typical acylation conditions, gave rise to a complex mixture of reaction products (**Scheme 2.8**). The main obtained products were the enolacyl derivatives **2.25** and **2.26**.
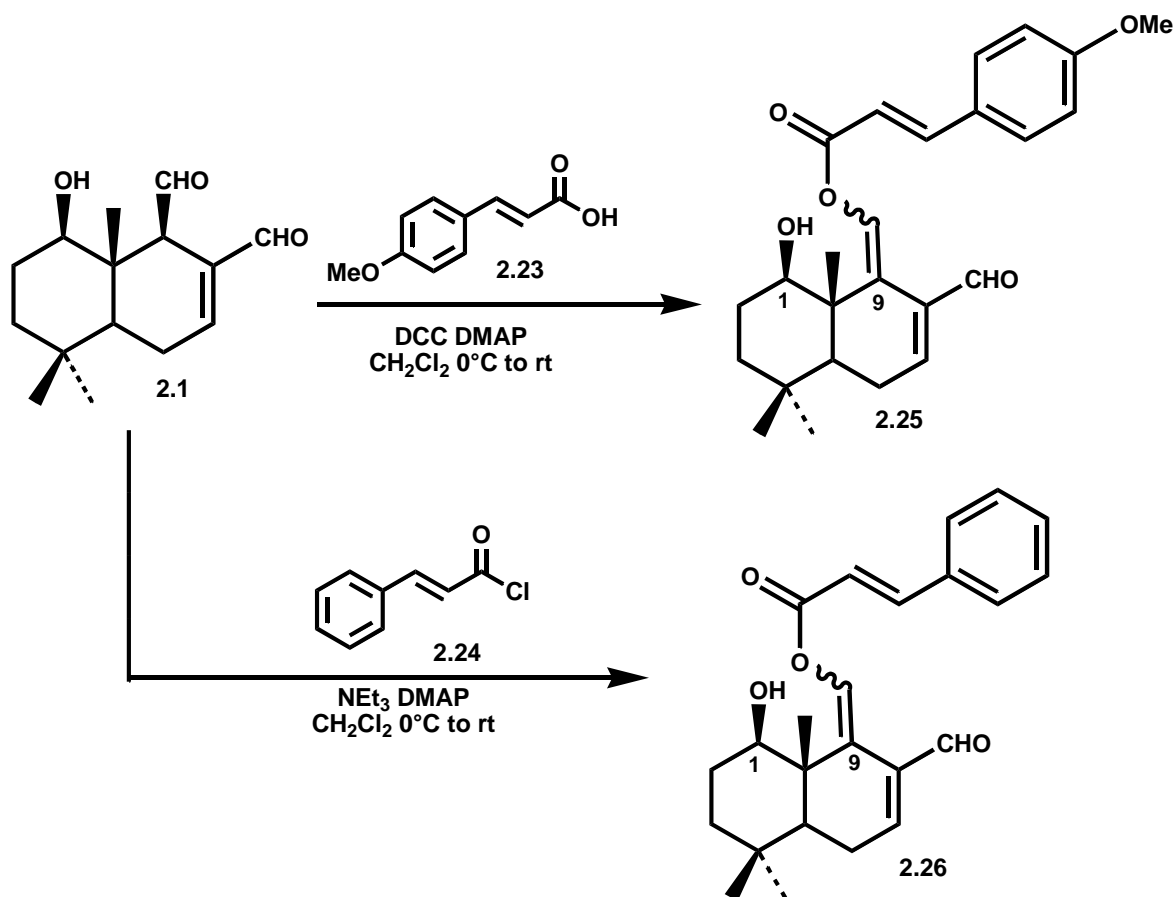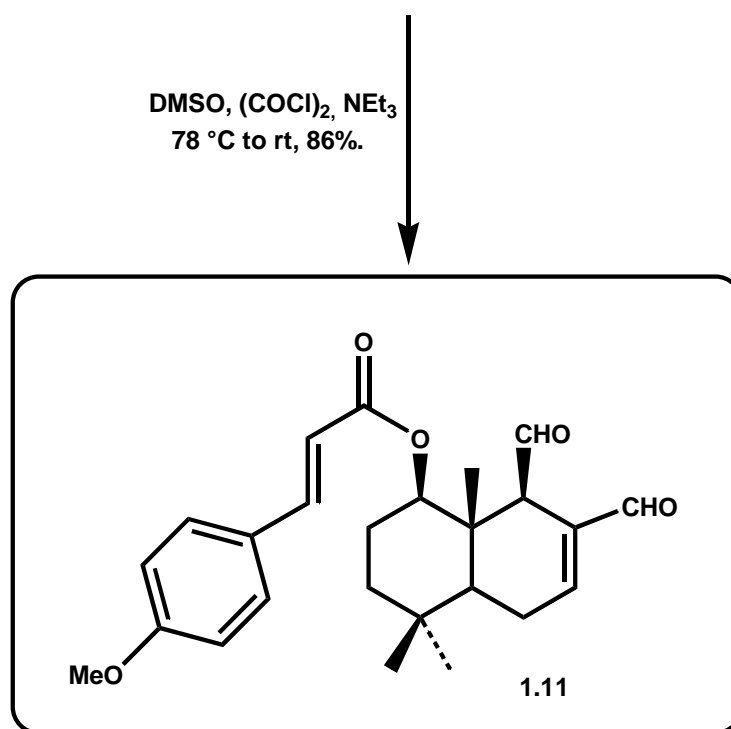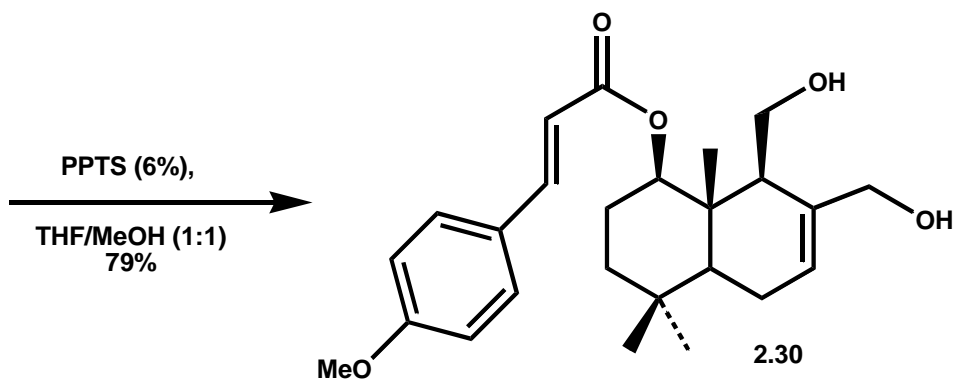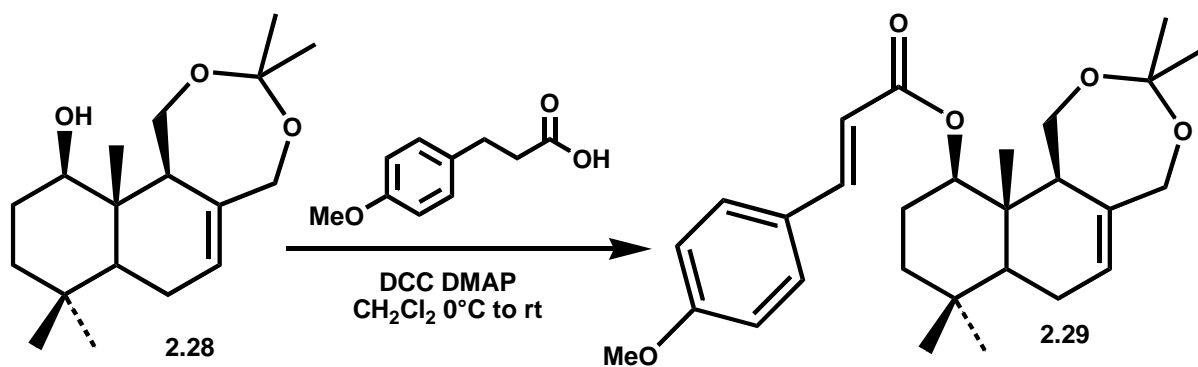
SCHEME 2.8

Very low yields of the desired acylated product at C-1 were achieved. In both cases, enolization at C-9 and subsequent acylation of the resulting enol was faster than acylation at C-1, even controlling reaction times and conditions. Therefore, the synthetic scheme, previously developed to obtain 1(R)-hydroxypolygodial **2.1**, was modified starting from diol **2.22**, as shown in the following scheme. Diol **2.22** was protected as acetonide, then protective group on secondary alcohol was removed giving compound **2.28**. (**Scheme 2.9**)
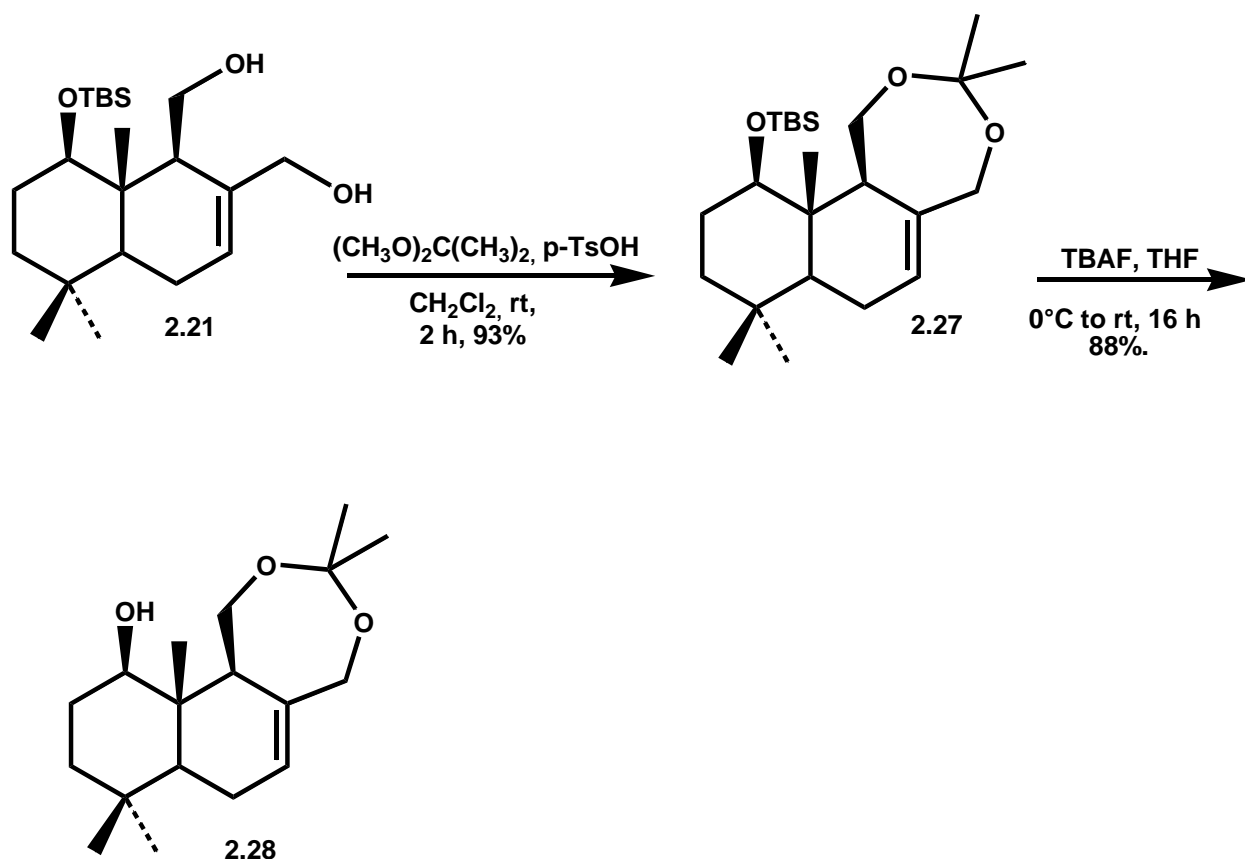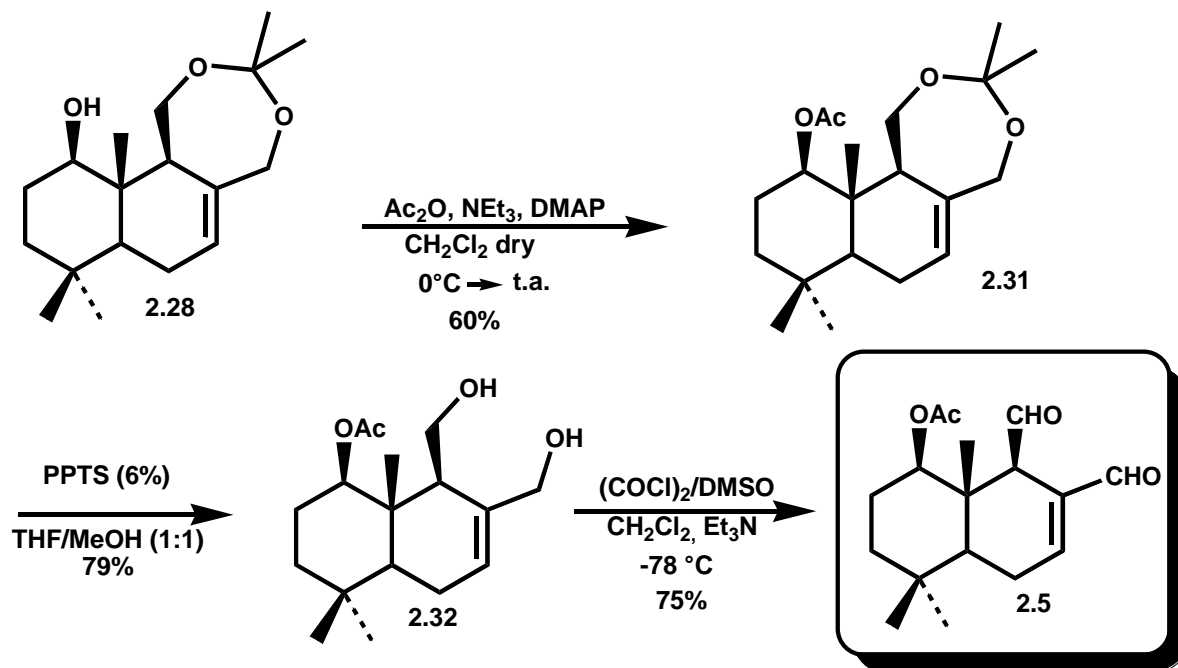
SCHEME 2.9

The transformation of **2.28** into the desired ester **2.29** was quantitatively carried out by treatment of **2.28** with *p*-methoxycinnamic acid **2.24** in the presence of DCC and DMAP. The acetonide **2.29** was finally deprotected using pyridinium *p*-toluenesulfonate (PPTS) in THF/MeOH to afford **2.30** along with unreacted starting **2.29**. The recovered **2.29** was expose again to the deprotection conditions affording **2.30** in 79% overall yield. Oxidation of primary alcohols in **2.30** to the corresponding dialdehyde using Swern conditions completed the synthesis of 1-β-(p-methoxycinnamoyl) polygodial (**2.3**). (**Scheme 2.10**)
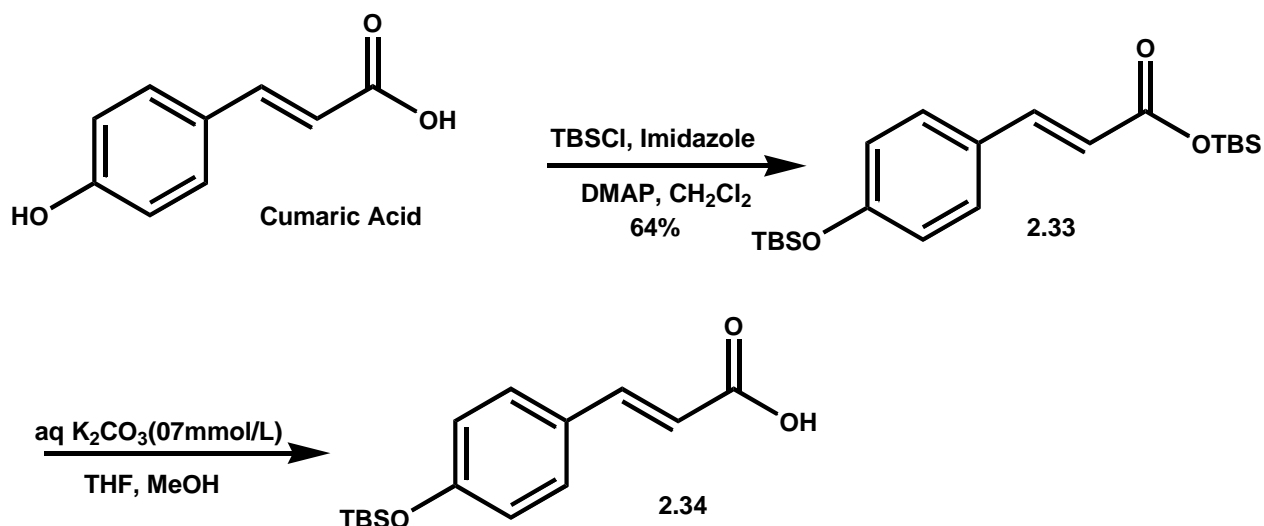
SCHEME 2.10

- *Synthesis of acetyl derivative **2.5*** -The protected diol **2.28** has been transformed into corresponding acetyl derivative under Scheuer condition and after deprotection of acetonide and oxidation of the resulting diol **2.32**, C-1acetyl derivative **2.5** has been obtained in good yield (**Scheme 2.11**).
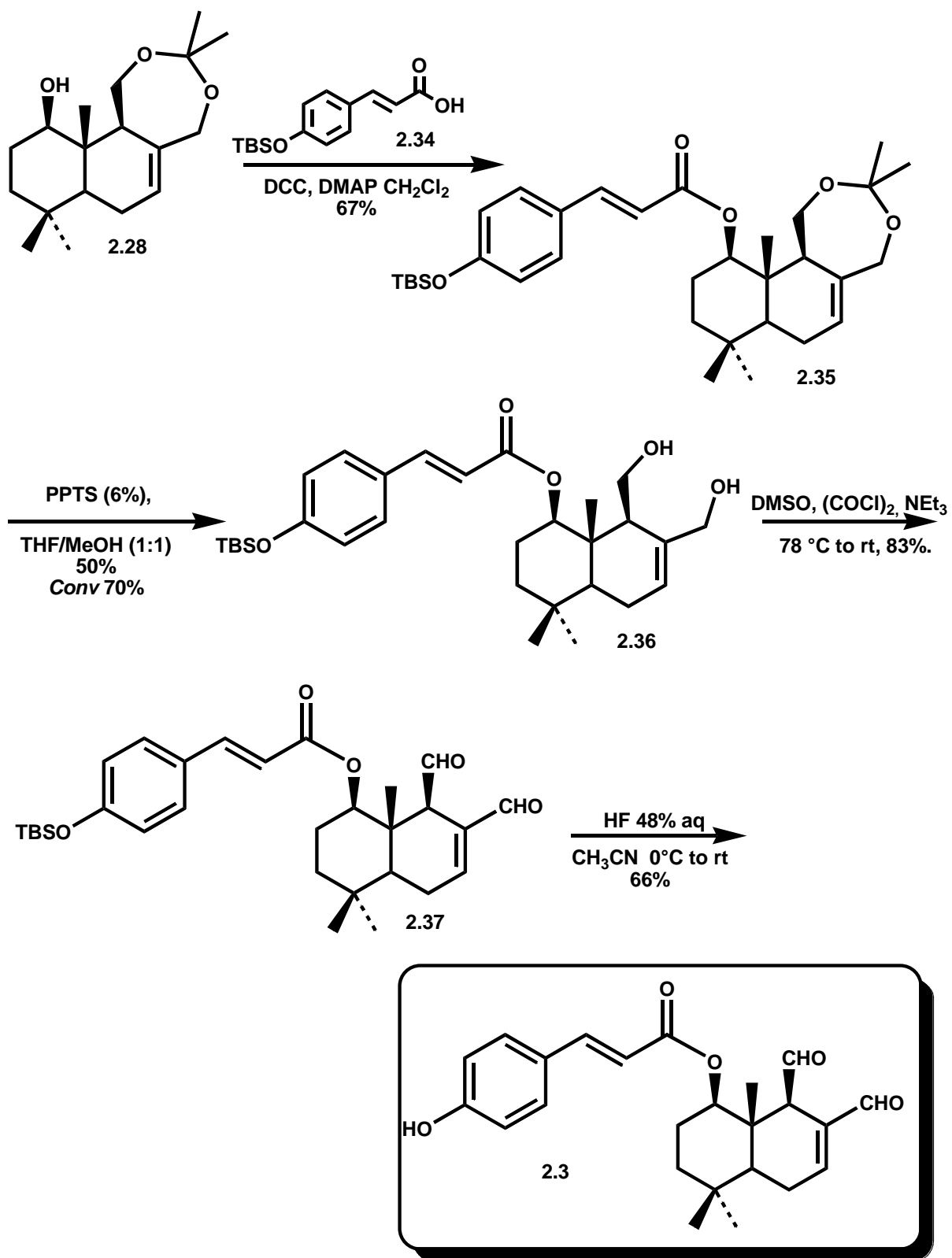

SCHEME 2.11

- *Synthesis of 1-β-p cumaroil polygodial **2.3*** Starting from acetonide **2.28** other polygodial C-1 derivatives have been prepared.

1-β-p cumaroyl polygodial **2.3** has been prepared staring from cumaric acid and protecting both hydroxyl and carboxyl moiety as TBSCl, then a selective deprotection yielded **2.34** (**Scheme 2.12**).
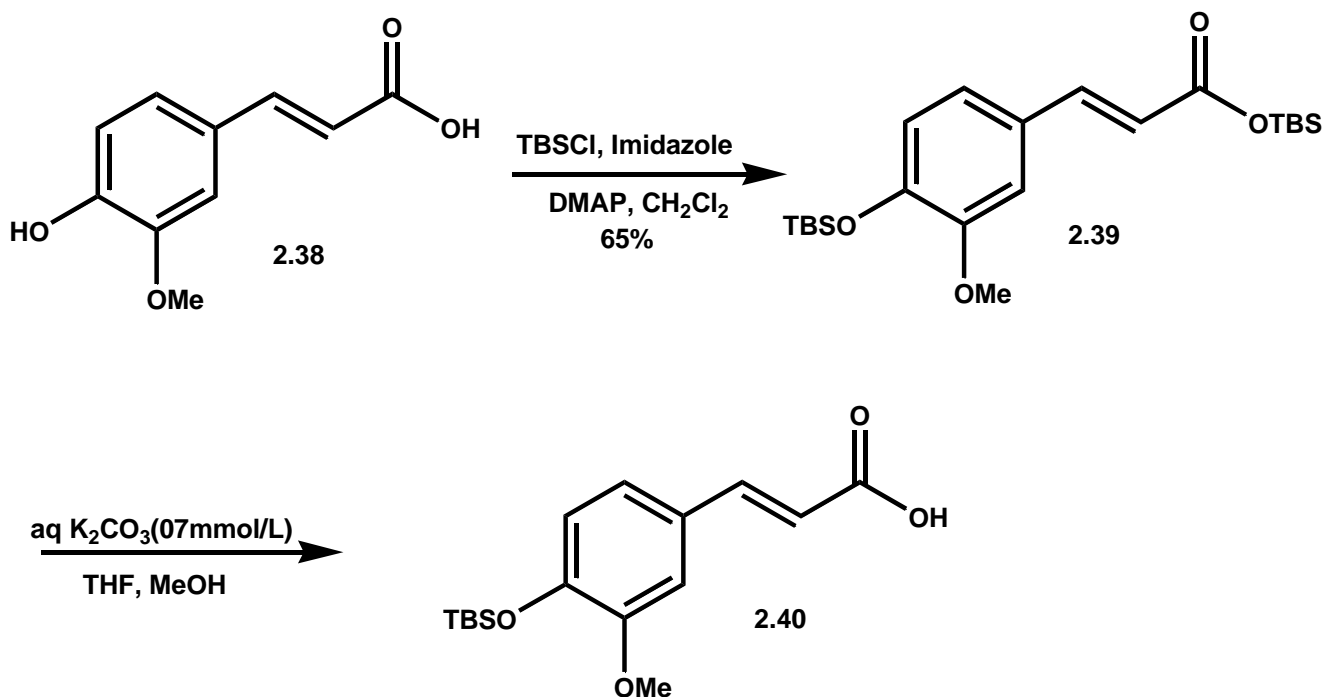

SCHEME 2.12

Once prepared the silyl derivative, acylation performed on acetonide **2.28** provided adduct **2.35** in good yield, the diol was deprotected ,oxidated to the corresponding dialdehydes by Swern oxidation and TBS group was removed by HF48% aq, affording the wanted product **2.3** (**Scheme 2.13**).

Reagents and conditions for Scheme 2.13:

2.28 + 2.34 → 2.35: DCC, DMAP CH₂Cl₂, 67%

2.35 → 2.36: PPTS (6%), THF/MeOH (1:1), 50%, Conv 70%

2.36 → 2.37: DMSO, (COCl)₂, NEt₃, 78 °C to rt, 83%.

2.37 → 2.3: HF 48% aq, CH₃CN 0°C to rt, 66%

SCHEME 2.13

• *Synthesis of hybrid structure 2.4* Finally we planned the preparation of a hybrid compound containing both a vanyllic and a dialdehyde moiety, in order to study their eventual synergy in bioactivity. The preparation of this hybrid was done following a similar procedure described above. Fenol function in **2.38** was selectively protected by reaction with TBDSCl followed by selective deprotection of the carboxylic acid (**Scheme 2.14**).

SCHEME 2.14

Then, compound **2.40** was coupled with **2.28** using DCC. Deprotection gave diol **2.42** which was oxidized to dialdehyde **2.43.** Hybrid vanyldialdehyde **2.4** was obtained by deprotection of fenolic function. (**Scheme 2.15**)
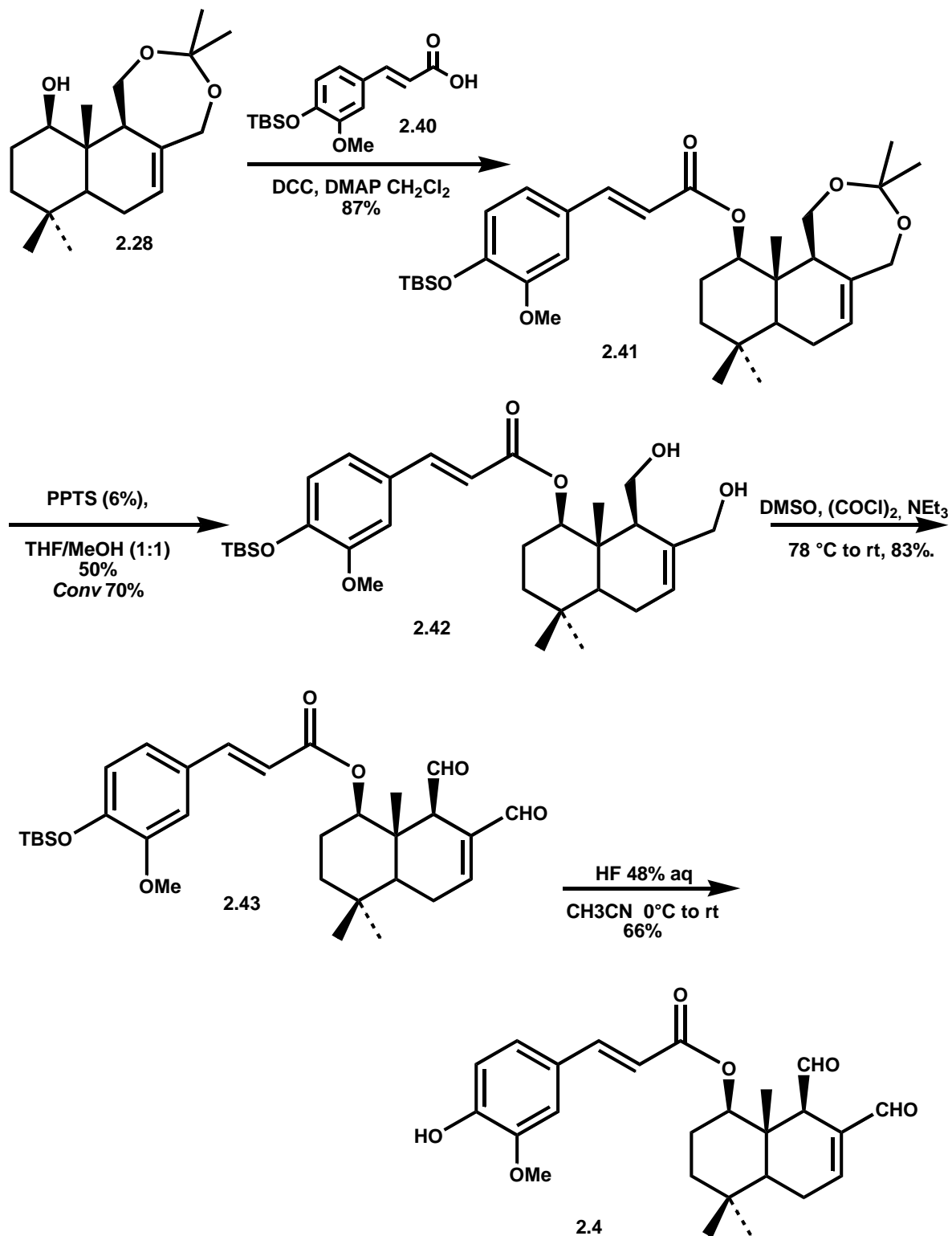
SCHEME 2.15

2.3 TOTAL SYNTHESIS OF POLYGODIAL C1 DERIVATIVES - CONCUSIONS

In this first part C-1 polygodial derivatives have been prepared. One of these, 1-β-(p-methoxycinnamoyl) polygodial (**2.3**) is a natural product from *Drymis winterii* bark.

All of these compounds (**Figure 2,1** and **2.2**) will be assayed on TRPV1 Receptor, on both the new TRPA1 receptor and on some tumor line cells.
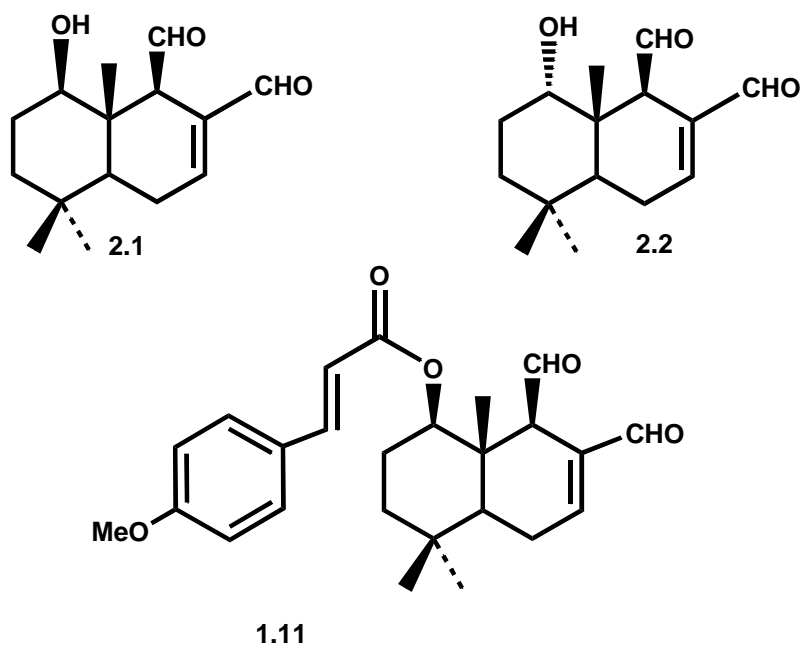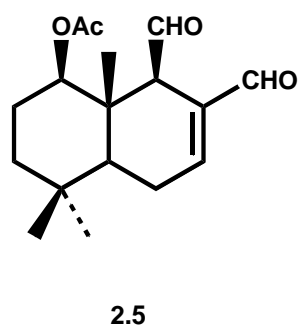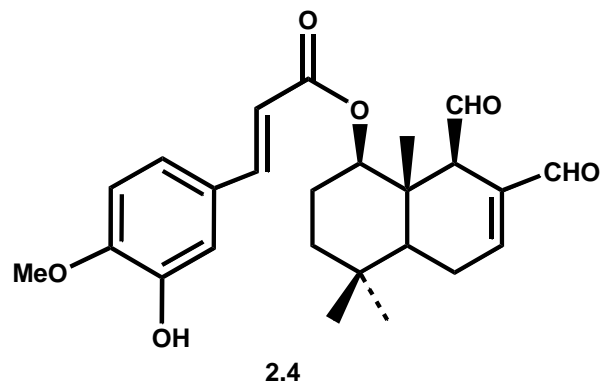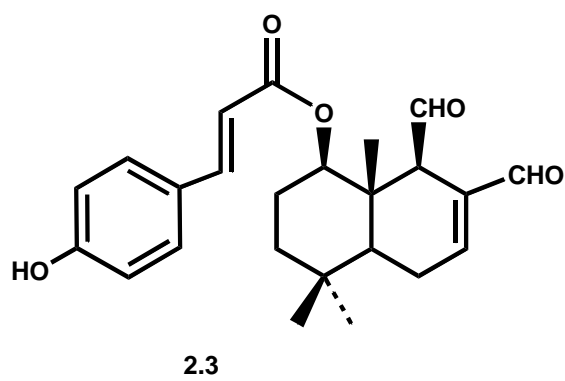

FIGURE 2.1

2.3

2.4

2.5

FIGURE 2.2

### 2.4 *ENANTIOSELECTIVE TOTAL SYNTHESIS OF C-3 POLYGODIAL DERIVATIVES*

This section is dedicated to enantioselective synthesis C-3 functionalised polygodial derivative in order to evaluate both their antiproliferative and receptorial activity (see **Figure 2.3**).

2.4.1 *A NEW STRAIGHTFORWARD SYNTHETIC STRATEGY: CYCLISATION BY FREE RADICAL CHEMISTRY*

In order to prepare C-3 functionalised drimanic backbone we needed to develop a different strategy.

In fact, we found in literature that the Diels Alder reaction performed on hydroxydiene **2.10** and DMAD **2.11** was achieved with no diastereoselectivity, in particular it has been obtained a mixture of (1:1) ratio of the two possible byciclic compounds. This means that even though the hydroxyl group is protected as bulky silylether,[54] it is not able to control the stereochemistry of the diene-dienofile approach. (**Scheme 2.16**)

---

[54] Mori, K.; Watanabe, H. *Tetrahedron* **1986**, *42*, 273.

2.44

2.46   **1 : 1**

+

2.47

+

COOMe

COOMe

2.11

110 °C

2.45

2.48   **1 : 1**

+

2.49
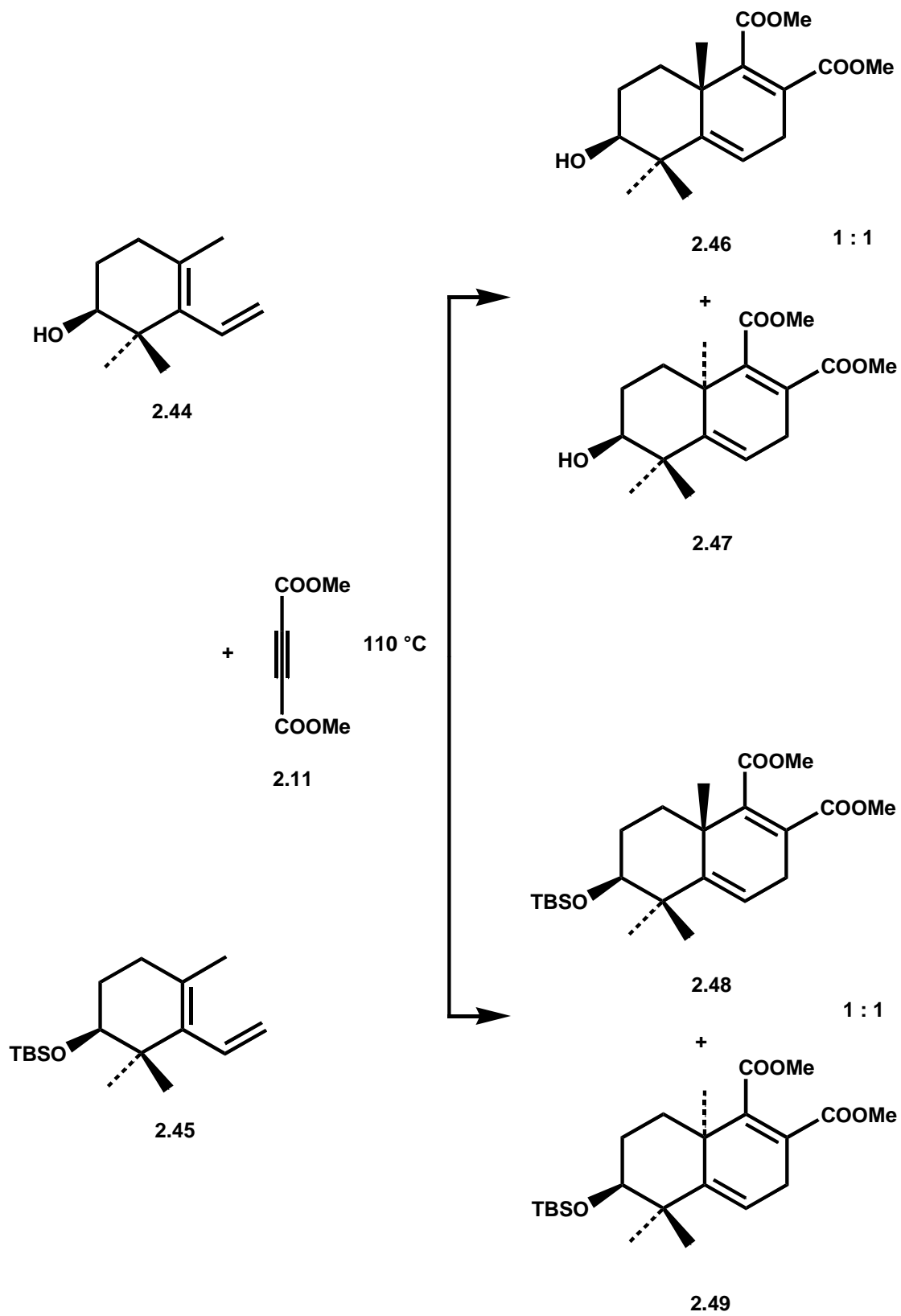
SCHEME 2.16

As previously described, the Diels Alder reaction was achieved to synthesize C-1 functionalised drimane. In fact in this case we obtained an high diastereoselectivity due to the presence of a bulky sylil ether very close to the reaction centre and this is important in the stereocontrol of reaction. (**Scheme 2.17**)
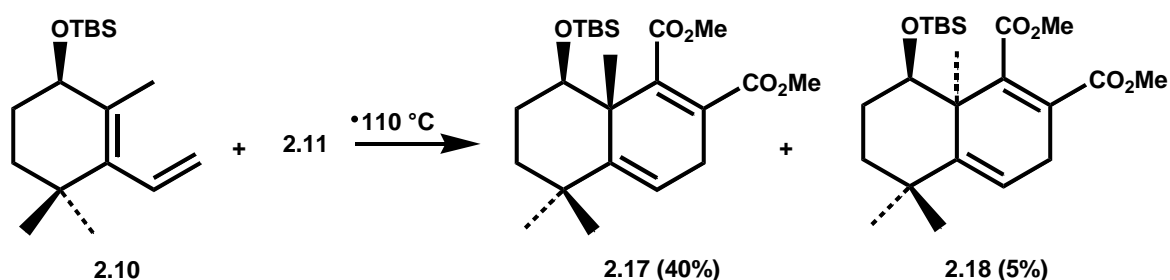
SCHEME 2.17

To prepare C-3 derivative we need a new strategy to achieve the enantioselective construction of byciclic backbone.

Our attention was attracted to some biomimetic methods described in literature to gain cyclic natural product, based on both cationic and radical chemistry.

The aim is to mimic some natural transformation such as biosynthesis of lanosterol from squalene, that taking place in only two steps: the enantioselective epoxidation of squalene followed by the stereoselective cascade cyclization of 2,3-oxidosqualene. In particular, the enzyme-catalyzed cyclization of (S)-2,3-oxidosqualene into lanosterol has received considerable attention.[55]

[55] a) Abe,I.; Rohmer,M.; Prestwich, G. D. *Chem. Rev.* **1993**, 93, 2189.b) Wendt, K. U.; Schulz, G. E.; Corey, E. J.; Liu, D. R. *Angew. Chem. Int. Ed*. **2000**, 39,2812.

Because of this, Goldsmith, Van Tamelen, and Corey, among others, have exploited the acid-induced cascade cyclization of epoxypolyprenes as a very useful procedure in the building of polycyclic terpenoids through carbocationic chemistry. This method involves certain drawbacks, however, such as the need to attach extra groups to the polyene substrate to stabilize carbocationic intermediates and control the termination steps. [56]

An alternative concept, radical cascade cyclization, introduced by Breslow[57] and Julia[58] more than thirty years ago, has also proven to be an excellent method for the stereoselective synthesis of polycyclic compounds from different acyclic precursors.

This concept was never applied to the cyclization of epoxypolyprenes during the last century, probably owing to the lack of a suitable protocol for the radical opening of epoxides. Nevertheless, the titanocene(iii)-based procedure discovered by Nugent and RajanBabu[59] and the catalytic version subsequently developed by Gansauer[60] and co-workers has filled this gap, thus opening up the possibility of mimicking lanosterol synthase with free-radical chemistry.

Recently Barrero et al, taking inspiration from these previous works about free radical chemistry, have developed a very straightforward procedure for the synthesis of terpenoids with a wide range of carbocyclic skeletons.

The titanocene-catalyzed cascade cyclisation of epoxipolyenes, which are easily prepared from commercially available polyprenoids, has proven to be a useful procedure for the synthesis of C_{10}, C_{15}, C_{20}, and C_{25} terpenoids, including monocyclic, bicyclic, and tricyclic natural products.

---

[56] a) Goldsmith, D. J., *J. Am. Chem. Soc*. **1962**, 3913 b) Van Tamelen, E. E. *Acc. Chem. Res.* **1975**, 8, 152 c). Huang, A. X.; Xiong, Z.; Corey, E. J. *J. Am. Chem. Soc*. **1999**, 121, 9999 d) Zhang, J.; Corey, E. J., *Org. Lett*. **2001**, 3, 3215.

[57] a) Breslow, R.; Barrett, E., Mohacsi, E., *Tetrahedron Lett*. **1962**, 3, 1207 b) Breslow, R.; Olin, S. S.; Groves J. T. *Tetrahedron Lett*. **1968**, 1837.

[58] Lallemand, J. Y.; Julia, M.; Mansuy, D. *Tetrahedron Lett*. **1973**, 14, 4461.

[59] a) Nugent, W. A.; RajanBabu, T. V. *J. Am. Chem. Soc*. **1988**, 110, 8561 b) RajanBabu, T. V.; W. Nugent, A. *J. Am. Chem. Soc*. **1994**, 116, 986.

[60] a) Gansauer, A.; Pierobon, M.; Bluhm, H. *Angew. Chem. Int. Ed.* **1998,** 37, 101 c) Gansauer, A.; Pierobon, M., Bluhm, H. *Synthesis* **2001**, 2500 e) Gansauer, A.; Bluhm H.; Rinker, B.; Narayan, S.; Schick, M. Lauterbach ,T., Pierobon, M. *Chem. Eur. J.* **2003**, 9, 531.

In the scheme below (Scheme **2.18**) is reported the radical cyclisation of racemic 10-11-epoxyfarnesylacetate **2.50**. The titanocene complex used is Cp_2TiCl_2, a Ti (IV) complex that gave, in the medium by reaction with Mn dust, the catalytic Ti (III) species being able to start the radical cascade with the epoxide opening.[61]
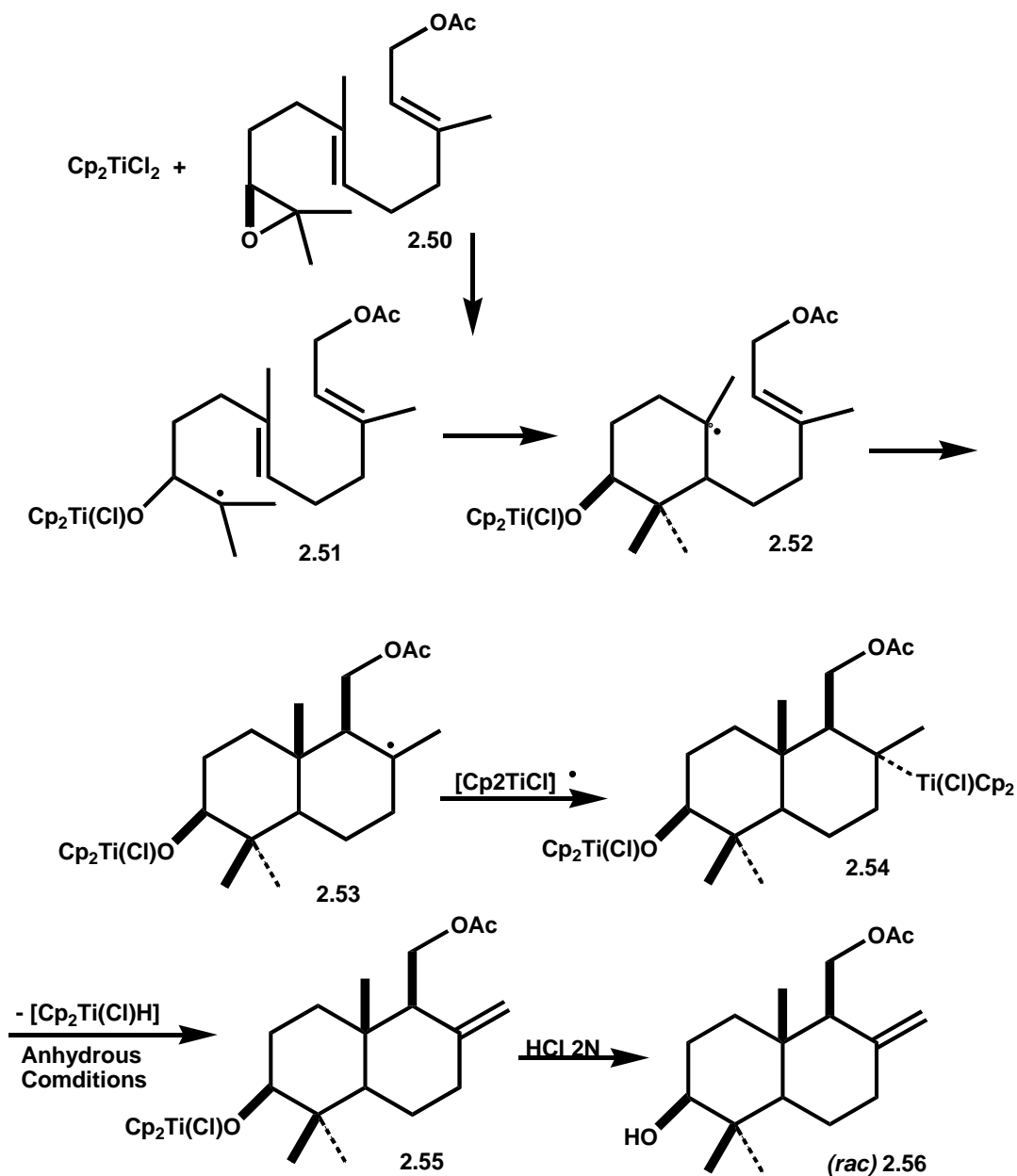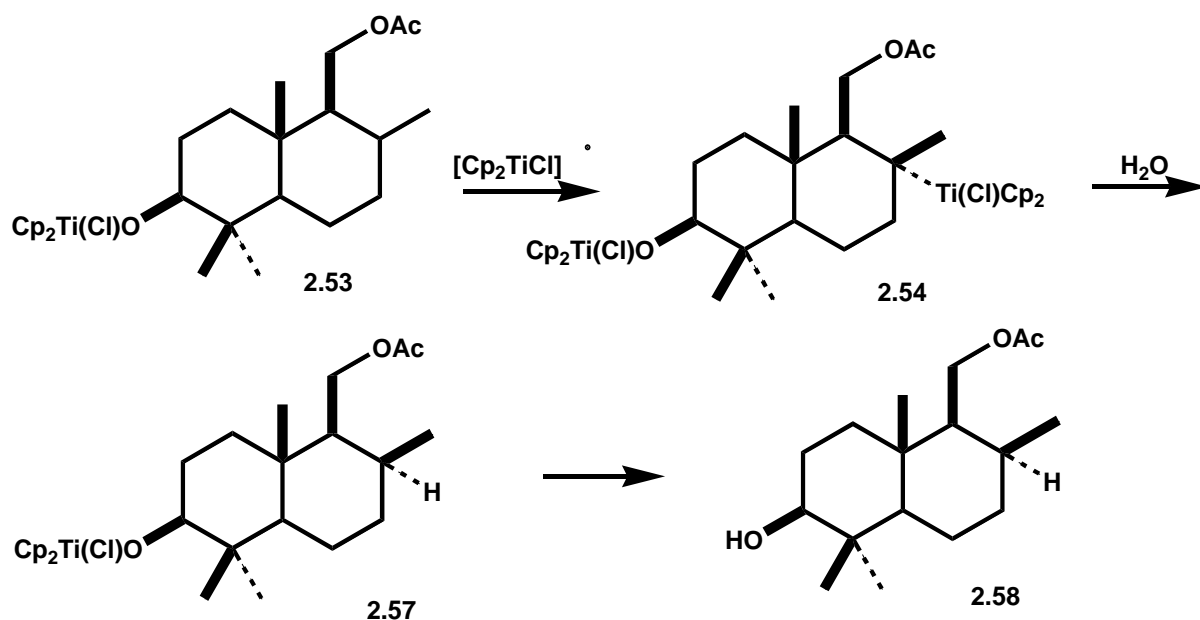

SCHEME 2.18

[61] Justicia, J, Rosales, A; Bueuel, E; Oller-LÛpez, J; Valdivia, M; Hadour, A; Oltra, E; Barrero, A.F; Curdenas,D; . Cuerva.M. *Chem. Eur. J.* **2004,** 10, 1778.

Both theoretical and experimental evidence suggests that this cyclisation takes place in a nonconcerted fashion via discrete carbon-centered radicals. Nevertheless, the termination step of the process seems to be subjected to a kind of water-dependent control, which is unusual in free-radical chemistry.

The catalytic cycle is based on the use of the novel combination $Me_3SiCl/2,4,6$-collidine to regenerate the titanocene catalyst. In practice this procedure has several advantages: it takes place at room temperature under mild conditions compatible with different functional groups, uses cheap reagents, and its end step can easily be controlled to give exocyclic double bonds by simply excluding water from the medium.

The reaction is totally stereospecific giving *trans* junction in the decaline backbone with equatorial orientation of acetylcarbinolic group, is highly regioselective giving the endocyclic alkene as main product with an acceptable yield.

Because of presence of highly reactive intermediate in the medium, other collateral reactions can occur, one of which depends on presence of water in a non perfect anhydrous medium, as shown below. (**Scheme 2.19**)
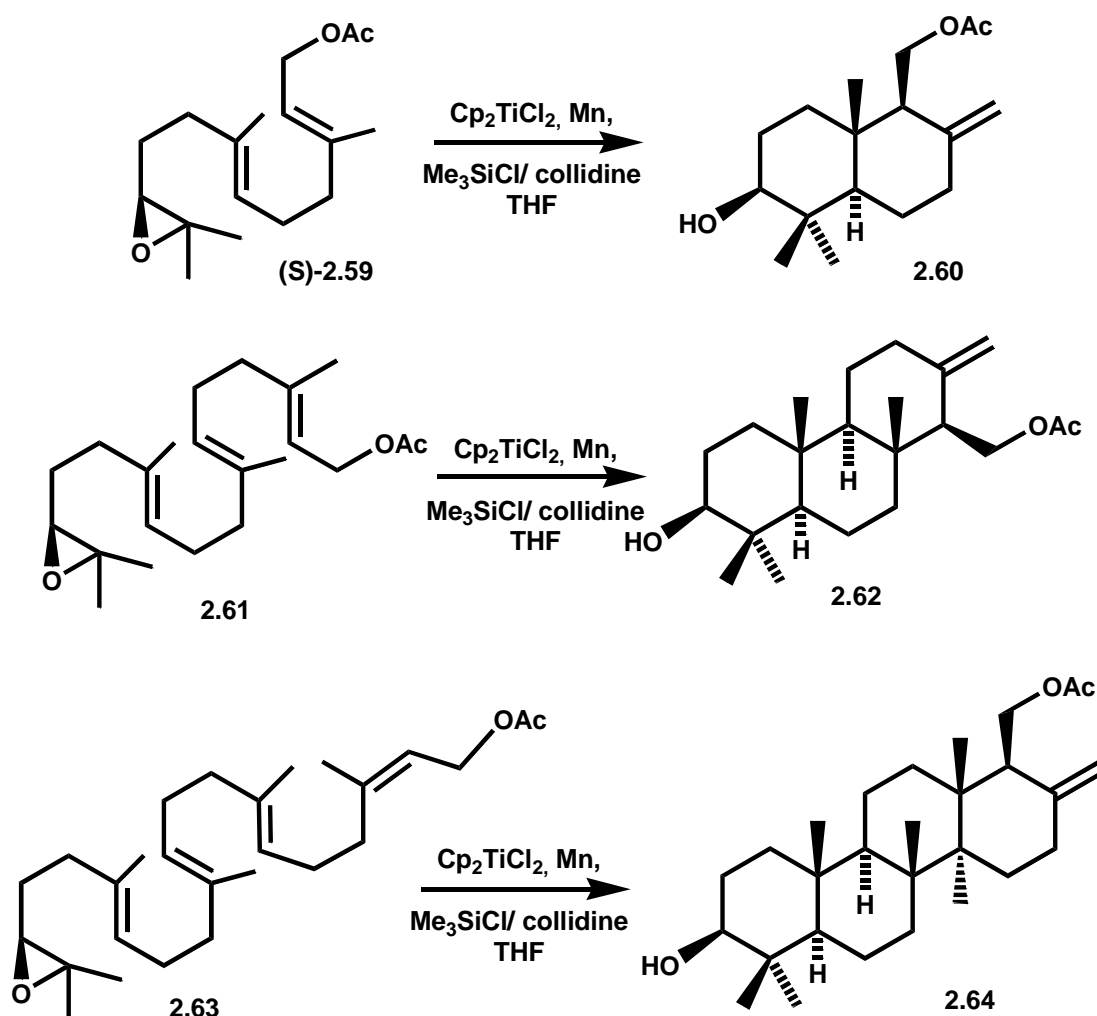

SCHEME 2.19

It has been proposed this following catalytic cycle for the Ti (III) mediated cyclisation.[11] (**Scheme 2.20**)
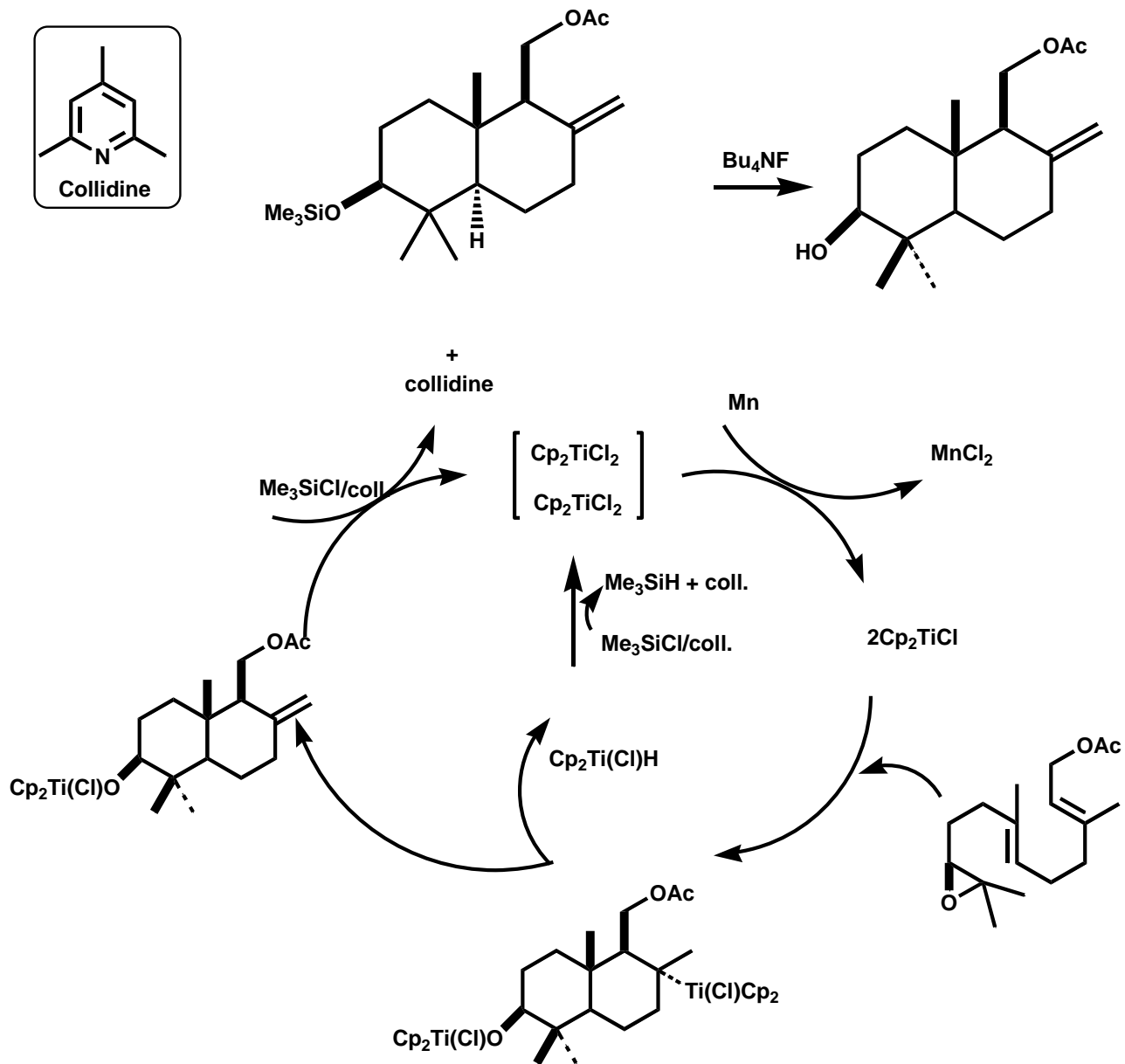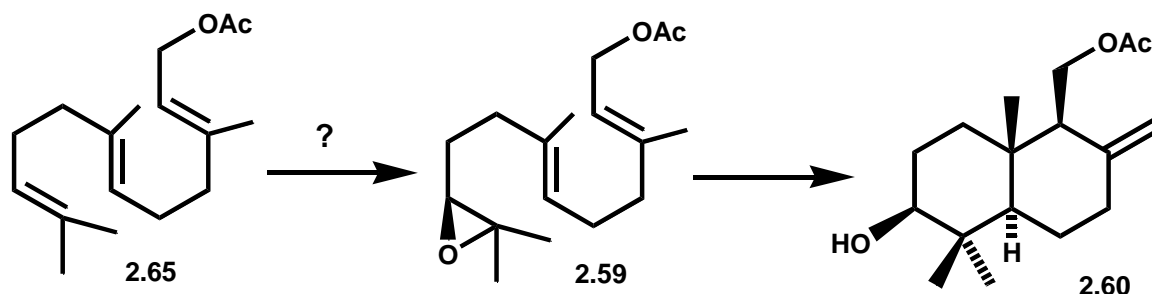

SCHEME 2.20

This approach was initially proposed for the synthesis of racemic compounds. Therefore, once chosen this strategy as the proper one for our purposes, we planned an enantioselective variant simply starting from a chiral epoxypolyene obtained at the beginning of the synthesis *via* asymmetric catalysis.

Hopefully, this method allows us to obtain several polycyclic backbones simply using a proper length epoxypolyene as starting material. (**Scheme 2.21**)

SCHEME 2.21- *Our proposed enantoselective synthesis of polycyclic backbones*

The decalinic backbone **2.60** is given by radical cyclisation of chiral epoxyde **2.59,** obtained by chiral induction starting from the commercially available *all trans* farnesylacetate **2.65**. (**Scheme 2.22**)

SCHEME 2.22

As first attempt, we thought of prepare the chiral epoxide using an enantioselective protocol that could provide a direct epoxidation of the terminal double bond

Jacobsen epoxydation could be a good chance to prepare a chiral epoxide from a terminal alkene. Furthermore, Jacobsen epoxidation could give a low *ee.* as in total synthesis of α-ambrinol.[62] (**Scheme 2.23**)
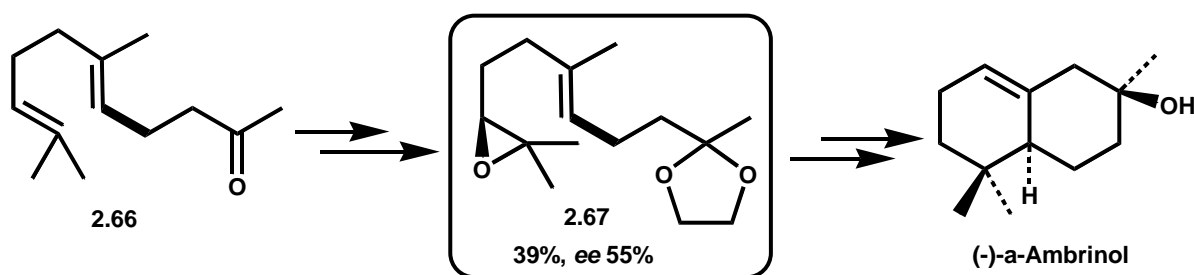
SCHEME 2.23

---

[62] Justicia, J.; Campana, A. G.; Bazdi, B.; Robles, R.; Cuerva, J.M.; Oltra, J.E. *Adv. Synth. Catal*. **2008,** *350,* 571.

On the other hand, it could be possible to prepare a chiral epoxide **2. 59** via S_N2 ring closing from diol **2.68**, obtained from **2.68** *via* Sharpless Asymmetric Dihydroxylation. This reaction provide *ee* higher than direct Jacobsen epoxydation.[63] (**Scheme 2.24**)
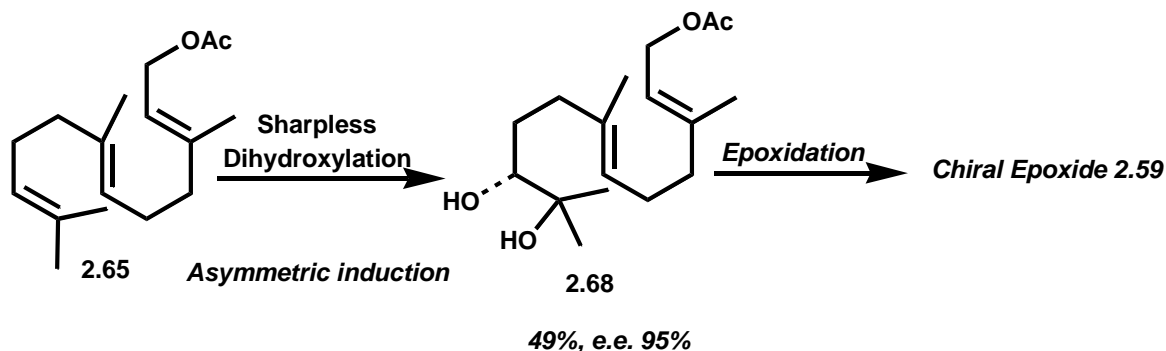
SCHEME 2.24

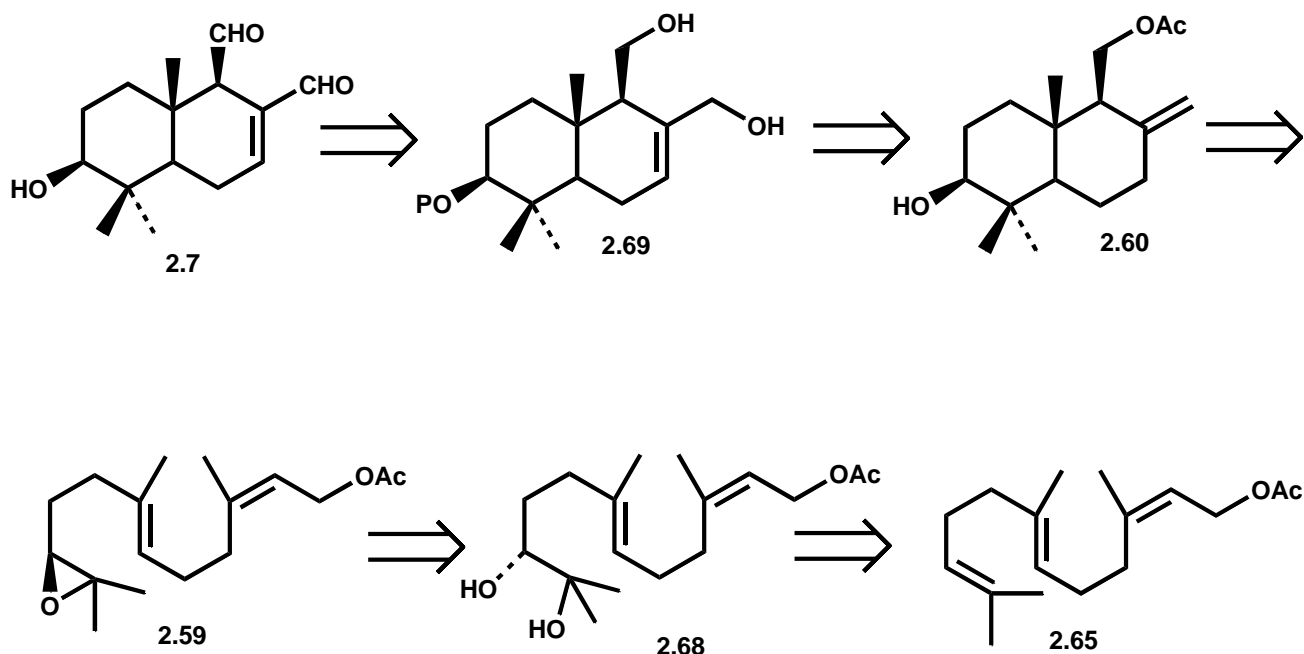## 2.5 TOTAL SYNTHESIS OF 3-HYDROXYPOLYGODIAL AND ITS ANALOGUES

2.5.1 RETROSYNTETIC SCHEME

This retrosyntetic analysis shows that $3-\beta$-hydroxypolygodial **2.7** was obtained from diol **2.69** via Swern oxidation.

Diol **2.69** was obtained *via* epoxide with a proper elaboration of the key intermediate **2.60**, in which has been protected the C-3 OH.

The key intermediate **2.60** was prepared starting from epoxyfarnesilacetate **2.59**. This was obtained from the commercially available *trans trans* farnesylacetate **2.65** via Sharpless dihydroxylation of $\Delta^{10,11}$ double bond. (**Scheme 2.25**)

---

[63] Vidari, G.; Dappiaggi, A.; Zanoni, G.; Garlaschelli, G. *Tetrahedron Lett.* **1993**, *34*, 648

SCHEME 2.25

2.5.2 _SYNTHESIS OF 3-β-HYDROXYPOLYGODIAL_

The construction of the 3-hydroxy drimane skeleton was based on the titanium-catalyzed radical cyclization of epoxypolyprenes recently developed by Barrero et al. In order to obtain an enantioselective process, we applied this titanocene catalyzed cyclisation to an epoxide with high optical purity.

To this purpose we prepared (10S)-10,11-epoxy-farnesyl acetate **2.59** starting from (2E,6E)-farnesyl acetate **2.65**. The synthesis begins with a chiral induction on the starting material.

Highly enantioselective dihydroxylation of (2E,6E)- farnesyl acetate employing AD-mix-β afforded diol **2.68**.

The enantiomeric excess was determined as 93% by 1H NMR analysis of the corresponding mono-(S)-MTPA ester of diol.[64]

The diol **2.68** was converted in the corresponding epoxide **2.70** achieving a mesylation of the primary alcohol followed by by reaction with K_2CO_3 in CH_3OH.
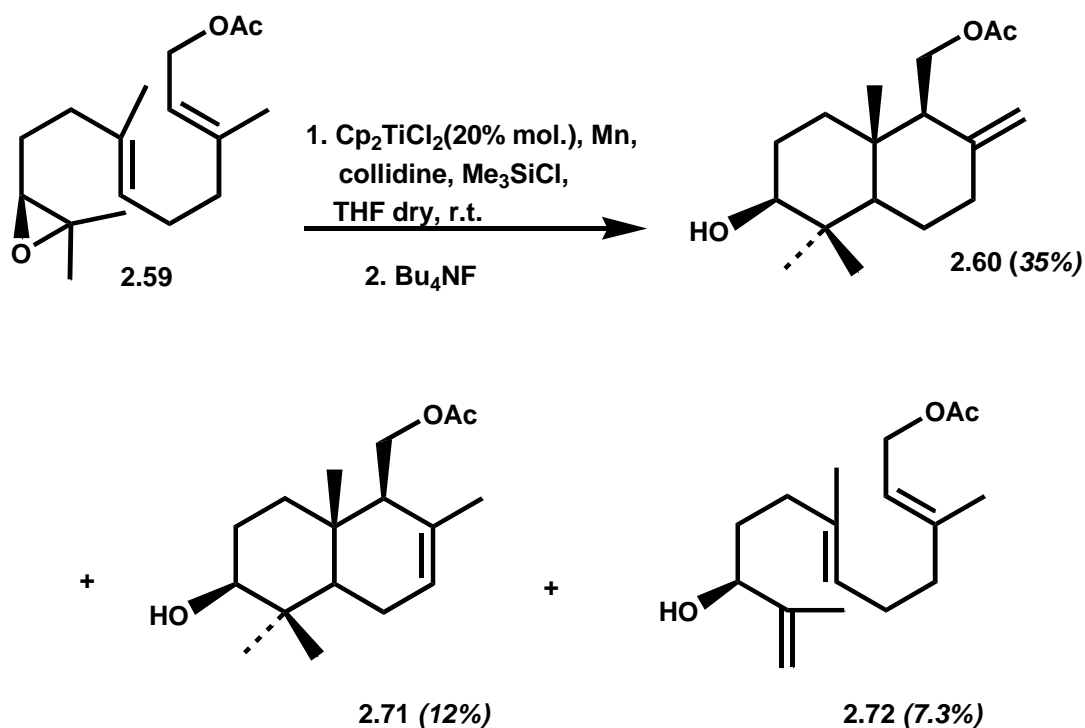
---

[64] Huang, A. X.; Xiong, Z.; Corey, E. J. _J. Am. Chem. Soc._ **1999**, 121, 9999-10003.

The (10S)-10,11-oxidofarnesol obtained (**2.70**) was subject to acetylation affording **2.59**. (**Scheme 2.26**)
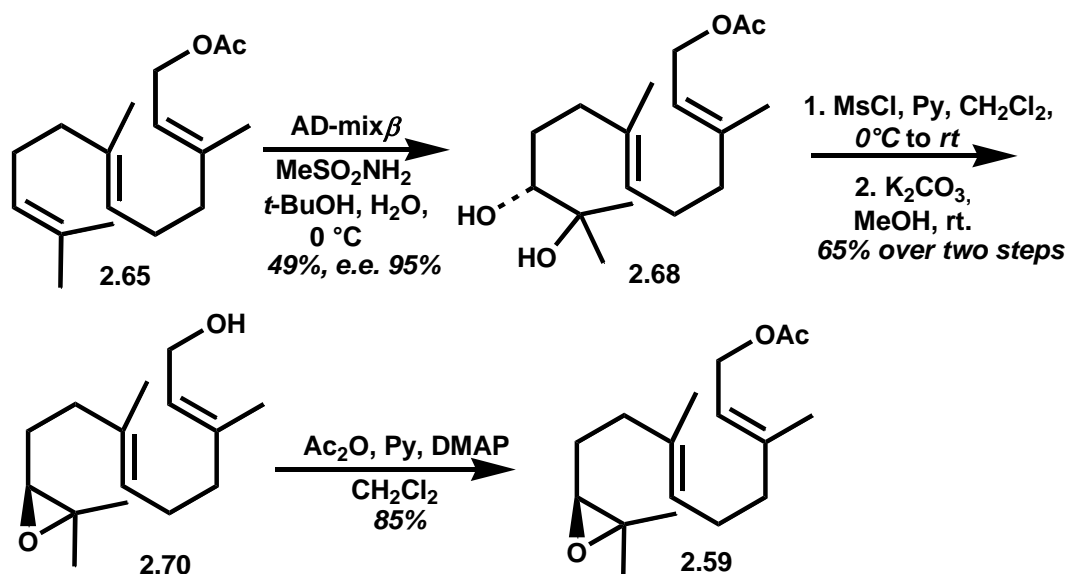
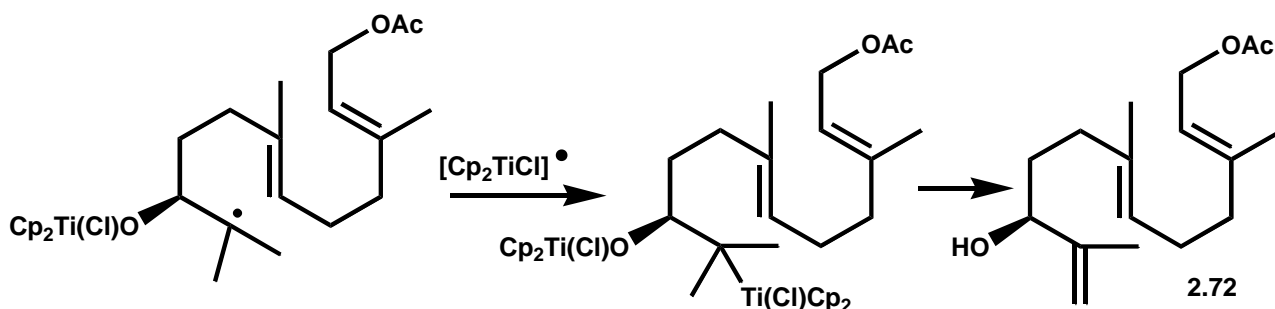**SCHEME 2.26**

(10S)-10,11-epoxy-farnesyl acetate **2.59** is the proper substrate that undergo to Titanocene-catalyzed cyclization that afforded a mixture of products in which the major product was **2.60** (34%). (**Scheme 2.27**)

SCHEME 2.27

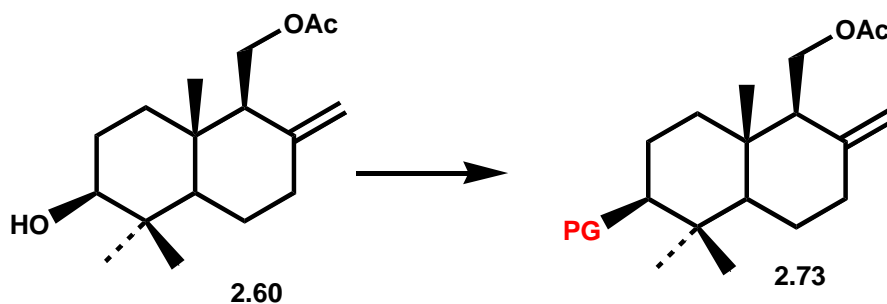From a very complex mixture we have identified three products, the main one with exocyclic double bond **2.60**, then its regioisomer **2.71** and a further product **2.72** derived by elimination of [Cp$_2$Ti(Cl)H] with an acid *quenching*. (**Scheme 2.28**)

SCHEME 2.28

At this point we need to protect the C-3 OH in **2.60** as reported in **Scheme 2.29** and **Table 2.1**).
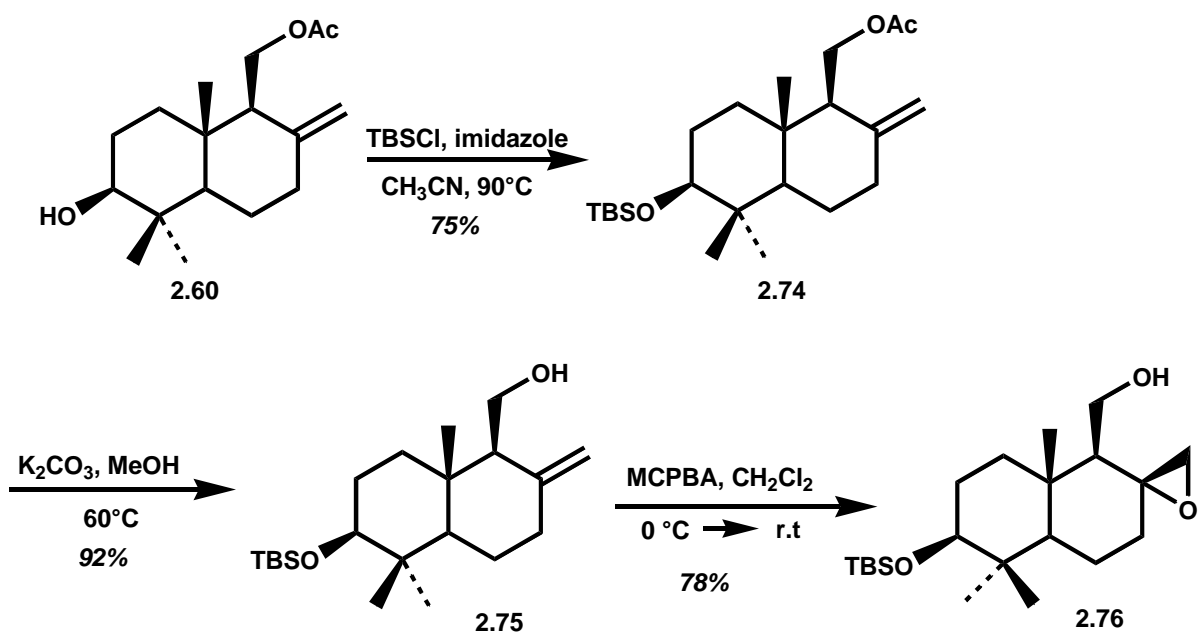

SCHEME 2.29

Table 2.1

PG *	Base	Additive	Solvent	T (°C)	2.73 (%)
BnBr	TBAI	/	THF	0 to rt	/
BnBr	/	Ag$_2$O	CH$_3$CN	90	/
TBDPSiCl	DBU		CH$_2$Cl$_2$	r.t	/
TBDPSiCl		AgNO$_3$/DMAP	THF	rt	/
TBDPSiCl	Imidazole	/	CH$_3$CN	90	30
TBDMSiCl	Imidazole	/	CH$_3$CN	90	75

*PG= *Protecting group*

The best way to perform this protection was using tert-butyldimethylsilylchloride (TBDSiCl) as protecting group. The C3-OH in **2.60** was protected as silylether with tert-butyldimethylsilylchloride (TBSCl) provided protected 3-hydroxy drimane derivative **2.74** in 75% yield.

The acetyl group in **2.74** was removed by treatment with K$_2$CO$_3$ in MeOH affording **2.75**. The introduction of allylic alcohol was obtained through acid catalyzed opening of the epoxide **2.76** easily prepared from **2.75**.

In fact, treatment of **2.75** with m-CPBA afforded epoxide **2.76** as a single isomer. (**Scheme 2.30**)

SCHEME 2.30

The opening of **2.76** into **2.77** the corresponding allyl alcohol was subject to several trials. Camphorsulfonic acid (CSA) treatment in CH₃CN/DMSO (10:1) as solvent at room temperature successfully yielded the desired diol **2.77** in 58% yield together with diol **2.78** (20%) and with a small amount of deprotected diol **2.79**. Reactions performed at lower temperatures (10-15 °C) resulted in lower yield of the desired diol **2.77**. (**Scheme 2.31**)
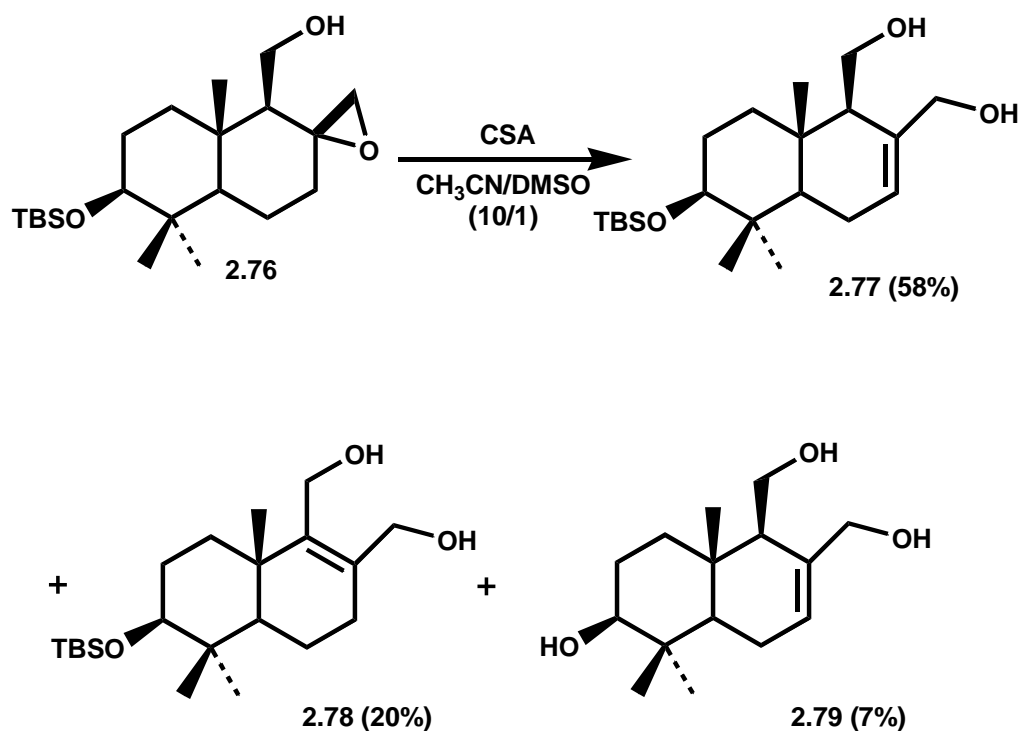
SCHEME 2.31

The final stages of the synthesis are shown in the following **Scheme 2.32** . Oxidation of both primary alcohol functionalities of the diol **2.77**, conducted under Swern conditions, gave **2.80** in excellent yield (76%). At the end, removal of the TBS protective group (HF, CH₃CH aq) in **2.80** afforded 3-β-hydroxypolygodial **2.7**.
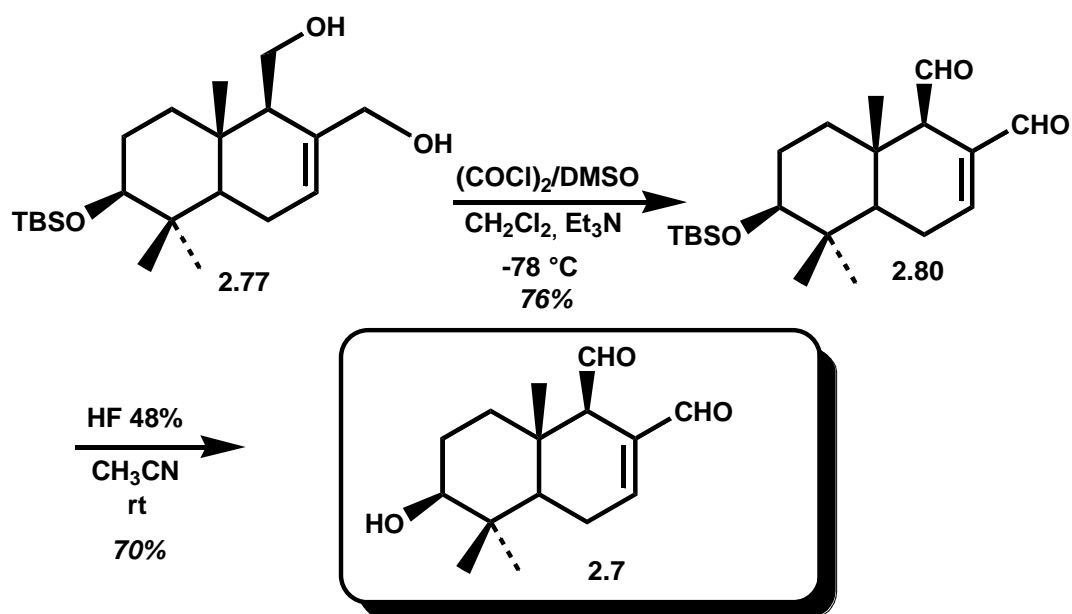
SCHEME 2.32

2.5.3 DIRECT ACYLATION OF C3-OH:SYNTHESIS OF 3- β ACETOXYPOLYGODIAL AND 3-β (*p*-METHOXYCINNAMOIL)POLYGODIAL

From 3- β-hydroxypolygodial **2.7** were prepared the natural product from Canella winteriana 3- β-acetoxypolygodial **2.8** and 3-*β-p*-methoxycinnamoil polygodial **2.9**.

In this case direct acylations were successful, without formation of secondary products.

- 3- β-acethoxypolygodial, a natural product from *Canella winteriana,* was prepared by reaction of **2.7** with acetic anhydride, under Scheuer conditions.[65] No epimerisation of C-9 centre has been observed. (**Scheme 2.33**)

---

[65] Terem, B.; Scheuer, P. J. *Tetrahedron* **1986**, *42*, 4409.
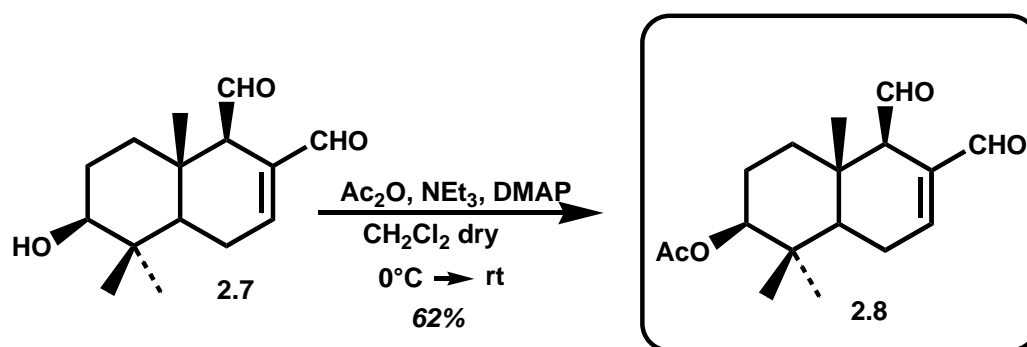
SCHEME 2.33

- 3-β-p-methoxycinnamoil polygodial was prepared with a protocol involving a coupling between p-methoxy carboxylic acid **2.23** and **2.7**, using DCC as coupling agent in combination with a catalytic amount of DMAP.

The reaction gave the wanted product with a good yield and with no epimerization at C-9 stereocentre. (**Scheme 2.34**)
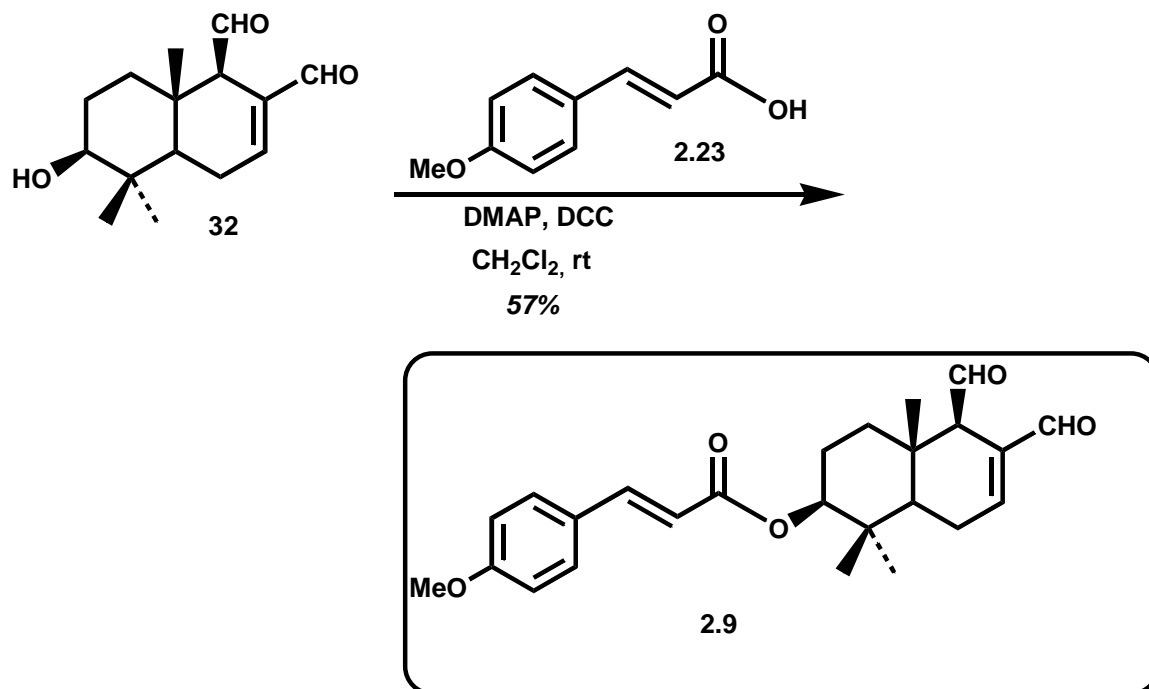
SCHEME 2.34

2.6 TOTAL SYNTHESIS OF POLYGODIAL C3 DERIVATIVES–CONCUSIONS

In this part synthesis of C-3 polygodial derivative, 3(R)-hydroxypolygodial, the natural product from *Canella winteriana* 3- β-acetoxypolygodial, and 3-β-(p-methoxycinnamoyl) polygodial, an analogue of the natural product from Drymis winterii bark, has been shown.(**Figure 2.3**)

Our purpose is to assay their activity towards both vanilloid receptor TRPV1 and new TRPA1 receptor. In addition, some antiproliferative assays will be done.
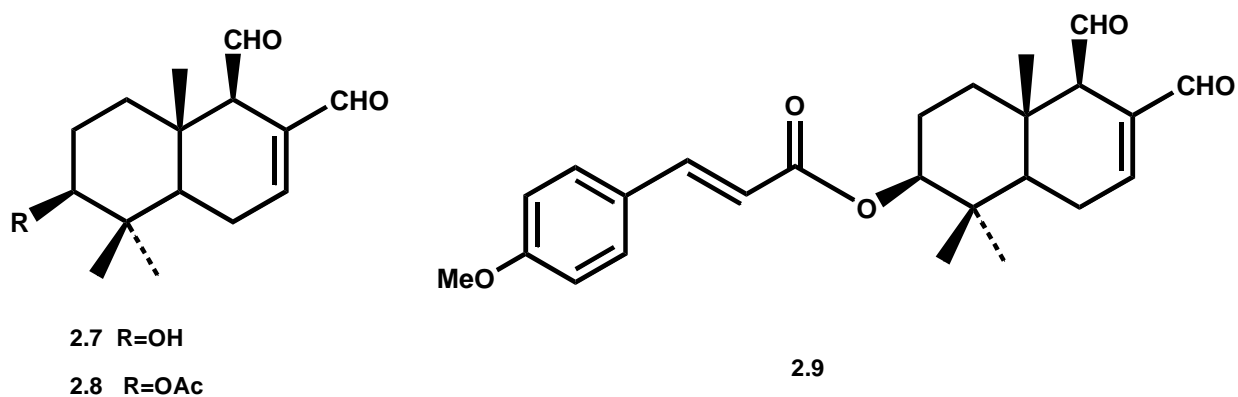

2.7 R=OH

2.8 R=OAc

2.9

**FIGURE 2.3**

2.7 EXPERIMENTAL

2.7.1. *GENERAL*

All reactions were carried out under a dry N_2 atmosphere using freshly distilled solvents, unless otherwise noted. Tetrahydrofuran (THF) was distilled from sodium-benzophenone complex. Dichloromethane was distilled from calcium hydride. Glassware was flame-dried (0.05 Torr) prior to use. Starting materials and reagents purchased from commercial suppliers were generally used without purification. Reaction temperatures were measured externally; reactions were monitored by thin layer chromatography on Merck silica gel plates (0.25 mm) and visualized by UV light and spraying with phosphomolybdic acid, p-anisaldehyde or $Ce(SO_4)_2$ solutions and drying. Flash chromatography was performed on Merck silica gel 60 (particle size: 0.040e0.063 mm). Yields refer to chromatographically and spectroscopically (1H and 13C NMR) pure materials. The NMR spectra were recorded at room temperature on a Bruker DRX 400, a Bruker DRX 300 or a Bruker AV 250 spectrometers. Chemical shifts are reported relative to the residual solvent peak ($CHCl_3$: d¼7.26, $^{13}CDCl_3$: d¼77.0). Assignments in the 13C NMR spectra were confirmed by DEPT spectroscopy experiments. ESIMS spectra were performed on a Micromass Quattro micro API mass spectrometer equipped with an electrospray ionization source operating in positive mode. IR spectra were obtained at a resolution of 2.0 cm^{-1} with a Vector 22 Bruker Spectrometer. Optical rotations were measured with a JASCO DIP-1000 polarimeter. Elemental analyses were performed on Flash EA 1112 (Thermo Electron Corporation) analyzer.

2.7.2. SYNTHESIS OF 1(R)HYDROXYPOLYGODIAL
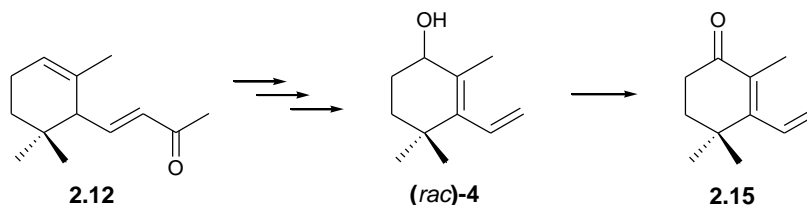
SCHEME 2.35. 2.15 was prepared starting from racemic compound 4, easily prepared starting from following a protocol by Nicolaou et al.[66]

- **2,4,4-Trimethyl-3-vinyl-cyclohex-2-enone (2.15).** To a solution of the alcohol (*rac*)-4 (0.543 g, 3.26 mmol) in dry CH_2Cl_2 (17 mL), 4 Å molecular sieves (1.09 g) and PDC (2.45 g, 6.52 mmol) were added at room temperature under a nitrogen atmosphere. The mixture was stirred for 1 h, then diluted with diethyl ether (100 mL) and allowed to stir for additional 1 h. Filtration through a short pad of silica gel (particle size 0.063 – 0.200 mm) and $CaSO_4$ (10% in weight) afforded a solution, which was concentrated in vacuo. The residue was flash-chromatographed (10% – 40% diethyl ether in petroleum ether) to afford pure enone **2.15** (0.488 g, 2.97 mmol, 91%) as a colorless oil.

-
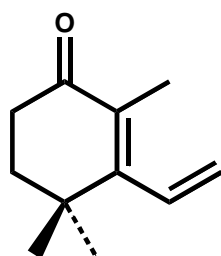
Compound 2.15: R_f = 0.56 (30% diethyl ether in petroleum ether). ^{1}H NMR (CDCl3, 400 MHz): *δ* 1.14 (6H, s, $(CH_3)_2C$), 1.81 (3H, s, $CH_3C=$), 1.84 (2H, t, *J* = 6.7 Hz, H-3 and H-3' overlapped), 2.50 (2H, t, *J* = 6.7 Hz, H-2 and H-2' overlapped), 5.17 (1H, dd, *J* = 1.9, 17.8 Hz, *H*HC=CH), 5.49 (1H, dd, *J* = 1.9, 11.8 Hz, H*H*C=CH), 6.33 (1H, dd, *J* = 11.8, 17.8 Hz, H_2C=C*H*).

[66] Nicolaou, K.C.; Li, W.S. *Chem. Soc. Chem. Commun.* **1985** 421

^{13}C NMR (CDCl$_3$, 100 MHz): δ 13.3 (CH$_3$), 27.2 (x 2) (CH$_3$), 34.3 (CH$_2$), 35.2 (C), 37.2 (CH$_2$), 120.7 (CH$_2$), 129.8 (C), 133.9 (CH), 161.5 (C), 199.6 (C). IR (CHCl$_3$): ν = 2964, 2929, 1660 cm^{-1}. EIMS: m/z (%) = 164(100), 149(4), 107(15). Anal. Calcd. for C$_{11}$H$_{16}$O: C, 80.44; H, 9.82. Found: C, 80.32; H, 9.87.

- **(R)-2,4,4-Trimethyl-3-vinyl-cyclohex-2-enol (2.16).** A solution of BH$_3$·THF (1 M in THF, 1.87 ml, 1.87 mmol) was slowly added (1.2 mmol/h by siringe-pump) to a warm solution (35 °C) of dienone **2.15** (342.0 mg, 2.08 mmol) and (S)-methyl-oxazaborolidine (1 M in toluene, 2.29 mL, 2.29 mmol) in 2.1 mL of dry THF, under a nitrogen atmosphere. After the completion of the addition, TLC analysis showed the disappearance of the starting material. The reaction mixture was then cooled and quenched with water at 0 °C. The THF was removed in vacuo and (R)-4 was extracted with diethyl ether (3 x 10 mL). The combined organic extracts were dried (MgSO$_4$), filtered and evaporated in vacuo. Flash-chromatography of the crude (20% – 60% diethyl ether in petroleum ether) afforded pure (R)-**2.16** (324.0 g, 1.95 mmol, 94%) as a white amorphous solid.
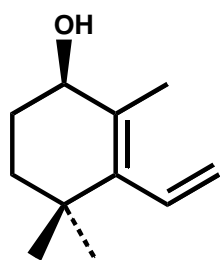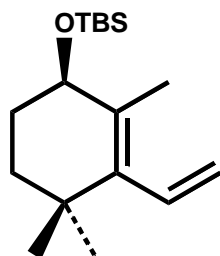

Compound (R)-2.16: R_f = 0.35 (30% diethyl ether in petroleum ether). ^{1}H NMR (CDCl$_3$, 400 MHz): δ 0.99 (3H, s, CH$_3$), 1.02 (3H, s, CH$_3$), 1.41 (1H, m, H-3), 1.43 (1H, br s, OH), 1.63 (1H, m, H-3'), 1.71 (1H, m, H-2), 1.81 (3H, s, CH$_3$C=), 1.90 (1H, m, H-2'), 3.98 (1H, m, H-1), 5.01 (1H, dd, J = 2.5, 17.7 Hz, HHC=CH), 5.29 (1H, dd, J = 2.5, 11.4 Hz, HHC=CH), 6.19 (1H, dd, J = 11.4, 17.7 Hz, H$_2$C=CH). ^{13}C NMR (CDCl$_3$, 100 MHz): δ 18.2 (CH$_3$), 27.1 (CH$_3$), 28.4 (CH$_2$), 28.7 (CH$_3$), 34.2 (C), 34.4 (CH$_2$), 70.0 (CH), 118.8 (CH$_2$), 129.2 (C), 134.8 (CH), 142.1 (C). [α]$_D^{25}$ = + 43.1 (c = 1.0, CHCl$_3$). EIMS: m/z

(%) = 166(8), 149(100), 148(50), 133(9), 90(20). Anal. Calcd. for $C_{11}H_{18}O$: C, 79.46; H, 10.91. Found: C. 79.32; H. 10.60.

HPLC conditions for ee determination: column: Chiralcel OD; solvent: 1% propan-2-ol in hexane; flow rate: 1 mL/min; detector: UV detector (254 nm); retention time: (*R*): 10.37 min; (*S*): 9.21 min.

(*R*)-*tert*-Butyl-dimethyl-(2,4,4-trimethyl-3-vinyl-cyclohex-2-enyloxy)-silane (**2.10**). To a solution of (*R*)-**2.16** (1.45 g, 8.72 mmol) in dry CH_2Cl_2 (18 mL) were sequentially added, at room temperature, imidazole (1.78 g, 26.1 mmol) and TBSCl (3.93 g, 26.1 mmol). The resulting mixture was left stirring overnight and then diluted with H_2O. The separated aqueous layer was extracted with CH_2Cl_2 (3 x 40 mL) and the combined organic phases were dried ($MgSO_4$), filtered and evaporated in vacuo. The crude product was flash-chromatographed (0% – 30% ethyl ether in petroleum ether) to afford 2.39 g (8.52 mmol, 98%) of pure colourless oil **2.10**.

Compound 2.10: R_f = 0.62 (0.5% diethyl ether in petroleum ether). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 0.08 (3H, s, CH_3Si), 0.09 (3H, s, CH_3Si), 0.91 (9H, s, $(CH_3)_3$CSi), 0.96 (3H, s, CH_3), 1.02 (3H, s, CH_3), 1.37 (1H, ddd, J = 3.1, 9.8, 13.0 Hz, H-3), 1.59 – 1.68 (2H, m, H-3' and H-2 overlapped), 1.72 (3H, s, CH_3), 1.80 (1H, m, H-2'), 4.01 (1H, t-like, J = 5.6 Hz, H-1), 5.00 (1H, dd, J = 2.6, 17.7 Hz, *H*HC=CH), 5.26 (1H, dd, J = 2.6, 11.3 Hz, H*H*C=CH), 6.18 (1H, dd, J = 11.3, 17.7 Hz, H_2C=C*H*). ^{13}C NMR ($CDCl_3$, 100 MHz): δ – 4.6 (CH_3), – 4.2 (CH_3), 18.1 (CH_3), 18.2 (C), 26.0 (x 3) (CH_3), 28.1 (CH_3), 28.2 (CH_3), 29.3 (CH_2), 34.2 (C), 35.3 (CH_2), 71.2 (CH), 118.5 (CH_2), 130.7 (C), 135.3 (CH), 140.7 (C). $[\alpha]_D^{25}$ = + 2.4 (c = 1.0, $CHCl_3$). Anal. Calcd. for $C_{17}H_{32}OSi$: C, 72.79; H, 11.50. Found: C, 72.46; H, 11.36.

(8*R*,8a*S*)-8-(*tert*-Butyl-dimethyl-silanyloxy)-5,5,8a-trimethyl-3,5,6,7,8,8a-hexahydro-naphthalene-1,2-dicarboxylic acid dimethyl ester (2.10). A mixture of the diene **2.10** (187.0 mg, 0.667 mmol) and freshly distilled DMAD (**2.11**) (369.0 μL, 3.00 mmol) was heated in a reacti-vial at 110 °C for 48 h, under a nitrogen atmosphere. After cooling, a first flash-chromatography of the reaction mixture (2% – 40% ethyl acetate in petroleum ether) furnished 28.0 mg (0.100 mmol, 15%) of starting material 13, 13.0 mg (0.031 mmol, 5%) of the minor adduct 15 as an oil, a mixture of the major adduct 14 and DMAD (5), and 16.4 mg (0.057 mmol, 8.5%) of the pure triene 16. The fraction containing the major adduct 14 was re-purified (5% – 40% diethyl ether in petroleum ether). Pure **2.17a** (114.0 mg, 40%) was obtained as pale yellow oil.
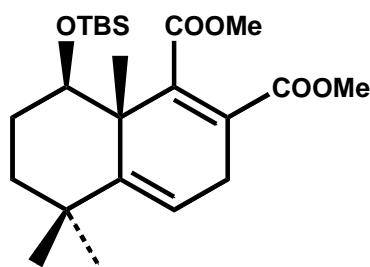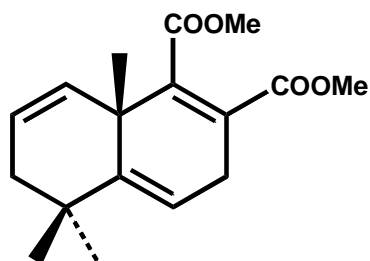

Compound 2.17a: R_f = 0.40 (20% diethyl ether in petroleum ether). ^{1}H NMR (CDCl$_3$, 400 MHz): δ 0.06 (3H, s, CH$_3$Si), 0.09 (3H, s, CH$_3$Si), 0.89 (9H, s, (CH$_3$)$_3$CSi), 1.08 (3H, s, CH$_3$), 1.16 (3H, s, CH$_3$), 1.31 (3H, s, CH$_3$), 1.34 (1H, m, H-3), 1.47 (1H, ddd, J = 4.5, 4.5, 13.6 Hz, H-3'), 1.73 – 1.79 (2H, m, H-2 and H-2' overlapped), 2.78 (1H, dd, J = 2.1, 22.4 Hz, H-7), 3.04 (1H, dd, J = 5.8, 22.4 Hz, H-7'), 3.69 (3H, s, CO$_2$CH$_3$), 3.78 (3H, s, CO$_2$CH$_3$), 4.17 (1H, dd, J = 5.8, 8.4 Hz, H-1), 5.70 (1H, dd, J = 2.1, 5.8 Hz, H-6). ^{13}C NMR (CDCl$_3$, 100 MHz): δ – 3.0 (CH$_3$), – 2.6 (CH$_3$), 18.9 (C), 21.7 (CH$_3$), 26.4 (x 3) (CH$_3$), 26.8 (CH$_2$), 28.5 (CH$_2$), 31.3 (CH$_3$), 32.2 (CH$_3$), 35.4 (C), 36.1 (CH$_2$), 46.3 (C), 51.9 (CH$_3$), 52.5 (CH$_3$), 74.1 (CH), 119.0 (CH), 126.6 (C), 148.0 (C), 148.8 (C), 166.4 (C), 169.2 (C). $[\alpha]_D^{25}$ = – 50.7 (c = 0.95, CHCl$_3$). IR (CHCl$_3$): ν = 2954, 2930, 1729 (br) cm^{-1}. ESIMS: m/z 423 [M + H]$^+$. Anal. Calcd. for C$_{23}$H$_{38}$O$_5$Si: C, 65.36; H, 9.06. Found: C, 65.84; H, 9.04.
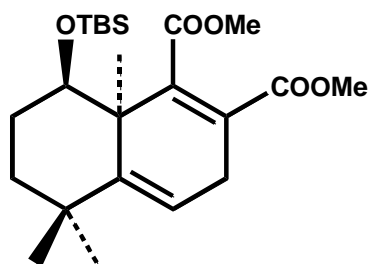
Compound 2.17 B: R_f = 0.80 (20% ethyl acetate in petroleum ether). ^{1}H NMR (CDCl$_3$, 400 MHz): δ 0.03 (3H, s, CH$_3$Si), 0.04 (3H, s, CH$_3$Si), 0.80 (9H, s, (CH$_3$)$_3$CSi), 1.14 (3H, s, CH$_3$), 1.16 (1H, m, H$_3$), 1.19 (3H, s, CH$_3$), 1.42 (3H, s, CH$_3$), 1.58 (1H, m, H-2), 1.82 (1H, ddd, J = 3.8, 13.1, 13.1 Hz, H-3'), 2.01 (1H, dddd, J = 1.6, 3.8, 14.0, 14.0 Hz, H-2'), 2.89 (1H, dd, J = 2.8, 23.3 Hz, H-7), 3.03 (1H, dd, J = 4.6, 23.3 Hz, H-7'), 3.70 (3H, s, CO$_2$CH$_3$), 3.71 (3H, s, CO$_2$CH$_3$), 4.14 (1H, br s, H-1), 5.68 (1H, dd, J = 2.8, 4.6 Hz, H-6). ^{13}C NMR (CDCl$_3$, 100 MHz): δ − 5.5 (CH$_3$), − 3.4 (CH$_3$), 18.2 (C), 25.1 (CH$_2$), 25.2 (CH$_3$), 25.8 (x 3) (CH$_3$), 27.9 (CH$_2$), 31.4 (CH$_3$), 33.2 (CH$_3$), 33.9 (CH$_2$), 35.5 (C), 45.6 (C), 51.7 (CH$_3$), 51.9 (CH$_3$), 72.3 (CH), 116.7 (CH), 131.4 (C), 142.9 (C), 144.6 (C), 168.3 (C), 168.5 (C). $[\alpha]_D^{30}$ = − 29.4 (c = 1.0, CHCl$_3$). IR (CHCl$_3$): ν = 2950, 2929, 1717 (br), 1095 cm^{-1}. ESIMS: m/z 445 [M + Na]$^+$.
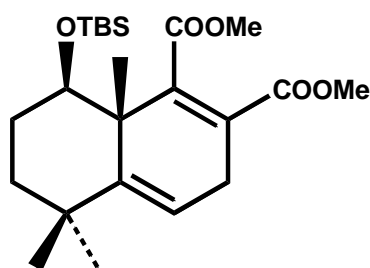

Compound 2.17 C: R_f = 0.69 (30% diethyl ether in petroleum ether). ^{1}H NMR (CDCl$_3$, 300 MHz): δ 1.17 (3H, s, CH$_3$), 1.25 (3H, s, CH$_3$), 1.40 (3H, s, CH$_3$), 1.82 (1H, dd, J = 6.3, 16.9 Hz, H-3), 1.98 (1H, br d, J = 16.9 Hz, H-3'), 2.78 (1H, dd, J =1.0, 21.9 Hz, H-7), 3.23 (1H, dd, J = 6.0, 21.9 Hz, H-7'), 3.73 (3H, s, CO$_2$CH$_3$), 3.81 (3H, s, CO$_2$CH$_3$), 5.48 (1H, dd, J = 2.7, 10.0 Hz, H-1), 5.72 (1H, br d, J = 6.0 Hz, H-6), 5.77 (1H, br dd, J = 6.3, 10.0 Hz, H-2). ^{13}C NMR (CDCl$_3$, 100 MHz): δ 26.6 (CH$_2$), 26.8 (CH$_3$), 28.8 (CH$_3$), 29.5 (CH$_3$), 35.5 (C), 39.4 (CH$_2$), 41.2 (C), 51.9 (CH$_3$), 52.1 (CH$_3$), 116.8 (CH), 126.7 (CH), 126.8 (C), 129.5 (CH), 147.1 (C), 150.3 (C), 166.1 (C), 169.2 (C). EIMS: m/z (%) = 290(7.5), 275(12), 243(100), 231(24), 199(23), 171(20), 157(17), 142(14).

(1*R*,8*R*,8a*S*)-8-(*tert*-Butyl-dimethyl-silanyloxy)-5,5,8a-trimethyl-1,5,6,7,8,8a-hexahydro-naphthalene-1,2-dicarboxylic acid dimethyl ester (2.18).

To a solution of **2.17 A** (110.0 mg, 0.260 mmol) in dry THF (2.6 mL) was added DBU (35.0 μl, 0.234 mmol) under a nitrogen atmosphere. The mixture was heated at 40 °C for 4 h and then cooled at room temperature. The resulting orange solution was diluted with Et$_2$O and filtered through a pad of silica gel (particle size 0.063 – 0.200 mm); the filter cake was washed thoroughly with Et$_2$O. The combined filtrate and washings were concentrated in vacuo. The residue was purified by flash-chromatography (15% – 30% diethyl ether in petroleum ether) affording **2.18** (99.0 mg, 0.234 mmol, 90%) and its epimer **2.19** (5.5 mg, 0.013 mmol, 5%) as colourless oils.
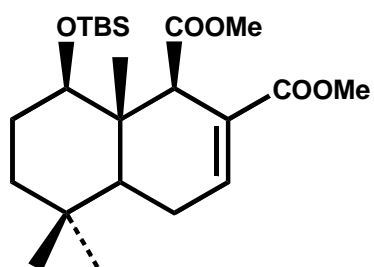

Compound 2.18: R_f = 0.23 (20% diethyl ether in petroleum ether). ^{1}H NMR (CDCl$_3$, 400 MHz): δ 0.05 (3H, s, CH$_3$Si), 0.07 (3H, s, CH$_3$Si), 0.90 (9H, s, (CH$_3$)$_3$CSi), 1.13 (3H, s, CH$_3$), 1.19 (3H, s, CH$_3$), 1.21 (3H, s, CH$_3$), 1.36 (1H, ddd, J = 5.6, 10.6, 12.5 Hz, H-3), 1.58 (1H, ddd, J = 4.7, 4.7, 12.5 Hz, H-3'), 1.68 – 1.80 (2H, m, H-2 and H-2' overlapped), 3.48 (1H, br d, J = 2.4 Hz, H-9), 3.68 (3H, s, CO$_2$CH$_3$), 3.70 (3H, s, CO$_2$CH$_3$), 3.90 (1H, dd, J = 5.2, 8.7 Hz, H-1), 5.98 (1H, d, J = 6.2 Hz, H-6), 6.94 (1H, dd, J = 2.4, 6.2 Hz, H-7). ^{13}C NMR (CDCl$_3$, 100 MHz): δ – 3.4 (CH$_3$), – 3.2 (CH$_3$), 14.6 (CH$_3$), 18.7 (C), 26.3 (x 3) (CH$_3$), 28.3 (CH$_2$), 31.3 (CH$_3$), 31.8 (CH$_3$), 36.0 (C), 36.5 (CH$_2$), 46.5 (C), 51.6 (CH$_3$), 51.8 (CH$_3$), 53.1 (CH), 77.7 (CH), 118.2 (CH), 125.1 (C), 133.7 (CH), 160.0 (C), 167.3 (C), 173.0 (C). [α]$_D^{25}$ = – 87.7 (c = 1.0, CHCl$_3$). IR (CHCl$_3$): v = 2954, 2929, 2858, 1733, 1710, 1279, 1253 cm^{-1}. ESIMS: m/z 445 [M + Na]$^+$. Anal. Calcd. for C$_{23}$H$_{38}$O$_5$Si: C, 65.36; H, 9.06. Found: C, 65.24; H, 9.03.
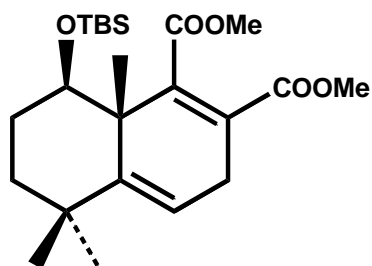
Compound 2.19: R_f = 0.28 (20% diethyl ether in petroleum ether). ^{1}H NMR (CDCl$_3$, 400 MHz): δ 0.05 (6H, s, (CH$_3$)$_2$Si), 0.91 (9H, s, (CH$_3$)$_3$CSi), 1.13 (3H, s, CH$_3$), 1.15 (3H, s, CH$_3$), 1.17 (3H, s, CH$_3$), 1.41 – 1.52 (2H, m, H-3 and H-3' overlapped), 1.65 (1H, m, H-2), 1.76 (1H, m, H-2'), 3.58 (3H, s, CO$_2$CH$_3$), 3.76 (3H, s, CO$_2$CH$_3$), 3.78 (1H, s, H-9), 3.93 (1H, dd, J = 4.8, 11.2 Hz, H-1), 5.96 (1H, d, J = 6.0 Hz, H-6), 7.06 (1H, d, J = 6.0 Hz, H-7). ^{13}C NMR (CDCl$_3$, 100 MHz): δ – 5.3 (CH$_3$), – 3.6 (CH$_3$), 18.1 (C), 18.4 (CH$_3$), 25.8 (x 3) (CH$_3$), 27.8 (CH$_2$), 30.7 (CH$_3$), 32.0 (CH$_3$), 35.4 (C), 36.7 (CH$_2$), 44.4 (C), 47.5 (CH), 51.3 (CH$_3$), 51.7 (CH$_3$), 74.0 (CH), 117.2 (CH), 122.5 (C), 135.4 (CH), 162.0 (C), 167.0 (C), 172.0 (C). $[\alpha]_D^{28}$ = – 325.2 (c = 0.7, CHCl$_3$). IR (CHCl$_3$): ν = 2954, 2927, 1735, 1716 cm^{-1}. ESIMS: m/z 445 [M + Na]$^+$.

(1R,4aS,8R,8aS)-8-($tert$-Butyl-dimethyl-silanyloxy)-5,5,8a-trimethyl-1,4,4a,5,6,7,8,8a-octahydro-naphthalene-1,2-dicarboxylic acid dimethyl ester 2.20. To a stirred vigorously suspension of **2.18** (794.0 mg, 1.88 mmol) and a catalytic amount of 10% Pd/C in MeOH (21 mL), in a conical flask, was introduced H$_2$ gas at room temperature. After the disappearance of starting material (1 h), the reaction mixture was filtered through a Celite$^®$ bed and the filtrate was concentrated in vacuo. The residue was flash-chromatographed (10% – 40% diethyl ether in petroleum ether) affording pure **2.20** (679.0 mg, 1.60 mmol, 85%) as colourless oil.

Compound 2.20: R_f = 0.33 (30% diethyl ether in petroleum ether). ^{1}H NMR (CDCl$_3$, 400 MHz): δ 0.04 (3H, s, CH$_3$Si), 0.09 (3H, s, CH$_3$Si), 0.85 (3H, s, CH$_3$), 0.90 (9H, s, (CH$_3$)$_3$CSi), 0.91 (3H, s, CH$_3$), 0.99 (3H, s, CH$_3$), 1.17 (1H, dd, J = 6.0, 10.7 Hz, H-5), 1.26 (1H, m, H-3), 1.43 (1H, ddd, J = 3.3, 3.3, 13.6 Hz, H-3'), 1.58 (1H, dddd, J = 3.3, 11.2, 13.4, 13.6 Hz, H-2), 1.71 (1H, dddd, J = 3.3, 4.2, 4.2, 13.4 Hz, H-2'), 2.15 − 2.28 (2H, m, H-6 and H-6' overlapped), 3.21 (1H, m, H-9), 3.61 (3H, s, CO$_2$CH$_3$), 3.66 (3H, s, CO$_2$CH$_3$), 3.74 (1H, dd, J = 4.2, 11.2 Hz, H-1), 7.02 (1H, m, H-7). ^{13}C NMR (CDCl$_3$, 100 MHz): δ − 2.9 (CH$_3$), − 2.3 (CH$_3$), 9.2 (CH$_3$), 18.9 (C), 21.8 (CH$_3$), 24.5 (CH$_2$), 26.6 (x 3) (CH$_3$), 28.5 (CH$_2$), 32.5 (C), 32.6 (CH$_3$), 39.4 (CH$_2$), 43.4 (C), 48.1 (CH), 51.6 (CH$_3$), 51.7 (CH$_3$), 56.6 (CH), 80.6 (CH), 129.6 (C), 140.3 (CH), 167.3 (C), 173.3 (C). $[\alpha]_D^{25}$ = − 39.6 (c = 1.0, CHCl$_3$). IR (CHCl$_3$): ν = 2952, 1721 (br), 1256 cm^{-1}. ESIMS: m/z 425 [M + H]$^+$. Anal. Calcd. for C$_{23}$H$_{40}$O$_5$Si: C, 65.05; H, 9.49. Found: C, 65.13; H, 9.39.
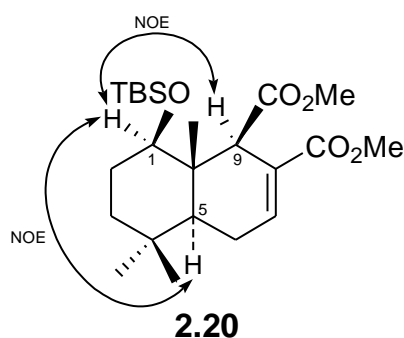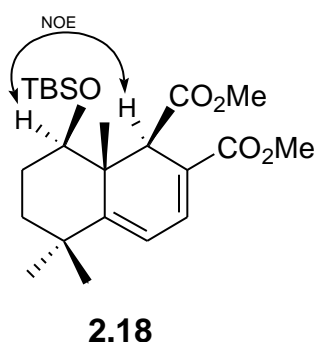

FIGURE 2.4-NOE Analysis

Hydrogenation of **2.18** (H$_2$, Pd/C, MeOH) afforded compound **2.20** (85% yield) (Scheme 4) whose stereochemistry was established via ^{1}H NMR NOE measurements (**Figure 2.4**). Irradiation of H-1 (δ 3.74 ppm) led to enhancements of the signals due to H-5 (δ 1.17 ppm) and H-9 (δ 3.21 ppm).

(1*R*,4a*S*,8*R*,8a*S*)-[8-(*tert*-Butyl-dimethyl-silanyloxy)-1-hydroxymethyl-5,5,8a-trimethyl-1,4,4a,5,6,7,8,8a-octahydro-naphthalen-2-yl]-methanol.(2.21)

To a stirring solution of **2.20** (193.0 mg, 0.454 mmol) in 2 mL of dry THF, cooled to -78 °C, was added dropwise a solution of diisobutylaluminum hydride (DIBAL-H) (1.5 M in toluene, 6.0 mL, 9.0 mmol) under a nitrogen atmosphere. The solution was allowed to warm to room temperature and stir overnight. Then, it was cooled to -78 °C and 5 mL of MeOH/H$_2$O (1/1) solution were carefully added. The resulting mixture was concentrated and dried under high vacuum. The residue was suspended in MeOH, filtered through a Celite® bed and the pad was thoroughly washed with MeOH. The filtrate was concentrated in vacuo to afford the crude diol 20 as a white amorphous solid, which was used without any further purification. For a complete characterization, flash-chromatography (50% – 90% diethyl ether in petroleum ether) of a small aliquot of crude gave pure **2.21**.
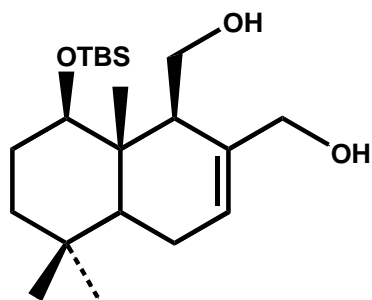
Compound 2.21: R_f = 0.20 (40% diethyl ether in petroleum ether). ^{1}H NMR (CDCl$_3$, 400 MHz): δ 0.11 (6H, s, (CH$_3$)$_2$Si), 0.84 (3H, s, CH$_3$), 0.85 (3H, s, CH$_3$), 0.87 (3H, s, CH$_3$), 0.92 (9H, s, (CH$_3$)$_3$CSi), 1.15 (1H, dd, J = 4.8, 11.2 Hz, H-5), 1.28 (1H, ddd, J = 3.6, 13.6, 13.6 Hz, H-3), 1.39 (1H, ddd, J = 3.2, 3.2, 13.6 Hz, H-3'), 1.51 (1H, dddd, J = 3.2, 3.6, 4.0, 13.6 Hz, H-2), 1.65 (1H, dddd, J = 3.2, 11.2, 13.6, 13.6 Hz, H-2'), 1.91 − 2.04 (2H, m, H-6 and H-6' overlapped), 2.26 (1H, m, H-9), 2.89 (2H, br s, 2 x OH), 3.55 (1H, dd, J = 4.0, 11.2 Hz, H-1), 3.72 (1H, dd, J = 7.2, 10.4 Hz, H-11), 3.92 (1H, d, J = 12 Hz, H-12), 4.30 (1H, d, J = 12 Hz, H-12'), 4.55 (1H, dd, J = 2.4, 10.4 Hz, H-11'), 5.80 (1H, m, H-7). ^{13}C NMR (CDCl$_3$, 100 MHz): δ − 4.1 (CH$_3$), − 3.6 (CH$_3$), 9.1 (CH$_3$), 18.2 (C), 22.3 (CH$_3$), 23.2 (CH$_2$), 26.1 (x 3) (CH$_3$), 29.1 (CH$_2$), 32.8 (C), 33.1 (CH$_3$), 40.0 (CH$_2$), 41.7 (C), 49.4 (CH), 53.3 (CH), 62.9 (CH$_2$), 67.1 (CH$_2$), 82.9 (CH), 127.0 (CH), 137.9 (C). $[\alpha]_D^{25}$ = − 20.4 (c = 1.0, CHCl$_3$). IR (CHC1$_3$): ν = 3474 (br) cm^{-1}. ESIMS: m/z 369 [M + H]$^+$. Anal. Calcd. for C$_{21}$H$_{40}$O$_3$Si: C, 68.42; H, 10.94. Found: C, 68.54; H, 10.89.

(1R,4aS,8R,8aS)-8-($tert$-Butyl-dimethyl-silanyloxy)-5,5,8a-trimethyl-1,4,4a,5,6,7,8,8a-octahydro-naphthalene-1,2-dicarbaldehyde (2.22). To a stirring solution of oxalyl chloride (377.7 mg, 2.98 mmol, 0.260 mL) in 7 mL of dry CH$_2$C1$_2$ cooled to -78 °C, were added dropwise 464.9 mg (5.95 mmol, 0.549 mL) of DMSO under a nitrogen atmosphere. After 5 min, a solution of 137.0 mg (0.372 mmol) of **2.21** in 2.5 mL of CH$_2$C1$_2$/DMSO (3/1) was added via cannula. After 1 h, freshly distilled triethylamine 1.40 g (13.8 mmol, 1.93 mL) was added and the resulting mixture was allowed to stir at -78 °C for 5 min and then warm to room temperature. Then it was passed through a short pad of silica gel (particle size 0.040 – 0.263 mm) eluting with ethyl acetate, under N$_2$, and the eluent was concentrated to give a yellow oil. The oil was purified by

flash-chromatography (40% – 60% diethyl ether in petroleum ether) under N_2 to give 118.0 mg (0.324 mmol, 87% for two steps) of **2.22** as a white amorphous solid.
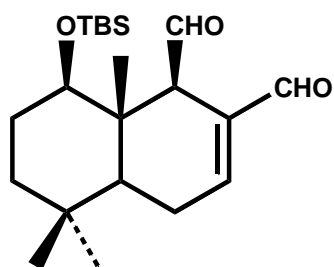
Compound 2.22: R_f = 0.50 (50% diethyl ether in petroleum ether). ^{1}H NMR (CDCl$_3$, 400 MHz): δ 0.12 (3H, s, CH$_3$Si), 0.14 (3H, s, CH$_3$Si), 0.83 (3H, s, CH$_3$), 0.90 (9H, s, (CH$_3$)$_3$CSi), 0.91 (3H, s, CH$_3$), 0.95 (3H, s, CH$_3$), 1.26 (1H, dd, J = 4.9, 11.1 Hz, H-5), 1.33 (1H, ddd, J = 4.2, 13.2, 13.2 Hz, H-3), 1.50 (1H, ddd, J = 3.2, 3.2, 13.2 Hz, H-3'), 1.56 − 1.71 (2H, m, H-2 and H-2' overlapped), 2.26 – 2.42 (2H, m, H-6 and H-6' overlapped), 3.30 (1H, br s, H-9), 3.65 (1H, dd, J = 4.5, 10.8 Hz, H-1), 7.03 (1H, m, H-7), 9.32 (1H, s, OHCC-8), 10.0 (1H, d, J = 3.6 Hz, OHCC-9). ^{13}C NMR (CDCl$_3$, 100 MHz): δ − 3.7 (CH$_3$), − 3.1 (CH$_3$), 9.9 (CH$_3$), 18.3 (C), 22.4 (CH$_3$), 24.6 (CH$_2$), 26.1 (x 3) (CH$_3$), 28.0 (CH$_2$), 32.6 (C), 32.8 (CH$_3$), 39.8 (CH$_2$), 44.8 (C), 49.0 (CH), 59.4 (CH), 81.7 (CH), 141.2 (C), 152.0 (CH), 192.7 (CH), 201.0 (CH). $[\alpha]_D^{25}$ = + 45.3 (c = 1.0, CHCl$_3$). IR (CHC1$_3$): ν = 2929, 2856, 1715, 1682, 1255 cm^{-1}. ESIMS: m/z 387 [M + Na]$^+$. Anal. Calcd. for C$_{21}$H$_{36}$O$_3$Si: C, 69.18; H, 9.95. Found: C, 69.29; H, 9.81.

(1R,4aS,8R,8aS)-8-Hydroxy-5,5,8a-trimethyl-1,4,4a,5,6,7,8,8a-octahydro-naphthalene-1,2-dicarbaldehyde [1-(R)-hydroxypolygodial] (2.1). To a solution of **2.22** (60.0 mg, 0.165 mmol) in acetonitrile (5.60 mL), in a silicon vessel, 48% aqueous hydrofluoric acid (1.7 mL) was added at room temperature. The mixture was stirred at room temperature overnight and then NaHCO$_3$ was carefully added until the mixture was neutralized. The organic solvent was removed in vacuo and the residue aqueous layer was extracted with ethyl acetate (3 x 4 mL). The combined organic extracts were dried (Na$_2$SO$_4$), filtered and evaporated in vacuo.

Flash-chromatography of the crude (30% – 60% ethyl acetate in petroleum ether) under N_2 afforded pure **2.1** (32.6 mg, 0.130 mmol, 79%) as a white amorphous solid.
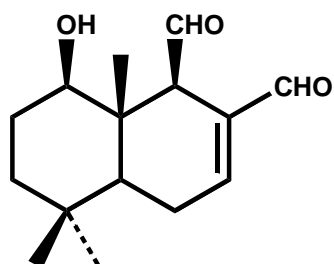
Compound 2.1: R_f = 0.50 (50% ethyl acetate in petroleum ether). ^{1}H NMR (CDCl$_3$, 400 MHz): δ 0.88 (3H, s, CH$_3$), 0.91 (3H, s, CH$_3$), 0.94 (3H, s, CH$_3$), 1.25 (1H, dd, J = 4.9, 11.4 Hz, H-5), 1.36 (1H, m, H-3), 1.51 (1H, ddd, J = 3.2, 3.2, 13.5 Hz, H-3'), 1.56 – 1.66 (2H, m, H-2 and H-2'), 2.37 (1H, dddd, J = 2.4, 3.8, 11.4, 20.0 Hz, H-6), 2.45 (1H, m, H-6'), 3.20 (1H, br s, H-9), 3.62 (1H, dd, J = 7.6, 8.2 Hz, H-1), 7.09 (1H, m, H-7), 9.37 (1H, s, OHCC-8), 9.82 (1H, d, J = 3.2 Hz, OHCC-9). ^{13}C NMR (CDCl$_3$, 62.5 MHz): δ 9.3 (CH$_3$), 22.0 (CH$_3$), 24.8 (CH$_2$), 27.8 (CH$_2$), 32.7 (CH$_3$), 32.8 (C), 39.6 (CH$_2$), 43.5 (C), 48.5 (CH), 59.8 (CH), 79.8 (CH), 139.5 (C), 153.1 (CH), 192.9 (CH), 203.9 (CH). $[\alpha]_D^{25}$ = – 8.2 (c = 1.0, CHCl$_3$). IR (CCl$_4$): ν = 3513 (br), 2960, 2928, 2873, 2855, 2739, 1722, 1690 cm^{-1}. ESIMS: m/z 273 [M+Na]$^+$. Anal. Calcd. for C$_{15}$H$_{22}$O$_3$: C, 71.97; H, 8.86. Found: C, 71.77; H, 8.91.

(1S,4aS,8R,8aS)-8-Hydroxy-5,5,8a-trimethyl-1,4,4a,5,6,7,8,8a-octahydro-naphthalene-1,2-dicarbaldehyde [1-(R)-hydroxyisotadeonal] (2.2). To a solution of 2.1 (5.5 mg, 0.022 mmol) in CH$_2$Cl$_2$ (2.5 mL) basic Al$_2$O$_3$ (10.0 mg) was added at room temperature. The mixture was stirred for 20 h and then filtered on a short pad of silica gel. The pad was thoroughly washed with ethyl acetate, under N_2, and the filtrate was concentrated in vacuo to afford a crude mixture containing **2.2** and **2.1** in a ratio of 4.4:1 (determined by ^{1}H NMR). The crude was purified by flash-chromatography (30% – 70% ethyl acetate in petroleum ether) under N_2 to give 4.5 mg (0.018 mmol, 82%) of **2.2**, as white amorphous solid, and 1.0 mg (0.004 mmol, 18%) of **2.1.**
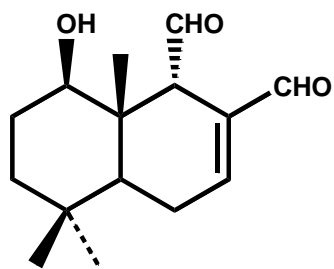
Compound 2.2: R_f = 0.57 (50% ethyl acetate in petroleum ether). ^{1}H NMR (CDCl$_3$, 400 MHz): δ 0.91 (3H, s, CH$_3$), 0.93 (3H, s, CH$_3$), 0.95 (3H, s, CH$_3$), 1.20 − 1.33 (2H, m, H-3 and H-3' overlapped), 1.49 (1H, dd, J = 5.0, 11.7 Hz, H-5), 1.62 (1H, dddd, J = 4.0, 4.0, 4.0, 13.0 Hz, H-2), 1.73 (1H, dddd, J = 3.3, 11.2, 11.2, 13.0 Hz, H-2'), 2.28 (1H, dddd, J = 2.4, 2.4, 11.7, 20.5 Hz, H-6), 2.56 (1H, ddd, J = 5.0, 5.0, 20.5 Hz, H-6'), 3.53 (1H, dd, J = 4.0, 11.2 Hz, H-1), 3.66 (1H, br s, H-9), 7.11 (1H, dd, J = 2.4, 5.0 Hz, H-7), 9.44 (1H, s, OHCC-8), 9.89 (1H, d, J = 2.5 Hz, OHCC-9). ^{13}C NMR (CDCl$_3$, 100 MHz): δ 14.8 (CH$_3$), 22.1 (CH$_3$), 25.3 (CH$_2$), 27.5 (CH$_2$), 32.3 (CH$_3$), 32.7 (C), 39.6 (CH$_2$), 43.1 (C), 43.6 (CH), 54.9 (CH), 74.7 (CH), 137.5 (C), 152.7 (CH), 192.9 (CH), 203.1 (CH). $[\alpha]_D^{25}$ = − 98.1 (c = 0.15, CHCl$_3$). IR (CHCl$_3$): ν = 3467 (br), 2976, 2929, 2896, 1717, 1682 cm^{-1}. ESIMS: m/z 273 [M + Na]$^+$.

2.7.3. <u>SYNTHESIS OF 3-(S)-HYDROXYPOLYGODIAL</u>

5.2 Starting material. The epoxide **2.59** ($[\alpha]_D^{22}$ = −2.9 c = 2.4, CHCl$_3$) was prepared according to a known procedure starting from (2E, 5E)-farnesyl acetate (**2.65**).[13] The enantiomeric excess was determined as 95% by ^{1}H-NMR analysis of the corresponding mono-(S)-MTPA ester of diol **2.68**.[67]

Preparation of ((2S, 4aS, 5S, 8aR)-decahydro-2-hydroxyl-1,1,4a-trimethyl-6- methylenenaphthalen-5-yl) methyl acetate (2.60).

Strictly deoxygenated THF (16.7 mL) was added to a mixture of Cp$_2$TiCl$_2$ (106.6 mg, 0.42 mmol) and Mn dust (940.6 mg, 17.1 mmol) under an Ar atmosphere and the suspension was stirred at room temperature until it turned green (after about 15 min). A solution of epoxide **2.65** (600 mg, 2.14 mmol) in THF dry (1.67 mL) was added *via cannula* to this suspension and then collidine (2.26 mL) and Me$_3$SiCl (1.10 mL) were added sequentially. The solution was stirred overnight. The reaction was then quenched with aq. HCl 2N (50 mL) and was stirred until no effervescence and a change in color from green to brown were observed. THF was removed under *vacuum* and the residue aqueous layer was extracted with EtOAc. The combined organic layers were dried (anhydrous MgSO$_4$) and concentrated under reduced pressure. TBAF dry (1M in THF, 10.6 mmol, 10.6 mL) was added to the residue and the solution was stirred under N$_2$ atmosphere for 3h. THF was removed under *vacuum* and the residue was diluted with EtOAc and H$_2$O. The aqueous layer was extracted with AcOEt, the combined organic phases were washed with brine, dried (anhydrous Na$_2$SO$_4$) and solvent removed. The residue was flash chromatographed on silica gel (10% → 50% AcOEt in petroleum ether) giving 207.2 mg of **2.60** (34 %) as a yellow oil;

---

[67] Huang, A. X.; Xiong, Z.; Corey, E. J. *J. Am. Chem. Soc*. **1999**, 121, 9999.
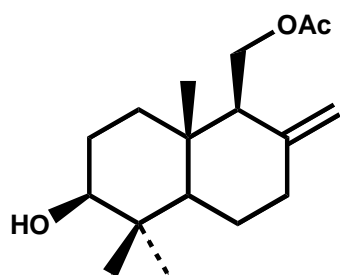
Compound 2.60 [Found: C, 73.13; H, 9.87. $C_{17}H_{28}O_3$ requires C, 72.82; H, 10.06]; R_f (30%EtOAc/hexane) 0.40; $[\alpha]_D^{27}$ = + 18.0 (c =1.7, CHCl$_3$). ^{1}H-NMR (CDCl$_3$, 400 MHz): δ 4.86 (1H, bs), 4.53 (1H, bs), 4.31 (1H, dd, J =11.0, 3.9 Hz), 4.18 (1H, dd, J =11.0, 8.6 Hz), 3.27 (1H, dd, J= 10.7, 3.9 Hz), 2.45 – 2.38 (1H, m), 2.09 – 1.97 (2H, m), 2.01 (3H, s), 1.80 – 1.30 (6H, m), 1.12 (1H, dd, J= 12.5, 2.4 Hz), 1.00 (3H, s), 0.78 (3H, s), 0.75 (3H, s). ^{13}C-NMR (100 MHz CDCl$_3$): δ 171.6 (s), 146.4 (s), 107.8 (t), 78.8 (d), 61.7 (t), 54.7 (d), 54.5 (d), 39.4 (s), 38.9 (s), 37.6 (t), 37.2 (t), 28.5 (q), 27.9 (t), 23.7 (t), 21.3 (q), 15.7 (q), 15.3 (q). ES-MS (m/z) = 281 (M+H$^+$), 221 (M- AcOH +H$^+$).

Synthesis of compound 2.74: To a solution containing **2.60** (175 mg, 0.62 mmol), imidazole (493 mg, 7.24 mmol), CH$_3$CN (3 mL) under N$_2$ atmosphere, TBSCl (467 mg, 3.1 mmol) was added. The solution was heated to 90 °C and stirred for 3h. CH$_3$CN was removed under N$_2$ flux, the residue was diluited with H$_2$O and the aqueous layer was extracted with AcOEt , The combined organic phases were dried (Na$_2$SO$_4$), filtered and evaporated *in vacuo*. The crude product was flash chromatographed (5% $\rightarrow$ 40% Et$_2$O in petroleum ether) to afford 185 mg (75%) of **1.74** as colorless oil.
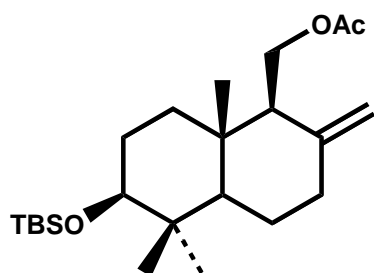
Compound **2.74** [Found C, 70.45; H, 11.02. $C_{23}H_{42}O_3Si$ requires C, 70.00; H, 10.73] R_f (15% EtOAc/hexane) 0.91 ; $[\alpha]_D^{25}$ = + 26.0 (c = 0.4, CHCl$_3$). ^{1}H-NMR (CDCl$_3$, 400 MHz):δ 4.85 (1H, bs), 4.52 (1H, bs), 4.32 (1H, dd, J =11.2, 3.7 Hz), 4.16 (1H, dd, J =11.2, 9.0 Hz), 3.22 (1H, dd, J = 10.2, 5.6 Hz), 2.43–2.36 (1H, m), 2.05–2.01 (1H, m), 2.00 (3H, s), 1.99–1.97 (1H, m), 1.75–1.71 (1H, m), 1.69–1.67 (1H, m), 1.60–1.54 (2H, m), 1.44–1.28 (2H, m), 1.09 (1H, dd, J = 12.5, 2.5 Hz), 0.91 (3H, s), 0.88 (9H, s), 0.74 (6H, bs), 0.04 (3H, s), 0.03 (3H, s). ^{13}C-NMR (100 MHz CDCl$_3$): δ 171.3 (s), 146.4 (s), 107.3 (t), 79.0

(d), 61.5 (t), 54.5 (d), 54.2 (d), 39.7 (s), 38.6 (s), 37.5 (t), 36.9 (t), 28.7 (q), 28.1 (t), 25.9 (q, 3C), 23.7 (t), 21.1 (q), 18.1 (s), 15.9 (q), 15.1 (q), -3.8 (q), -4.9 (q). ES-MS (*m/z*) = 395(M+H$^+$).

5.5 Synthesis of compound 2.75: To a solution of **2.74** (185 mg, 0.47 mmol) in MeOH (2.5 mL), at room temperature, K$_2$CO$_3$ (78 mg, 0.56 mmol) was added. The reaction mixture was heated to reflux and stirred for 1h. MeOH was removed *in vacuo*, the residue was diluited with H$_2$O and the aqueous layer was extracted with AcOEt, The combined organic phases were washed with aq. HCl 1N, dried (anhydrous Na$_2$SO$_4$), filtered and evaporated *in vacuo*. The crude product was flash chromatographed (5% → 20% AcOEt in petroleum ether) to afford 152 mg (92%) of **2.75** as colorless oil**.**
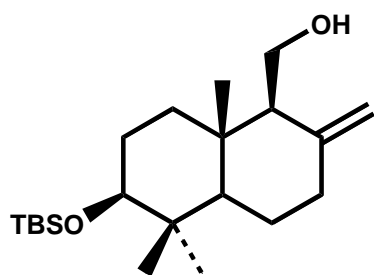
Compound 2.75.[Found: C, 71.35; H, 11.24. C$_{21}$H$_{40}$O$_2$Si requires C, 71.53; H, 11.43] R$_f$ (15% EtOAc/hexane) 0.62 [α]$_D^{25}$ = + 10.1 (c = 0.6, CHCl$_3$). ^{1}H-NMR (CDCl$_3$, 400 MHz): δ 4.93 (1H, bs), 4.63 (1H, bs), 3.85–3.70 (2H, m), 3.24–3.20 (1H, m), 2.42 (1H, ddd, *J* = 13.0, 4.2, 2.4 Hz), 2.00 (1H, ddd, *J* = 13.0, 12.8, 4.9 Hz), 1.93–1.89 (1H, m), 1.77–1.71 (1H, m), 1.67–1.63 (1H, m), 1.58–1.54 (1H, m), 1.45–1.36 (1H, m), 1.32–1.28 (1H, m), 1.09 (1H, dd, *J* = 12.5, 2.8 Hz), 0.91 (3H, s), 0.88 (9H, s), 0.73 (6H, s),0.04 (3H, s), 0.03 (3H, s) ppm. ^{13}C-NMR (100 MHz CDCl$_3$): δ 147.5 (s), 106.5 (t), 79.1 (d), 58.9 (d), 58.8 (t), 54.4 (d), 39.7 (s), 38.6 (s), 37.8 (t), 36.9 (t), 28.7 (q), 28.1 (t), 25.9 (q), 23.9 (t), 18.1 (s), 15.9 (q), 15.3 (q), –3.8 (q), –4.9 (q) ppm. ES-MS *(m/z)* = 353 - (M+H$^+$).

Synthesis of epoxide 2.76: A solution of **2.75** (152 mg, 0.43 mmol) in CH$_2$Cl$_2$ dry (3.2 mL), under N$_2$ atmosphere, was cooled to 0°C and m-CPBA (77 mg, 0.991 mmol) was added. After 5 min, the solution was warmed to room temperature and stirred for 1h. Then, the solution was cooled to 0°C, a 10% solution of Na$_2$SO$_3$ (12 mL) was added and the resulted mixture was

stirred at room temperature for 1h. The aqueous layer was extracted with AcOEt, the combined organic layers were washed with $NaHCO_3$, dried (anhydrous Na_2SO_4), filtered and evaporated in vacuo. The crude product was flash chromatographed (5% → 40% AcOEt in petroleum ether) to afford 124 mg (78%) of pure **2.76** as a white solid**.**
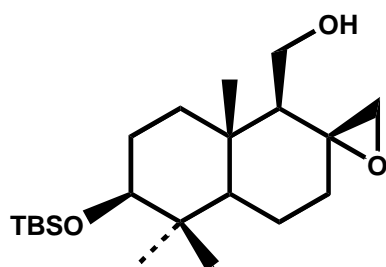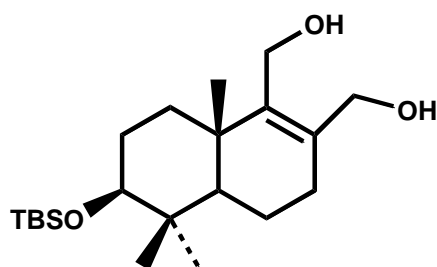
Compound 2.76.[Found: C, 68.61; H, 11.22. $C_{21}H_{40}O_3Si$ requires C, 68.42; H, 10.94] R_f (20% EtOAc/hexane) 0.49; $[\alpha]_D^{25}$ = −11.8 (c = 1.0, $CHCl_3$). ^{1}H-NMR ($CDCl_3$, 400 MHz): δ 3.59 (1H, ddd, J = 10.2, 10.2, 3.3 Hz), 3.40 (1H, dd, J= 10.4, 10.2 Hz), 3.23–3.21 (1H, m), 3.18 (1H, dd, J = 3.6, 2.1 Hz), 3.06 (1H, d, J =10.2 Hz, OH), 2.70 (1H, d, J = 3.6 Hz), 2.00–1.92 (1H, m,), 1.87–1.85 (1H, m), 1.85–1.80 (1H, m), 1.70–1.62 (1H, m), 1.61-1.54 (3H, m), 1.43–1.39 (1H, m), 1.34–1.30 (1H, m,), 1.04 (1H, dd, J = 12.4, 2.3 Hz), 0.93 (3H, s), 0.88 (9H, s), 0.84 (6H, s), 0.76 (3H, s), 0.04 (6H, s) ppm. ^{13}C-NMR (100 MHz $CDCl_3$): δ 78.9 (d), 61.6 (s) , 58.8 (t), 54.2 (d), 53.9 (d), 51.7 (t), 39.6 (s), 38.8 (s), 36.7 (t), 36.2 (t), 28.7 (q), 27.6 (t), 25.8 (q), 21.5 (t), 18.1 (s), 16.0 (q), 15.7 (q), -3.8 (q), -5.0 (q) ppm. ES-MS (m/z)= 391 (M+Na$^+$).

Synthesis of diol 2.77: To a solution of **2.76** (55 mg, 0.149 mmol) in a 10:1 mixture of CH_3CN (16 mL) and DMSO (1.6 mL), under N_2 atmosphere, at room temperature, was added CSA (34.6 mg, 0.149 mmol) and the solution was stirred at room temperature overnight. The CSA was quenched with saturated $NaHCO_3$ solution and the mixture was concentrated in vacuo. The residue was diluted with H_2O and AcOEt and the aqueous layer was extracted with AcOEt. The combined organic phases were dried (anhydrous Na_2SO_4), filtered and evaporated in vacuo. The crude product was flash chromatographed (from 15% AcOEt in petroleum ether to pure AcOEt) to afford 32 mg (58%) of **2.77** as white matt solid**,** 12.6 mg (23%) of $\Delta^{8,9}$ double bond isomer **2.78** as a white solid.and a 7% of deprotected diol as white solid **2.79**
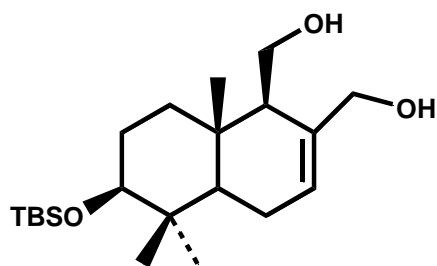
Compound 2.77: [Found: C, 68.67; H, 11.35. $C_{21}H_{40}O_3Si$ requires C, 68.42; H, 10.94] R_f (40% EtOAc/hexane) 0.41; $[\alpha]_D^{23}$ = + 1.9 (c = 1.0, CHCl$_3$). ^{1}H-NMR (CDCl$_3$, 400 MHz): δ 5.81 (1H, bs); 4.35 (1H, d, *J* = 12.2 Hz), 3.99 (1H, d, *J* = 12.2 Hz), 3.90 (1H, dd, *J* = 11.2, 2.0 Hz), 3.69 (1H, dd, *J* = 11.2, 8.2 Hz), 3.21 (1H, dd, *J* = 11.1, 4.4 Hz), 2.77 (2H, bs), 2.12–2.06 (1H, m), 2.07–1.99 (2H, m), 1.66–1.57 (2H, m), 1.33–1.21 (3H, m), 0.88 (12H, bs), 0.82 (3H, s), 0.75 (3H, s), 0.05 (3H, s), 0.03 (3H, s) ppm. ^{13}C-NMR (75 MHz CDCl$_3$): δ 136.7 (s), 127.7 (d), 79.2 (d), 67.3 (t) ,61.4 (t), 54.4 (d), 48.9 (d), 39.2 (s), 37.4 (t), 35.3 (s), 28.3 (q), 27.7 (t), 25.8 (q x 3), 23.4 (t), 18.0 (s), 15.6 (q), 14.6 (q), –3.9 (q), –5.0 (q) ppm. ES-MS (*m/z*)= 391 (M+Na$^+$), 351.;

Compound 2.78 : [Found: C, 68.01; H, 11.16. $C_{21}H_{40}O_3Si$ requires C, 68.42; H, 10.94] R_f (40% EtOAc/hexane) 0.23; ^{1}H-NMR (CDCl$_3$, 400 MHz): δ 4.22 (1H, d, *J* = 11.7 Hz), 4.17 (1H, d, *J* = 11.6 Hz), 4.11 (1H, d, *J* =11.7 Hz), 4.01 (1H, d, *J* = 11.6 Hz), 3.22 (1H, dd, *J* = 11.0, 5.1 Hz), 2.26–2.22 (2H, m), 1.84 (1H, ddd, *J* = 13.2, 3.0, 3.0 Hz), 1.79–1.35 (6H, m), 0.99 (3H, s), 0.93 (3H, s), 0.89 (9H, s), 0.78 (3H, s), 0.04 (3H, s), 0.03 (3H, s) ppm. ^{13}C-NMR (100 MHz CDCl$_3$): δ 145.9 (s), 136.2 (s), 79.1 (d), 63.9 (t), 58.0 (t), 50.5 (d), 39.3 (s), 37.8 (s), 34.3 (t), 31.6 (t), 28.4 (q), 27.9 (t), 25.9 (q x 3), 20.4 (q), 18.6 (t), 18.1 (s), 15.9 (q), –3.8 (q), –4.9 (q) ppm. ES-MS (*m/z*)= 391 (M+Na$^+$).
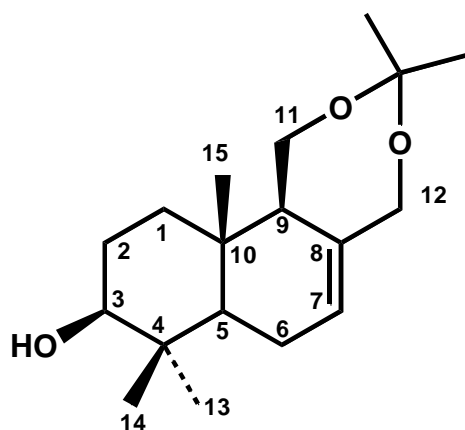
Compound 2.79 has been characterised as acetonide 2.79a[1]H-NMR (CDCl$_3$, 400 MHz): δ 5.63 (1H, s, H-7), 4.30 (1H, d, J=13 Hz, H-12 o H'-12), 3.76 (1H, d, J=13 Hz, H-12 o H'-12), 3.66(2H, m, J=11.7, 4.6 Hz, H-11 o H'-11), 3.27 (1H, dd, J=11.1, 4.3Hz, H-3), 2.03 (3H,m, H-9), 1.91(1H, m, H-1 o H'-1), 1.67-1.54 (3H, m, H-1 o H'-1, H-2 o H'-2), 1.34 (3H, s, CH$_3$), 1.32 (3H, s, CH$_3$), 0.98 (3H, s, *CH$_3$*), 0.87(3H, s, *CH$_3$*), 0.82(3H, s, *CH$_3$*).^{13}C-NMR (100MHz CDCl$_3$): δ 135.5 (d)125.1(s), 101.4(s), 78.8 (d), 66.9 (t), 60.7 (t), 60.38 (d), 54.3 (d), 49.5 (d), 38.6 (s), 37.4 (d), 34.6 (s), 28.1(q), 27.3(t), 24.8 (q), 24.3(q), 23.1 (t), 15.4 (q), 14.7 (q).

$[\alpha]_D^{25}$ = +35.62 (c = 0.85, CHCl$_3$).

ES-MS (*m/z*)= 277.32 (M+Na$^+$).

Synthesis of compound 2.80: To a stirring solution of oxalyl chloride (84.0 mg, 0.66 mmol) in CH$_2$Cl$_2$ (0.4 mL), under N$_2$ atmosphere, cooled to −78 °C, DMSO (103 mg, 1.32 mmol) was added dropwise. After 5 minutes, a solution of **2.77** (30.5 mg, 0.0827 mmol) in a 3/1 mixture of CH$_2$Cl$_2$ (0.6 mL) and DMSO (0.2 mL) was added *via cannula* and the solution was stirred for 2h. Dry NEt$_3$ (309 mg, 3.06 mmol) was added and the resulting mixture was allowed to stir at −78 °C for 10 min. The reaction mixture was warmed to room temperature and stirred for further 45 min. Then the mixture was passed through a short pad of silica gel (particle size 0.040–0.263 mm) eluting with ethyl acetate, under N$_2$, and the eluent was concentrated to give a yellow oil. The oil was purified by flash chromatography (20%→ 40% diethyl ether in petroleum ether) under N$_2$ to give 23 mg (76%) of **2.80** as a white amorphous solid.
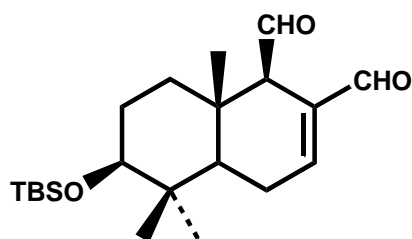
Compound 2.80 [Found: C, 68.82; H, 10.34. $C_{21}H_{36}O_3Si$ requires C, 69.18; H, 9.95] R_f (60% Et_2O/hexane) 0.72; $[\alpha]_D^{25}$ = −41.4 (c = 0.5, $CHCl_3$). IR ($CHCl_3$, cm^{-1}) = ν 2938, 2860, 1718, 1682. ^{1}H-NMR ($CDCl_3$, 400 MHz): δ 9.51 (1H, d, J = 4.4 Hz), 9.46 (1H, bs), 7.14–7.12 (1H, m), 3.23 (1H, dd, J = 9.8, 5.2), 2.78–2.74 (1H, m), 2.56–2.35 (2H, m), 1.82 (1H, ddd, J = 13.2, 3.0, 3.2 Hz), 1.65–1.53 (2H, m), 1.49 (1H, ddd, J = 13.2, 12.6, 4.6 Hz), 1.25–1.21 (1H, m), 0.94 (3H, s), 0.93 (3H, s), 0.89 (3H, s), 0.88 (9H, s), 0.05 (3H,s), 0.03 (3H, s) ppm. ^{13}C-NMR (100 MHz $CDCl_3$): δ 201.7 (d), 193.1 (d), 154.3 (d), 138.1(s), 78.8 (d), 60.2 (d), 48.5 (d), 39.4 (s), 37.3 (t), 36.5 (s), 28.4 (q), 27.0 (t), 25.8 (q x 3), 25.2(t), 18.0 (s), 15.8 (q), 15.3 (q), −3.9 (q), −5.0 (q) ppm. ES-MS (*m/z*) = 365 (M+H$^+$), 233.

Synthesis of 3(*S*)-hydroxypolygodial (2.7): To a solution of **2.80** (23 mg, 0.063 mmol) in CH_3CN (2.3 mL), in a silicon vessel, 48% aqueous hydrofluoric acid (0.64 mL) was added at room temperature. The mixture was stirred at room temperature for 4h and then $NaHCO_3$ was carefully added until the mixture was neutralized. The organic solvent was removed *in vacuo* and the residual aqueous layer was extracted with ethyl acetate. The combined organic extracts were dried (Na_2SO_4), filtered and evaporated *in vacuo*. Flash chromatography of the crude (30→50% ethyl acetate in petroleum ether) under N_2 afforded 11 mg of **2.7** (70%) as a white amorphous solid.
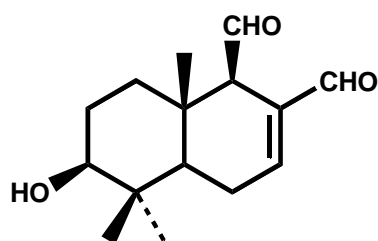
Compound 2.7 [Found: C, 71.82 ; H, 8.98. $C_{15}H_{22}O_3$ requires C, 71.97; H, 8.86] R_f (50% EtOAc/hexane) 0.23; $[\alpha]_D^{25}$ = −77.7 (c = 0.5, $CHCl_3$). IR ($CHCl_3$, cm^{-1}) = ν 3508, 2968, 2938, 2860, 2732, 1722, 1681. ^{1}H-NMR ($CDCl_3$, 400

MHz):δ 9.51 (1H, d, J = 4.4 Hz), 9.47 (1H, bs), 7.16–7.14 (1H, m), 3.30 (1H, dd, J = 11.0, 4.1 Hz), 2.80–2.76 (1H, m), 2.57–2.49 (1H, m), 2.46–2.36 (1H, m), 1.88–1.84 (1H, m), 1.73–1.59 (2H, m), 1.52–1.49 (1H, m), 1.27–1.23 (1H, m), 1.04 (3H, s), 0.94 (3H, s), 0.93 (3H, s) ppm. ^{13}C-NMR (100 MHz, CDCl$_3$):☐☐☐ 201.6 (d), 193.0 (d), 154.0 (d), 138.1 (s), 78.3 (d), 60.0 (d), 48.4 (d), 38.8 (s), 37.3 (t), 36.5 (s), 27.9 (q), 26.7 (t), 25.0 (t), 15.4 (q), 15.2 (q) ppm. ES-MS (*m/z*) = 289 (M+K$^+$), 273 (M+Na$^+$), 251 (M+H$^+$).

Synthesis of 3β-acetoxypolygodial (2.8): To a solution of **2.7** (5.8 mg, 0.023 mmol) in CH$_2$Cl$_2$ (1 mL) acetic anidride (2.8 µL, 0.030 mmol) was added. The solution was cooled to 0°C. and dry NEt$_3$ (3.8 µL, 0.028 mmol) and DMAP, in catalytic amount, were sequentially added. The solution was warmed to room temperature and stirred for 1h. The reaction mixture was quenched with HCl 0.2N (1mL) and stirred for few minutes. The organic and aqueous layers were separated and the last one was extracted with CH$_2$Cl$_2$. The resulting organic layer were washed with H$_2$O and the resulting aqueous layer was extracted again with CH$_2$Cl$_2$. The combined organic extracts were dried over Na$_2$SO$_4$, filtered and concentrated in *vacuo*. Flash chromatography of the crude (20% - 70% AcOEt / petroleum ether) under N$_2$ afforded 4.2 mg of **2.8** (62%) as a white amorphous solid.
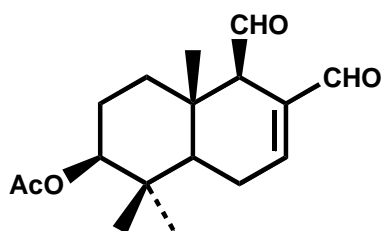
Compound 2.8 [Found: C, 69.98 ; H, 8.39. C$_{17}$H$_{24}$O$_4$ requires C, 69.84; H, 8.27]; R$_f$ (50% EtOAc/hexane) 0.60; $[\alpha]_D^{21}$ = −17.0 (c = 0.2, CHCl$_3$). IR (CHCl$_3$, cm^{-1}) = ν 2967, 1726, 1682, 1644, 1243. ^{1}H-NMR (CDCl$_3$, 400 MHz): δ 9.53 (1H, d, J = 4.3 Hz), 9.48 (1H, s), 7.14–7.12 (1H, m), 4.55 (1H, dd, J =11.2, 4.3 Hz), 2.82–2.80 (1H, m,), 2.56–2.48 (1H, m), 2.46–2.40 (1H, m), 2.06 (3H, s), 1.91–1.88 (1H, m), 1.78–1.58 (3H, m), 1.35 (1H, dd, J = 11.6, 4.9 Hz), 1.00 (3H, s), 0.96 (3H, s), 0.92 (3H, s) ppm. ^{13}C-NMR (75 MHz CDCl$_3$): δ

201.3 (d), 192.9 (d), 170.7 (s), 153.3 (d), 138.2 (s), 79.7 (d), 59.9 (d), 48.6 (d), 37.7 (s), 37.0 (t), 36.4 (s), 27.9 (q), 24.7(t), 23.2 (t), 21.2 (q),16.5 (q), 15.2 (q) ppm. ES-MS (m/z) = 315 (M+Na$^+$), 293 (M+H$^+$).

Synthesis of compound 2.9 To a solution of 3-β-hydroxypolygodial **25** (7.1 mg, 0.03 mmol), in CH$_2$Cl$_2$ anhydrous (0.4 ml) at room temperature and under N$_2$ p-methoxycinnamic acid **2.23** (16 mg, 0.09 mmol)was added. The mixture was cooled to 0 °C. and a solution of DCC (1 M in CH$_2$Cl$_2$, 0.14 mmol, 0.14 mL) and DMAP (0.73 mg, 0.006 mmol) were added The mixture was warmed to room temperature and left for 3h under stirring. The mixture was then filtered on silica using Ethyl Acetate.

Flash cromatography of the crude15% →50% of ethyl ether in Petroleum ether affoded 7.0 mg(57%) of **2.9** as white solid.
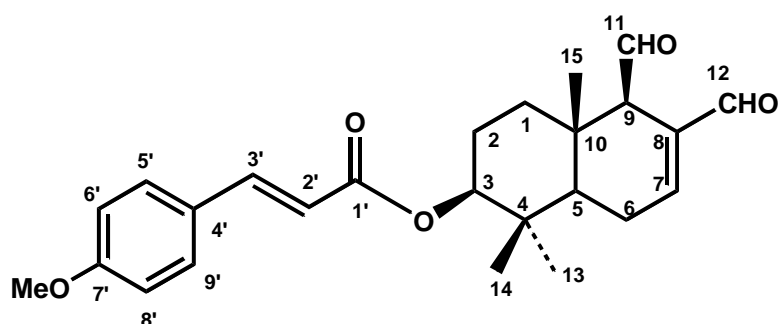
Compound 2.9 ^{1}H-NMR (CDCl$_3$, 400 MHz): δ 9.55 (1H, d, J= 4.1 Hz, H-11), 9.49 (1H, s, H-12), , 7.64 (1H, d, J= 15.9Hz, H'-3), 7.48(2H, d, J=8.7Hz, H'-5 e H'-9), 7.14 (1H,m, H-7), 6.90 (1H, d, J=8.7 Hz, H'-7), 6.30 (1H, d, J= 15.9 Hz, H'-2), 4.68 (1H, m, H-3), 3.34 (3H, s, OCH$_3$), 2.85 (1H, m, H-9), 2.57 (1H, m, H-6 o H'-6), 2,45 (1H, m, H-6 o H'-6), 1.94 (1H,m, H-1 o H'1), 1.85-1.66 (3H, m, H-2 e H'-2, H-1 o H'-1), 1.43 (1H, dd, J= 11.5, 4.9 Hz, H-5), 1.10 (3H, s, CH$_3$), 1.01(3H, s, CH$_3$), 0.99 (3H, s, CH$_3$).

^{13}C-NMR (75 MHz CDCl$_3$): δ 201.3 (d), 192.9 (d), 169.9 (s), 161.4 (s), 156.7 (s), 153.4 (d), 144.5 (d), 143.4 (s), 138.2(s), 129.7 (d), 127.1 (s), 115.7 (d), 114.3 (d), 79.5 (d), 59.9 (d),55.5 (q), 48.6(d), 38,0(s), 37.0(t), 36.4 (s), 28.0 (q), 24.8(t), 23.4(t), 16.7(q), 15.3(q).

$[\alpha]_D^{25}$ = -7.0 (c = 0.5, CHCl$_3$).

ES-MS (m/z)= 433.3 (M+Na$^+$), 411.4 (M+H$^+$).

CHAPTER 3

APPROACH TO THE SYNTHESIS OF DITERPENOIDIC AND SESTERTERPENOIDIC DIALDEHYDES

3.2 **OUTLINE**

In this chapter an approach to the enantioselectve syntheses of both diterpenoidic and seserterpenoidic unsaturated dialdehydes 3-hydroxy isocopalendial **3.1** and of 3-hydroxyscalaradial **3.2** is described. (**Figure 3.2**) Compounds **3.1** and **3.2** are structural analogues of occurring natural product and ent-isocopalendial1.5, scalaradial 1.6 and deacethoxyscalaradial 1.7. (**Figure 3.1**)
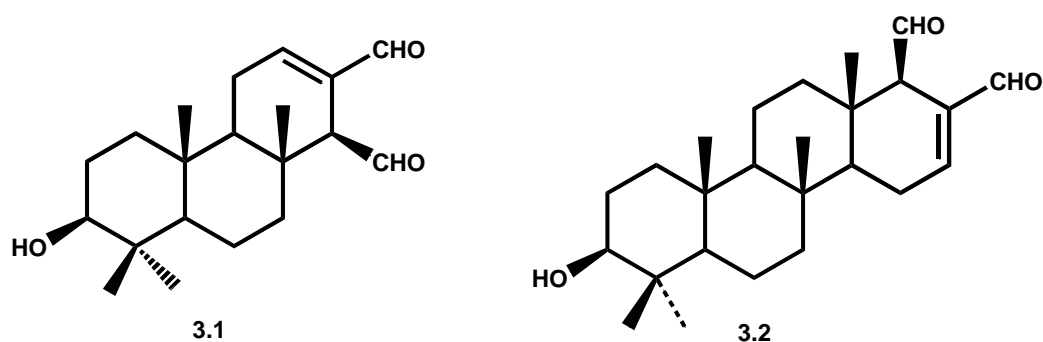
Figure 3.1 Our Synthetic Targets
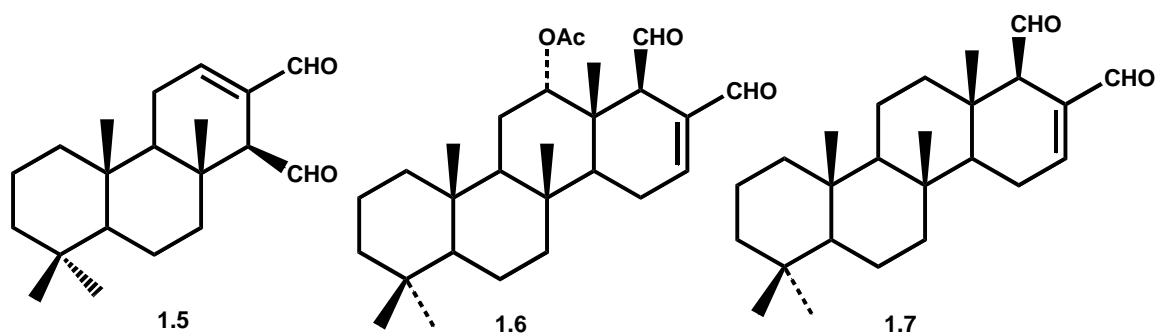
Figure 3.2 Some natural occurring *ent* isocopalane and scalarane compounds

In order to prepare the epoxypolyenes chains we developed an elongation chain protocol that allowed us to prepare epoxypolyene chains starting from cheap starting materials, to introduce a chiral epoxide at the beginning of our route and that is a versatile method to prepare any wanted length chains.

3.1.1 *PREPARATION OF EPOXYPOLYENES CHAINS: PRELIMINARY STUDIES*

In order to prepare dialdehydes with isocopalic or scalaranic skeleton we thought to use as key step the radical cyclisation previously used to prepare C-3 functionalised drimane (*see chapter 2*), but in these cases we need to build the epoxypolyene precursors. (**Scheme 3.1**)
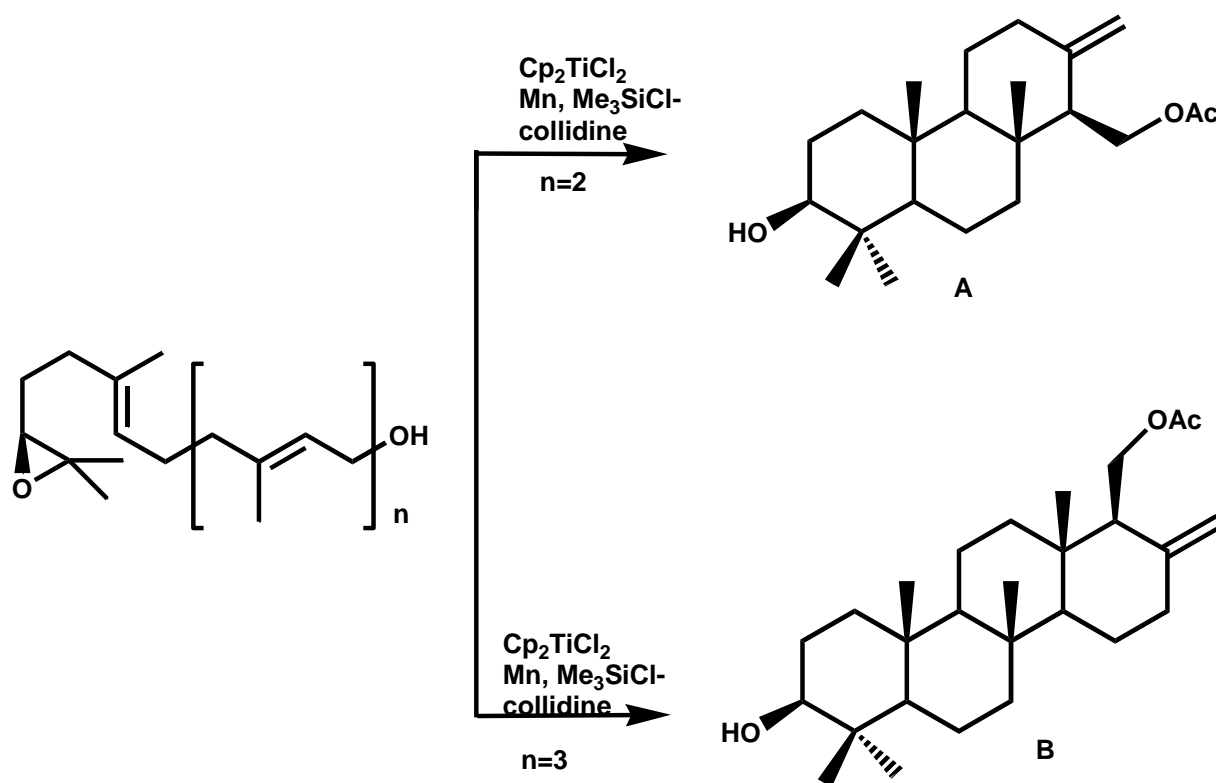
SCHEME 3.1

In fact, the acyclic starting material from which to prepare C 20 epoxide (n=2 ; **Scheme 3.1**) is geranylgeraniol **3.5**, a very expensive diterpene (1g = 472 € , Sigma Aldrich) and a proper acyclic chain from which to prepare C 25 epoxide (n=3 ; **Scheme 3.1**) sesterterpene is not commercially available.

In addition, we needed to introduce a chiral pathway at the beginning of our synthetic route and, as previously described in C-3 drimane functionalized synthesis, we achieved it *via* Sharpless asymmetric dihydroxylation on a proper length polyene precursor. (in **Scheme 3.2** the asymmetric induction is shown for synthesis of **2.59**, C15 chiral epoxide)
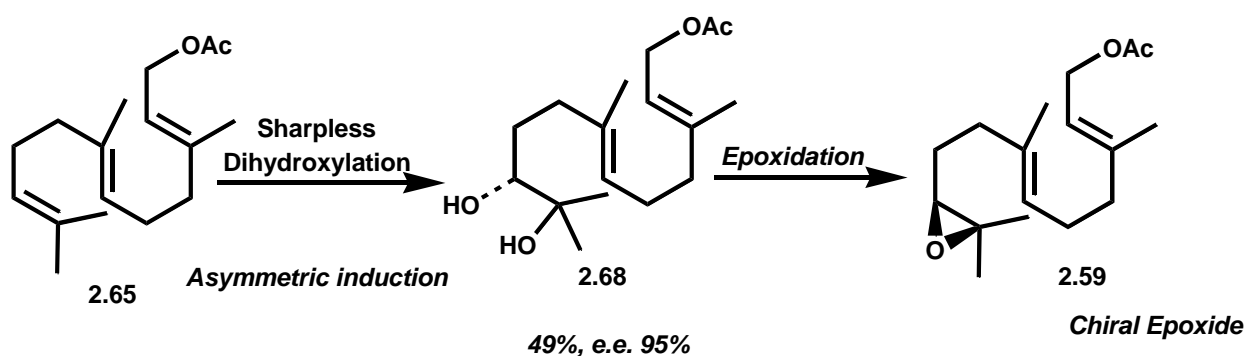
SCHEME 3.2 *Asymmetric induction for synthesis of **2.59**, C15 chiral epoxide*

Furthermore, Sharpless dihydroxylation is not a chemospecific reaction. In fact, this reaction furnishes dihydroxylate products deriving from each double bond in the molecule (the allylic one is unreactive due to electron influence on the acetyl group).

For instance, Sharpless asymmetric dihydroxylation performed on farnesylacetate **2.65** gave a mixture of diols: we obtained the wanted $\Delta^{10,11}$ diol **2.68**, $\Delta^{6,7}$ diol **3.3** coming out from the dihydroxylation of $\Delta^{6,7}$ double bond and **3.4** coming out from dihydroxylation of both $\Delta^{6,7}$ and $\Delta^{10,11}$ double bonds. In addition we recovered starting material **2.65**. (**Scheme 3.3**)
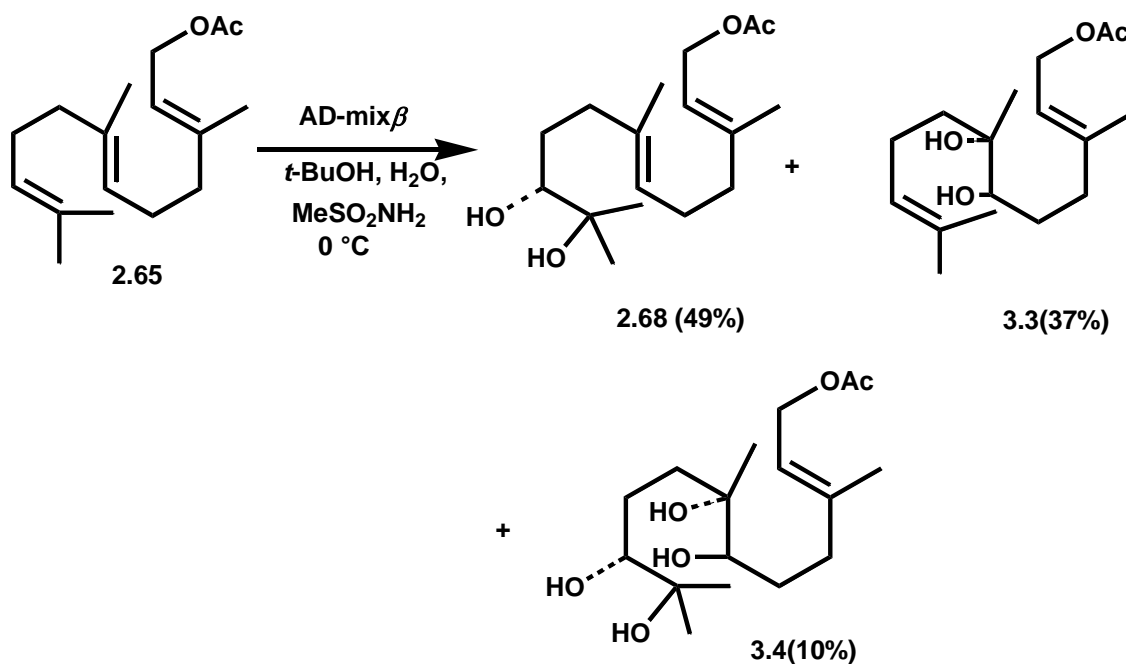
SCHEME 3.3

The same reaction performed on the expensive C 20 geranylgeraniol **3.5** gave the wanted diol **3.7** as major product, in even low yield. (**Scheme 3.4**)
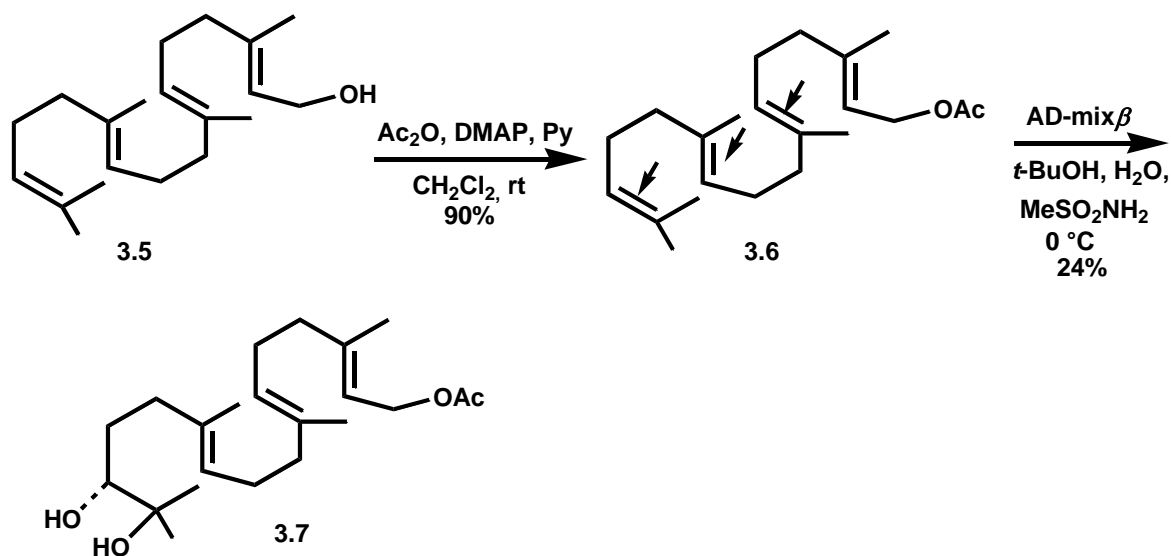
SCHEME 3.4

Dihydroxylation performed on cheaper (1g 7€ ;Sigma Aldrich) geranylacetate **3.8** give the wanted diol **3.9** with both high yield and ee. (**Scheme 3.5**)
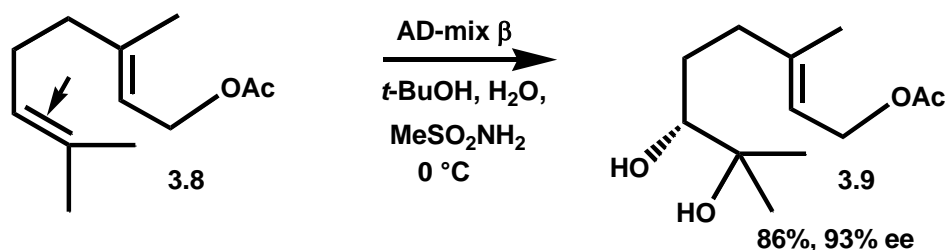
SCHEME 3.5

The most convenient way should give the chiral epoxide in excellent yield, high *ee* using a cheap starting material. All these condition are satisfied in the preparation of geranyl epoxide so we decided to use geranylacetate (**3.8**) as starting material to build the needed epoxipoliene by elongation procedure.

In order to develop a proper elongation chain protocol to prepare both C 20,C 25 polienic chain, to perform the radical cyclisation, we needed to plan a versatile method that allows the introduction of a chiral epoxide at the beginning of the route and store up it until the end.

Preparation of polyene chains is a very common topic in literature and we envisioned two interesting strategies:

- a convergent synthesis performed using as key step an *allyl-allyl* coupling between two polienic fragments[68]

- a linear elongation chain strategy, performed with ethylacetoacetate as a C-5 building block [69].

We adapted these routes to our purposes introducing, *via* asymmetric induction, a chiral epoxide that will be useful, once obtained the proper length chain, to perform our radical cyclisation.

[68] Yu,X.Z.; Zhang,H.; Jun Xiong,F.; Xiang Chen,X.; Chen. F. *Helvetica Chimica Acta* **2008**, 91 , 1967.

[69] Jin,Y.; . Roberts,F.; t M. Coates.R. *Org. Synth.* **2007**, *84*, 43.

3.2 *A CONVERGENT SYNTHESIS OF EPOXYPOLYENE CHAINS*

At the beginning of this investigation about elongation chain methods, it has been planned a convergent synthesis to prepare the wanted epoxypolyenes chains.

This route allows to prepare the wanted chain using an *allyl-allyl* coupling between two synthons, both prepared from geranylacetate, a very cheap substrate.

The planning is to prepare two C-10 building block, one prepared introducing at first a chiral epoxide and then an EWG group, as we have the nucleophilic moiety after deprotonation, the second one containing an allylic bromide. It should react with the nucleophilic moiety coming from deprotonation on acidic α protons in the first synthon.

3.2.2 *RETROSYNTETIC ANALYSIS*

The synthesis of the C20 epoxypolyprenyl **2.61** chain is illustrated in **Scheme 3.6**.The convergent synthetic strategy features the iterative *allyl-allyl* coupling of monomers easily derived from geranilacetate (**3.8**).

The first monomer contain a chiral epoxide and an EWG group that makes acide its α protons (**3.10**), the second one contain one allylic bromide **3.11**. It should react with the nucleophilic moiety coming from deprotonation on acidic α protons in **3.10**.

As EWG group we thought to introduce a p-toluensulfone easily prepared via bromination perfomed on epoxigeraniol **3.12**. The epoxide **3.12** was prepared *via* asymmetric catalysis from (all-E)-geranylacetate.

The second monomer **3.11** was obtained from (all-E)-geranylacetate **3.8** *via* allylic oxidation of terminal methyl group.

At the end, the repetitive reductive elimination of the p-toluenesulfonyl (Ts) groups should lead to the wanted epoxypoliene chain **2.61**.

2.61

3.10   +   **3.11**

3.12   **3.13**

3.8
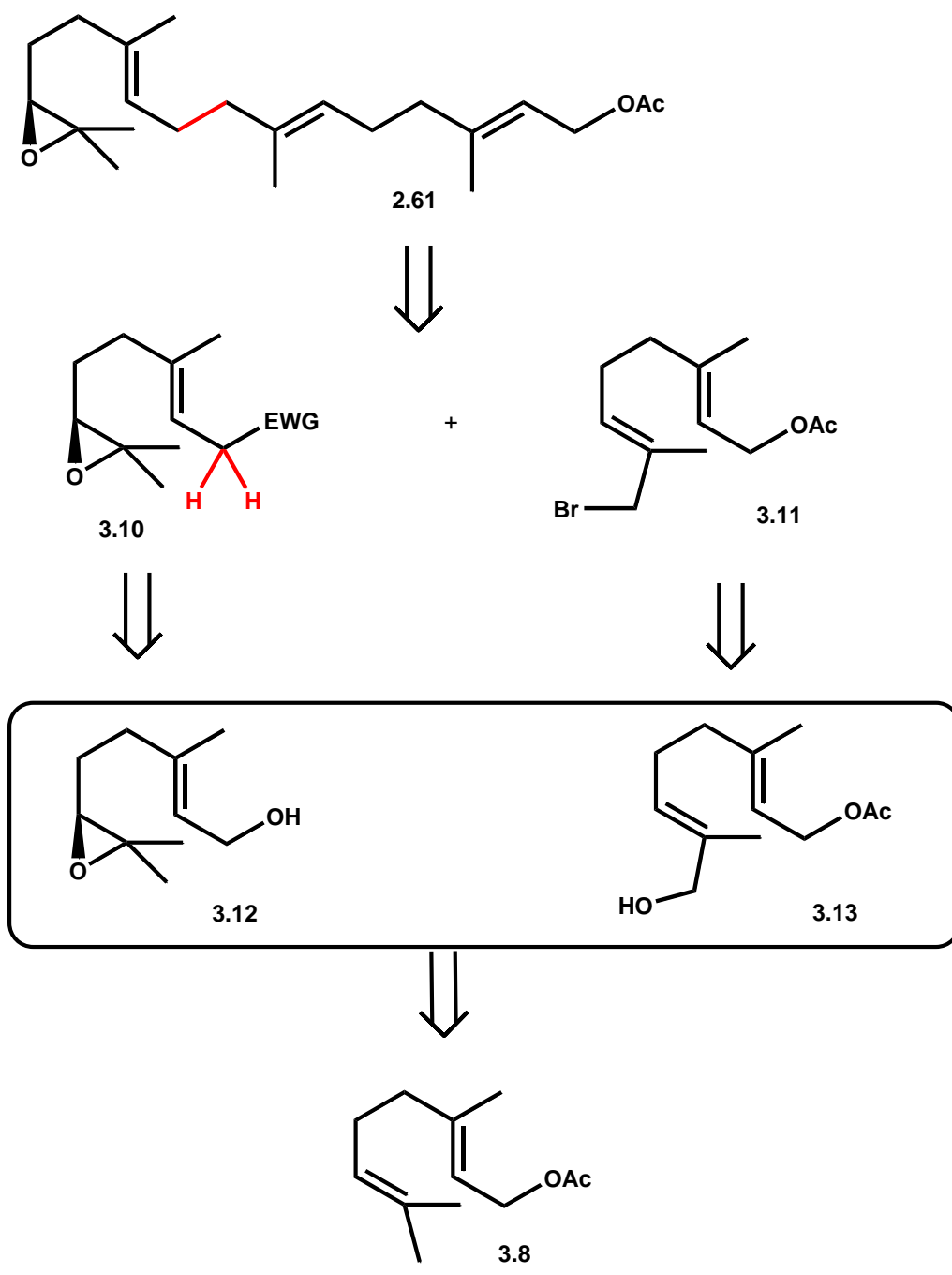
SCHEME 3.6

3.2.2 *SYNTHETIC SECTION*

This section is dedicated to preparation of the two C-10 fragments **3.10** and **3.11** in order to achieve the allyl allyl coupling to obtain the C-20 epxipolyene **2.61**.

3.2.3 *PREPARATION OF SYNTHONS 3.16 AND 3.11*

In order to achieve an enantioselective synthesis of epoxypolyenes chains, we introduced the chirality at the beginning of our synthetic route with Sharpless asymmetric dihydroxylation. This reaction performed on geranyl acetate **3.8** gave the diol **3.14** as single product with hight *ee.* The enantiomeric excess was determined as 93% by 1H NMR analysis of the corresponding mono-(S)-MTPA ester of diol.[70]

AD-mix β is a mixture containing $K_2OsO_2(OH)_4$ as source of OsO_4, $K_3Fe(CN)_6$, as oxidant, K_2CO_3 and the chiral catalyst $(DHQD)_2PHAL$.

The diol **3.14** was converted into (*S*)-2,3-epoxygeraniol (**3.12**) with a mesylation of the secondary alcohol followed by an S_N2 ring closing. Removal of acetyl group occurs for the basic conditions of the ring closing step. (**Scheme 3.7**)
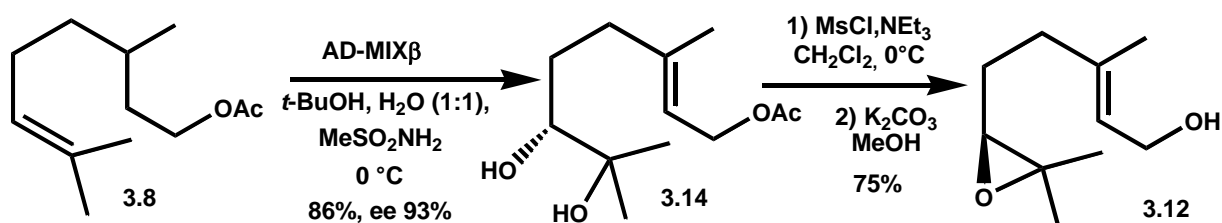
SCHEME 3.7

---

[70] Huang, A. X.; Xiong, Z.; Corey, E. J. *J. Am. Chem. Soc.* **1999**, 121, 9999-10003.

The (*S*)-2,3-epoxygeraniol **3.12** was converted into corresponding bromide **3.15** by mesylation of the primary alcohol and then by treatment with a solution 2M of LiBr in THF. (**Scheme 3.8**)

The allylic bromide **3.15** is a very instable compound, It must be used as crude because it is prone to decomposition on silica and it has to be stored at -20 °C not more than one night.

The obtained bromide **3.15** was treated, as crude, with sodium p-toluen sulfinate PhMeSO$_2$⁻Na⁺ to afford sulfone **3.16**, the nucleophilic synthon required by this convergent protocol.
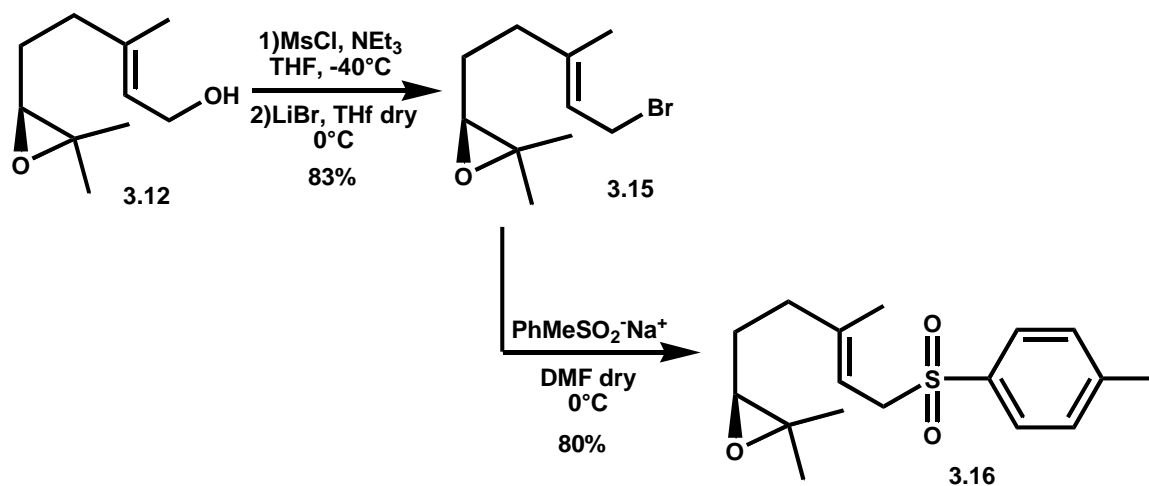
SCHEME 3.8

The second building block was obtained performing the oxidation of the terminal methyl group in geranylacetate **3.8** .

The mechanism proposed for this reaction (*Sharpless et al*)[71] is an addition of selenium oxide to the double bond in **3.17** forming an allyl selenic acid **3.18**. This undergo to a sigmatropic rearrangiament to give a selenium (II) ester **3.19** that seems to be more stable. Carbonyl products **3.20** formed in SeO_2 oxidations may in part arise directly from the selenium (II) ester. The main product comes from hydrolysis of the Se-O bond in the selenium (II) ester and is the allilic alcohol **3.21** (**Scheme 3.9**)
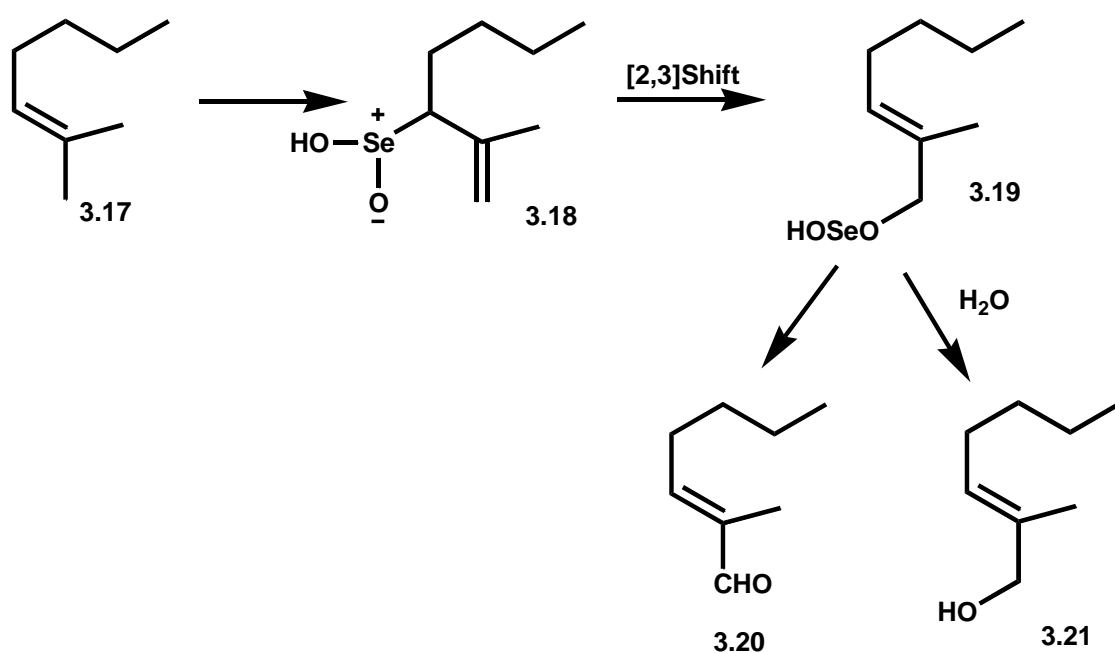
SCHEME 3.9 - *Allylic oxidation: Sharpless proposed mechanism*

We performed this reaction on geranylacetate **3.8** with selenium dioxide and *tert*-butylhydroperoxide in presence of salycilic acid. This reaction afforded the wanted allylic alcohol **3.13** and a small amount of an α,β unsatured aldehyde **3.21**.

[71] K. B. Sharpless, R. *F.* Lauer *Journal of the American Chemical Society* **1972***,* 7154.

The allylic alcohol obtained was converted into bromide **3.10** by mesylation and then by treatment with a 2M solution of LiBr in THF. (**Scheme 3.10**)
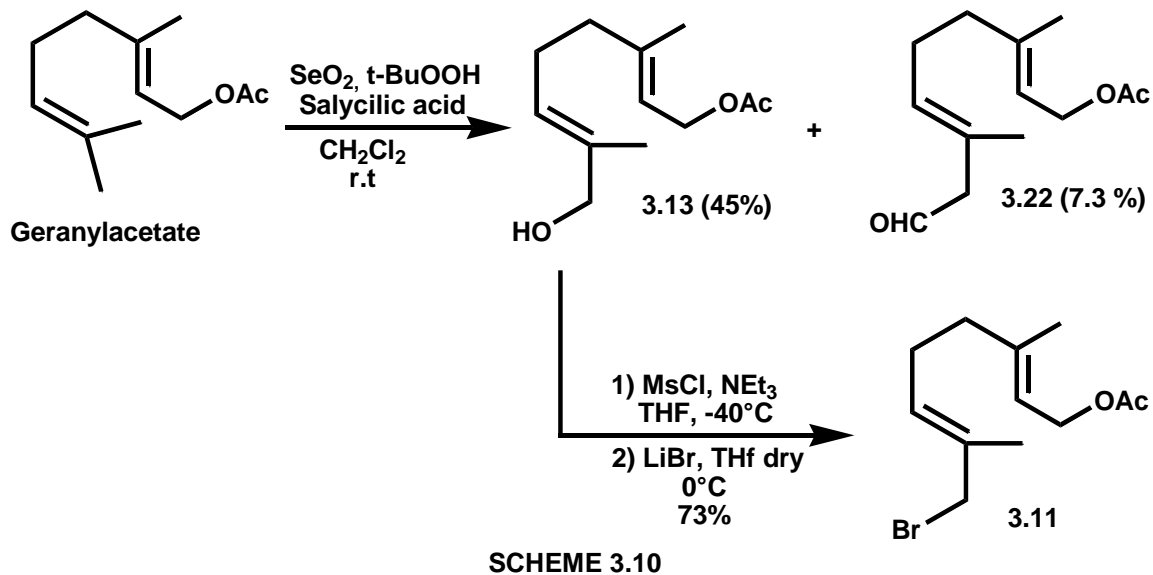
SCHEME 3.10

3.2.4 *ALLYL ALLYL COULING AND REDUCTIVE DESULFONYLATION*

With the two monomers **3.16** and **3.11** in hand we performed the *allyl allyl* coupling using KHMDS as a non nucleophilic base. The reaction gave the C20 skeleton **3.23** with 65% of yield. (**Scheme 3.11**)

SCHEME 3.11
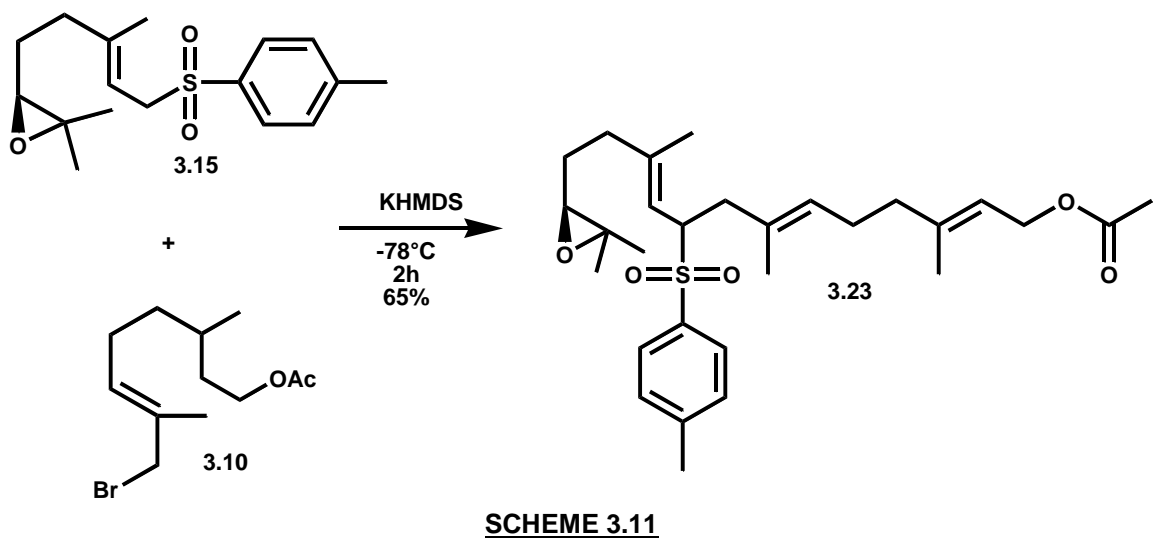
Once obtained this fragment **3.23** we attempt to eliminate the sulfonyl group. We checked in literature about methods to perform this reaction and we found that the best one for our purposes uses $Li(C_2H_5)_3BH$ (Super Hydride) in presence of 10% of $Cl_2Pd(dppp)$.

In presence of an acetyl group in the molecule this system directs the reaction to the removal of the acetyl group (**3.24**), in addition we recover 33% of starting material (**3.23**). No desulfonilated compound has been observed. (**Scheme 3.12**)
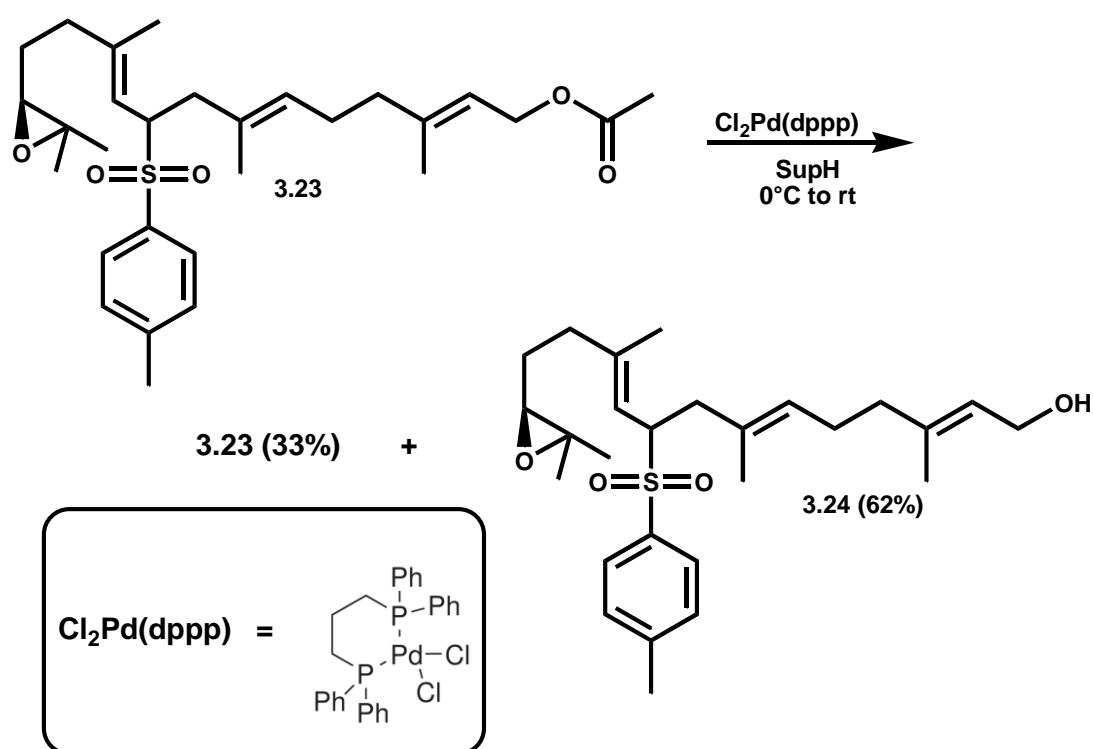
SCHEME 3.12

Therefore, to perform this reaction we need to remove the acetyl group from the molecule and we achieved it using basic conditions (K_2CO_3 and a mixture of MeOH and H_2O as solvent).[72] The reaction gave only the deacetilated sulfone in good yield without further purification. (**Scheme 3.13**)
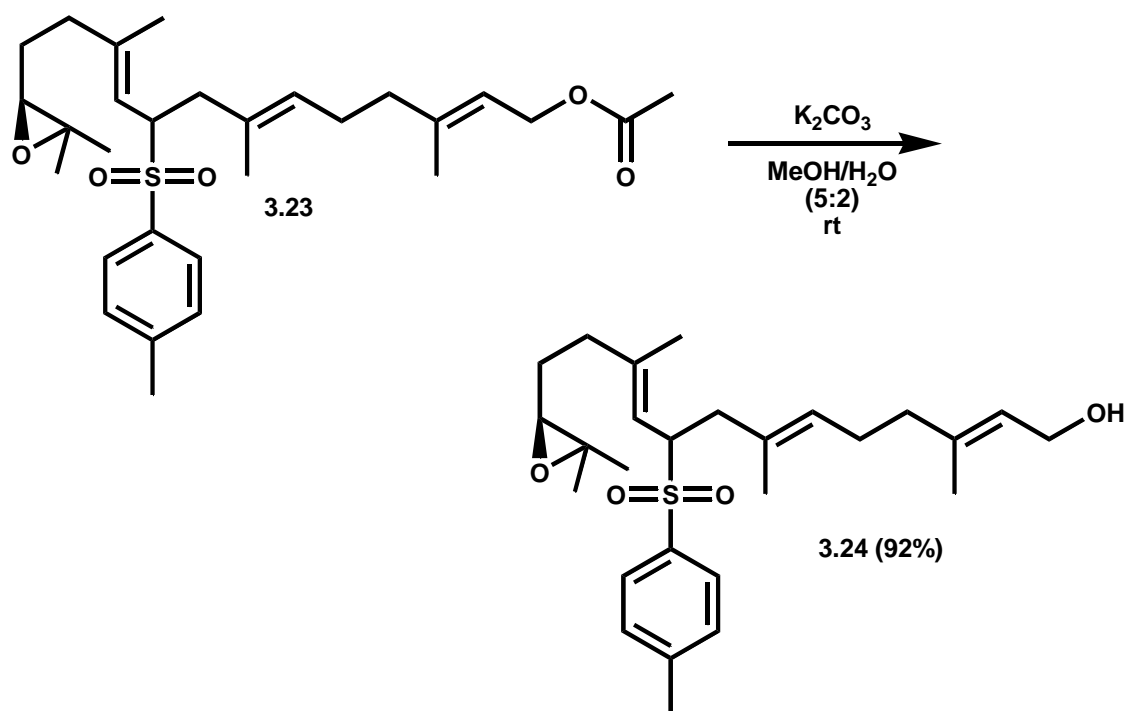
SCHEME 3.13

Desulfonilation has then been achieved on the deacetilated molecule and it has been obtained a little amount of desulfonolated product **3.25** with recovered starting material. (**Scheme 3.14**)

---

[72] Minuth, .; Irmak, M .; Groschner, A.; Lehnert, T.; and Boysen M. *Eur. J. Org. Chem.* **2009**, 997.
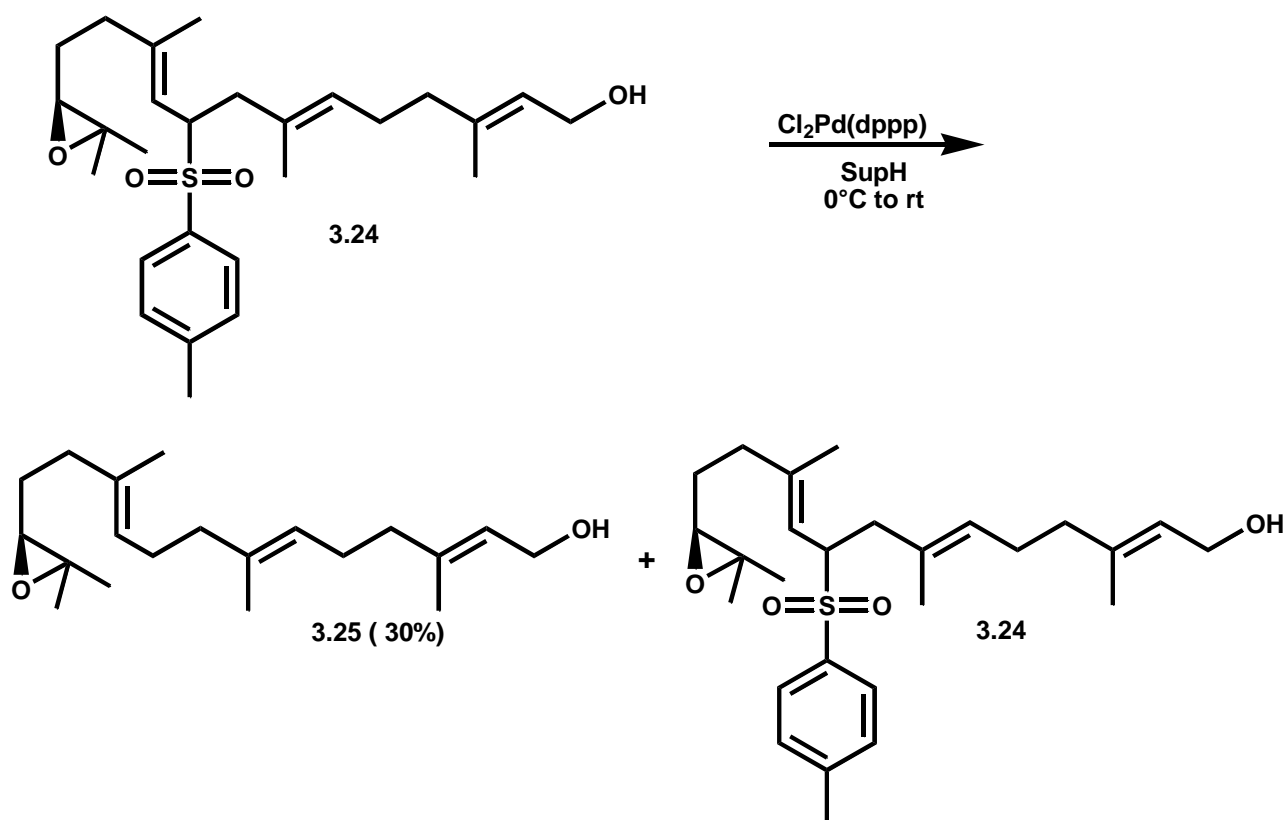
SCHEME 3.14

In order to optimize the reaction some conditions (like temperature and amount of catalyst and Super Hydride) have been changed, without good results.

Thus, we thought to perform the radical cyclistation directly on the sulfonilated substrate.

in literature some studies about cationic cyclisations performed on *all E* polyprenols having a benzensulfonyl moieties in the framework are reported.[73]

One of these showed polyprenols chains of different lengths containing two consecutive prenyl sulfone moieties at the tail end and these chains underwent to controlled electrophilic cyclization only at the carbon-carbon double bonds that were remote from the flat and rigid benzenesulfonyl groups.[5b]

[73] a) Kulcilki, V; Ungur N, and F.Vlad, P.. *Tetrahedron* **1998,** 54, 11925.b) Kuk, J; Kim, Heejung, B; Jung, S.; , Park, J. *J. Org. Chem.* **2008**, *73*, 1991.

In addition a mixture of isomers with two different location of double bond is reported. (**Scheme 3.15**)
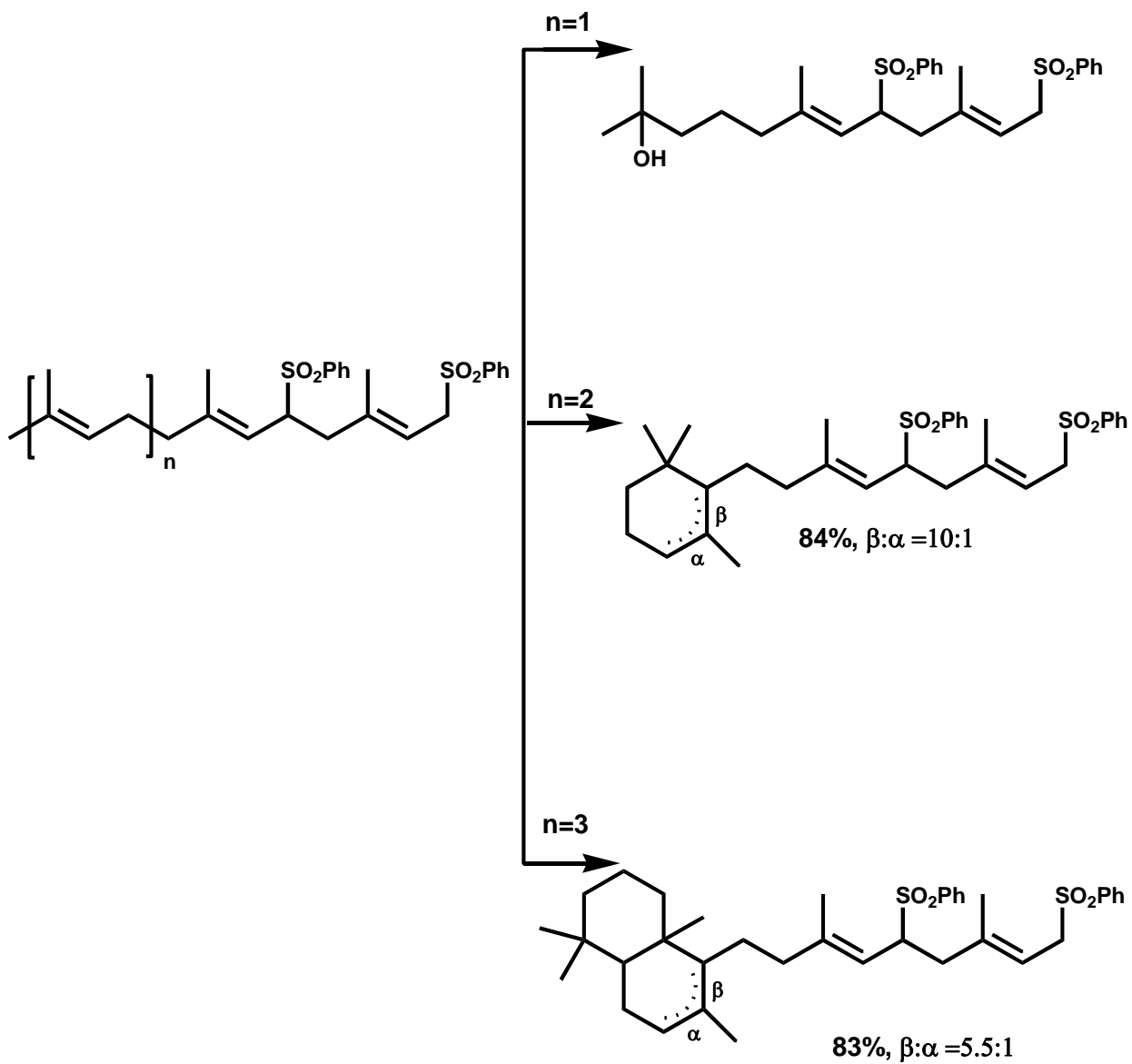
SCHEME 3.15

It has been concluded that a sulfonyl group in the molecule could inhibit the cyclisation so it has been considered a linear approach to synthesize the epoxypolienic chain.

This convinced us that convergent way was not suitable for our purposes, so we turned to a linear elongation approach.

3.3 <u>A LINEAR SYNTHESIS OF C-20 AND C-25 EPOXYPOLYENIC CHAINS.</u>

The second way to prepare C 20 C 25 chains is a synthetic route that allow us to adding isoprenic moiety trough a proper elaboration of epoxygeranylacetate **3.12**.

Although numerous methods for isoprenoid chain extensions have been envisioned,[74] We considered the using of Weiler's acetoacetate dianion alkylation-enol phosphate coupling methods, a procedure that effects isoprene chain extension of (*E, E*)-farnesol with high E selectivity at both the 2,3 and 6,7 double bonds, satisfactory overall yield.[75]

We adapted this route to our pourposes using geranylacetate **3.8**, as starting material in order to obtain both high yield and *ee* with asymmetric induction.

3.3.2 <u>RETROSYNTHETIC ANALYSIS</u>

In the retrosynthetic scheme developed it has been shown that both C_{20} and C_{25} chains could be obtained with a progressive addition of isoprenic unity.

The isocopalic dialdehyde **3.1** comes from elaboration of **2.62**, obtained from acyclic precursor **2.61** by radical cyclisation. Compound **2.61** has been obtained by progressive elongation chain performed starting from C_{10} alcohol **3.26**.

3.26 has been obtained from β keto ester **3.27** by elaboration of its β carbonyl group and then reduction of ester moiety.

---

[74] Cainelli, G.; Cardillo, G. *Acc. Chem. Res*. **1981**, *14*, 89.

[75] a) Sum, F. W.; Weiler, L. *Can. J. Chem.* **1979**, *57*, 1431. b) Sum, F. W.; Weiler, L. *Tetrahedron*, **1981**, *37*, 303-317. c) Alderdice, M.; Sum, F. W.; Weiler, L. *Org. Synth. Coll. Vol. 7*, 351-356. d). Eis, K.; Schmalz, H.-G. *Synthesis* **1997**, 202-

3.27 is a key intermediate of the elongation chain protocol and it has been obtained by a coupling performed between bromide **3.26** and ethyl acetoacetate The bromide was obtained from the corresponding epoxi alcohol, obtained with asymmetric catalysis from geranylacetate **3.8**. (**Scheme 3.16**)
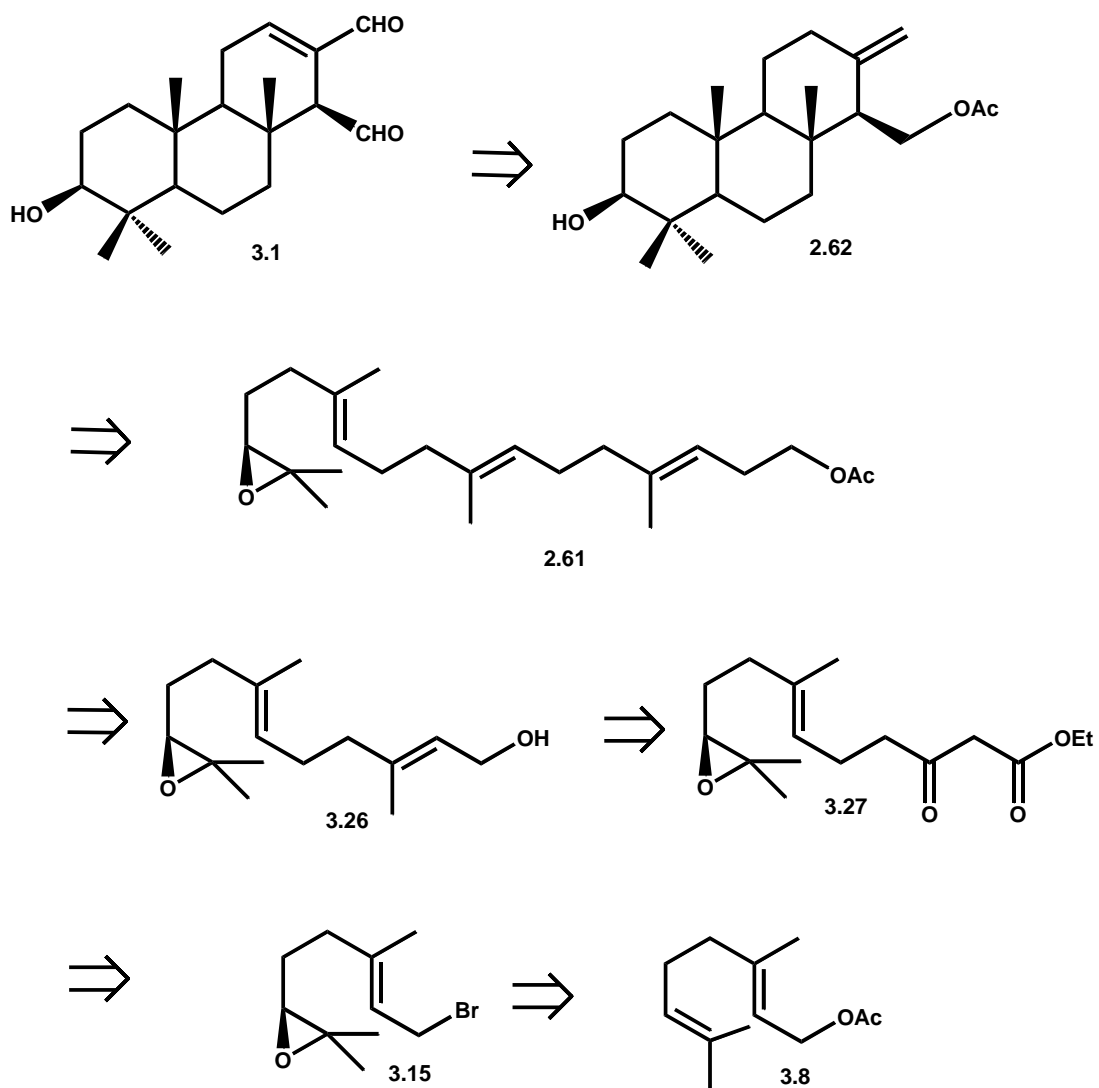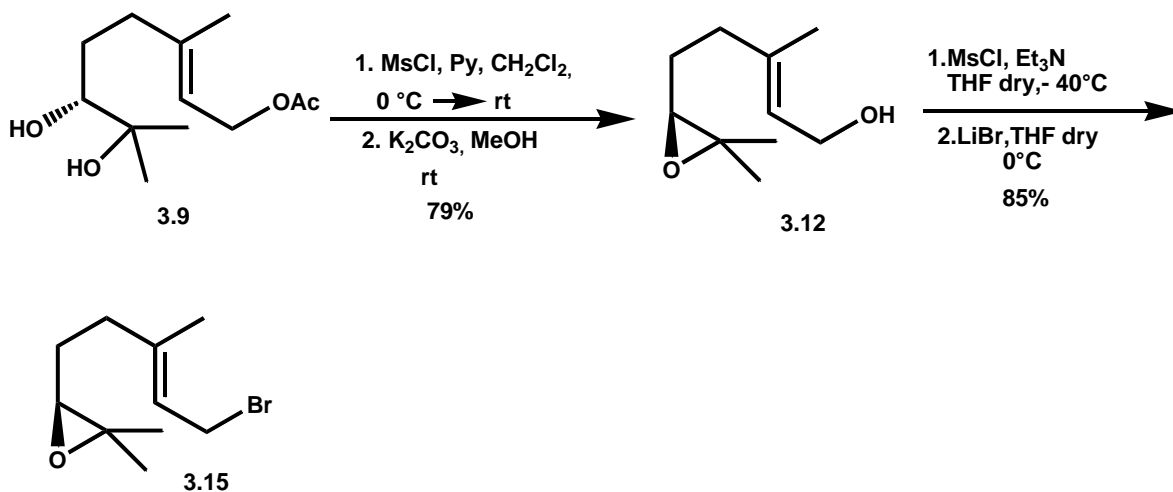

SCHEME 3.16

3.3.2 <u>SYNTHETIC SECTION</u>

As we planned, we induced the chirality at the beginning of the synthesis with an asymmetric dihydroxilation of geranylacetate **3.8** achieved using the proper chiral catalyst that afforded the wanted diol **3.9** with a 83% of yield and 95% of *ee.* (**Scheme 3.17**)
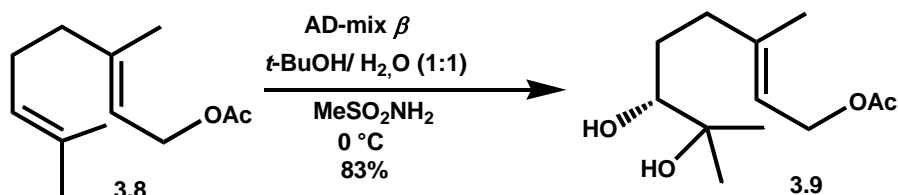
<u>SCHEME 3.17</u>

The diol was converted into (*S*)-2,3-epoxygeraniol by mesylation of the secondary alcohol followed by an SN_2 ring closing to afford the epoxide **3.12.** Removal of acetyl group occurs due to basic conditions of the ring closing step. The (*S*)-2,3-epoxygeraniol was converted into corresponding bromide (**3.15**) by mesylation of the primary alcohol and then by treatment with LiBr in THF. (**Scheme 3.18**). This allyl bromide is a very instable compound, it is used as crude because it is prone to decomposition on silica and it has to be stored at -20°C not more than one night.

<u>SCHEME 3.18</u>

Then we perform the key reaction to make the chain elongation. This reaction is carried out using ethyl acetoacetate as building block by its conversion into corresponding dianione by reaction with sodium hydride first and then butyllithyum to deprotonated the methyl group.

The dianion reacts with the allylic bromide to afford the dicarbonilic compound X as a only product. It is important to remark that the epoxide doesn't take part to this reaction. (**Scheme 3.19**)
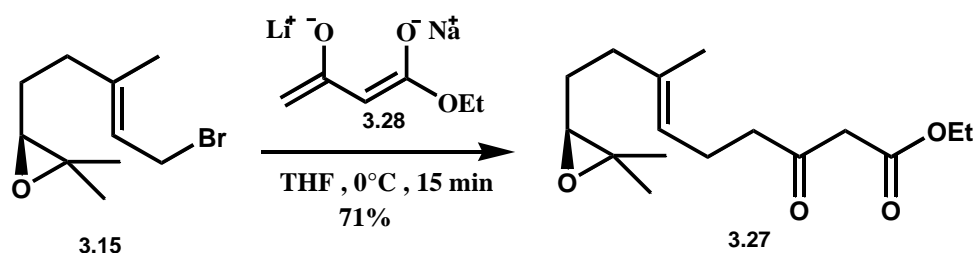
SCHEME 3.19

Once obtained the dicarbonilic compound we need to replace a methyl group instead of the β carbonyl group, For this purpose we considered two strategies :

- *methylation with organocuprate compounds*
- *methylation via sp2-sp3 cross coupling*

3.3.5 METHYLATION WITH ORGANOCUPRATE REAGENTS

In order to introduce the methyl group in **3.27** we activated this compound towards reaction with organocuprate reagents by converting it into corresponding enol phosphate and

The reaction of β-keto ester **3.27** with diethylchlorophosphate gave the wanted product **3.29** in good yield. The enolphosphate **3.29** obtained was used without further purification because it is not stable on silica.

Once obtained this substrate, the coupling reaction to introduce a methyl group was performed.

The proper organocuprate compound Me_2CuLi has been formed in situ by reaction between CuI and MeLi . (**Scheme 3.20**)

Many attempts were carried out, using the copper salt as received, drying[76] or purifying[77] the copper salt CuI, but we didn't have any good result, affording a very unclear mixture inseparable by column.
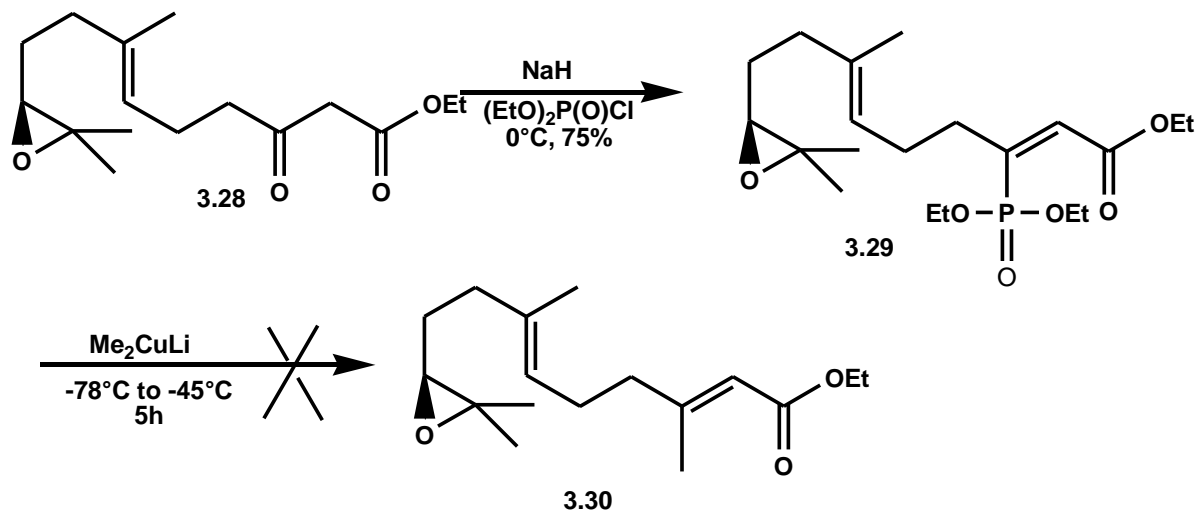
SCHEME 3.20

3.3.6 *METHYLATION VIA sp²-sp³ CROSS- COUPLING*

As second chance to introduce a methyl group in **3.27** we considered an sp^2-sp^3 cross coupling. To perform this reaction we need to convert the β-keto ester **3.27** into corresponding enoltriflate.

As first attempt, the reaction was performed using DIPEA as base and Tf_2O as trifling agent at 0°C,[78] but the reaction was drawn to formation of collateral product: in fact, the epoxide has been both transformed into corresponding ketone by rearrangement and opened to secondary alcohol due to the presence of traces of triflic acid formed in the medium. (**Scheme 3.21**)

[76] Breit, B, Demel, P. *Tetrahedron* **2000**, 56, 2833-2846

[77] Kauffman, G Fang, L. *Inorganic Synthesis* chapter Two-Transition metal complexes and compounds, 101-103 .

[78] Specklin, S.; Bertus,P.; Weibel, J.M.; and Pale, P. *J. Org. Chem.* **2008**, *73*, 7845–7848

3.27

LiOTf
DIPEA
Tf$_2$O
CH$_2$Cl$_2$
0°C

3.31(50%)

+

3.32 (30%)
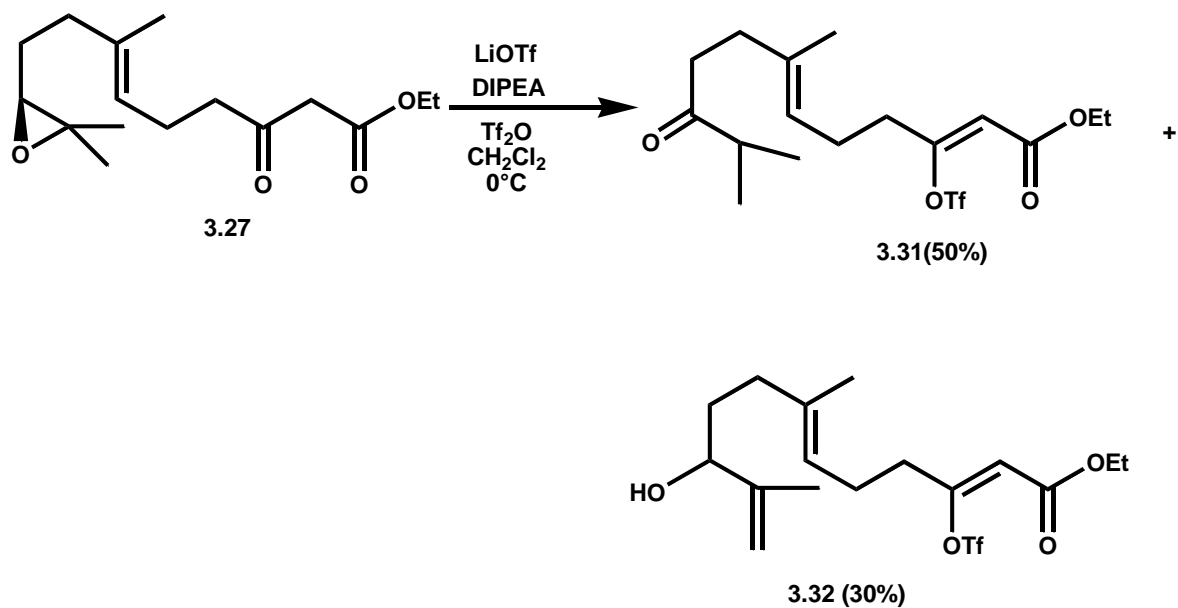
SCHEME 3.21

As second possibility to form enoltriflate we used KHMDS as non nucleophilic base and PhN(Tf)$_2$ (*N*-fenil bis-trifluorometan sulfonimmide) as triflate donor.[79] The reaction, performed at -78°C gave the wanted product in good yield. (**Scheme 3.22**)

3.27

KHMDS
PhN(Tf)$_2$
THF
-78°C → rt
84%

3.33
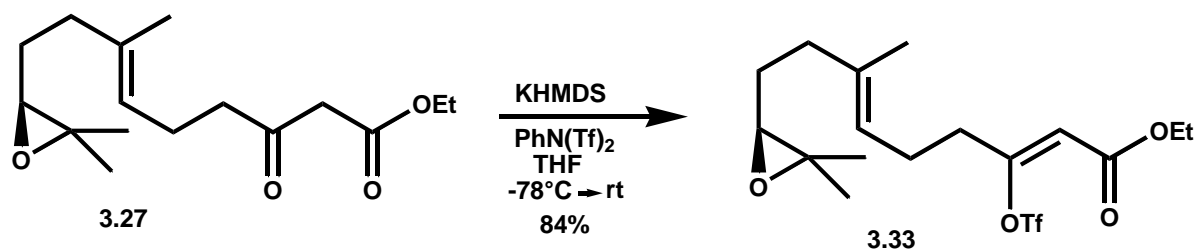
SCHEME 3.22

Once obtained the enoltriflate **3.33** performed Suzuki's cross coupling reaction catalysed by Pd (0) using methylboronic acid B(OH)$_2$Me as donor of methyl group. The palladium complex used is (PhCN)$_2$PdCl$_2$ (bis(benzonitrile)

---

[79] Crisp, G. T.; Meyer, A. G. *J. Org. Chem.* **1992**, 57, 6972.

palladium (II) chloride), which gave the Pd(0) complex Pd(AsPh$_3$)$_4$ reacting in situ with AsPh$_3$.

Ag$_2$O take faster the reaction between boronic acid and vinyl triflate and K$_3$PO$_4$ allows a rapid consumption of triflate.

The reaction performed on **3.33** gave the coupled product **3.34** in good yield.[80] (**Scheme 3.23**)
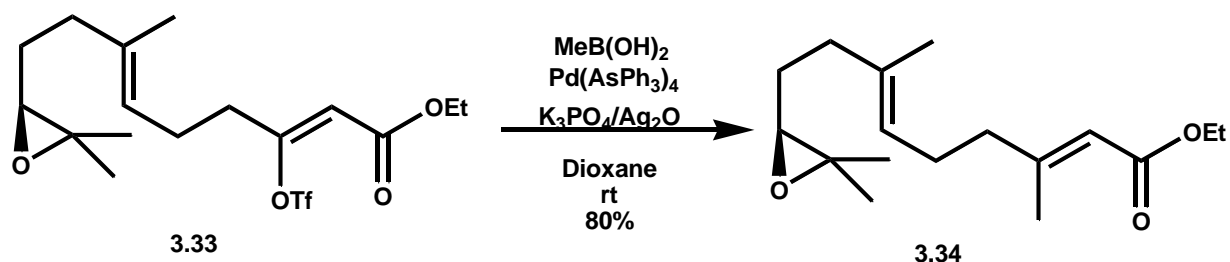
SCHEME 3.23
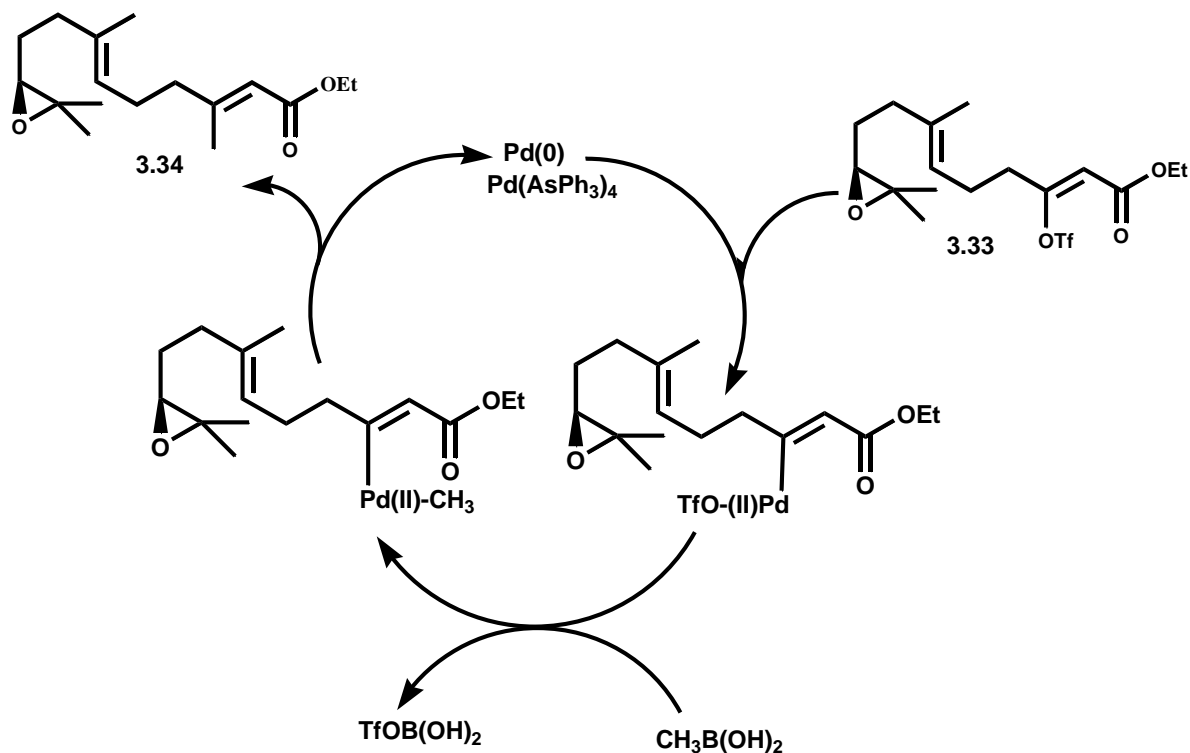
SCHEME 3.24 - *Catalytic Cycle Of Suzuki Cross Coupling*

[80] a) Mu, Y.; Gibbs, R. *Tetrahedron Letters* **1995**, 36, 5669-5672. b) Muay Y.; Eubanks, L.; Poulter, C.; and Gibbs. R. *Bioorg. Med. Chem.* **2002**, 10, 1207–1219.

3.3.5. <u>REDUCTION STEP:A METHODOLOGIC STUDY</u>

In order to obtain the C15 alcohol we need to reduce the conjugate ester group to the corresponding allylic alcohol. To perform this we have to consider the chemoselectivity of the reaction because, in the same framework we have two potential electrophilic sites: the secondary carbon of the epoxide and the ester carbonyl group that is conjugate with a double bond.
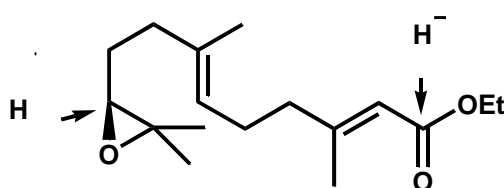
At first, we attempted the reaction with DIBALH, but the reaction performed at different conditions (**Table3.1**) gave the wanted product **3.26**, furthermore epoxide ring opening (**3.35**) occurred in any case. (**Scheme 3.25**)
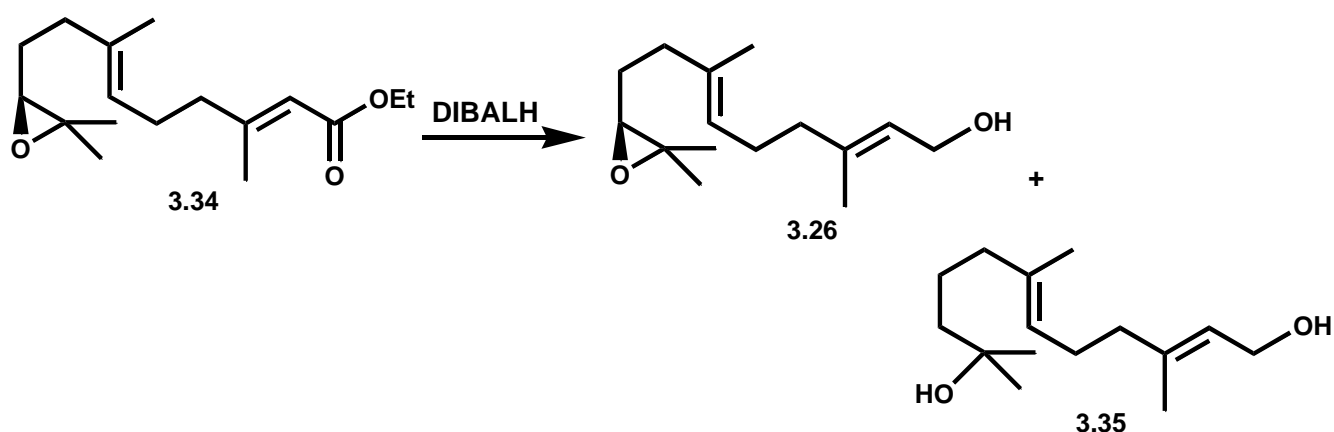
SCHEME 3.25

Table 3.1

Exp	H⁻	eq	T (°C)	Time (min)	Solvent	Yield 3.26 (%)	Yield 3.35 (%)	Yield 3.34 (%)
1	DIBALH	3	-78	30	Toluene	60	24	/
2	DIBALH	2	-78	30	Toluene	38	1.4	41
3	DIBALH	2.5	-78	30	Toluene	45	2.3	13
4	DIBALH	2.5	-20	30	THF	70	20	/

In order to optimize this reaction we decide to prepare a model substrate very similar to the our one, with both an epoxide ring and a $\alpha.\beta$ unsatured ester.
We prepared it starting from geranic acid, we performed a Fisher esterification and then an epoxidation with MCPBA. (**Scheme 3.26**)
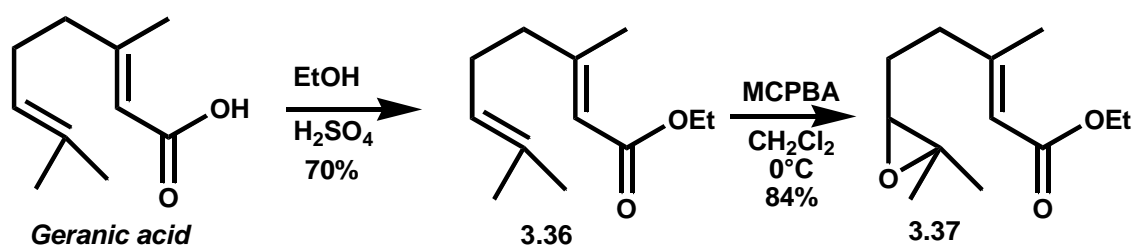
SCHEME 3.26

Once obtained our model compound we tried several borohydride (**Figure 3.3**)

L-Selectride   SuperHydride   9-BBN
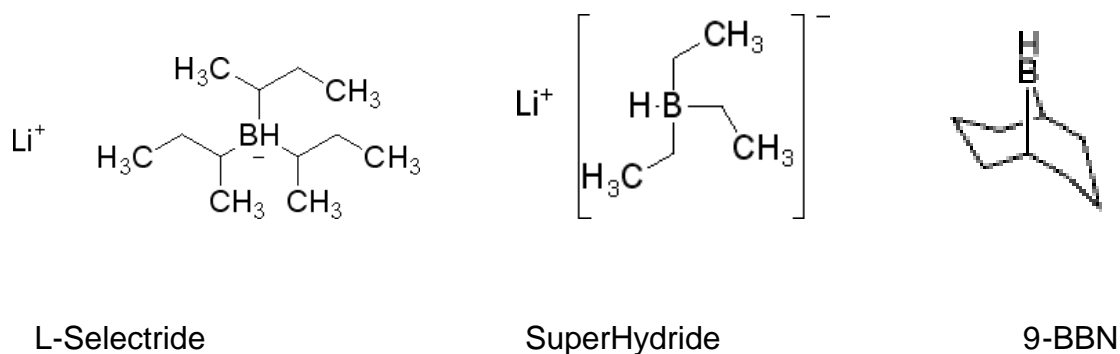
FIGURE 3.3
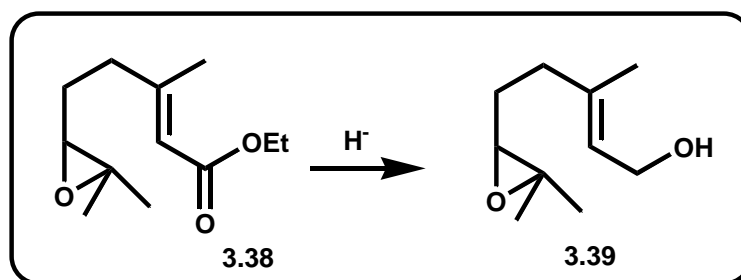
SCHEME 3.27

Table 3.2

Exp	H⁻	eq	T (°C)	Time (min)	Solvent	Yield 29 (%)
1	9-BBN	2.2	60	overnight	THF	/
2	L-Selectride	3	-78	30	THF	/
3	SuperH	3	-78	30	THF	35
4	SuperH	3	-10	30	THF	78

Both 9BBN and L-Selectride didn't give any result: the reaction was very slow probably due to steric hindered of the structure. The reaction performed on the model substrate gave the best result using Super Hydride, affording 78% of allylic alcohol without epoxide opening.(**Scheme 3.27**, **Table 3.2**)

Then we performed the reaction on C15 chain and we obtained the wanted alcohol in good yield and high conversion. (**Scheme 3.28**)
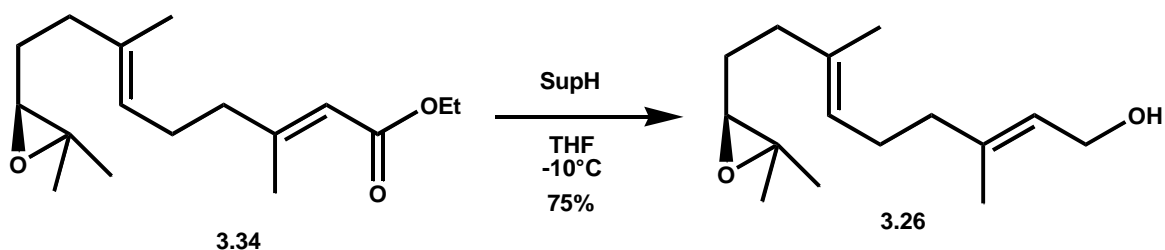
SCHEME 3.28

3.4 SYNTHESIS OF C-20 DITERPENE EPOXYGERANYLGERANIOL AND CYCLISATION

Once obtained C-15 chain we reiterated the elongation chain procedure to obtain C-20 skeleton,and we prepared (S)(-) epoxygeranylgeraniol an acyclic diterpene isolated from fruits of *Pterodon pubescens* and shows a strong *antifeedant* activity against infection *by Schitosma mansoni* [81]

The epoxy alcohol **3.26,** obtained by elongation chain from geranylacetate, was converted in the corresponding bromide **3.40** by mesylation of the primary alcohol and then by reaction by Lithium bromide 2M solution in THF. LiBr was dried under hight vacuum at 150°C overnight. (**Scheme 3.29**)
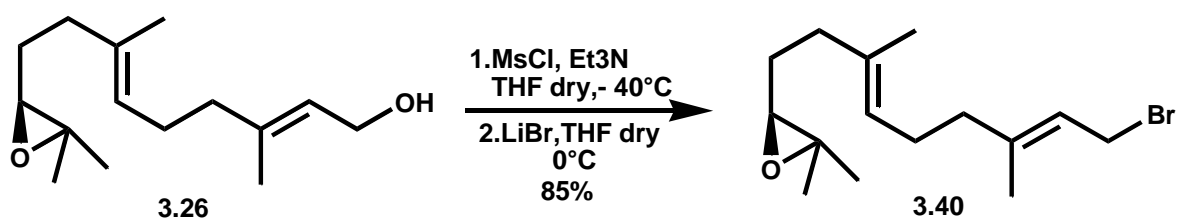
SCHEME 3.29

---

[81] Mors, W.; Dos Santos, M.; Monteiro,H; Gilbert.B. *Science* **1967**, 157 950.

The obtained bromide **3.40**, the electrophilic substrate for the elongation chain key step, we performed this elongation chain using Ethyl acetoacetate previously converted in the corresponding dianion, first by reaction with sodium hydride and then with BuLi, affording the dicarbonilic compound **3.42**. (**Scheme 3.30**)
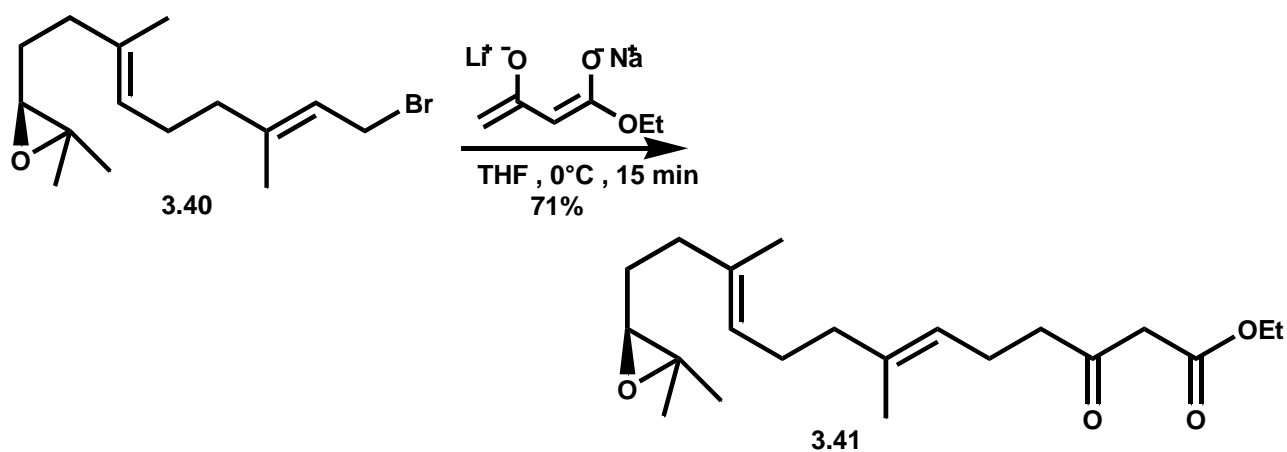
SCHEME 3.30

The dicarbonilic compound **3.41** was then converted in the corresponding enol triflate **3.42** which is activated toward sp^2-sp^3 Suzuki cross coupling. (**Scheme 3.31**)
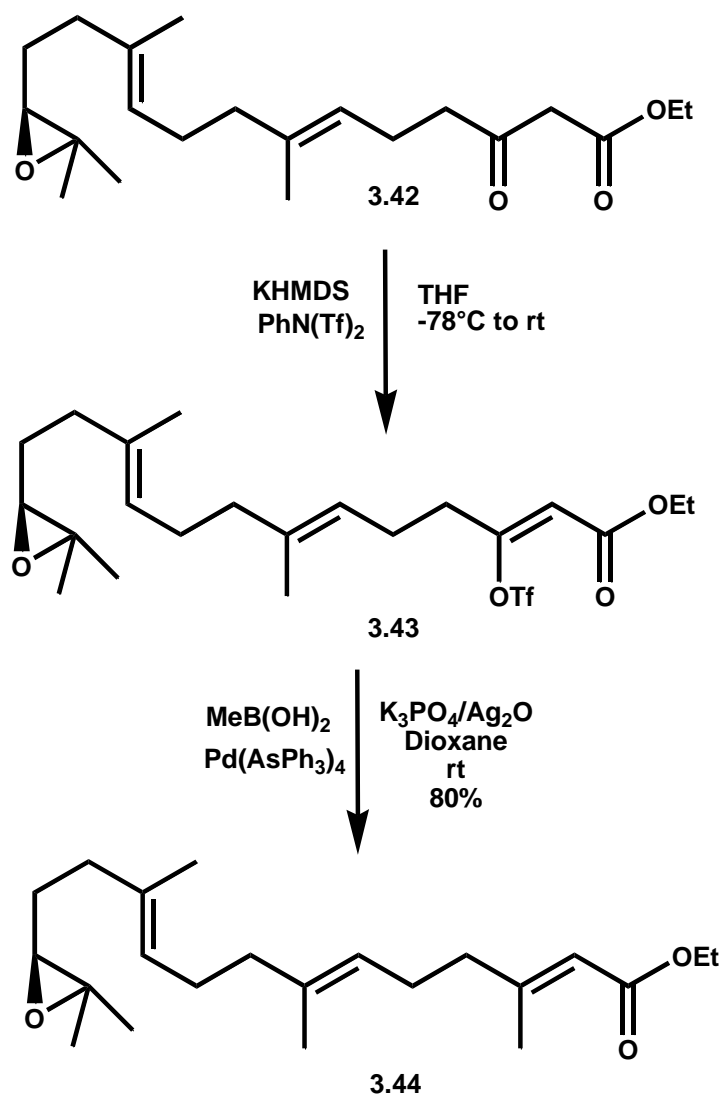
SCHEME 3.31

At the end, the final step, a reduction performed on **3.44** with SuperHydride (Triethylborohydride) at -10°C in 30 mins gave the natural product (-) (S) epoxygeranylgeraniol **3.45**.(**Scheme 3.32**)
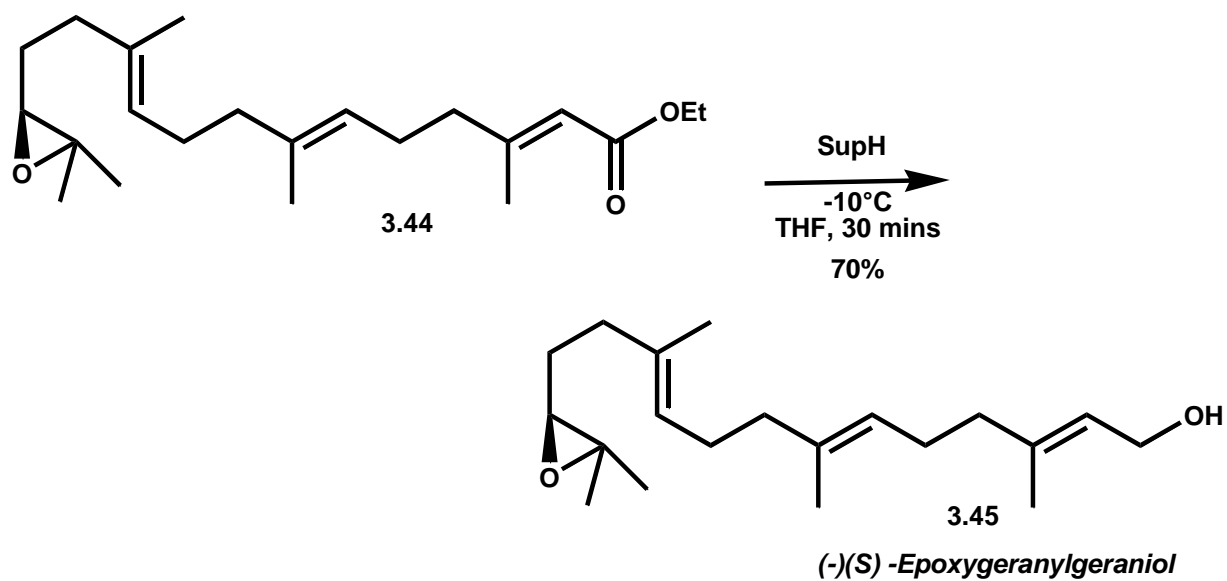
SCHEME 3.32

Acetylation has been performed on epoxialcohol **3.45** and acetylated product **2.61** has been obtained in good yield.

Radical cyclisation has been performed on **2.61** affording 30% of isocopalic compound **2.62**. The hydroxyl group has been protected as TBSCl and protected compound **3.46** has been deacetylated affording compound **3.47.**

This compound will be elaborated to obtain 3-hydroxyisocopalendial **3.1 (Scheme 3.33)**

Ac₂O, Py, DMAP / CH₂Cl₂ — 80%

Cp₂TiCl₂(20% mol.), Mn, collidine, Me₃SiCl, THF dry, r.t.; Bu₄NF — 30%

TBSCl, imidazole / CH₃CN, 90°C — 70%

K₂CO₃, MeOH / 60°C — 60%
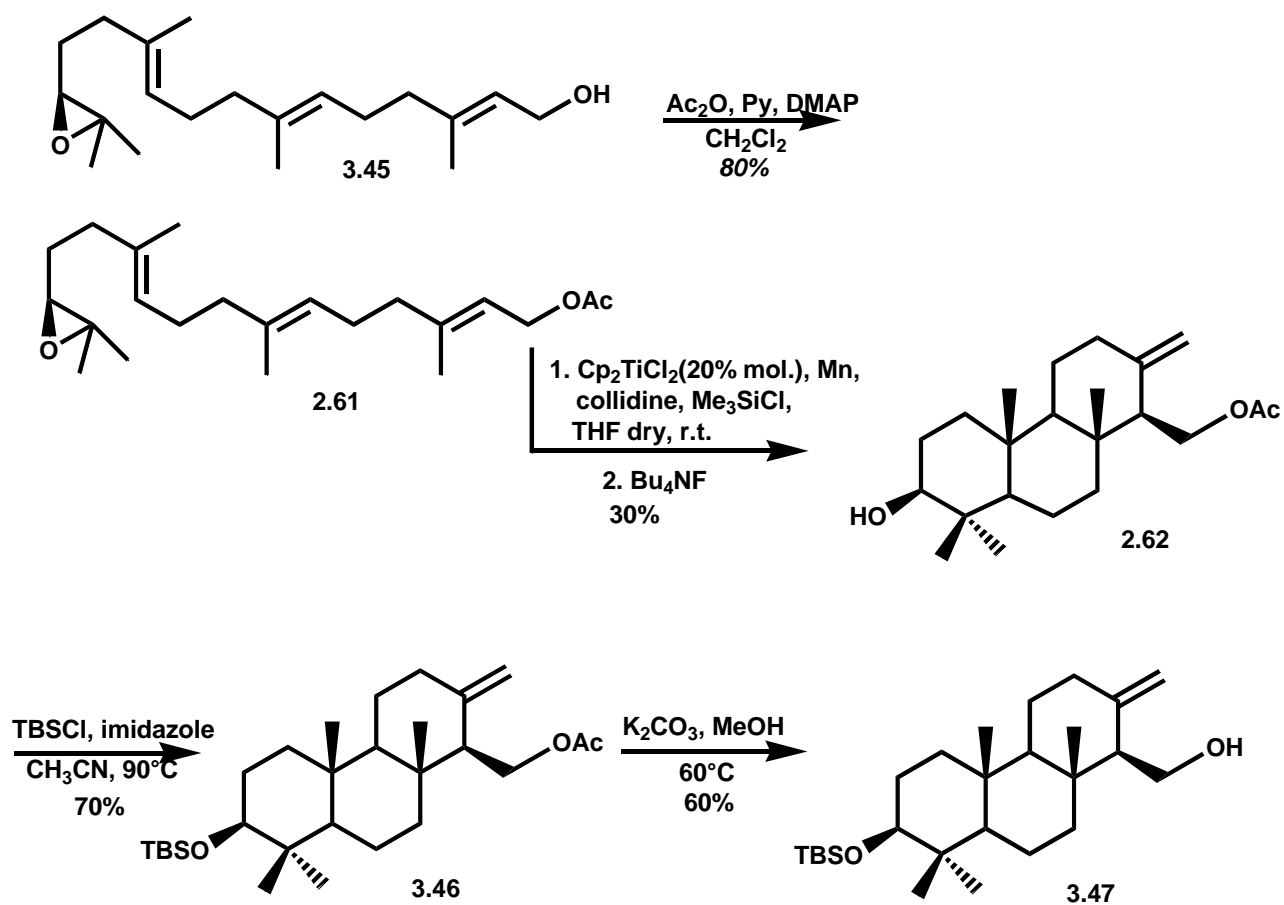
SCHEME 3.33

3.5 CONCLUSIONS

Two strategies for preparation of epoxypolyenic chains have been developed. The linear elongation chain afforded the first precursor (S)-epoxygeranylacetate in good yield.

With cyclisation of **2.62** we obtained the isocopalane backbone that could be elaborated to obtain the iscocopaledial analogue **3.1**(**Scheme 3.34**).
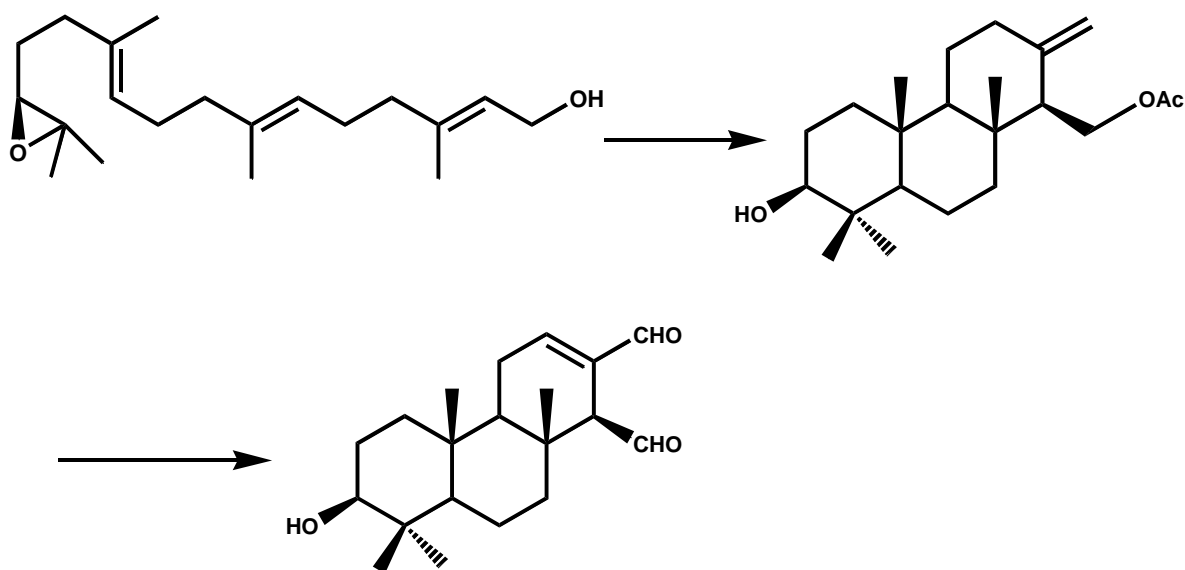
SCHEME 3.34

This methodology allows to build any wanted lenght chain: reiterating this procedure on alcohol **2.61** we are able to obtain the C25 chain,the acyclic precursor of scalaranic backbone.
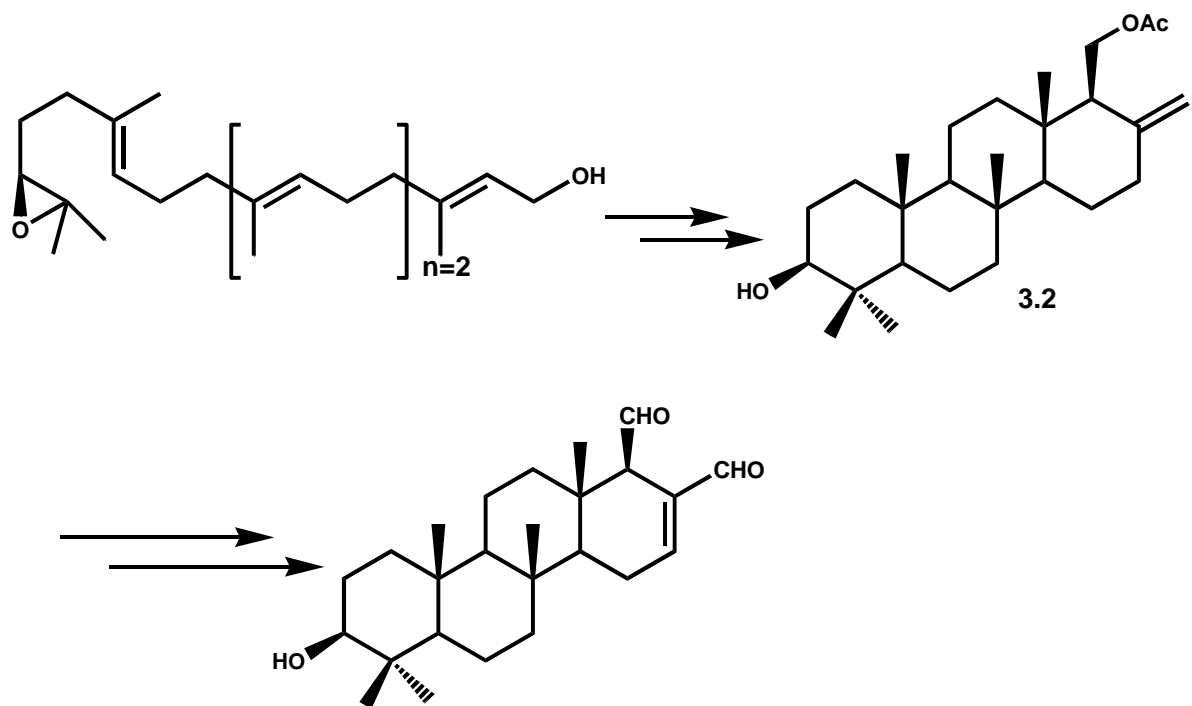
SCHEME 3.35

- EXPERIMENTAL

SHARPLESS DIHYDROXYLATION ON GERANILACETATE

A flame-dried, 1L round-bottomed flask, equipped with a magnetic stirring bar, is charged with t-BuOH/ H_2O (1:1; 130 mL) AD-Mix β (36 g). The solution is cooled to 0°C and $MeSO_2NH_2$ (2.4 g, 25.5 mmol) and geranylacetate (5 g, 25.5 mmol, 5.5 mL) are added. The mixture is stirred for 48h, then $Na_2S_2O_3$ (36 g). is added and warmed to room temperature in 2h.The solution turns to a green color.

The mixture is diluted with H_2O, extracted with CH_2Cl_2, the organic layer were dried over Na_2SO_4, filtered and evaporated in *vacuum.* Crude product was purified on silica (40% to 70% AcOEt in Petroleum ether) affording4.88 g (yield 83%) of pure **3.14** as a yellow oil.
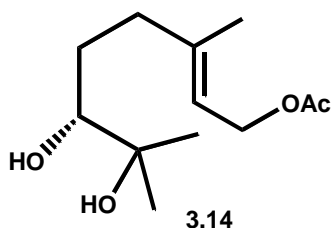
Compound 3,14 ^{1}H-NMR (400 MHz, CDCl$_3$): δ=5.39 (t,1H); 4.59 (d, 2H); 3.32(dd, 1H) ;2.33 (m, 1H); 2.10 (m, 1H); 2.05 (s, 3H); 1.72 (s, 3H); 1.60 (m, 1H); 1.45 (m, 1H), 1.21 (s, 3H); 1.16 (s, 3H).

$[\alpha]^{25}= +25.2$ (c =1.0, CHCl$_3$).

- **EPOXYDATION OF 3.14**

A flame-dried, 100mL round-bottomed flask, equipped with a magnetic stirring bar, is charged with a solution of **3.14** (430 mg, 1.86 mmol) in CH_2Cl_2 dry (5 mL), and Pyridine dry (0.6 mL, 7.44 mmol) is added. The mixture is cooled to 0°C and MsCl (0.35 mL, 4.46 mmol) is added The resulting mixture is left under stirring at room temperature overnight Then MeOH (21 mL) and K_2CO_3 (5 g) are added and the solution left under stirring overnight again. MeOH was evaporated under *vacuum*, and the resulting mixture diluted with H_2O, and extracted with CH_2Cl_2

The resulting organic layers are washed with CuSO$_4$ 1M and brine, dried over Na$_2$SO$_4$, filtered and evaporated in *vacuum.*

Crude product is purified on silica (30% to 60% EtOAc in Petroleum ether) affording 79% (255 mg) of pure **3.12** as a yellow oil.
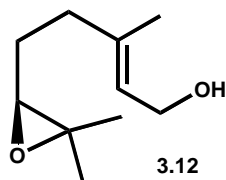
Compound 3.12 yellow oil ^{1}H-NMR (400 MHz, CDCl$_3$): δ= 5.43 (t, 1H); 4.13 (d, 2H); 2.70 (t, 1H); 2.16 (m, 2H); 1.67 (s, 3H); 1.65 (m, 2H); 1.29 (s, 3H); 1.24 (s, 3H).

^{13}C-NMR (400 MHz, CDCl$_3$): δ=138.51 (s); 124.22 (d); 64.22 (d); 59.30 (s); 58.60 (t); 36.37 (t); 27.23(t), 24.95 (q); 18.85(q); 16.36(q).

$[\alpha]^{25}$= -80 (c =1.4, MeOH).

ESI-MS : *m/z* 171.49 [M + H]$^+$, 193.52 [M + Na]$^+$.

- **BROMINATION OF 3.12**

A flame-dried, 100-mL, two-necked round-bottomed flask, equipped with a magnetic stirring bar, a rubber septa, and a nitrogen inlet connected to a mineral oil bubbler, is charged with **3.12** (404 mg, 2.37 mmol) and 12 mL of THF. Triethylamine (0.66 mL, 4.74 mmol) is added and the solution was stirred and cooled in a dry ice/acetonitrile bath (temperature 45 °C to 47 °C), while methanesulfonyl chloride (0.24 mL, 3.08 mmol) is added *via* syringe over 5 min. A white solid precipitates during the addition, and the resulting suspension is stirred at 45 °C to 47 °C for 45 min. A room temperature solution 2M of lithium bromide (823 mg, 9.48 mmol) in THF is prepared in a separate flask (previously dried at 150°C under *vacuum* overnight) and added by cannula transfer over 5 min. The dry ice/acetonitrile bath is removed, and the suspension is allowed to warm to 0 °C with an ice-bath. The yellowish mixture is stirred at 0°C for 1 h. The reaction mixture is poured into ice water. The aqueous layer is separated and extracted with hexane The combined organic extracts are sequentially washed with ice-cold saturated NaHCO$_3$ and brine dried over anhydrous MgSO$_4$ filtered, and concentrated by rotary evaporation and vacuum drying to give 470 mg, (2.02 mmol) of bromide as a light yellow oil which is used in the next step without purification.

Compound 3.15 yellow oil^{1}H-NMR (400 MHz, CDCl$_3$): δ= 5.58 (t, 1H); 4.02 (d, 2H); 2.70 (t, 1H); 2.23 (m, 2H); 1.75(s, 3H); 1.66 (m, 2H); 1.31 (s, 3H); 1.27 (s, 3H).

SYNTHESIS OF EPOXY GERANYL TOLYL SULFONE 3.16

A flame-dried, 50-mL, two-necked, round-bottomed flask, equipped with a magnetic stirring bar, a rubber septa, and a nitrogen inlet connected to a mineral oil bubbler, is charged with sodium p-toluenesulfinate (327 mg , 1.84 mmol) and DMF (2mL) and the mixture is cooled to 0°C Bromide **3.15** (330 mg 1.41 mmo) is added. The resulting mixture is stirred for 2 h at room temperature then poured into brine (200 ml), and extracted with CH$_2$Cl$_2$ filtered, and concentrated by rotary evaporation and vacuum drying to give 335 mg, of sulfone 3.16 as a white solid which is used in the next step without purification
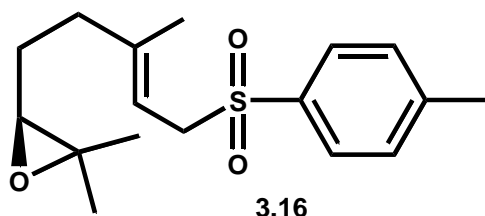
Compound 3.16 White solid. M.p. 44.0 – 44.58 from hexane (44-45.8).[82] IR(KBr): 1315, 1149 (SO$_2$). ^{1}H-NMR(CDCl$_3$): 7.74 (d, ^{3}J=6.4, 2 arom. H); 7.32 (d, ^{3}J= 6.4, 2 arom. H); 5.18 (t,^{3}J= 6.3, C=CH); 5.04 (m, C=CH); 3.79 (t, ^{3}J = 6.3, TsCH$_2$); 2.70 (t, C=CH);)2.45 (s, Me-Ar); 2.02 (m, 2 CH$_2$); 1.69 (s,Me); 1.59 (s, Me); 1.34 (s, Me). ESI-MS: 309.14 (M+H$^+$)

[82] N. K. N. Yee, R. M. Coates, *J. Org. Chem*. **1992**, 57, 4598.

SYNTHESIS OF ALLYLIC ALCOHOL 3.13

Salicylic acid (140mg, 1.02 mmol), SeO_2 (45.2 mg, 0.407 mmol) and 70% t-BuOOH (3.44 g, 38.18 mmol, 4.88 mL) are stirred in CH_2Cl_2 (50 ml) for 15 min at 0°C., then geranylacetate (1 g, 5.09 mmol) is added. After being stirred for another 20 h at room temperature the mixture is concentrated under reduce pressure below 30°C. EtOAc is added, and the organic layer are washed with 10% KOH, H_2O, and brine dried over $MgSO_4$, and concentrated in vacuo. The crude product was purified on silica (10% to 50% EtOAc in Petroleum ether) to afford **3.13** (70%) and **3.21** (15%)
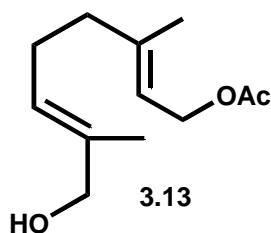
Compound 3.13 Colorless oil. IR (film): 3427 (OH). ^{1}H-NMR(CDCl$_3$): δ 5.34 – 5.37 (m, 2 C=CH); 4.58 (d, ^{3}J.= 7.3, CH_2OAc); 3.99 (s, CH_2O); 2.17 (m, CH_2); 2.09 (m, CH_2, COMe); 1.71 (s, Me); 1.67 (s, Me). ^{13}C-NMR δ 172.29 (s) 141.77 (s) 135.24 (s); 124.96 (d); 118.59 (d); 68 (t); 61 (t), 39.04 (t); 25.65 (t); 20.6 (q); 16.33 (q); 13.65 (q).
ESI-MS: 152 (2, [M-HOAc]$^+$).
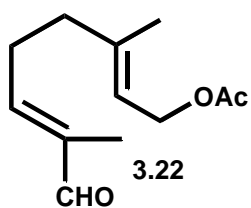
Compound 3.22 Colorless oil ^{1}H-NMR(CDCl$_3$): δ 9.3 (s, 1H); 6.4 (t, 1H), 5.3 (t, 1H) 4.6 (d, 2H); 2.4 (q, 2H); 2.2 (t, 2H); 2.0 (s, 3H COCH$_3$); 1.73 (s, 2xCH$_3$). ^{13}C-NMR δ 197.0 (s), 172.29 (s) 141.77 (s) 135.24 (s); 124.96 (d); 118.59 (d); 61 (t), 39.04 (t); 25.65 (t); 20.6 (q); 16.33 (q); 13.65 (q).
ESI-MS: 211.12 [M+H]$^+$

- **BROMINATION OF 3.13**

A flame-dried, 100-mL, two-necked round-bottomed flask, equipped with a magnetic stirring bar, a rubber septa, and a nitrogen inlet connected to a mineral oil bubbler, is charged with **3.13** (334 mg, 1.57 mmol) and 8 mL of THF. Triethylamine (0.43 mL, 3.14 mmol) is added and the solution was stirred and cooled in a dry ice/acetonitrile bath (temperature 45 °C to 47 °C), while methanesulfonyl chloride (0.16 mL, 2,05 mmol) is added *via* syringe over 5 min. A white solid precipitates is formed during the addition, and the resulting suspension is stirred at 45 °C to 47 °C for 45 min. A room temperature solution 2M of lithium bromide (547 mg, 6.3 mmol) in 3.15 THF is prepared in a separate flask (previously dried at 150°C under *vacuum* overnight) and added by cannula transfer over 5 min. The dry ice/acetonitrile bath is removed, and the suspension is allowed to warm to 0 °C with an ice-bath. The yellowish mixture is stirred at 0°C for 1 h. The reaction mixture is poured into ice water. The aqueous layer is separated and extracted with hexane The combined organic extracts are sequentially washed with ice-cold saturated $NaHCO_3$ and brine dried over anhydrous $MgSO_4$ filtered, and concentrated by rotary evaporation and vacuum drying to give 470 mg, (2.02 mmol) of bromide as a light yellow oil which is used in the next step without purification

Compound 3.13 Yellow oil. ^{1}H-NMR(CDCl$_3$): δ 5.55 (t, C=CH); 5.37 (t, C=CH); 4.58 (d, ^{3}J.= 7.3, CH$_2$OAc); 3.95 (s, CH$_2$Br); 2.17 (m, CH$_2$); 2.09 (m, CH$_2$, COMe); 1.71 (s, Me); 1.67 (s, Me). ^{13}C-NMR δ 172.29 (s) 141.77 (s) 135.24 (s); 124.96 (d); 118.59 (d); 68 (t); 61 (t), 39.04 (t); 25.65 (t); 20.6 (q); 16.33 (q); 13.65 (q).

- **ALLYL-ALLYL COUPLING**

To a stirred soln. of 3.13 (376 Mg, 1.36 mmol) and 3.16 (3.35 mg, 1.13 mmol) in. THF dry (10 ml) is added KHMDS (0.5 M in toluene 1.24 mmol; 2.5 mL) at -78°C under N_2 , and the mixture is stirred for 16 h, then poured into brine and extracted with EtOAc (3x10 ml). The organic layer is washed with H_2O (3x20 ml) and brine (20 ml), dried ($MgSO_4$), and evaporated under reduced pressure. The crude product is purified on silica (20% to 50% EtOAc/Petroleum ether) to give **3.23** (280 mg, 65%).

3.23
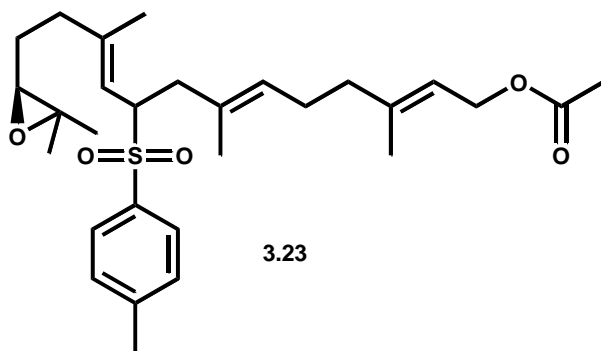
Compound 3.23 Colorless oil. IR(film): 1738 (CO), 1312,1232 (MeCOOR), 1144 (SO_2). ^{1}H-NMR($CDCl_3$): 7.70 (d, ^3J=8.2, 2 arom. H); 7.29 (d, ^3J= 8.2, 2 arom.H); 5.29 (t, ^3J=7.1, C=CH); 5.11 (t, ^3J =6.6, C=CH); 4.87 (d, ^3J =10.4, C=CH); 4.55 (d, ^3J = 7.1, CH_2OAc); 3.87 (m, $CHSO_2$); 2.85 (dd, ^3J =2.4, ^2J =12.8, 1H of CH_2); 2.62 (t, HC-O); 2.43 (s, Me-Ar); 2.25 (dd, ^3J =3.2, ^2J =12.8, 1 H of CH_2); 1.93 – 2.06 (m, 4 CH_2 , COMe); 1.19, 1.52, 1.59, 1.66 (4s, 5Me). ^{13}C-NMR($CDCl_3$): 171.02 (CO); 144.81 (arom. C); 144.23 (=C); 141.78 (2 =C); 135.04 (SO_2Ar); 131.82 (Me_2C=CH); 130.43 (2 arom. CH); 129.30 (arom. CH); 129.25 (arom. CH); 127.50 (=CH); 123.64 (=CH); 118.43 (=CH);117.39 (=CH); 67.1 (CH); 63.46 (CH_2O); 61.28 (SO_2CH); 39.67 (=C(Me)CH_2CH); 39.18 (=C(Me)CH_2); 37.54 (ArCHCH_2); 26.32 (=CHCH_2); 26.23 (=CHCH2); 25.67 (Me); 21.62 (Me-Ar); 21.02 (COMe); 17.64 (Me); 16.40 (Me); 16.39 (Me); 15.93 (Me).

ESI MS= 503.27 [M+H]$^+$

DEACETYHYLATION OF 3.23

A mixture of compound **3.23** (77 mg, 0.15 mmol) and potassium carbonate (42.4 mg, 0.30 mmol) in MeOH/H2O (5:2, 21 mL) is stirred overnight at room temperature The reaction mixture is. concentrated under vacuum, diluted with H_2O and extracted with CH_2Cl_2 dried ($MgSO_4$), and evaporated under reduced pressure affording 65,3 mg of clean product **3.24**.

3.24 (62%)
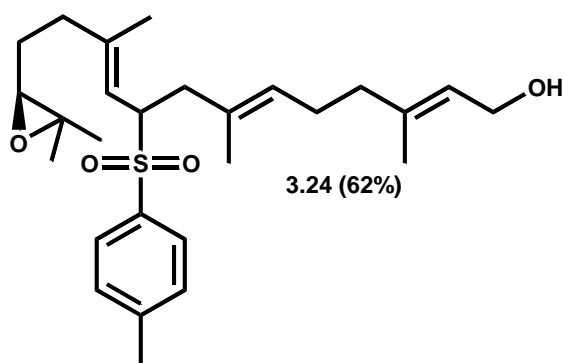
Compound 3.24 Colorless oil. IR(film): 1144 (SO_2). ^{1}H-NMR($CDCl_3$): 7.70 (d, ^3J=8.2, 2 arom. H); 7.29 (d, ^3J= 8.2, 2 arom.H); 5.29 (t, ^3J=7.1, C=CH); 5.11 (t, ^3J =6.6, C=CH); 4.87 (d, ^3J =10.4, C=CH); 4.20 (d, ^3J = 7.1, CH_2OH); 3.87 (m, $CHSO_2$); 2.85 (dd, ^3J =2.4, ^2J =12.8, 1H of CH_2); 2.62 (t, *H*C-O); 2.43 (s, Me-Ar); 2.25 (dd, ^3J =3.2, ^2J =12.8, 1 H of CH_2); 1.93 – 2.06 (m, 4 CH_2); 1.19, 1.52, 1.59, 1.66 (4s, 5Me). ^{13}C-NMR($CDCl_3$); δ 144.81 (arom. C); 144.23 (=C); 141.78 (2 =C); 135.04 (SO_2Ar); 131.82 (Me_2C=CH); 130.43 (2 arom. CH); 129.30 (arom. CH); 129.25 (arom. CH); 127.50 (=CH); 123.64 (=CH); 118.43 (=CH);117.39 (=CH); 67.1 (CH); 59.46 (CH_2O); 61.28 (SO_2CH); 39.67 (=C(Me)CH_2CH); 39.18 (=C(Me)CH_2); 37.54 (ArCHCH_2); 26.32 (=CHCH_2); 26.23 (=CHCH_2); 25.67 (Me); 21.62 (Me-Ar); 21.02 (COMe); 17.64 (Me); 16.40 (Me); 16.39 (Me); 15.93 (Me).
ESI MS= 461 [M+H]$^+$

DESULFONILATION OF 3.24

Without further purification,**3.24** is dissolved in dry THF (0.75 mL) in the presence of [Pd(dppp)Cl$_2$] (4.4 mg, 0.0074 mmol) at 0°C under N$_2$. LiHBEt$_3$ (1.0 M in THF, 0.185 mL, 0.185 mmol) is added dropwise over 10 min. The resulting mixture was allowed to warm to room temperature. over 3 h and then stirred for another 7 h. The reaction is quenched with NH$_4$Cl and extracted with AcOEt The combined org. phase is washed with brine dried (MgSO$_4$), and evaporated in *vacuum.*

The crude product is purified on silica (30% to 80% EtOAc/Petroleum ether) to give, 30% of **3.25**.

3.25 (30%)
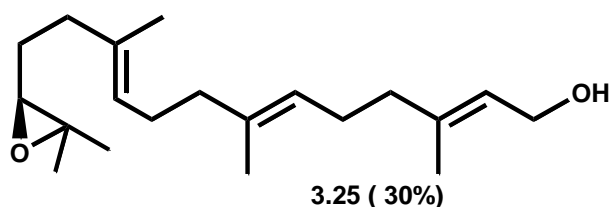
Compound 3.25 Colorless oil. IR(film): 3331 (OH). 1H-NMR (CDCl$_3$): 5.42 (t, ^3J = 6.6, C=CH); 5.11 – 5.09 (m, 2C=CH); 4.15 (d, ^3J = 6.6, CH$_2$O); 2.62 (t, HC-O); 2.13 – 1.96 (m,6 CH$_2$); 1.68 – 1.58 (m, 5 Me); ^{13}C-NMR(CDCl$_3$): 139.84 (=C); 135.42 (=C); 134.94 (=C); 131.27 (=C); 124.27 (=CH); 123.77 (=CH); 123.34 (=CH); 67.1 (CH); 59.42 (CH$_2$O); 39.76 (=C(Me)CH$_2$); 39.74 (=C(Me)CH$_2$); 39.58 (=C(Me)CH$_2$); 26.78 (=CHCH$_2$); 26.72 (=CHCH$_2$); 27.35 (COCH$_2$); 25.72 (Me); 17.70 (Me); 16.31 (2 Me); 16.04 (Me). EI-MS: 307.25. [M+H]$^+$

$[a]^{30}_D$ = + 0.9 (c = 0.2, MeOH).

PREPARATION OF β-KETO ESTER 3.27

A flame-dried, 50-mL, two-necked, round-bottomed flask, equipped with a magnetic stirring bar, a rubber septa, and a nitrogen inlet connected to a mineral oil bubbler, is charged with powder sodium hydride (159.58 mg,6.65 mmol). Tetrahydrofuran (4 mL) is added, and the hydride suspension is stirred and cooled in a 0 °C ice-bath as ethyl acetoacetate (0.77mL, 6.04 mmol) is added dropwise via syringe over 15 min. The light yellow solution of the sodium enolate is stirred at 0 °C for an additional 15 min. A solution of *n*-BuLi in hexane (1.6 M in hexane, 6.35 mmol, 4 mL) is then added by syringe over 15 min. The

resulting orange solution of acetoacetate dianion is stirred at 0 °C for 15 min. A room temperature solution of bromide **3.15** (470 mg, 2.01 mmol)) in 1 mL of THF is added by cannula transfer over 10 min. The resulting orange suspension is stirred at 0 °C for 15 min, aqueous 1N HCl, is added, and the aqueous layer is separated and extracted with ehyl acetate The combined organic extracts are washed sequentially with saturated aqueous NaHCO$_3$ and brine dried over anhydrous MgSO4, filtered, and concentrated by rotary evaporation and vacuum drying to give 21.6 g of crude product. The excess ethyl acetoacetate i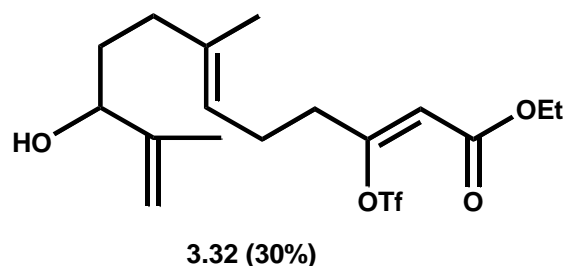s removed under vacuum. The residue is loaded into a column of silica gel and eluted with (10% to 70% of AcOEt in hexane) affording 406 mg, (yield 71%). of pure **3.27** as a yellow oil.

3.27
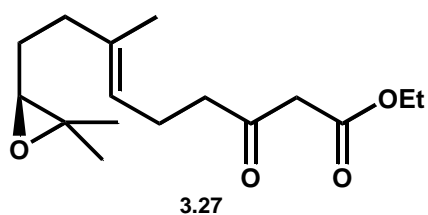
Compound 3.27 ^{1}H-NMR (400 MHz, CDCl$_3$): δ 5.03 (t, 1H); 4.07 (q,2H); 3.33 (s,2H), 2.60 (t,1H); 2.40 (t, 2H); 2.20 (q, 2H), δ 2.01 (m, 2H); 1.53 (m, 5H), 1.19–1.15 (m, 9H).

^{13}CNMR(400MHz,CDCl$_3$):δ 202.83(s); 167.58 (s); 136.17 (s); 123.24 (d); 64.41 (d); 61.66 (t); 58.62 (s); 49.76 (t); 43.27 (t); 36.71 (t); 27.77 (t); 25.27 (t), 22.49 (q); 19.16 (q); 16.38 (q); 14.52 (q).

ESI-MS : m/z 283.55 [M + H][+], 305.54 [M + Na] [+]; 323.55 [M(H$_2$O) + Na]; 587.67 [2M + Na] [+].

$[\alpha]_D^{27}$ = -2.15 (c = 4, CHCl$_3$).

IR: 1714, 1743, 2920 cm

SYNTHESIS OF COMPOUND 3.29

A flame-dried, 50-mL, round-bottomed flask, equipped with a magnetic stirring bar, under nitrogen, is charged with sodium hydride (5mg, 0.20 mmol). THF (1mL) is added, and the mixture is stirred and cooled at 0 °C with an ice-bath. β-Keto ester **3.27** (45 mg, 0.16 mmol) in 1 mL of THF is added slowly via cannula

over 20 min, and the reaction mixture is stirred at rt for 20 min or until gas evolution ceases. The clear orange solution is then stirred at 0 °C, and neat diethyl chlorophosphate (47 mg, 66 μL, 0.27 mmol) is added by syringe over 5 min. The mixture is stirred at 0 °C for 15 min, after which time the reaction is quenched by adding saturated aqueous NH4Cl (3 mL). The aqueous layer is separated and extracted with ethyl acetate (3 x 15 mL).

The combined organic extracts are washed sequentially with saturated aqueous NaHCO3 and brine, dried over anhydrous MgSO4, filtered, and concentrated by rotary evaporator and vacuum drying to give the crude enol phosphate **3.29** (68 mg) as a yellow oil
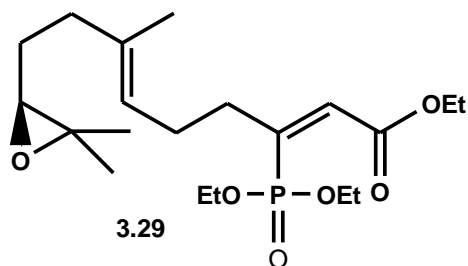
Compound 3.29 1H NMR (400 MHz, CDCl3) 5.35 δ (s, 1 H, vinyl *H*); 5.16–5.15 (t, 1 H, vinyl *H*), 4.26 (dq, *J* = 7.8, 7.1 Hz, 4 H, 2C*H*$_2$) 4.16 (q, *J* = 7.2 Hz, 2 H, C*H*$_2$), d 2.60 (t, 1H, C*H*); 2.46 (t, *J* = 7.6 Hz, 2 H,C*H*2), 2.27 (q, *J* = 7.3 Hz, 2 H, C*H*2), 1.95–2.07 (m, C*H*2) 1.27 (t, *J* = 7.1 Hz, 3 H, C*H*3), 1.36 (td, *J* = 7.1, 1.2 Hz, 6 H, 2C*H*3), 1.60 (s, 3 H, C*H*3), 1.61 (s, 3 H, C*H*3),

- **PREPARATION OF TRIFLATE 3.33 FIRST PROCEDURE**

A flame-dried, 50-mL, round-bottomed flask, equipped with a magnetic stirring bar, under nitrogen, is charged with dicarbonyl compound **3.27** (117 mg, 0.415 mmol) and lithium trifluoromethanesulfonate (129 mg, 0.831 mmol) in dichloromethane (12.45 mL) was cooled to 0 °C, and the DIPEA (79.5μL, 0.457 mmol) is then added. After 20 min of stirring at 0 °C, trifluoromethanesulfonic anhydride (77 μL, 0.457 mmol) is added, and the reaction mixture was further stirred at this temperature upon reaction completion (followed by TLC). A saturated solution of ammonium chloride (30 mL/mmol) and dichloromethane (10 mL/mmol) are then added, and the aqueous phase is extracted twice with dichloromethane. The resulting organic layers is dried over sodium sulfate and evaporated.

The crude is loaded into a column of silica gel and eluted with (0% -> 30% of AcOEt in hexane) affording 406 mg, (yield 71%). of pure **3.31** (50%) and **3.32** (30%) both as a yellow oils.
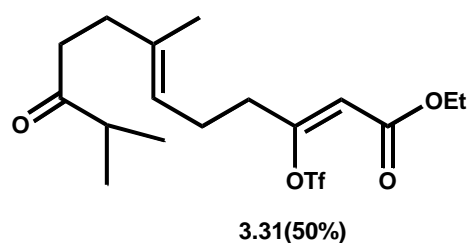
3.31(50%)

^{1}H-NMR (400 MHz, CDCl$_3$): δ 5.72 (s, 1H); δ 5.07 (t, 1H); δ 4.25 (q, 2H), 2.65–2.50 (m, 3H), δ 2.40 (t, 2H); δ 2.7 (q, 4H); δ 1.6 (s, 3H), δ 1.30 (t, 3H); δ 1.10 (s, 3H), δ 1.07 (s, 3H).
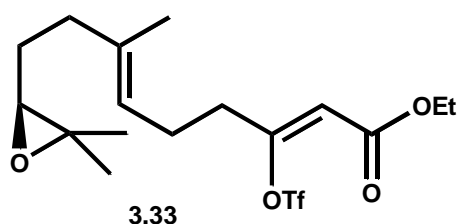
ESI-MS : *m/z* 415.36 [M + H]$^+$, 437. 41 [M + Na]$^+$.

3.32 (30%)

^{1}H-NMR (400 MHz, CDCl$_3$): 5.73 (s, 1H); 5.09 (t, 1H); 4.92 (s, 1H); 4.83 (s, 1H); 4.23 (q, 2H); 4.00 (t, 1H); 2.41 (t, 2H); 2.28 (q, 2H); 2.0 (m, 4H); 1.72 (s; 3H); 1.62 (s, 3H); 1.29 (q, 3H).

ESI-MS : *m/z* 415.36 [M + H]$^+$; 437.33 [M + Na]$^+$; 397.36 [M-(H$_2$O)] .

PREPARATION OF TRIFLATE SECOND POCEDURE

A flame-dried, 50-mL, round-bottomed flask, equipped with a magnetic stirring bar, under nitrogen, is charged with a solution of **3.27** (413 mg, 1.46 mmol) in THF dry (3.32 mL), and then is added KHMDS (4.39 ml, 2.194 mmol,0.5 M in toluene) at -78°C. While at -78 °C Tf$_2$NPh (784 mg, 2.194 mmol) is added, and the mixture is allowed to warm to room temperature overnight and turns from yellow to brown. The mixture was washed with water and twice with a 10% citric acid solution and dried and the solvent is removed under *vacuum*. The crude is purified by flash chromatography (5% to 25% ethyl acetate/hexane) and gave **3.33** as a clear, light yellow oil (509 mg, 1.23 mmol 84%).

Compound 3.33 ^{1}H-NMR (400 MHz, CDCl$_3$): δ 5.73 (s, 1H); 5.11(t, 1H); 4.23 (q, 2H); 2.67 (t, 1H); 2.40 (t, 2H); 2.28 (q, 2H); 2.15-2.11 (m, 2H); 1.62 (s, 3H); 1.29 (t, 3H), 1.25 (s, 6H).

^{13}C-NMR (400 MHz, CDCl$_3$): δ 162.66 (s); 158.50 (s); 137.54 (s); 121.40 (d); 118,00 (q, J=318 Hz); 112.30 (d); 64.67 (d); 61.46 (t); 58.68 (s); 34.67 (t); 33.00 (t); 27.51 (t); 25.00 (t); 24.55 (q); 18.90 (q); 16.38 (q); 14.19 (q).

ESI-MS : 437.57 [M + Na]$^{+}$;

$[\alpha]_D^{27}$ = 1.92 (c = 1.77, CHCl$_3$).

• **SUZUKI CROSS COUPLING ON VINYL TRIFLATE 3.33**

Vinyl triflate **3.33** (410.5 mg,1.00 mmol), MeB(OH)$_2$ (180 mg, 3.00 mmol), bis(benzonitrile) palladium (II) chloride (77 mg, 0.20 mmol), AsPh$_3$ (245 mg, 0.80 mmol), and Ag$_2$O (927 mg, 4.00 mmol) are placed in 10 mL of dioxane under nitrogen. Potassium phosphate (849 mg, 0.80 mmol) is then added, and the resulting mixture was stirred for 5 h at room temperature. The reaction was diluted with AcOEt washed with water dried over MgSO$_4$, and concentrated. The crude is purified by flash chromatography (0% -> 30% ethyl acetate /hexane) and gave **3.33** as a clear, colorless oil (223 mg, 1.23 mmol 80%).
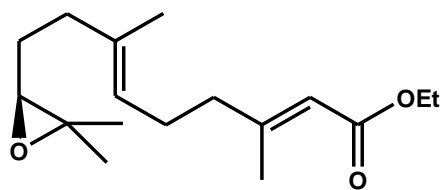
Compound 3.33: ^{1}H-NMR (400 MHz, CDCl$_3$): δ 5.65 (s,1H); 5.13 (t, 1H); 4.13 (q, 2H); 2.68 (t, 1H);2.15 (m, 9H); 1.41 (m, 5H);1.25 (m, 9H).

^{13}C-NMR (400 MHz, CDCl$_3$) : δ 167.05 (s); 159.73 (s); 135.49 (s); 123.73 (d); 115.93 (d); 64.30 (d); 59.67 (t); 58.51 (s); 41.04 (t); 36.51 (t); 29.90 (t); 26.13 (t); 25.08 (q); 18.99 (q); 18.95 (q); 16.22 (q); 14.53 (q).

ESIMS: m/z 303.61 [M + Na]$^+$.

[α]$_D^{27}$ = -80.8 (c = 0.7, CHCl$_3$).

- **REDUCTION OF 3.33 WITH DIBALH**

A flame-dried, 50-mL, two-necked, round-bottomed flask, equipped with a magnetic stirring bar, two rubber septa, and a nitrogen inlet connected to a mineral oil bubbler, is charged with ester **5** (127 mg, 0.451 mmol) and toluene (1.6 mL). The solution is cooled in a dry ice/acetone bath (temperature –78 °C), and diisobutylaluminum hydride (1M in toluene, 1.36 mL, 1.35 mmol) is added slowly via syringe through one of the septa over 20 min. After 30 min at –78 °C, the reaction is quenched by careful addition of methanol via a glass pipette over approximately 10 min. The mixture is allowed to warm to room temperature and poured into a mixture of saturated NH$_4$Cl (4 mL) and 1N HCl (4 mL) with stirring, and the stirring is continued for 30 min. The aqueous layer is separated and extracted with ethyl acetate. The combined organic extracts are sequentially washed with water and brine dried over anhydrous MgSO$_4$, filtered, and concentrated by rotary evaporator and vacuum drying. The crude is purified by flash chromatography (20% -> 60 % ethyl acetate / hexane) and yielded 60% **3.26** as a clear, colorless oil (63 mg, 0.265 mmol) and 24% of **3.35**
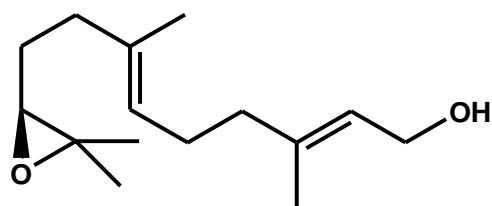
Compound 3.26 ^{1}H-NMR (400 MHz, CDCl$_3$): δ 5.41 (t, 1H); δ 5.17 (t, 1H); δ 4.16 (d, 2H); δ 2.71 (t,1H); δ 2.15-2.05 (m, 6H); δ 1.68 (s, 3H); δ 1.67-1.58 (m, 5H); δ 1.31 (s, 3H); δ 1.27 (s, 3H).

^{13}C-NMR (400 MHz, CDCl$_3$) : δ 139.33 (s); δ 134.48 (s); δ 124.70 (d); δ 123.85 (d); δ 64.35 (d); δ 59.44 (t); δ 58.60 (s); δ 39.56 (t); δ 36.48 (t); δ 27.42 (t); δ 26.31 (t); δ 25.01(q); δ 18.93 (q); δ 16.38 (q); δ 16.13 (q).

ESIMS: *m/z* 261.65 [M + Na] [+].

$[\alpha]_D^{27}$ = -5.0 (c =4.0, CHCl$_3$).
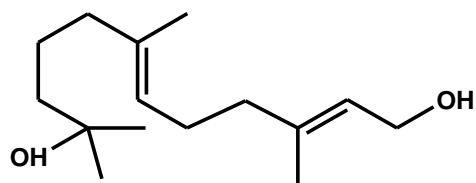
Compound 3.35 ^{1}H-NMR (400 MHz, CDCl$_3$): δ 5.38 (t, 1H); δ 5.08 (t, 1H); δ 4.13 (d, 2H); δ 2.11-1.97 (m, 6H); δ 1.65 (s, 3H); δ 1.57 (s, 3H); δ 1.40 (m, 4H); δ 1.18 (s, 6H).

^{13}C-NMR (400 MHz, CDCl$_3$) : δ 139.29 (s) ; δ 135.33 (s) ; δ 124.42 (d); δ 124.04 (d); δ 71.33 (s); δ 59.61 (t); δ 43.46 (t); δ 40.09 (t); δ 39.66 (t); δ 29.39 (x2) (q); δ 26.22 (t); δ 22.74 (t); δ 16.40 (q); δ 16.08 (q).

ESIMS: *m/z* 263.66 [M + Na] [+]

$[\alpha]_D^{27}$ = -0.78 (c =1.74, CHCl$_3$).

- **BROMINATION OF 3.12**

A flame-dried, 100-mL, two-necked round-bottomed flask, equipped with a magnetic stirring bar, a rubber septa, and a nitrogen inlet connected to a mineral oil bubbler, is charged with **3.35** (960 mg, 4.03 mmol)) and 20 mL of THF. Triethylamine (1.17 mL, 8.38 mmol) was added and the solution was stirred and cooled in a dry ice/acetonitrile bath (temperature 45 °C to 47 °C), while methanesulfonyl chloride (0.42 mL, 5.45 mmol) was added *via* syringe over 5 min. A white solid precipitates during the addition, and the resulting suspension is stirred at 45 °C to 47 °C for 45 min.

A room temperature solution 2M of lithium bromide (1.46 g, 16.8 mmol) in THF is prepared in a separate flask (previously dried at 150°C under *vacuum* overnight) and added by cannula transfer over 5 min. The dry ice/acetonitrile bath is removed, and the suspension is allowed to warm to 0 °C with an ice-bath. The yellowish mixture is stirred at 0°C for 1 h. The reaction mixture is poured into ice water. The aqueous layer is separated and extracted with hexane The combined organic extracts are sequentially washed with ice-cold saturated NaHCO$_3$ and brine dried over anhydrous MgSO$_4$ filtered, and concentrated by rotary evaporation and vacuum drying to give 470 mg, (1.02 g, 3.4 mmol), of bromide **3.40** as a light yellow oil which is used in the next step without purification
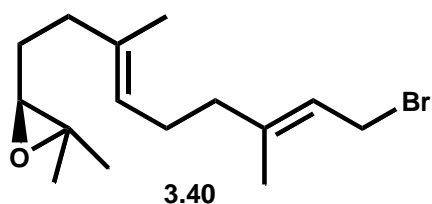
TLC R*f* = 0.72 (7:3 hexanes/ethyl acetate); ^{1}H NMR (500 MHz, CDCl$_3$) δ: 1.61 (br s, 6 H, 2C*H*3), 1.69 (s, 3 H, C*H*3), 1.74 (s, 3 H, C*H*$_3$), 1.97–2.13 (m, 8 H, 4C*H*$_2$), 4.03 (d, *J* = 8.3 Hz, 2 H, C*H*$_2$Br), 5.08–5.11 (m, 2 H, vinyl *H*), 5.54 (br t, *J* = 8.3 Hz, 1 H, vinyl *H*); 13C NMR (100 MHz, CDCl3) δ 16.2, 16.3, 17.9, 25.9, 26.3, 26.9, 30.0, 39.7, 39.9, 120.7, 123.6, 124.5, 131.6, 135.8, 143.8.

- **PREPARATION OF β-KETO ESTER 3.41**

A flame-dried, 50-mL, two-necked, round-bottomed flask, equipped with a magnetic stirring bar, a rubber septa, and a nitrogen inlet connected to a mineral oil bubbler, is charged with powder sodium hydride (218.6 mg, 9.11 mmol). Tetrahydrofuran (7.57 ml) is added, and the hydride suspension is stirred and cooled in a 0 °C ice-bath as ethyl acetoacetate (1.05 mL, 8.29 mmol) is added dropwise via syringe over 15 min. The light yellow solution of the sodium enolate is stirred at 0 °C for an additional 15 min. A solution of *n*-BuLi in hexane (2.5 M in hexane, 8.70 mmol, 3.48 mL) is then added by syringe over 15 min. The resulting orange solution of acetoacetate dianion is stirred at 0 °C for 15 min. A room temperature solution of bromide **3.40** (833 mg, 2.76 mmol) in 1.37 mL of THF is added by cannula transfer over 10 min. The resulting orange suspension is stirred at 0 °C for 15 min, aqueous 1N HCl, is added, and the aqueous layer is separated and extracted with ehyl acetate The combined organic extracts are washed sequentially with saturated aqueous NaHCO$_3$ and brine dried over anhydrous MgSO4, filtered, and concentrated by rotary evaporation and vacuum drying .The excess ethyl acetoacetate is removed under vacuum. The residue is loaded into a column of silica gel and eluted with (0% -> 20%of AcOEt in hexane) affording 985 mg, (2.81 mmol) (yield 70%). of pure **3.41** as a yellow oil.
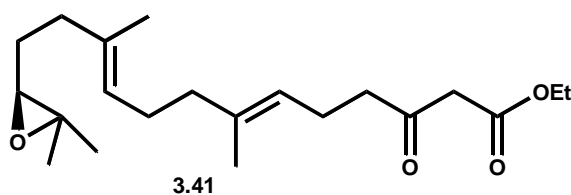
1H-NMR (300 MHz, CDCl3): 5.10 (m, 1H); 5.04 (m, 1H); 4.16 (m,2H); 3.43 (s, 2H); 2.70 (t, 1H, J = 6.2 Hz); 2.57 (m, 2H); 2.28 (m, 2H); 2.04 (m, 4H); 1.60 (s, 6H); 1.30 (s, 3H); 1.28 (t, 3H, J= 7.0 Hz); 1.27 (s, 3H);.13C-NMR (75 MHz, CDCl3): d 202.4 (s); 167.0 (s); d 136.3 (s); 133.9, (s); 124.5 (d); 122.0 (d); d 63.9 (d); 61.1 (t); 58.1 (s); 49.1 (t);42.8 (t); 39.3 (t); 36.1 (t); 27.2 (t); 26.3 (t); 24.7 (q); 21.9 (q) ,18.5 (q);15.8 (2C, q); 13.9 (q).

ESIMS (m/z)= 373 [M + Na+].

[α]$^{30}_D$ = 1.0 (c = 1.2, MeOH).

IR, cm^{-1}: 1745, 1716, 1640.

PREPARATION OF TRIFLATE 3.43

A flame-dried, 50-mL, round-bottomed flask, equipped with a magnetic stirring bar, under nitrogen, is charged with a solution of **3.41** (967 mg, 2.76 mmol) in THF dry (6.3 mL), and then is added KHMDS (8.28 ml, 4.14 mmol,0.5 M in toluene) at -78°C. While at -78 °C Tf$_2$NPh (1.48 g, 4.14 mmol) is added, and the mixture is allowed to warm to room temperature overnight and turns from yellow to brown. The mixture was washed with water and twice with a 10% citric acid solution and dried and the solvent is removed under *vacuum*. The crude is purified by flash chromatography (0% to 30% ethyl acetate/hexane) and gave **3.43** as a clear, light yellow oil (838.2 mg, 1.74 mmol 64% coversion 71%).

3.43
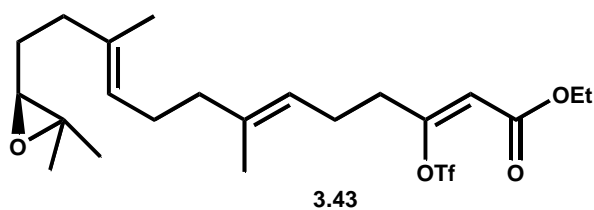
Compound 3.43 1H-NMR (400 MHz, CDCl3): δ 5.69 (s, 1H); 5.08 (t, 1H, J = 6.5 Hz);5.01 (t, 1H, J = 6.7 Hz); 4.18 (q, 2H, J = 7.1 Hz); 2.64 (t, 1H, J= 6.1 Hz); 2.35 (m, 2H); 2.23 (m, 2H) ;2.09 1.93; (m, 5H); 1.57(m, 2H); 1.57 (s, 6H); 1.24 (t, 3H, J = 7.1 Hz); 1.23 (s, 3H); 1.20(s, 3H). ^{13}C-NMR (75 MHz, CDCl$_3$): 162.4 (s); 158.4 (s); 138.0 (s); 134.3 (s); 124.4 (d); 120.6 (d); 112.0 (d); 64.0 (d); 61.1 (t); 58.2 (s); 39.4 (t); 36.2 (t); 34.5 (t); 27.4 (t); 26.4 (t); 24.8 (q); 24.3 (t); 18.6 (q); 15.9 (q); 15.8 (q); 13.9 (q).

ESIMS (m/z)= 505 [M + Na+], 373 [M - CF3SO2+ Na+].

[a]^{30}$_D$ = + 0.5 (c = 0.5, MeOH).

IR, cm^{-1}: 1728, 1678, 1429.

- **SUZUKI CROSS COUPLING ON 3.43**

Vinyl triflate **3.43** (748 mg, 1.55 mmol), MeB(OH)$_2$ (278.4 mg, 4.65 mmol), bis(benzonitrile) palladium (II) chloride (118.9 mg,0.31 mmol), AsPh$_3$ (379.7 mg, 1.24 mmol), and Ag$_2$O (1.44 g, 6.20 mmol) are placed in 16 mL of dioxane under nitrogen. Potassium phosphate (1.32 g,6.20 mmol) is then added, and the resulting mixture was stirred overnight at room temperature. The reaction was diluted with AcOEt washed with water dried over MgSO$_4$, and concentrated. The crude is purified by flash chromatography (0% -> 10% ethyl acetate /hexane) and gave **3.44** as a clear, colorless oil (438.0 mg, 1.26 mmol81%).

3.44
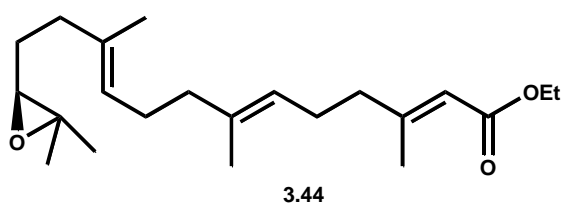
Compound 3.44 1H-NMR (400 MHz, CDCl$_3$): 5.66 (s, 1H); 5.14 (m, 1H); 5.08 (m,1H); 4.12 (q, 2H, J = 7.2 Hz); 2.70 (t, 1H, J = 8.0 Hz); 2.12 (s,3H); 2.04 (m, 4H); 1.95 (m, 4H); 1.59 (m, 2H); 1.57 (s, 3H); 1.56 (s, 3H); 1.26 (s, 3H); 1.23 (t, 3H, 1.2 Hz); 1.21 (s, 3H).

^{13}C-NMR (75 MHz, CDCl$_3$): δ 166.7 (s); 159.5 (s); 135.9 (s); 134.0 (s); 124.6 (d); d 122.9 (d); 115.5 (d); 64.2 (d); 59.5 (t); 58.4(s); 41.1 (t); 39.7 (t); 36.4 (t); 27.6 (t); 26.7 (t); 26.1 (t); 25.0 (q); 18.9 (q); 18.8 (q); 16.1 (q); 14.4 (q).

ESIMS (m/z)= 371 [M + Na+].

[a]^{30} $_D$ = + 0.75 (c = 0.6, MeOH).

- **REDUCTION OF 3.44 WITH SUPER HYDRIDE**

A 25 flamed two necked flask is charged with 3.44 (14 mg 0.040 mmol) and THF (6 mL). The mixture is cooled to -10°C and SupHydride (1M in THF , 0.120 mmol 0.120 mL) is added. The mix is left under stiffing for 20 min and then NH$_4$Cl is added. The aqueous layer is extracted with CH$_2$Cl$_2$ dried over

MgSO$_4$, and concentrated. The crude is purified by flash chromatography (20% to 50% ethyl acetate /hexane) and gave (S) epoxygeranylgeraniol **3.45** as a clear, colorless oil (9 mg, 0.03 mmol 73%). See spectral data for compound **3.25**.

- **PREPARATION OF 3.62**

Strictly deoxygenated THF (20 mL) is added to a mixture of [Cp$_2$TiCl$_2$] (0.5 mmol) and Mn dust (20 mmol) under an Ar atmosphere and the suspension is stirred at room temperature until it turns lime green (after about 15 min). Then, a solution of epoxide (2.5 mmol) and 2,4,6-collidine (20 mmol) in THF (2 mL), and Me$_3$SiCl (10 mmol) are added and the solution is stirred overnight. The reaction is then quenched with 2n HCl and extracted with EtOAc. The organic layer is washed with brine, dried (anhydrous Na2SO4), and the solvent removed.

The residue is dissolved in THF (20 mL) and stirred with Bu$_4$NF (10 mmol) for 2 h. The mixture is then diluted with EtOAc, washed with brine, dried (anhydrous Na$_2$SO$_4$), and the solvent removed. Product obtained is isolated by column chromatography of the residue on silica gel (hexane/EtOAc) and characterized by spectroscopic techniques.
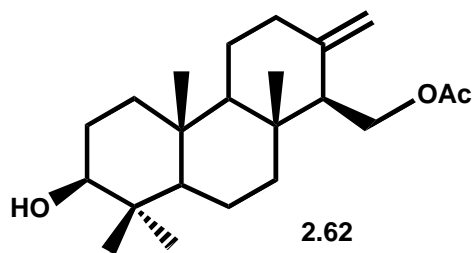
White solid; m.p. 132-135 8C; ^{1}H NMR (300 MHz, CDCl3): δ = 4.84 (br s, 1H), 4.51 (br s, 1H), 4.34 (dd, J =11.0, 3.6 Hz, 1H), 4.17 (dd, J = 11.0, 9.4 Hz, 1H), 3.21 (dd, J = 11.4, 4.8 Hz, 1H), 2.39 (br d, J = 12.7 Hz, 1 H), 2.10±1.90 (m, 1 H), 2.01 (s, 3H), 1.87±1.80 (m, 1 H), 1.75-1.25 (m, 12H), 0.98 (s, 3H), 0.81 (s, 3 H), 0.76 (s, 3 H), 0.74 ppm (s, 3H); ^{13}C NMR (75 MHz, CDCl3, DEPT): δ = 171.52, (C), 146.55 (C), 107.13 (CH2), 78.84 (CH), 61.52 (CH2), 59.64 (CH), 55.27 (CH), 55.02 (CH), 40.68 (CH2), 39.14 (C), 38.89 (C), 38.56 (CH2), 37.57 (C), 37.45 (CH2), 28.05 (CH3), 27.33 (CH2), 22.49 (CH2),21.20 (CH3), 18.70 (CH2), 16.37 (CH3), 16.06 (CH3), 15.37 (CH3); ESIMS m/z (%): 288 (1), 207 (17), 189 (14), 93 ppm (100); HRMS (FAB): calcd for C$_{22}$H$_{36}$O$_3$Na 371.2562, found 371.2561.
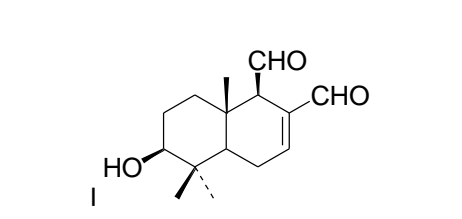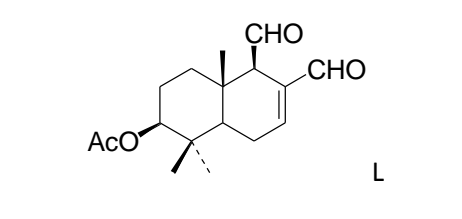
CHAPTER 4
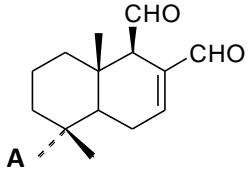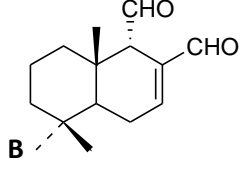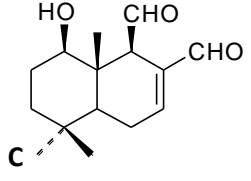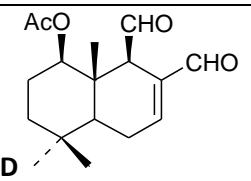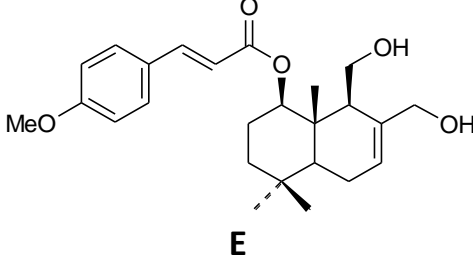
BIOACTIVITY ASSAYS
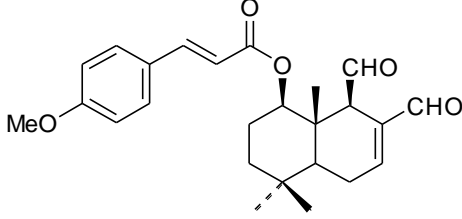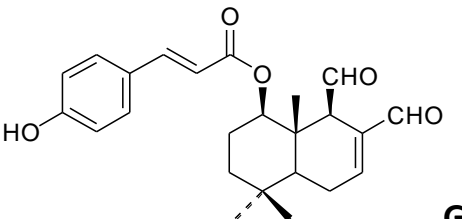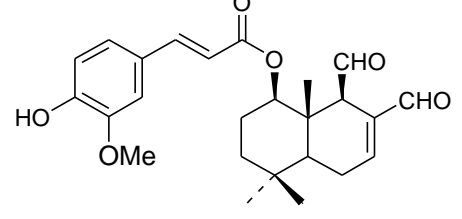
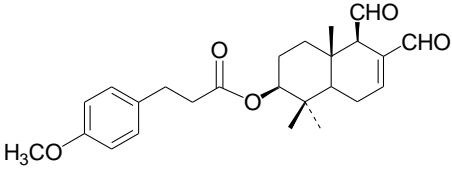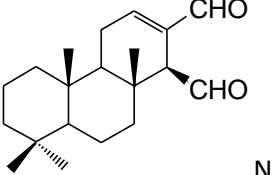**4.1 <u>ASSAYS ON TRPV1 RECEPTORS</u>.**

The assays on receptor TRP have been made in collaboration with Istituto di chimica biomolecolare of CNR, Pozzuoli (Na).

To assess vanilloid activity of the synthetized compounds assays were performed on TRPV1 vanilloid receptor in HEK cells transfected with the human TRPV1. Vanilloid activity was evaluated by measuring the entry of Ca^{2+} (the concentration of internal calcium $[Ca^{2+}]$ before and after the addition of test compounds). Data are expressed as the concentration exerting a half-maximal effect (EC50). The efficacy of the effect was determined by comparing it to the analogous effect observed with 4 μM ionomycin. In this assay, capsaicin induces a dose dependent increase of intracellular calcium (65% max Ionomycin ; pEC_{50} = 7.7) which is not observed in wild-type HEK-293 cells and is blocked by the vanilloid receptor antagonist capsazepine.

Material and Methods All prepared compounds have been assayed for TRPV1 sensitivity using fluorometric measurements of changes in intracellular calcium concentration. HEK293 (human embryonic kidney) cells were grown as monolayers in minimum essential medium supplemented with non-essential amino acids, 10% fetal calf serum and 2 mM glutamine, and maintained under 95%/5% O_2/CO_2 at 37 °C and stably transfected by using TRPV1 plasmids. Cells have been loaded for 1 h at 25 °C with 4 μM Fluo-4 methyl ester (Molecular Probes) in DMSO. $[Ca^{2+}]_i$ was determined before and after the addition of various concentrations of test compounds. After the loading, cells were washed with Tyrode pH 7.4, resuspended in Tyrode and transferred to the quartz cuvette of the fluorescence detector (Perkin–Elmer LS50B) under continuous stirring. Experiments were carried out by measuring cell fluorescence at 25 °C (λ_{EX} = 488 nm, λ_{EM} = 516 nm).

|	C μM	% max Ionomycin	Antagonist effect
A	10	37.3	activity was inhibited (50%) with the selective TRPV1 antagonist 5-iodo-resiniferatoxin (IRTX)
B	10	0	
C	10	0	
D	10	0	
E	10	0	

F	*1.72*	*90*	activity was inhibited (50%) with the selective TRPV1 antagonist 5-iodo-resiniferatoxin (IRTX)
G	*3.16*	*47.7*	activity resulted unaffected by the presence of the specific inhibitor
H	*5*	*89.3*	activity resulted unaffected by the presence of the specific inhibitor
I	*100*	*49*	activity was completely inhibited with the selective TRPV1 antagonist 5-iodo-resiniferatoxin (IRTX)
L	*-*	*-*	activity resulted unaffected using untransfetted cells

M	-	-	activity resulted unaffected using untransfetted cells
N	*10*	*100*	activity resulted unaffected by the presence of the specific inhibitor

4.2 ANTIPROLIFERATIVE ASSAYS

These assays have been made in collaboration with Prof.ssa Giuseppina Auore and co workers , Dipartimento di Farmacia Università degli Studi di Salerno. using the some synthesized molecole (**Scheme 4.1**)

4.1   **4.2**   **4.3**   **4.4**

4.5   **4.6**   **4.7**
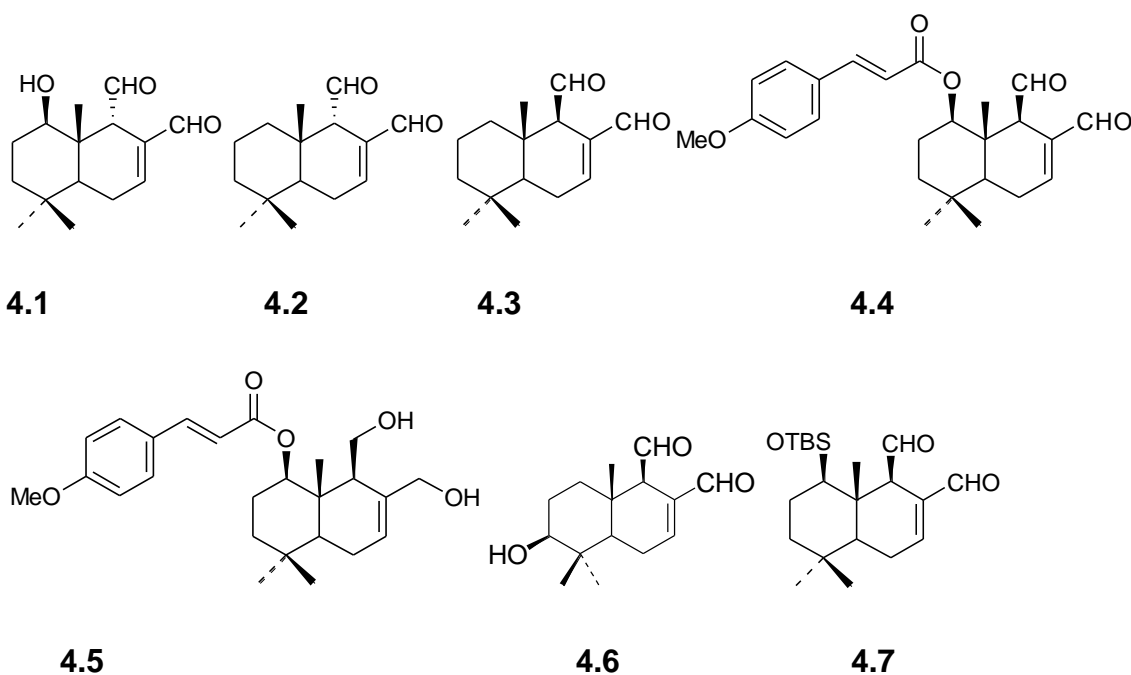
Scheme 4.1

These trials are been carried out using human tumor cells A 375 (Melanoma, **Figure A**), A 549 (lung, **Figure B**), MCF 7 (mammarian carcinoma, **Figure C**).
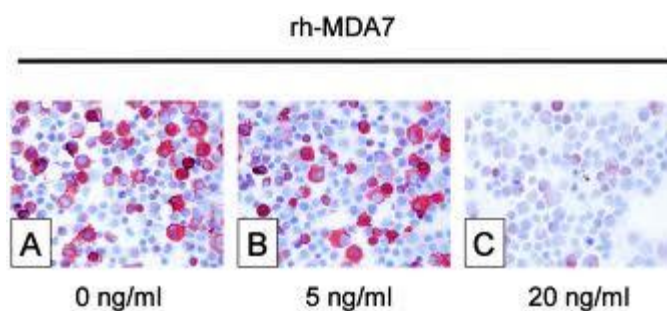
Figure A
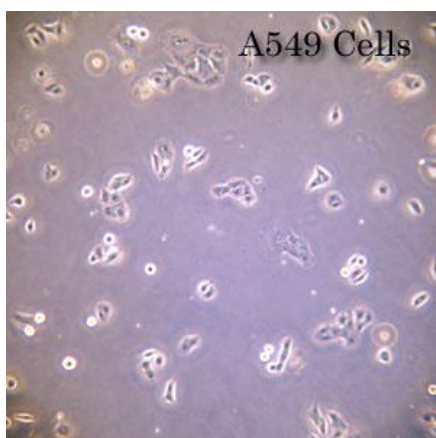
Figure B
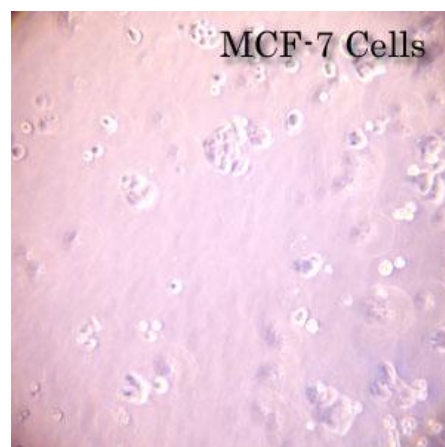
Figure C

Anti-proliferative activity

Material and Methods A 375, A 549 MCF 7 cell line (American Type Culture Collection, Rockville, MD), was grown in adhesion on Petri dishes and maintained with Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% foetal calf serum (FCS), 100 u/mL penicillin and 100 μg/mL streptomycin at 37 °C in a 5 % CO_2 atmosphere.

Cells (3.4×10^4) were plated on 96-well microtiter plates and allowed to adhere for 2 h. Thereafter, the medium was replaced with 90 μL of fresh medium and a 10 μL aliquot of serial dilutions compounds **4.1 4.7** (1-500 µM), alone, was added. Cells were incubated for 24, 48 and 72 h. Cell viability was assessed through MTT assay (Kinjo et al., 1994; Mosmann, 1983). Briefly, 25 μL of MTT (5 mg/mL) were added and the cells were incubated for an additional 3 h. Thereafter, cells were lysed and the dark blue crystals solubilised with 100 μL of a solution containing 50% (v:v) N,N-dimethylformamide, 20% (w:v) SDS with an adjusted pH of 4.5. The optical density (OD) of each well was measured with a microplate spectrophotometer (Titertek Multiskan MCC/340) equipped with a 620 nm filter. Cell viability in response to treatment was calculated as: % dead cells=100-(OD treated/OD control) x 100. (**see table 4.2**)

IC$_{50}$ [μM]			
Compounds	A-549	MCF-7	A-375
4.1	42.09±3.47	34.54±1.70	32.07±0.36
4.2	51.29±0.71	22.15±0.53	27.40±0.73
4.3	297.3±3.84	294.6±4.89	290.7±5.25
4.4	60.68±3.25	57.64±0.62	46.92±1.95
4.7	69.67±3.10	61.64±3.03	59.04±0.68

TABLE 4.2

Significant Antiproliferative Activity is shown for compounds 4.2 .

Surprisingly, Poligodial is not active while its epimer 4.2 is a compound with a significant antiproliferative activity, the most potent among the assayed dialdehydes.

THE UNIVERSITY *of* York

Department of Chemistry

A new approach to synthesis of α-alkylidene-γ-butyrolactones from transition metal carbenoids

Supervisors: **Professor Richard Taylor**

Dr. William Unsworth

February-September 2011

CHAPTER 5

α-alkylidene-γ-butyrolactones:natural sources, bioactivity and total synthesis

5.1 <u>OUTLINE</u>

The amount of research activity concerning α-methylene-γ-butyrolactones and α-alkylidene-γ-butyrolactones has increased dramatically in recent years. Traditional approaches to α-methylene-γ-butyrolactones and α-alkylidene-γ-butyrolactones are then reviewed together with novel approaches, including those from our own research group, reported more recently.

In addition I have perfomed a new synthetic route to synthesize these lactones involving Rh(II) chemistry.

5.2 <u>INTRODUCTION</u>

The α-methylene-γ-butyrolactone structural motif is found in a vast array of synthetically challenging and biologically significant natural products, many of which possess useful biological activities (e.g. anticancer, antimalarial, antiviral, antibacterial, antifungal, anti-inflammatory).[83,84] Of particular significance are natural products such as helenalin (**5.1**),[85] the anti-inflammatory active ingredient of Arnica, which is widely used in liniments and ointments for the treatment of strains, sprains, and bruises;[86] parthenolide (**5.2**),[87] a sesquiterpene lactone isolated from the medicinal herb feverfew, which possesses interesting anti-inflammatory, anticancer and antiviral properties; the hispitolides (e.g. hispitolide A, (**5.3**), a newly discovered family of compounds exhibiting activity against the hepatitis C virus (HCV) and arglabin (**5.4**), a tumor inhibitor. (**Figure 5.1**)

[83] H. M. R. Hoffmann, J. Rabe, Angew. Chem. 1985, 97, 96;Angew. Chem. Int. Ed. Engl. **1985**,24, 94.

[84] A. K. Picman, Biochem. Syst. Ecol. **1986**, *14*, 255.

[85] E. P. Clark, J. Am. Chem. Soc. 1936, 58, 1982.

[86] G. Lyss, T. J. Schmidt, H. L. Pahl, I. Merfort, Pharm. Pharmacol. Lett. **1999**, 9, 5.

[87] a) T. R. Govindachari, B. S. Joshi, V. N. Kamat, Tetrahedron **1965**, 21, 1509; b) L. Cretnik, M. ; Skerget, ZKnez, Sep. Purif. Technol. **2005**, 41, 13.

Helenalin 5.1
anti inflammatory

Parthenolide 5.2
Anti inflammatory
Anticancer
Antiviral

Hispitolide A
5.3
Antiviral

Arglabin 5.4
tumor inhibitor
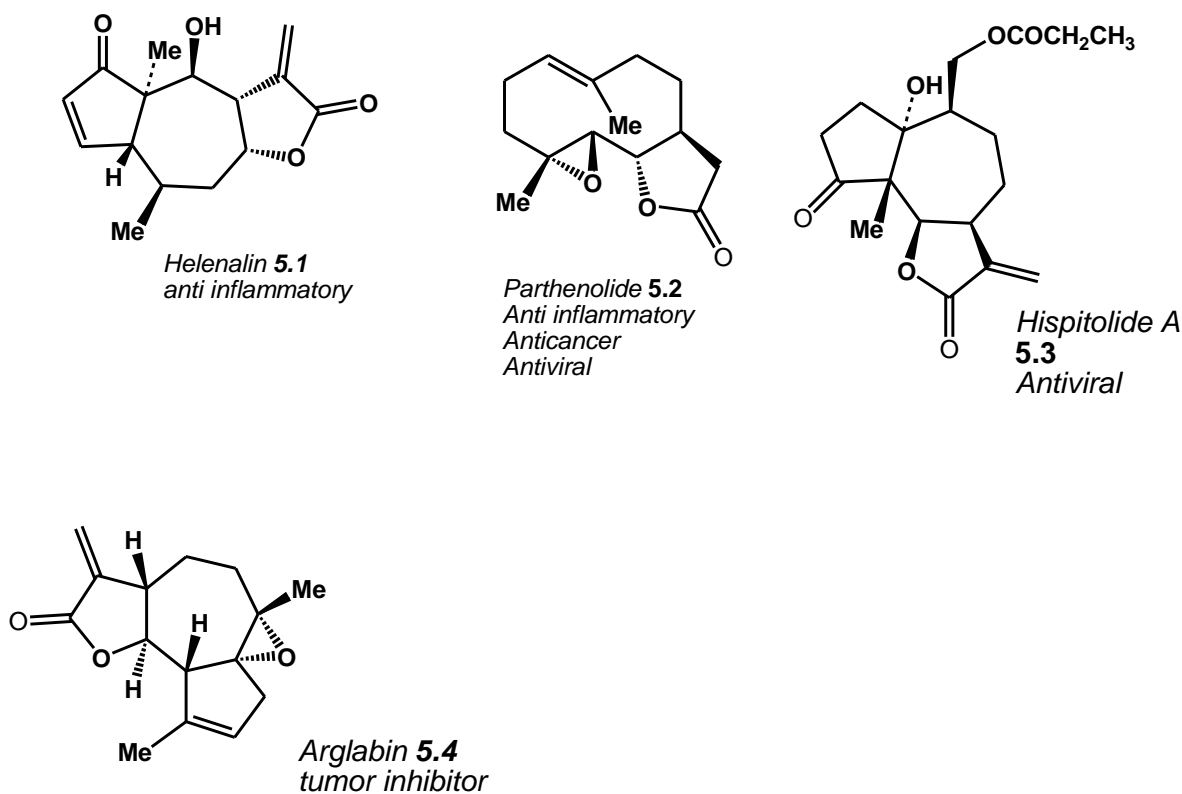
FIGURE 5.1

In addition, many α-alkylidene-γ-butyrolactones are known,[88] and the first α-methylene-γ-butyrolactone to be isolated was probably pyrethrosin (**5.5**) in 1891,[89] and the first alkylidene example was andrographolide (**5.6**) in 1911.[90] (Figure **5.2**). Since then this family has grown inexorably; in addition to reports describing the isolation of α-methylene- and α-alkylidene-γ-butyrolactones, there have been numerous publications covering their biosynthesis, biological properties, and medical applications.

---

[88] a) J.-H. Sheu, A. F. Ahmed, R.-T. Shiue, C.-F. Dai, Y.-H. Kuo, J. Nat. Prod. **2002**, 65, 1904; b) A. F. Ahmed, J.-H. Su, Y.-H. Kuo, J.-H. Sheu, J. Nat. Prod. **2004**, 67, 2079.

[89] a) H. Thoms, Ber. Dtsch. Pharm. Ges. **1891**, 1, 241; b) E. J. Gabe, S. Neidle, D. Rogers, C. E. Nordman, J. Chem. Soc. D **1971**, 559.

[90] a) K. Gorter, Recl. Trav. Chim. Pays-Bas **1911**, 30, 151; b) A. B. Smith III, B. H. Toder, P. J. Carroll, J. Donohue, J. Crystallogr. Spectrosc. Res. **1982**, 12, 309.

Pyrethrosin **5.5**
anti inflammatory

Andrographolide **5.6**
α-Glucosidase inhibitor
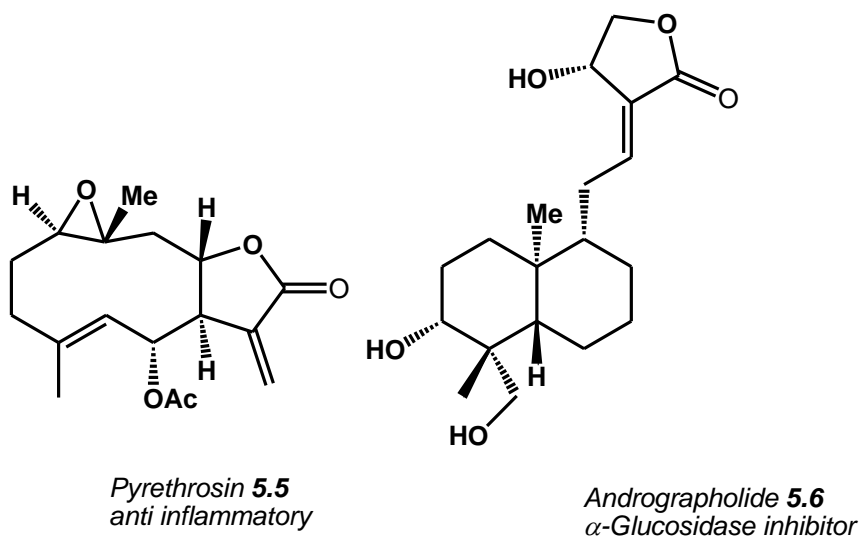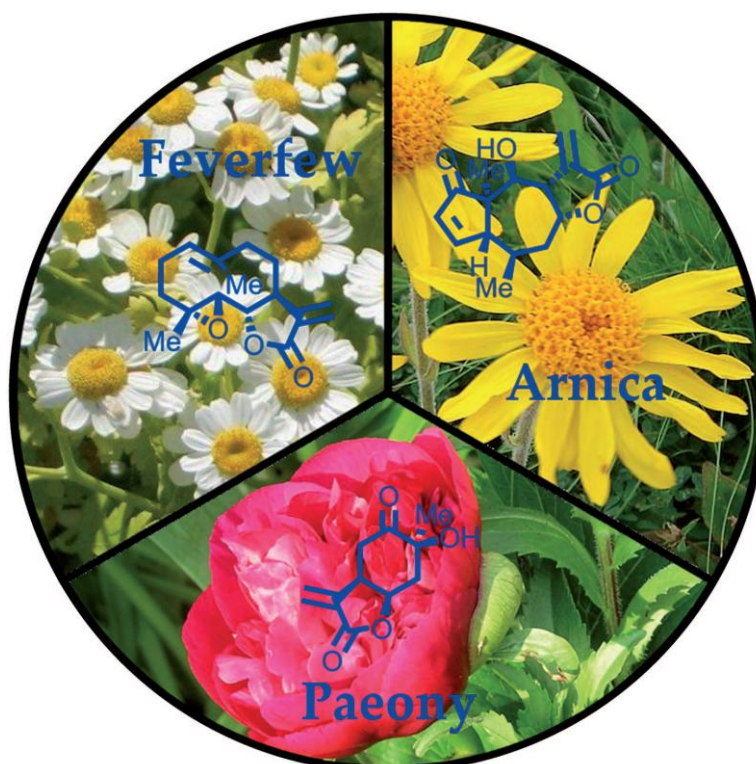
FIGURE 5.2

FIGURE 5.3 *some natural occurring α-alkylidene-γ-butyrolactones*

In the past ten years or so, there has been a renaissance of interest in the isolation and biological screening of α-methylene- and α-alkylidene-γ-butyrolactones as well as the development of new and improved synthetic approaches.

There have been almost 4000 α-methylene- and α-alkylidene-γ-butyrolactones isolated from natural sources. Given that the majority of these are α-methylene-γ-butyrolactone sesquiterpenes, it is possible to group novel structures into the standard [91] sesquiterpene categories plus another non terpenoidic category.

Germacranolides (with a 10-membered ring **7**, Figure **5.4**), which is represented by **5.8**, isolated in 2007 with other several sesquiterpene α-methylene-γ-butyrolactones from the aerial parts of *Tithonia diversifolia*, and shown to exhibit cytotoxic activity.
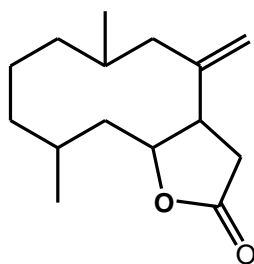
*Germacranolides type structure **5.7***
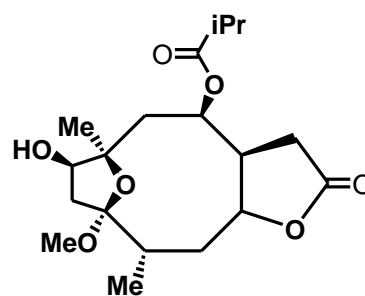
*Tithonia diversifolia **5.8***
constituent
Cytotoxic

FIGURE 5.4

Eudesmanolides (with a 6/6-fused bicyclic frame Figure **5.5**). This class usually exists as either a linear fusion of the two 6-membered rings and lactone or with the methylene lactone fused in the α-position to the bridgehead of the 6/6-ring An example of the former class is 5α hydroxy-eudesma-4,11-dien-12,8b-olide (**5.11**),[92] a compound isolated in 2007 from the Chinese herbs

[91] A. K. Picman, *Biochem. Syst. Ecol*. **1986**, 14, 255.

[92] C.-M. Wang, Z.-J. Jia, R.-L. Zheng, Planta Med. **2007**, 73, 180.

Carpesium macrocephalum and *Carpesium cernuum* and shown to be cytotoxic to human ovarian cell lines.

An example of the latter class is the sonchucarpolide derivative **5.12** which was isolated from *Centaurea spinosa* and shown to possess antibacterial activity (**Figure 5.5**).[93]

Eudesmanolides frame motif
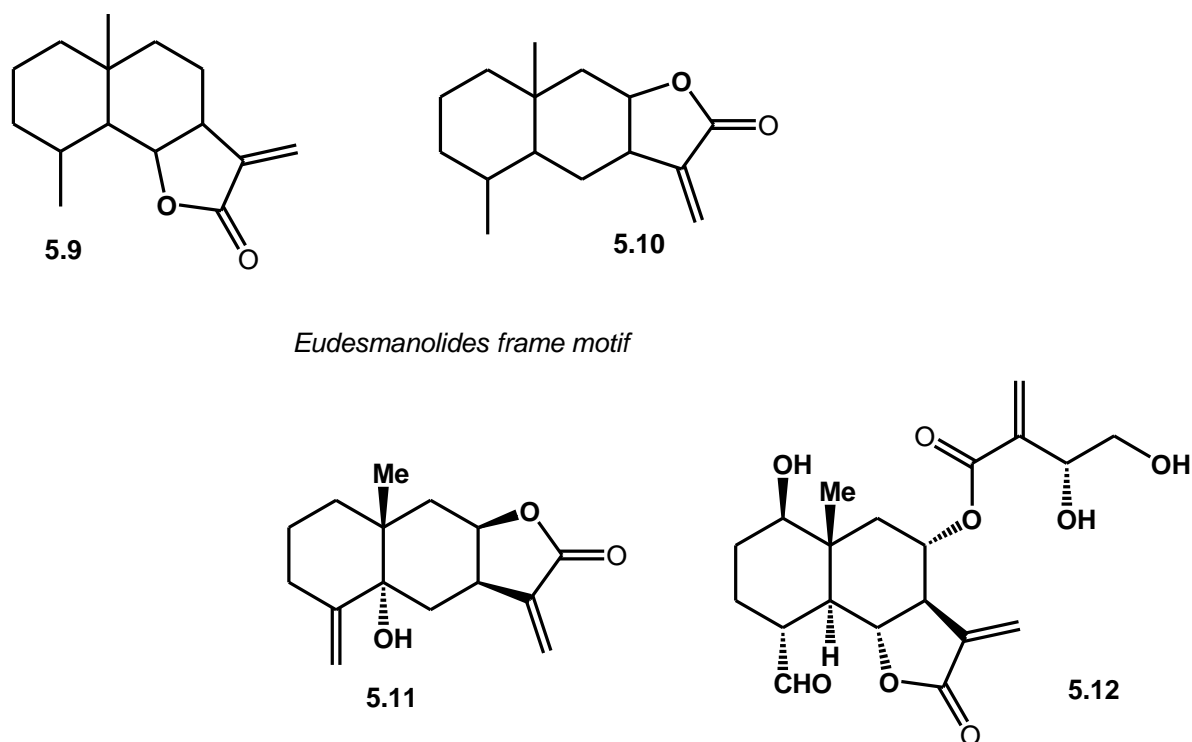
FIGURE 5.5

guaianolides and pseudoguaianolides (both with a fused 5/7-bicyclic system). In 2008, α-methylene-γ-butyrolactone guaianolides compounds **5.15** and **5.16** were isolated from *Pulicaria crispa*[94] and *Saussurea pulchella*,[95] respectively.(**Figure 5.6**)

[93] V. Saroglou, A. Karioti, C. Demetzos, K. Dimas, H. Skaltsa, J. Nat. Prod. **2005**, 68, 1404.

[94] M. Stavri, K. T. Mathew, A. Gordon, S. D. Shnyder, R. A. Falconer, S. Gibbons, Phytochemistry **2008**, 69, 1915.

[95] M. C. Yang, S. U. Choi, W. S. Choi, S. Y. Kim, K. R. Lee, J. Nat. Prod. **2008**, 71, 678.

5.13

5.14

Guaianolides -type motif

5.15

5.16
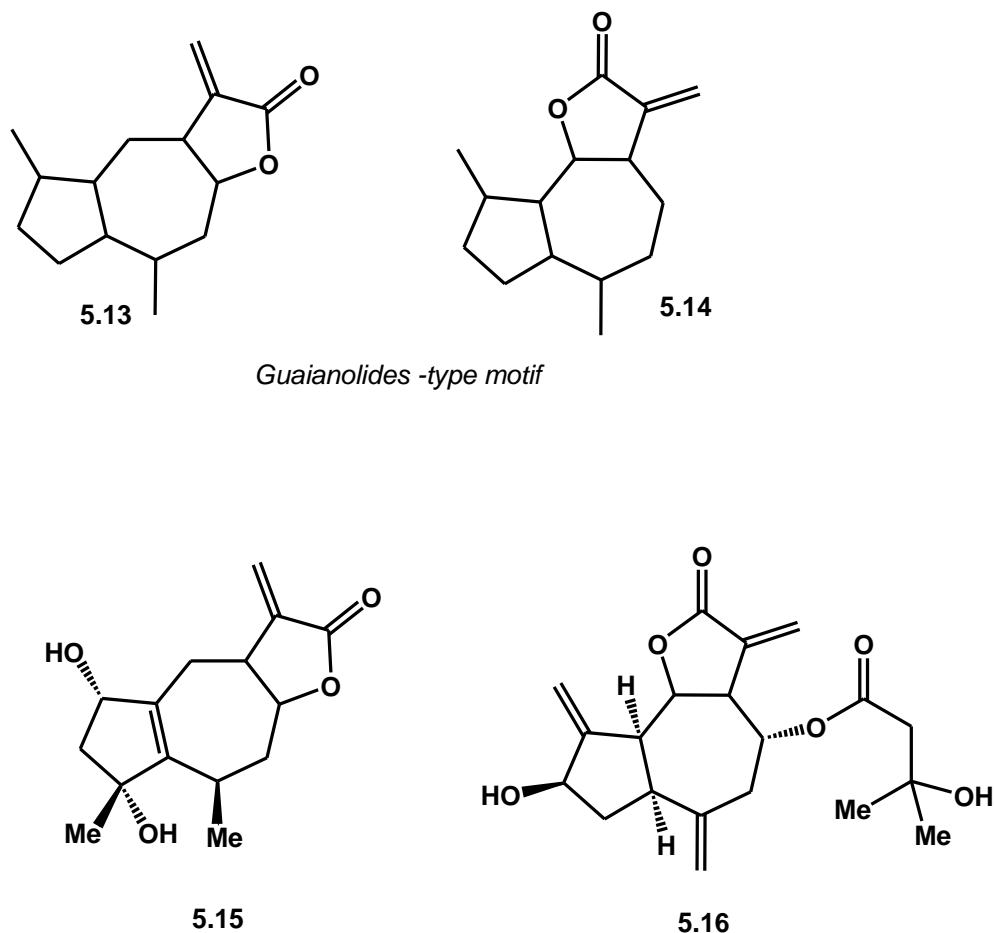
FIGURE 5.6

Pseudoguaianolides (**Figure 5.7**) are closely related to guaianolides in structural terms, differing only in the position of the one carbon substituent on the cyclopentane ring. In guaianolides the substituent is at the α position to the ring junction, whereas in pseudoguaianolides it is located at the ring junction itself. Recently isolated bioactive pseudoguaianolides include the hispitolide family **5.19**,[96] which exhibit potent activity against the hepatitis C virus, and cytotoxic parthenin analogues such as **5.20** isolated from *Parthenium hysterophorus*.[97]

[96] J.-F. Hu, R. Patel, B. Li, E. Garo, G. W. Hough, M. G. Goering, H.-D. Yoo, M. O_Neil-Johnson, G. R. Eldridge, *J. Nat. Prod.* **2007**, 70, 604.

[97] B. Das, V. S. Reddy, M. Krishnaiah, A. V. S. Sharma, K. R. Kumar, J. V. Rao, V. Sridhar, *Phytochemistry* **2007**, 68, 2029.

5.17

5.18

Pseudoguaianolides -type motif

*Hispitolide A **5.19**
antiviral*

*Parthenium hysterophorus
constituent A **5.19**
Cytotoxic*
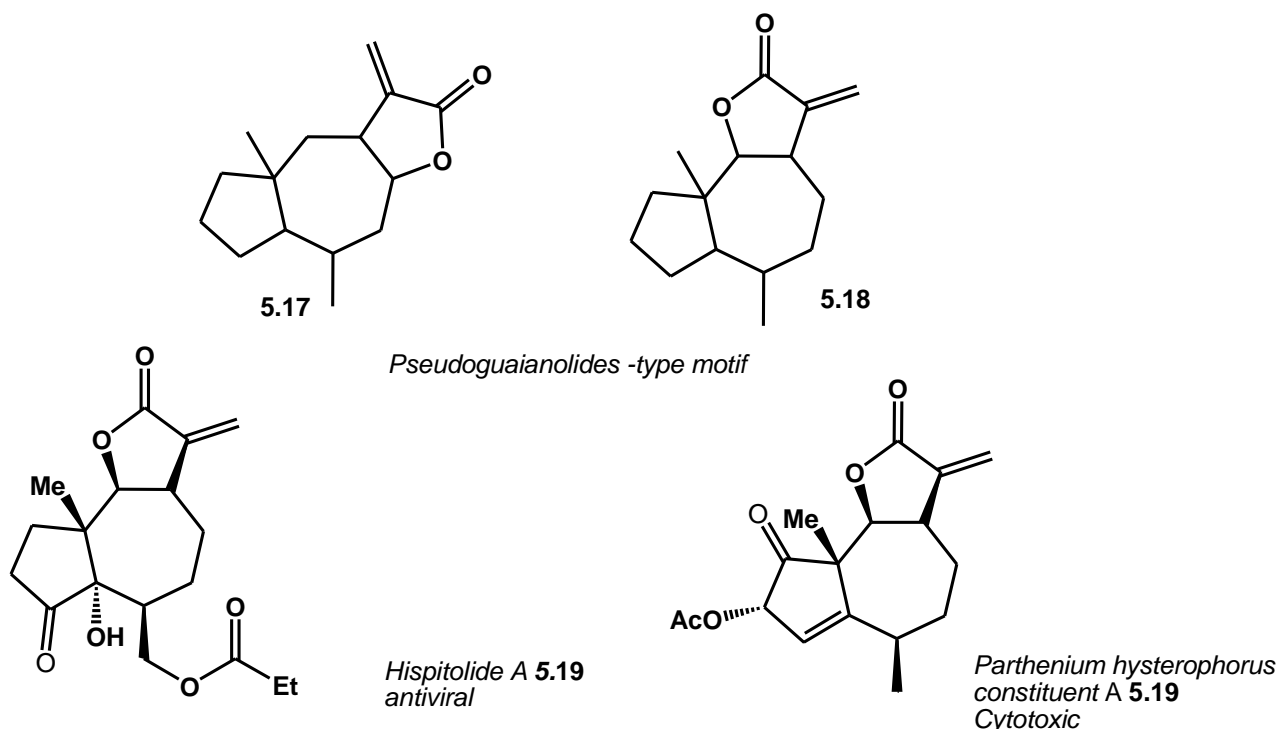
FIGURE 5.7

Cembranolides- There is a growing family of α-methylene-γ-butyrolactones based on the cembrane diterpene family known as cembranolides. These natural products, which are believed to be produced as defence chemicals, have mainly been isolated from marine soft corals of the genera *Lobophytum, Sinularia*, and *Sarcophyton*, and from gorgonians of the genus *Eunicea*. Soft coral of the genus *Lobophytum* has proved to be a particularly rich source of cembranolides.

Recently examples include michaolide A (**5.21**; plus 11 related family members) isolated from *Lobophytum michaelae* in 2007[98] and *crassumolide A* (**22**) isolated in 2008 from *Lobophytum crassum* together with four related novel compounds.[99] Both **5.21** and **5.22** display cytotoxic properties (e.g. against human colon and leukaemia cell lines).(**Figure 5.8**)

[98] L.-T. Wang, S.-K. Wang, K. Soong, C.-Y. Duh, *Chem. Pharm. Bull*. **2007**, 55, 766.

[99] C.-H. Chao, Z.-H.Wen, Y.-C.Wu, H.-C. Yeh, J.-H. Sheu. *J. Nat. Prod.* **2008**, 71, 1819.
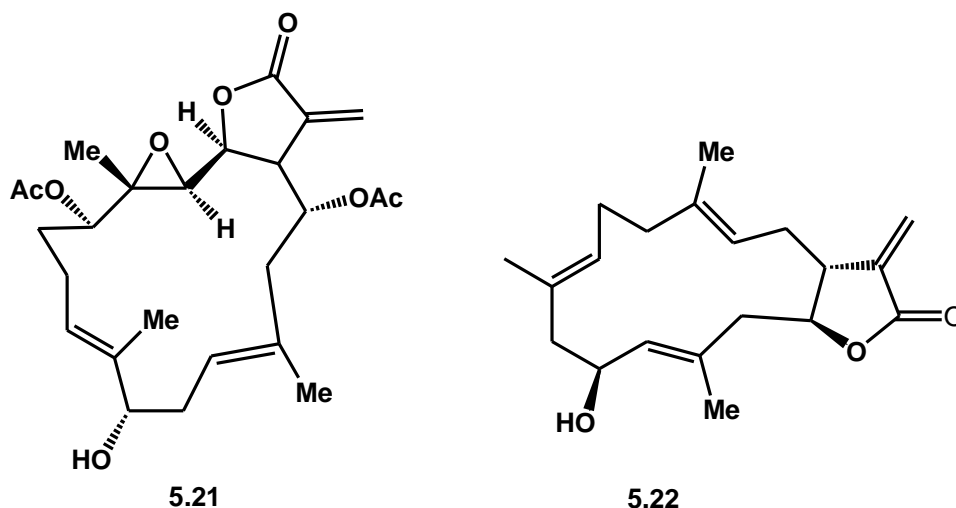
FIGURE 5.8

Non terpenoidic α-alkylidene-γ-butyrolactones

A large number of non-terpenoid, naturally occurring α-alkylidene-γ-butyrolactones are also known. Lignan representatives are growing in number and are illustrated by compounds **5.23–5.25**. Taiwanin A (**5.23**) was isolated from *Taiwania cryptomerioides* and shown to inhibit the growth of three types of human tumor cell;[100] more recent studies investigated the mode of action (induces cell-cycle arrest at the G2M phase and p53-dependant apoptosis).[101] The structurally related piperphilippinin family of lignans (e.g. piperphilippinin IV, **5.24**), was obtained from *Piper philippinum* in 2007,[102] and several members were found to possess antiplatelet aggregation activity. Similar compounds were isolated from *Phyllanthus acutissima*,[103] with acutissimalignan A (**5.25**) being the most noteworthy in a structural sense in view of its naphthaleno-γ-lactone tricyclic nucleus.(**Figure 5.9**)

[100] S.-T. Chang, D. S.-Y. Wang, C.-L. Wu, S.-G. Shiah, Y.-H. Kuo, C.-J. Chang, *Phytochemistry* **2000**, 55, 227.

[101] P.-J. Ho, C.-K. Chou, Y.-H. Kuo, L.-C. Tu, S.-F. Yeh, *Life Sci.* **2007**, 80, 493.

[102] Y.-C. Chen, C.-H. Liao, I.-S. Chen, *Phytochemistry* **2007**, 68, 2101.

[103] P. Tuchinda, J. Kornsakulkarn, M. Pohmakotr, P. Kongsaeree, S. Prabpai, C. Yoosook, J. Kasisit, C. Napaswad, S. Sophasan, V. Reutrakul. *J. Nat. Prod*. **2008**, 71, 655.

Taiwanin A **5.23**
Tumor inhibitor

Piperphilippinin IV **5.24**
Anticoagulant

Acutissimalignan A **5.25**
Cytotoxic
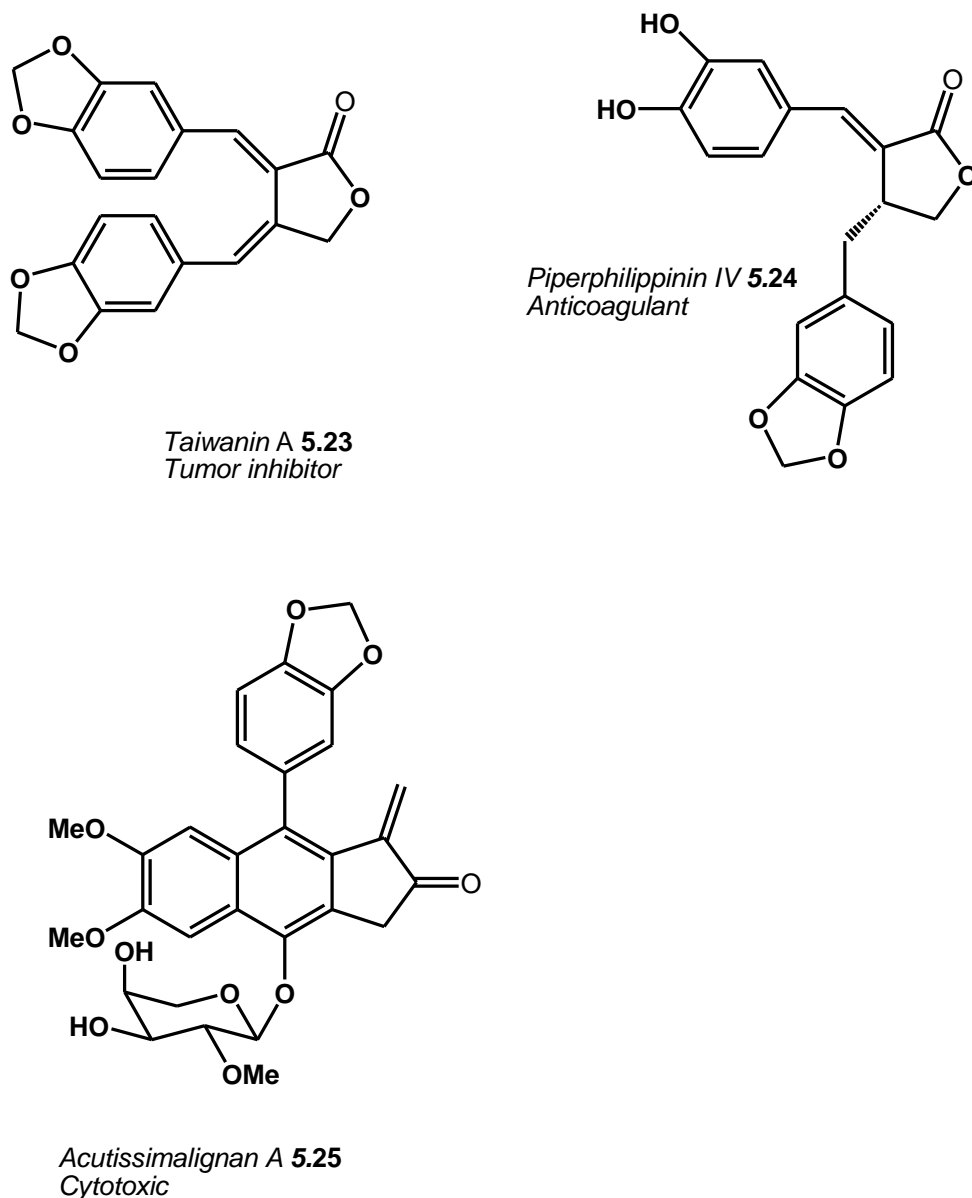
FIGURE 5.9

Several fairly simple naturally occurring α-alkylidene-γ-butyrolactones such as **5.26–5.28** have been isolated in the past five years. A family of novel compounds, including kotolactone A (**5.26**), was isolated from the stem wood of *Cinnamomum kotoense*,[104] and in 2008, two new monocyclic butanolides named subamolides D and E (**5.27**) [105] were isolated from the leaves of

---

[104] F.-C. Chen, C.-F. Peng, I.-L. Tsai, I.-S Chen, *J. Nat. Prod.* **2005**, 68, 1318.

[105] S.-Y. Kuo, T.-J. Hsieh, Y.-D. Wang, W.-L. Lo, Y.-R. Hsui, C.-Y. Chen, *Chem. Pharm. Bull.* **2008**, 56, 97.

Cinnamomum subavenium and found to possess potent activity against a strain of colon cancer.

In 2009, another monocyclic example was reported when 3-methylene-4-pentadecyldihydrofuran-2-one (**5.28**) was isolated from the juice of the ripe fruit of *Artabotrys odoratissimus* and found to possess good antifungal activity.(**Figure 5.10**)

It was proposed that the C20 natural product **5.28** was produced by a mixed isoprene/fatty acid biosynthetic pathway.[106]

Figure 5.10

Kotolactone A **26**

Subamolide D (Z)
Subamolide E (E) **27**
Anticancer

28
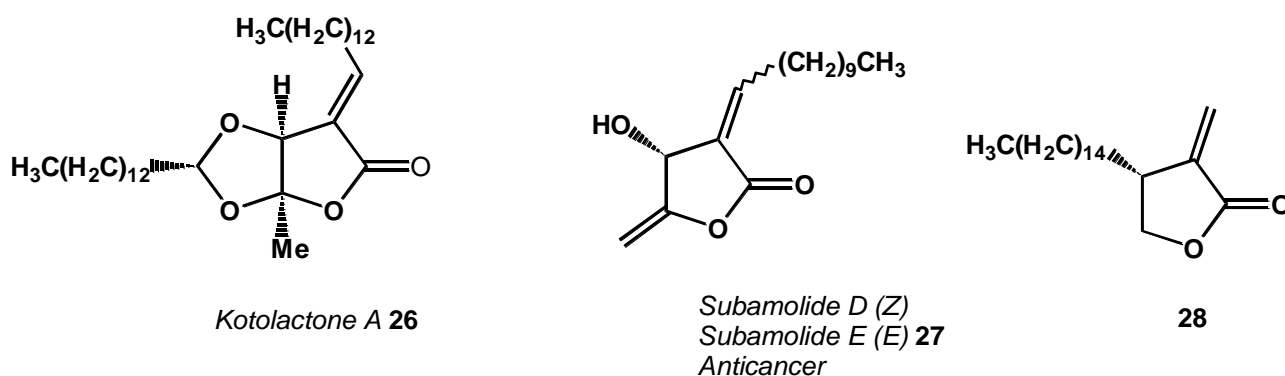
FIGURE 5.10

5.3 BIOLOGICAL ACTIVITY

The biological activities are wide-ranging and often potent. Of the newly discovered natural products there are numerous examples of compounds with cytotoxic/anticancer activity. Anti-inflammatory, antibacterial, anticoagulant and antifungal properties have also been found. Recently, the antiviral activity of α-methylene-γ-butyrolactones has been reported; for example, parthenolide **5.2** and the hispitolides **5.19**, both of which are active against the hepatitis C virus. In addition, the antiprotozoal, mantiparasitic, and insect antifeedant activities of sesquiterpene α-methylene-γ-butyrolactones have been reviewed.

---

[106] P. K. Bordoloi, P. D. Bhuyan, P. Boruah, M. Bordoloi, P. G. Rao,*Phytochem. Lett.* **2009**, 2, 22.

The allergenic properties of α-methylene-γ-butyrolactones, which are often present in pollen, should also be noted. Such compounds can cause allergenic contact dermatitis (ACD)[107] and photodermatosis.

It has been proposed that the α-methylene-γ-butyrolactones react with skin proteins in a Michael-type reaction, thereby forming antigenic compounds within epidermal cells.[108] α-methylene-γ-butyrolactones are excellent conjugate acceptors and this is believed to provide the basis of many of their biological activities (e.g. reaction with L-cysteinyl residues in proteins/enzymes). [109]

Finally, the over-the-counter herbal remedies arnica (treatment of strains, sprains, and bruises)[110] and feverfew (anti-inflammatory, anticancer, and antiviral properties)[111] deserve to be mentioned again in this section, as does arglabin (**5.4**), which in the prodrug form of the hydrochloride salt of the dimethylamino adduct, has been used against breast, lung, and liver cancer strains in Kazakhstan.[112]

5.4 BIOSYNTHESIS

The biosynthesis of sesquiterpenoid α-methylene-γ-butyrolactones proceeds along the terpene biosynthetic pathway via geranyl pyrophosphate (GPP) and then "head-to-tail" coupling with isopentenylpyrophosphate (IPP) to produce farnesyl pyrophosphate.

In standard sesquiterpenoid α-alkylidene-γ-lactone biosynthesis, FPP (**5.29**) then undergoes cyclization to (+)-germacrene A (**5.30**) and oxidative elaboration giving germacrene acid (**5.35**; **Scheme 5.1**).

---

[107] a) G. Dupuis, C. Benezra, *Allergenic Contact Dermatitis to Simple Chemicals: A Molecular Approach*, Marcel Dekker,
  New York, **1982**; b) A. K. Picman, F. Balza, G. H. N. Towers, *Phytochemistry* **1982**, 21, 1801.

[108] B. B. Patel, T. G. Waddell, R. M. Pagni, *Fitoterapia* **2001**, 72,511.

[109] a) H. N. Pati, U. Das, R. K. Sharma, J. R. Dimmock, *Mini-Rev. Med. Chem.* **2007**, 7, 131; b) Y. Higuchi, F. Shimoma, M. Ando, *J. Nat. Prod*. **2003**, 66, 810.

[110] G. Lyss, T. J. Schmidt, H. L. Pahl, I. Merfort, *Pharm. Pharmacol. Lett.* **1999**, 9, 5.

[111] c) M. T. Yip-Schneider, H. Wu, M. Ralstin, C. Yiannoutsos, P. A. Crooks, S. Neelakantan, S. Noble, H. Nakshatri, C. J. Sweeney, C. M. Schmidt, *Mol. Cancer Ther*. **2007**, 6, 1736; d) D.-R. Hwang, Y.-S. Wu, C.-W. Chang, T.-W. Lien, W.-C. Chen, U.-K. Tan, J. T. A. Hsu, H.-P. Hsieh, *Bioorg. Med. Chem*. **2006**, 14, 83.

[112] N. S. Zhangabylov, L. Y. Dederer, L. B. Gorbacheva, S. V. Vasil_eva, A. S. Terekhov, S. M. Adekenov, *Pharm. Chem. J.* **2004,** 38, 651.

More recently, extensive studies have been carried out by de Kraker and co-workers concerning the biosynthesis of sesquiterpene lactones in chicory (**Scheme 5.1**).[113] They obtained an enzyme from chicory roots and demonstrated that it converts germacrene acid (**5.31**) into costunolide (**5.32**).

Structurally, costunolide (**5.32**) is the simplest naturally occurring germacranolide, and it is further elaborated biosynthetically to give more complex germacranolides, as well as eudesmanolides and guaianolides.

In 2009 some theoretical studies were carried out to clarify the mechanism of the biosynthetic cyclization process which converts germacranolides into guaianolides and pseudoguaianolides.[114]

[113] J.-W. de Kraker, M. C. R. Franssen, M. Joerink, A. de Groot, H. J. Bouwmeester, *Plant Physiol*. **2002,** 129, 257.

[114] J. E. Barquera-Lozada, G. Cuevas, J. Org. Chem. 2009, 74, 874; see also A. Ortega, E. Maldonado, *Heterocycles* **1989**, 29, 635.
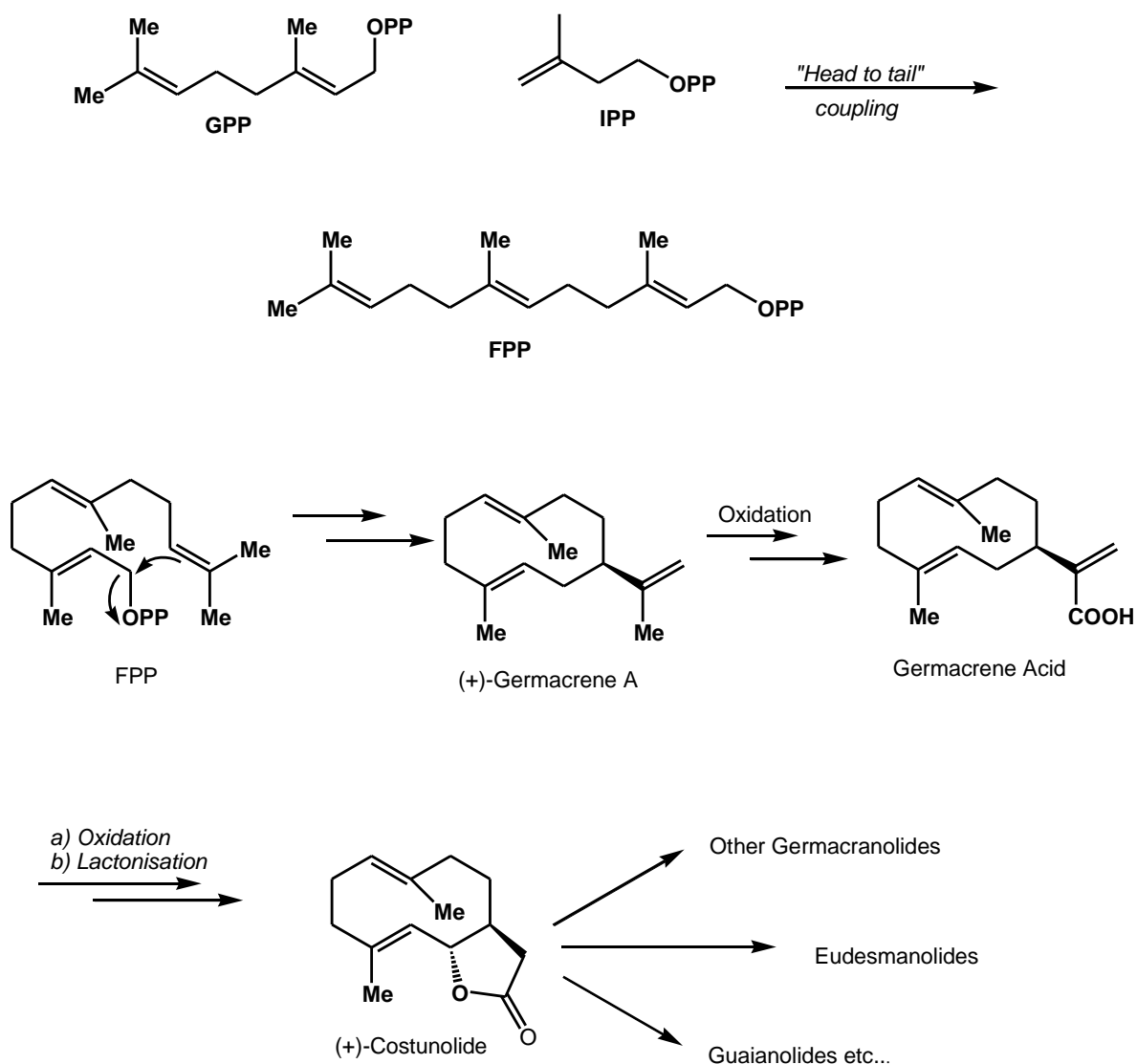
SCHEME 5.1

A non-FPP biosynthetic route is also possible and has been proposed for the biosynthesis of anthecotuloide (**5.34**),[115] an "irregular" sesquiterpene α-methylene-γ-butyrolactone isolated from *Anthemis cotula*.

It has been demonstrated that anthecotuloide (**5.34**) is formed via the intermediacy of the pyrophosphate **5.33**, resulting from a "head-to-head" coupling of geranyl pyrophosphate (GPP) with dimethylallylpyrophosphate (DMAPP). Subsequent oxidation of 5.**33** followed by lactonization produces anthecotuloide (**Scheme 5.2**).

---

[115] a) J. van Klink, H. Becker, S. Andersson, W. Boland, *Org.Biomol. Chem.* **2003**, 1, 1503; b) F. Bohlmann, C. Zdero, M. Grenz, *Tetrahedron Lett.* **1969**, 10, 2417.

SCHEME 5.2
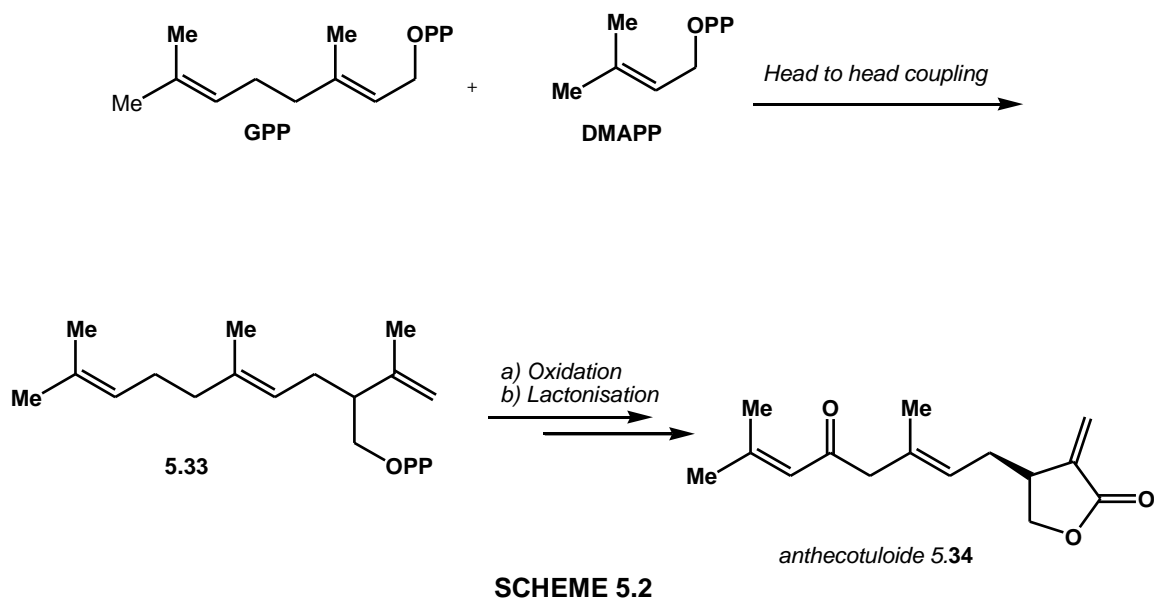
One other biosynthetic study concerns the biosynthesis of α-methylene-γ-butyrolactone (**5.37**) itself.[116]

Hutchinson and Leete studied the biosynthesis of tuliposide A (**5.36**), a 1-acylglucoside found in tulips, which is believed to undergo hydrolysis to generate α-methylene-γ-butyrolactone (tulipalin A, **5.37**) to protect the tulip bulbs from fungal infection (**Scheme 5.3**).

Using labeled pyruvate, they established that an initial condensation between pyruvate and acetyl coenzyme A ultimately generated γ-hydroxy-α-methylene-butanoic acid (**5.35**), the tuliposide A precursor.

[116] C. R. Hutchinson, E. Leete, *J. Chem. Soc. D* **1970**, 1189.

CH₃COSCoA + CH₃COCOOH ⟶

35

Glucose ⟶

Tuliposide A **5.36**

Tulipalin A **5.37**
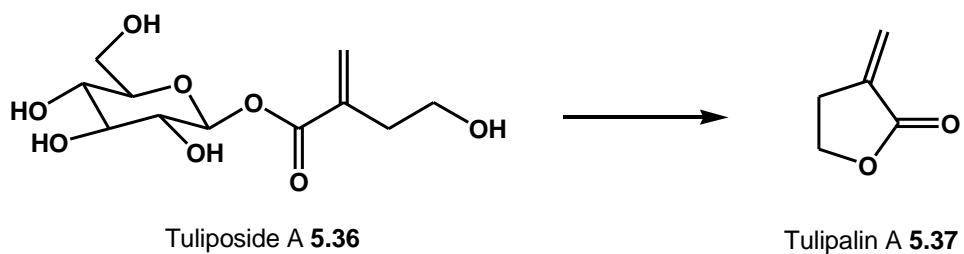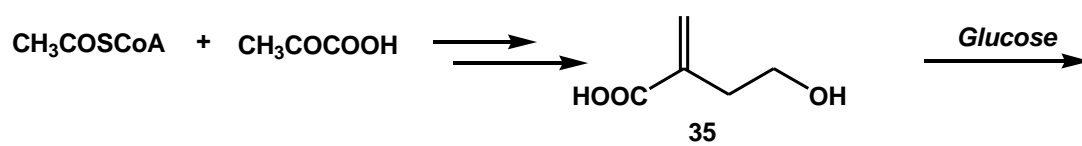
SCHEME 5.3

5.5 SYNTHESIS OF α-ALKYLIDENE-γ-BUTYROLACTONES

The synthesis of α-methylene-γ-butyrolactones has been extensively studied and well reviewed up to 1986. However, a wide array of new methods has been developed more recently.[117]

1 By Alkylidenation of g-Butyrolactones
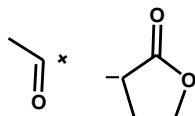
2 Lactonization Approaches
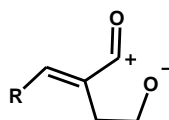
3. The Dreiding–Schmidt Organometallic Approach
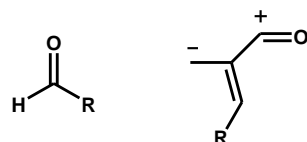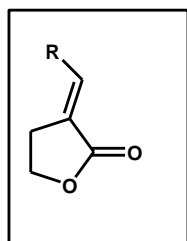
4. Other Metal-Promoted Approaches
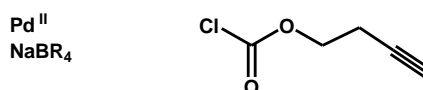
Pd^II
NaBR₄

5. Elaboration of Existing a-Methylene-g-butyrolactones

RHC=CH₂

6. Radical Cyclization Approaches

Bu₃SnH, AIBN

PhSe
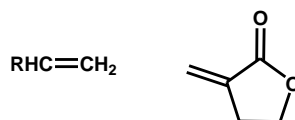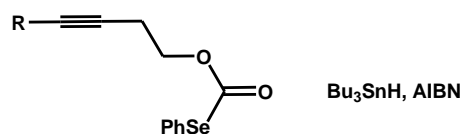
[117] Russell R. A. Kitson, Alessia Millemaggi, and Richard J. K. Taylor *Angew. Chem. Int. Ed.* **2009**, 48, 9426 – 9451 and references therein

5.5.1 By Alkylidenation of γ-Butyrolactones

One of the most commonly-used methods for the preparation of α-methylene-γ-butyrolactones, well-covered in earlier reviews,[118] involves the reaction of the γ−butyrolactone enolate or enolate equivalent with formaldehyde (or a formate ester followed by reduction) and subsequent dehydration of the resulting α-(hydroxymethyl)-γ-lactone, often by base-mediated elimination of a derived sulfonate ester. Metz and co-workers observed the spontaneous elimination of water when employing this method for their syntheses of the antileukaemia agents (-)-eriolanin (**5.39**) and (-)-eriolangin (**5.41**).[119] Treatment of γ-butyrolactone **38** with NaH followed by paraformaldehyde and heating to 100 °C in THF in a sealed tube gave the α methylene unit directly (**Scheme 5.4**)

[118] a) P. A. Grieco, *Synthesis* **1975**, 67. b) J. C. Sarma, R. P. Sharma, *Heterocycles* **1986**, 24, 441; c) N. Petragnani, H. M. C. Ferraz, G. V. J. Silva, *Synthesis* **1986**, 157.

[119] a) J. Merten, R. Frhlich, P. Metz, *Angew. Chem. Int. Ed*. **2004,** 43, 5991; b) J. Merten, A. Hennig, P. Schwab, R. Frhlich, S. V. Tokalov, H. O. Gutzeit, P.Metz, *Eur. J. Org. Chem*. **2006**, 1144.

5.39

(-)-Eriolanin

a) NaH,(CH$_2$O)n
THF, 100°C
Sealed tube

b) TBAF, THF

61% (2 steps)

5.38

5.40

5.41

(-)-Eriolangin
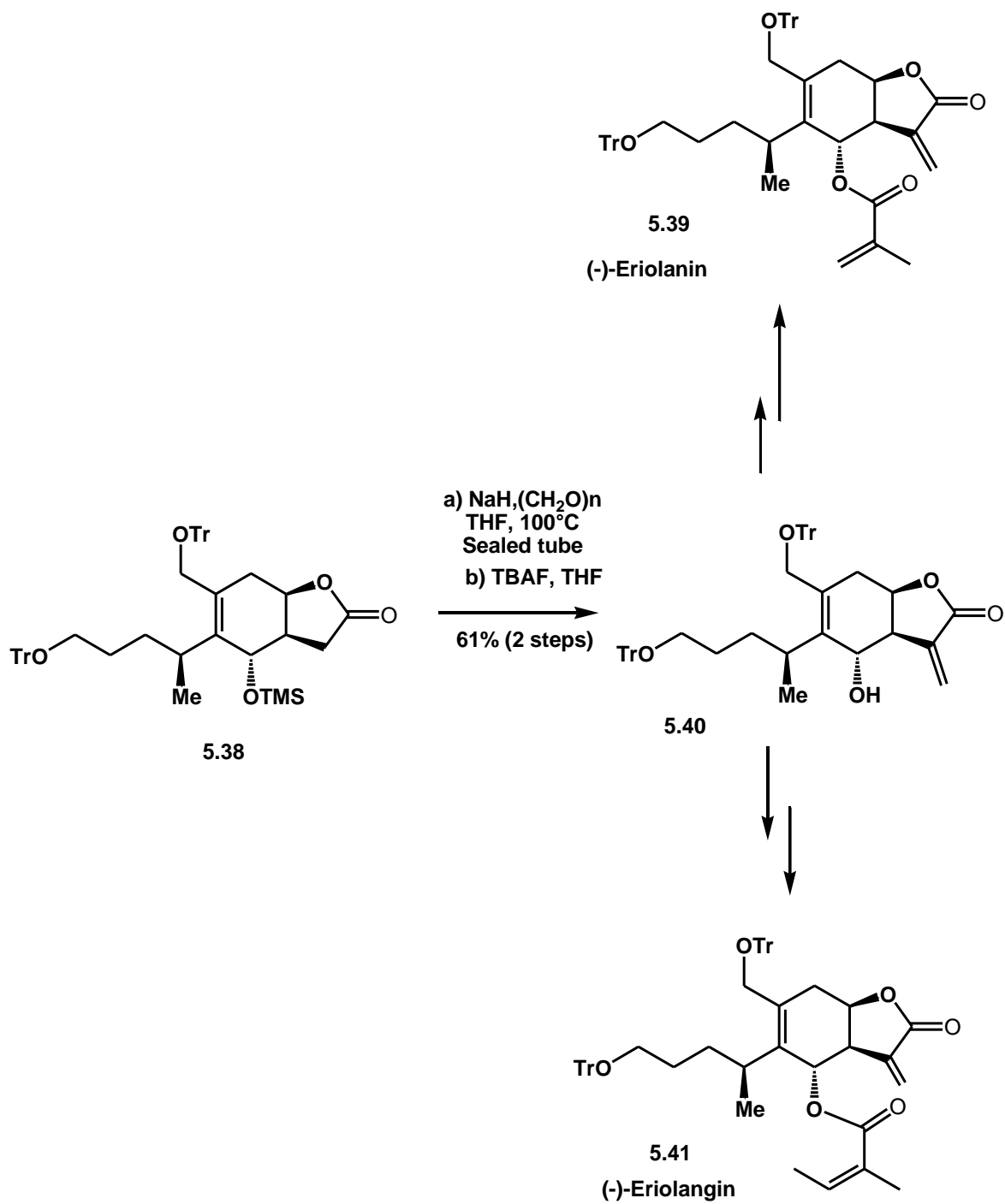
<u>**SCHEME 5.4**</u>

Eschenmoser's salt can be employed in place of formaldehyde and this approach was utilized by Reiser and co-workers in the total synthesis of the guaianolide arglabin (**5.4**), a farnesyl transferase inhibitor and promising antitumor agent (**Scheme 5.5**). The lithium enolate derived from γ–lactone **5.42** was trapped with Eschenmoser's salt to afford the tertiary amine **5.43** in good yield. Methylation and Hofmann elimination proceeded smoothly to give arglabin (**5.4**) in 80% yield.
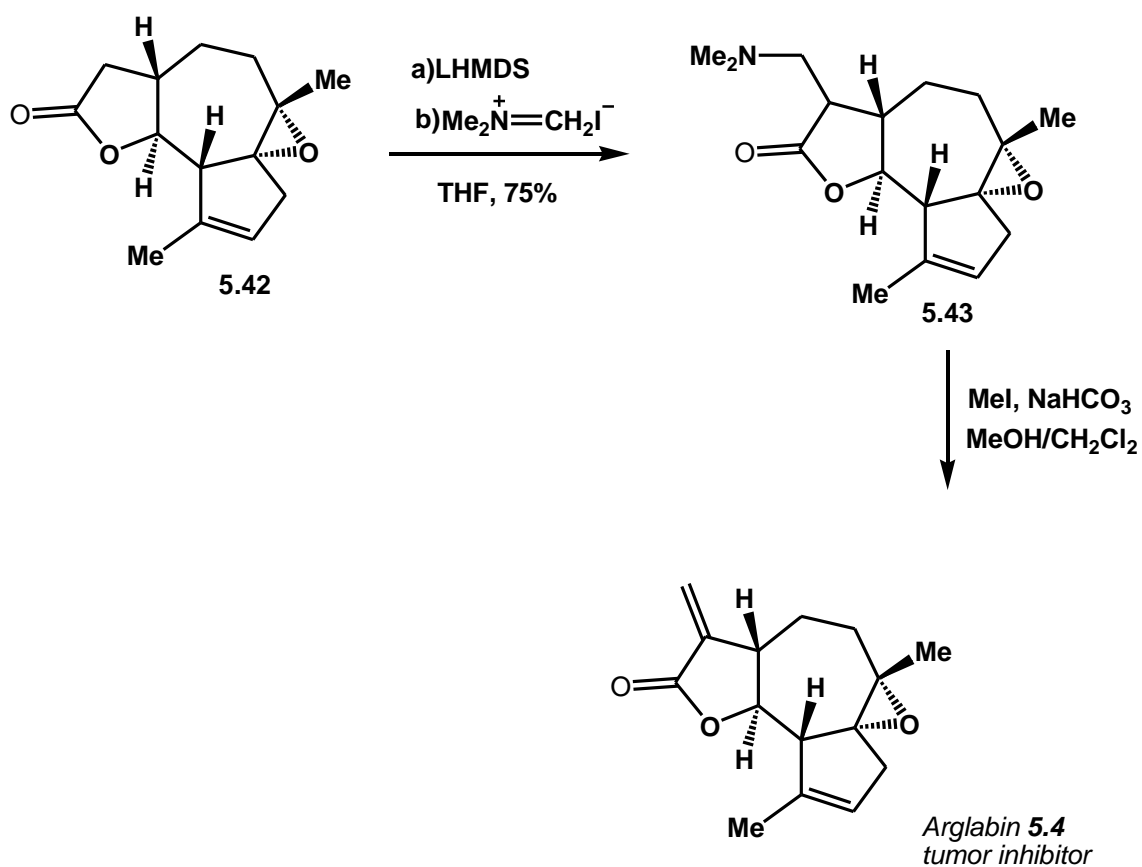
SCHEME 5.5

Another well-known route to α-methylene-γ-butyrolactones commences with an α-methyl lactone, and then utilizes a regioselective elimination reaction to introduce unsaturation. The most common variant of this type of approach proceeds by oxidation of an intermediate α-phenylselenide and subsequent β-

elimination. This approach was recently utilized by Oltra and co-workers in their synthesis of the eudesmanolide (+)-9β-hydroxyreynosin.[120]

The enolate derived from γ-lactone **5.44** was treated with phenylselenyl chloride; subsequent oxidation of the resulting selenide and selenoxide elimination afforded (+)-9βhydroxyreynosin (5.**45**) in 62% yield over the 2 steps (**Scheme 5.6**).
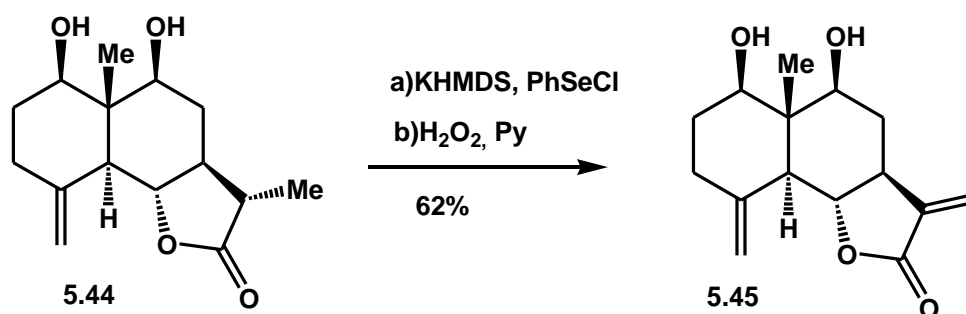
SCHEME 5.6

A different approach to α-alkylidene-γ-butyrolactones involves the direct alkylidation of an activated γ-lactone. Early examples involved Wittig and Horner‑Wadsworth‑Emmons (HWE) olefinations of α-phosphoranyl- or α-phosphono-γ-lactones with paraformaldehyde,[121] and these methods have since been utilized frequently.[122] The Wittig procedure was recently used by Jung and Murakami in their total synthesis of (-)-hedychilactone B (**5.48**; **Scheme 5.7**).[123]The aldehyde **5.46** was treated with α-(triphenylphosphoranyl)-γ-butyrolactone (**5.47**) to complete the total synthesis in 90% yield.

[120] A. F. Barrero, A. Rosales, J. M. Cuerva, J. E. Oltra, *Org. Lett.* **2003**, 5, 1935.

[121] H. M. R. Hoffmann, J. Rabe, *Angew. Chem*. **1985**, 97, 96; *Angew. Chem. Int. Ed. Engl.* **1985**, 24, 94.

[122] a)Albrecht, J. F. Koszuk, J. Modranka, M. R_zalski, U. Krajewska, A. Janecka, K. Studzian, T. Janecki, *Bioorg. Med.Chem*. **2008**, 16, 4872. b) G. E. Keck, R. L. Giles, V. J. Cee, C. A. Wager, T. Yu, M. B. Kraft, J. *Org. Chem.* **2008**, 73, 9675.

[123] M. E. Jung, M. Murakami, *Org. Lett.* **2007**, 9, 461.
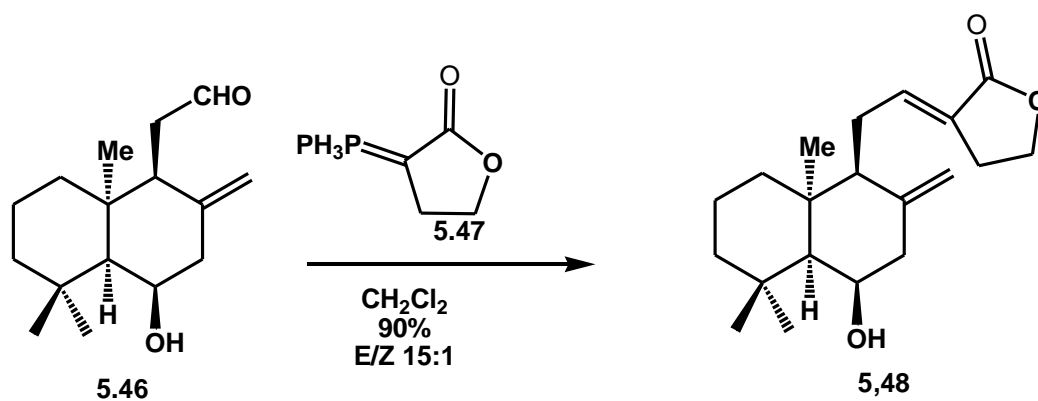
SCHEME 5.7

The phosphonate approach was employed by Akita and co-workers in the endgame of their total synthesis of (+)- pacovatinin (**5.52**; **Scheme 5.8**).[124] HWE olefination of aldehyde **5.49** with α-(diethylphosphono)- γ-butyrolactone (**5.50**) and KOtBu in DMSO gave a 63% yield of the desired isomer (E)-**5.51**, with 27% of the undesired (Z)-**5.51**. Cleavage of the TBS group in (E)-**5.51** was achieved with camphorsulfonic acid (CSA) in 92% yield and completed the total synthesis of (+)- pacovatinin (**5.52**).

---

[124] T. Miyake, K. Uda, M. Kinoshita, M. Fujii, H. Akita, *Chem. Pharm. Bull.* **2008**, 56, 398.
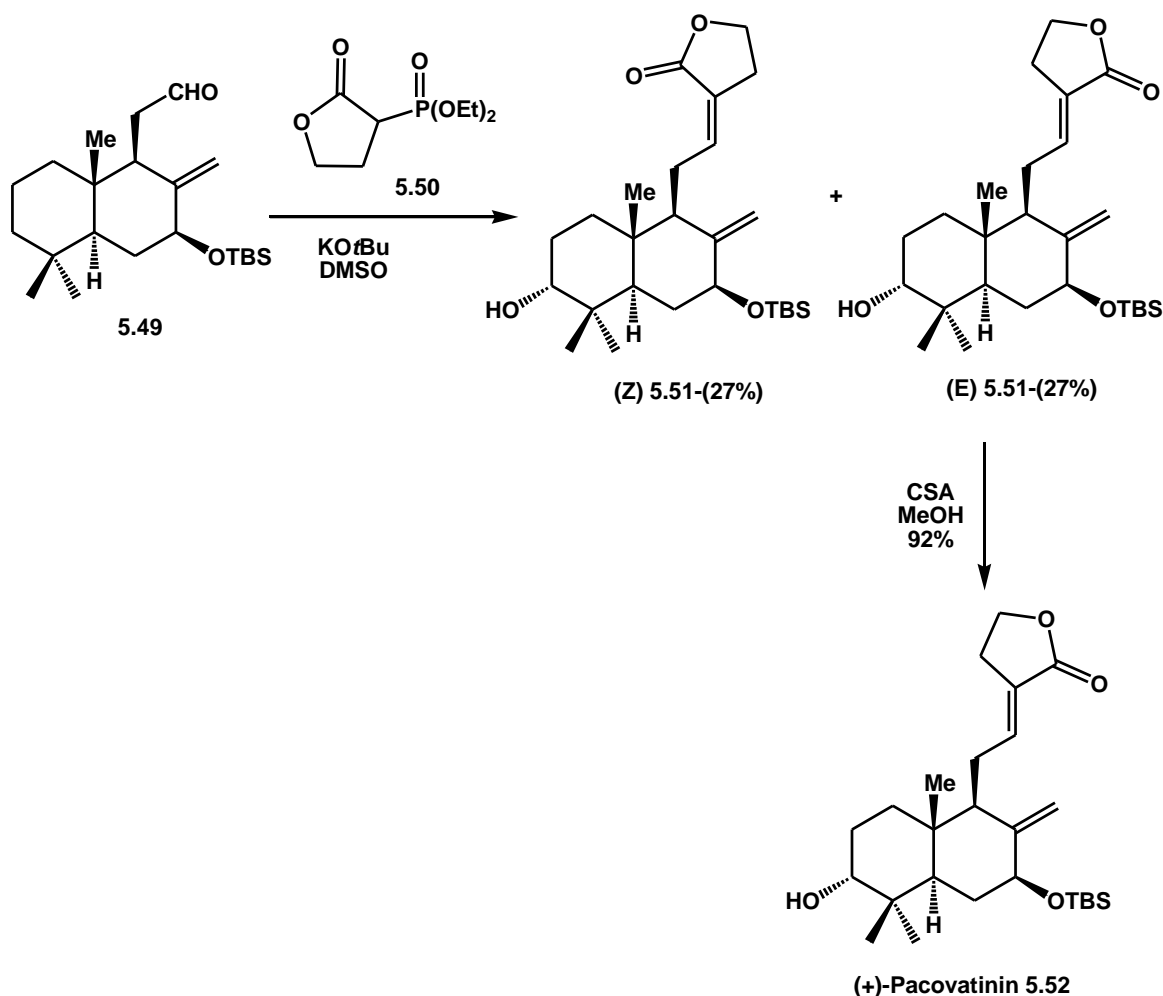
SCHEME 5.8

5.5.2 *Lactonization Approaches*

The construction of the α-methylene-γ-butyrolactone core by lactonization has been widely used and is well covered in earlier reviews.[125] Adam and co-workers developed an effective method for the preparation of optically active α-methylene-γ-butyrolactones by a lipase-catalyzed kinetic resolution (**Scheme 5.9**).[126] They submitted racemic γ-hydroxy esters rac-**5.53,** to enzyme catalyzed acetylation in the presence of lipase ChirazymeL-6. Excellent kinetic resolution was achieved, furnishing the ester (S)-(-)-**5.54** and the acetate (R)-(+)-**5.55** with excellent enantioselectivity at 50% conversion.

---

[125] a) P. A. Grieco, *Synthesis* **1975**, 67. b) J. C. Sarma, R. P. Sharma, *Heterocycles* **1986**, 24, 441; c) N. Petragnani, H. M. C. Ferraz, G. V. J. Silva, *Synthesis* **1986**, 157.

[126] W. Adam, P. Groer, C. R. Saha-Moller, *Tetrahedron: Asymmetry* **2000**, 11, 2239.

Cyclization of the optically active hydroxy ester (S)-(-)-**54** with trifluoroacetic acid afforded the α-methylene-γ-butyrolactone (S)-(+)-**5.56** in 89% yield (95% ee).
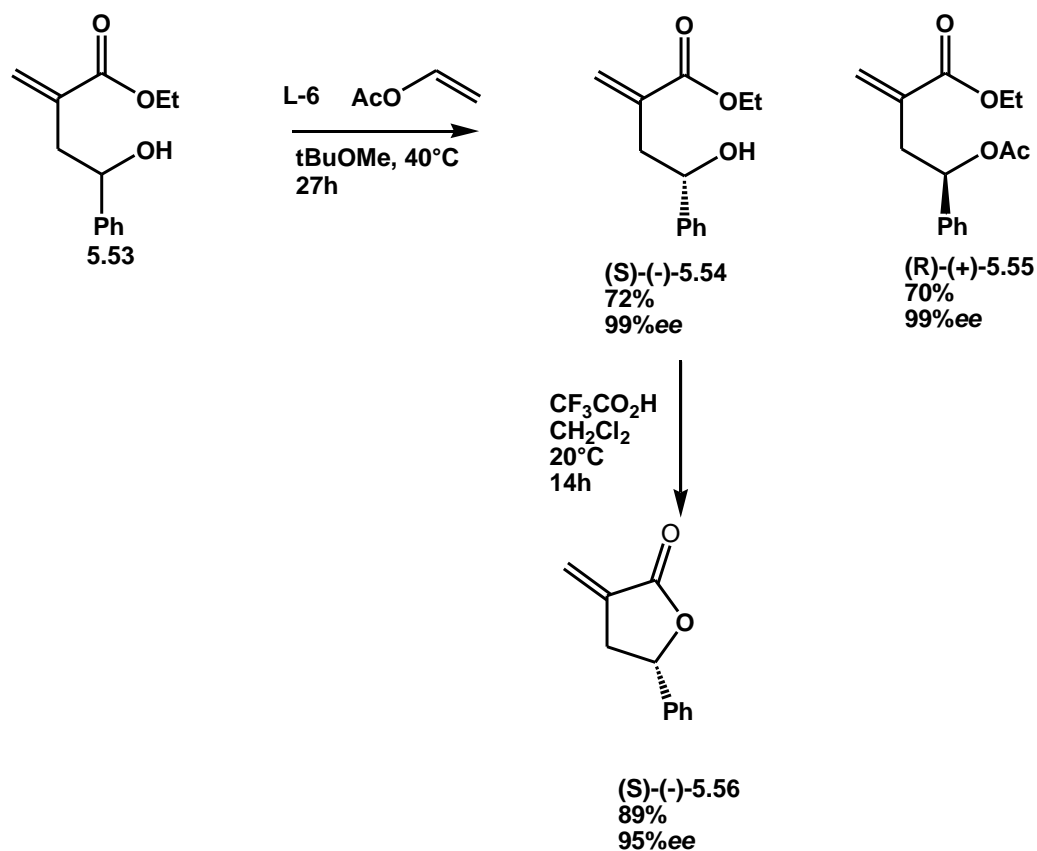
SCHEME 5.9

5.5.3 *The Dreiding–Schmidt Organometallic Approach*

In 1970, Dreiding et al. and Schmidt et al. developed a one-pot methodology for the preparation of α-methylene-γ-butyrolactones, which involved the treatment of 2-bromomethylacrylic esters with zinc to provide a functionalized organometallic reagent; addition of an aldehyde, followed by spontaneous cyclization produced α-methylene-γ-butyrolactones. The reaction proceeds via a six membered chair transition state and is cis-selective. This method provides one of the simplest and most direct methods for the preparation of α-methylene-γ-butyrolactones and it has been widely used and well reviewed. Dreiding-

Schmidt variants producing α-methylene-γ-butyrolactones have also been reported using chromium,[127] tin,[128] indium,[129] and Cu/Zn.[130]

Csuk's research group went on to develop an enantioselective Dreiding‑Schmidt variant, based on Oppolzer sultam methodology, for the asymmetric preparation of α-methylene-γ-butyrolactones **5.58** (Scheme **5.10**). Reaction between the enantiopure 2-bromomethyl-sultamamide **5.57** and a range of aldehydes using the zinc-silver/graphite reagent gave β-methyl-α-methylene-γ-butyrolactones such as **5.58** in high yields and moderate enantioselectivity. Similar examples were reported to produce the β-unsubstituted analogues.[131]

(-)-5.57

PhCHO
Zn/Ag-graphite

THF, 0°C
95%, 69%*ee*

(3S,4R)-5.58

(+)-5.57

PhCHO
Zn/Ag-graphite

THF, 0°C
96%, 90%*ee*

(3R,4S)-5.58
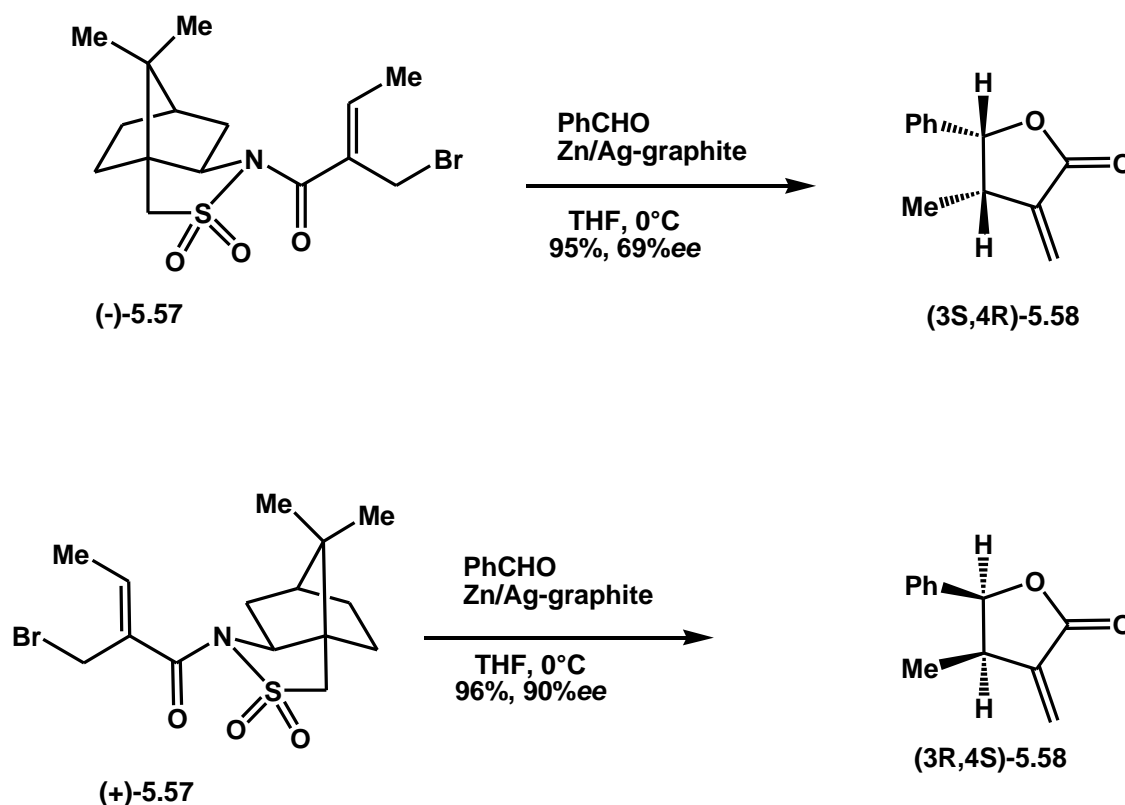
SCHEME 5.10

[127] Y. Okuda, S. Nakatsukasa, K. Oshima, H. Nozaki, *Chem. Lett.* **1985**, 481.

[128] a) K. Uneyama, K. Ueda, S. Torii, *Chem. Lett.* **1986**, 1201; b) Y.Masuyama, Y. Nimura, Y. Kurusu, *Tetrahedron Lett*. **1991**, 32, 225.

[129] a) V. J. Bryan, T.-H. Chan, *Tetrahedron Lett.* **1996**, 37, 5341; b) P. K. Choudhury, F. Foubelo,M. Yus, *Tetrahedron Lett.* **1998**, 39, 3581.

[130] A. R. Sidduri, P. Knochel, *J. Am. Chem. Soc.* **1992**, 114, 7579.

[131] R. Csuk, C. Schroder, S. Hutter, K. Mohr, *Tetrahedron: Asymmetry* **1997**, 8, 1411.

Finally, the classical Dreiding‑Schmidt process is still widely used. Trivedi and co-workers employed it in a highly stereoselective synthesis of steroid-derived spiro-α-methylene-γ-butyrolactones (Scheme **5.11**).[132] A range of steroidal α-hydroxyketones were utilized, and it was established that the α-hydroxy group was fundamental for the stereoselectivity, as the reaction proceeded via a transition state involving a chelate intermediate in which zinc is coordinated to both the hydroxy and carbonyl oxygen atoms. As an example, α–hydroxyketone **5.59** gave the spiro-α-methylene-γ-butyrolactone **5.61** in 76% yield as a single diastereomer.

SCHEME 5.11
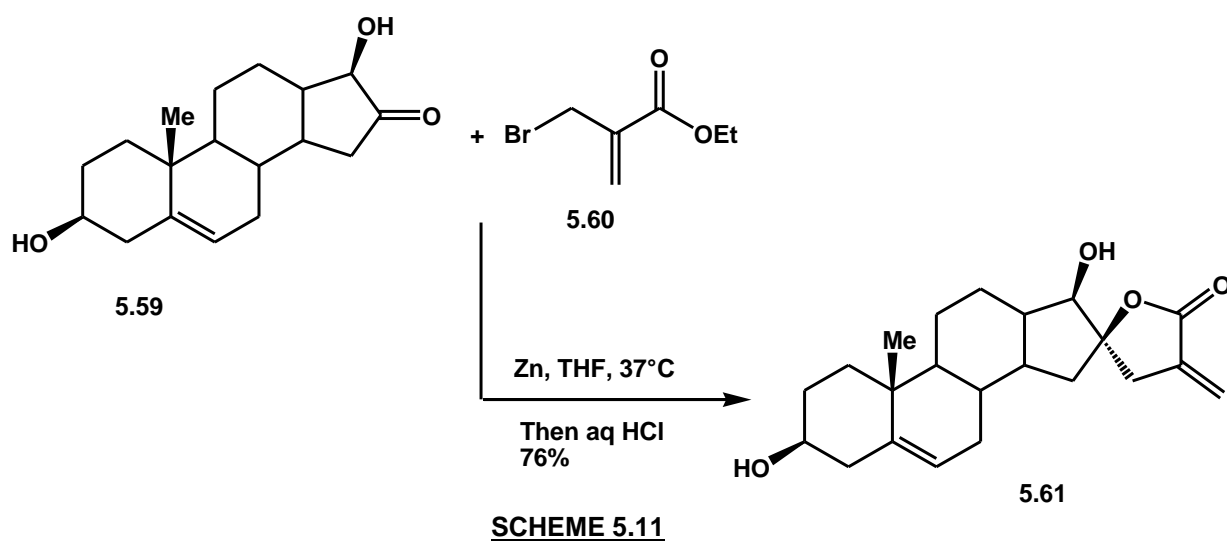
5.5.4 *Other Metal-Promoted Approaches*

In addition to the Dreiding‑Schmidt, a number of other organometallic approaches to α-methylene-γ-butyrolactones has been reported, and there have been many advances in this area in recent years.

Tungsten-promoted intramolecular alkoxycarbonylation approach to α-methylene-γ-butyrolactones was reported by Liu and co-workers (Scheme 5.**12**).[133]

[132] M. S. Sawant, R. Katoch, G. K. Trivedi, U. R. Desai, *J. Chem. Soc. Perkin Trans.* **1998**, 1 , 843.

[133] C.-C. Chen, J.-S. Fan, S.-J. Shieh, G.-H. Lee, S.-M. Peng, S.-L. Wang, R.-S. Liu, *J. Am. Chem. Soc*. **1996**, 118, 9279.

Treatment of propargyl chloride (**5.62**) with Na[CpW(CO)$_3$], followed by protonation under controlled conditions, gave the isolable η^3-γ-lactone **5.63** in 81% yield *(syn/anti* 62:38). A mechanistic proposal was presented to explain the observed products and stereoselectivity. The separated *syn*-η^3-tungsten intermediate **5.63** was then transformed into the corresponding α-methylene-γ-butyrolactone **5.64** by sequential treatment with nitrosonium tetrafluoroborate and sodium iodide (to generate the η^3-W(Cp)(NO)I species), followed by addition of an aldehyde. The aldehyde adds selectively at the γ-position to give, with benzaldehyde, the *anti*-disubstituted-γ-lactone **5.64** in 67% yield and with complete diastereoselectivity.
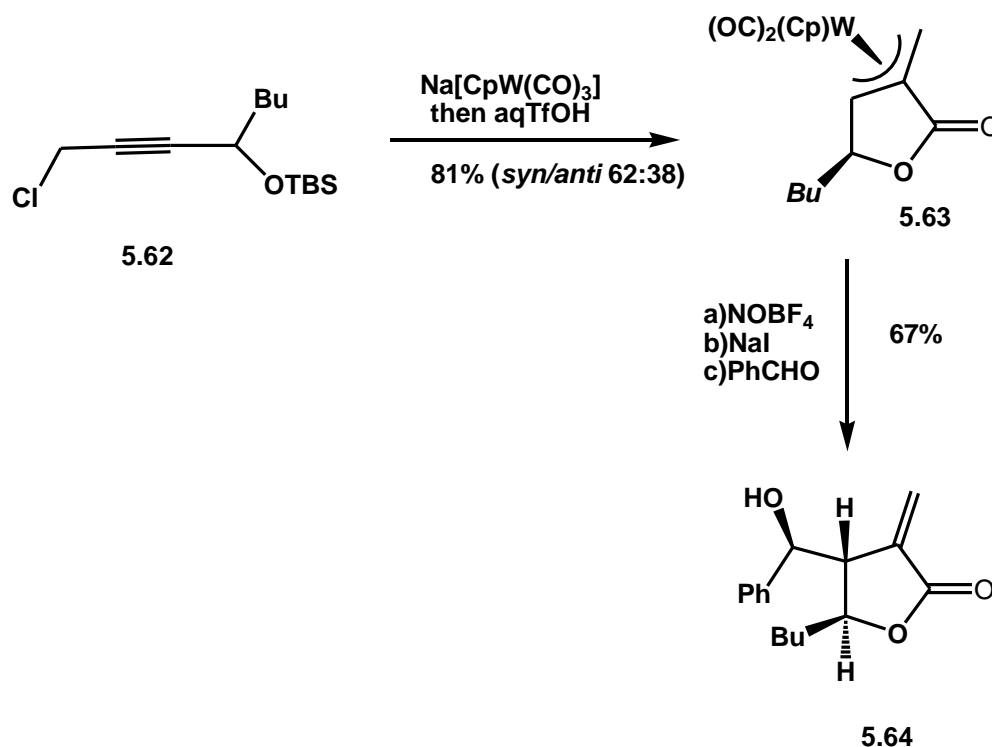
SCHEME 5.12

In 2003, Uenishi and Ohmi reported the conversion of hydroxylated vinyl bromides such as **5.65** (prepared by a Sakurai reaction of the corresponding 2-bromo-allylsilane and an aldehyde) into α-methylene-γ-butyrolactones **5.66** (Scheme **5.13**).[134] The transformation was mediated by stoichiometric amounts

[134] J. Uenishi, M. Ohmi, *Heterocycles* **2003**, 61, 365.

of [Ni(CO)$_2$(PPh$_3$)$_2$] and proceeded by a standard oxidative addition/CO insertion/reductive elimination sequence, with the nickel complex acting as the source of CO. The relative configuration of the starting material is retained in the product.

SCHEME 5.13
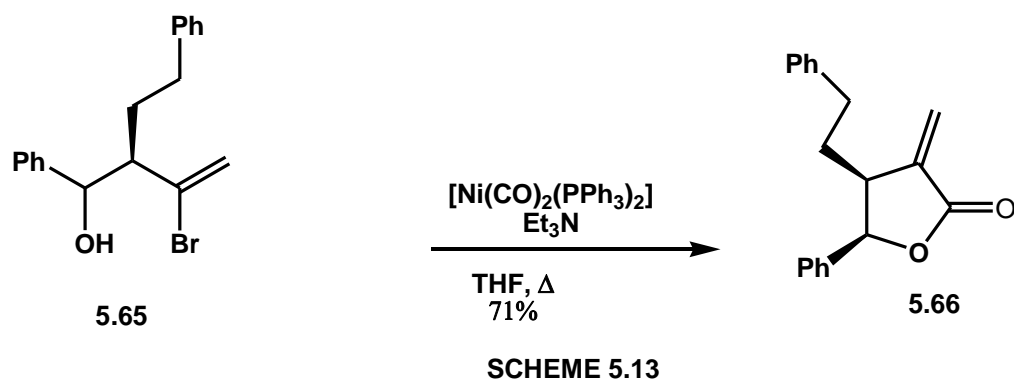
5.5.5 *Elaboration of Existing α-methylene-γ-butyrolactones*

The elaboration of simple α-methylene-γ-butyrolactones to give more complex α-alkylidene-γ-butyrolactone systems has been explored before,[135] but there have been major advances in this area in recent years. One of the most valuable methods for the construction of carbon‑carbon bonds is by olefin cross-metathesis,[136] and recently the groups of Howell and Cossy have extended the methodology to encompass exocyclic enone substrates.[137] In 2007, Howell's research group reported the coupling of α-methylene-γ-butyrolactone **37** with a range of terminal olefins (e.g. **5.67**) in the presence of 10 mol% Hoveyda‑Grubbs second generation catalyst (**5.68**) and 10 mol% 2,6-dichlorobenzoquinone to give cross-metathesis products such as **5.69** in excellent yields (Scheme 5.**14**).[54a,b]

Electron-deficient benzoquinones are essential additives as they prevent the formation of the undesired isomerized product **5.70**.

[135] a) J. C. Sarma, R. P. Sharma, *Heterocycles* **1986**, 24, 441; b) N. Petragnani, H. M. C. Ferraz, G. V. J. Silva, *Synthesis* **1986,** 157.

[136] S. J. Connon, S. Blechert, *Angew. Chem.* **2003**, 115, 1944; *Angew. Chem. Int. Ed*. **2003**, 42, 1900.

[137] a) R. Raju, A. R. Howell, *Org. Lett*. **2006**, 8, 2139; b) R. Raju, L. J. Allen, T. Le, C. D. Taylor, A. R. Howell, *Org. Lett*. **2007**, 9, 1699. c) J. Moise, S. Arseniyadis, J. Cossy, *Org. Lett.* **2007**, 9, 1695.

SCHEME 5.14

a) % amount in the absence of 2,6-dichloro-benzoquinone
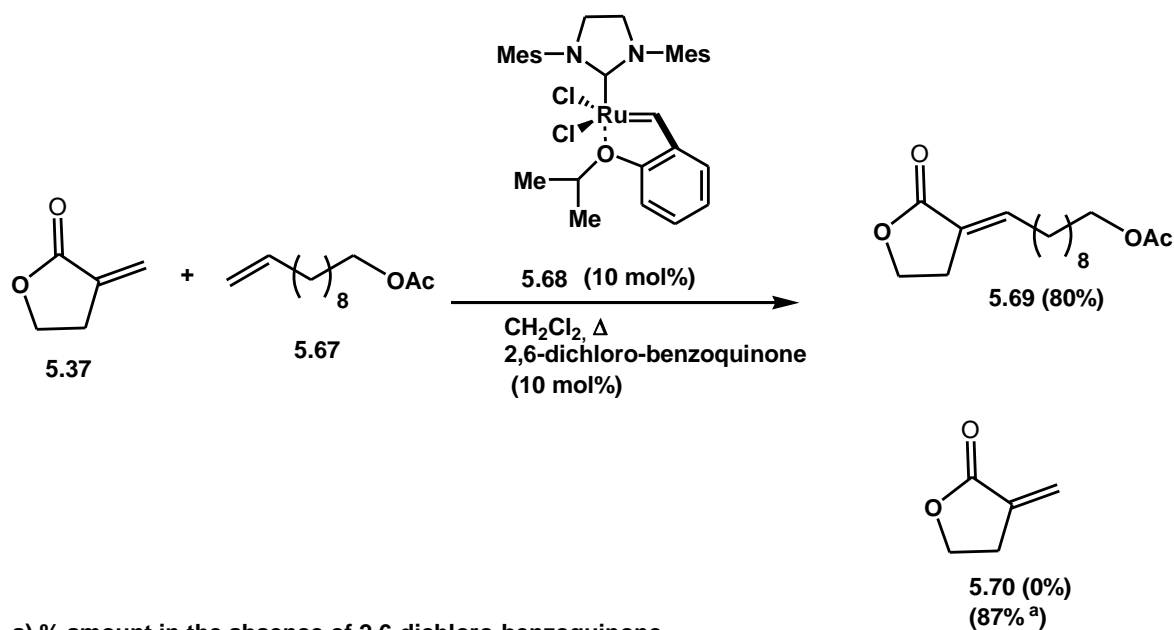
A similar approach has been reported by Cossy's research group.[54c] They developed a highly efficient cross-metathesis between α-methylene-γ-butyrolactone **5.37** and a large range of olefinic partners (Scheme **5.15**). The products (e.g. **5.73**) were obtained in moderate to excellent yields and with high E-stereoselectivity using a low loading (from 2.5 to 5 mol%) of the Grubbs second generation catalyst (**5.72**); again, an additive was required to minimize production of **5.70** (see Scheme **5.15**), and chlorocatecholborane proved most effective.
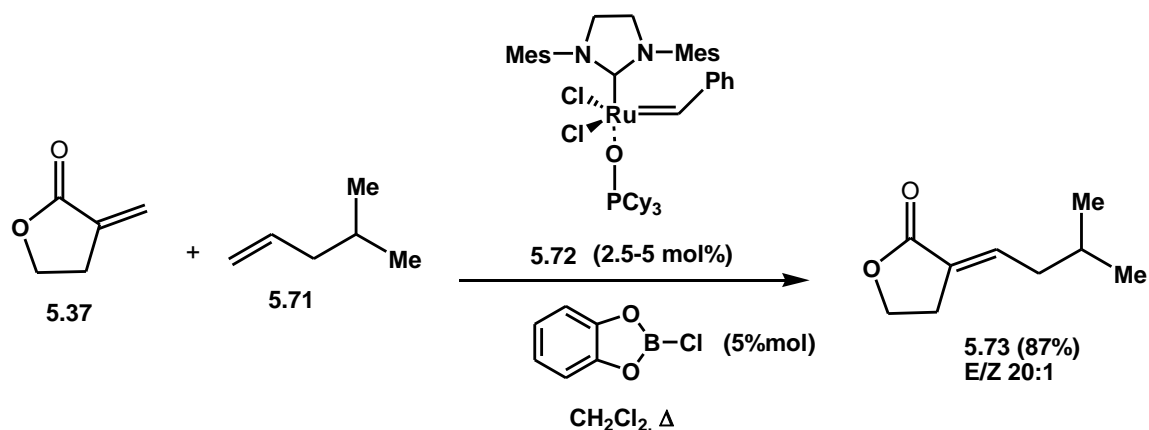
SCHEME 5.15

5.5.6 *Radical Cyclization Approaches*

There have been major advances in radical approaches to α-alkylidene-γ-butyrolactone. In 1990, Zard and Forbes described the use of (S)-alkoxycarbonyl dithiocarbonates **5.74** to prepare α-methylene-γ-butyrolactone (**5.37**, tulipalin A) in a yield of 38% over two steps (Scheme 5.**16**).[138] (S)-Alkoxycarbonyl dithiocarbonate **5.74**, derived from the corresponding homoallylic alcohol, phosgene, and EtOCS$_2$K, was irradiated with visible light in heptane under reflux.

Fragmentation/decarboxylation followed by 5-exo-radical cyclization and then radical recombination generated xanthate **5.75** in high yield. Elimination of the xanthate group was achieved by heating under vacuum in the presence of copper powder, which produced α-methylene-γ-butyrolactone (**5.37**).

5.74   **5.75**   **5.37**
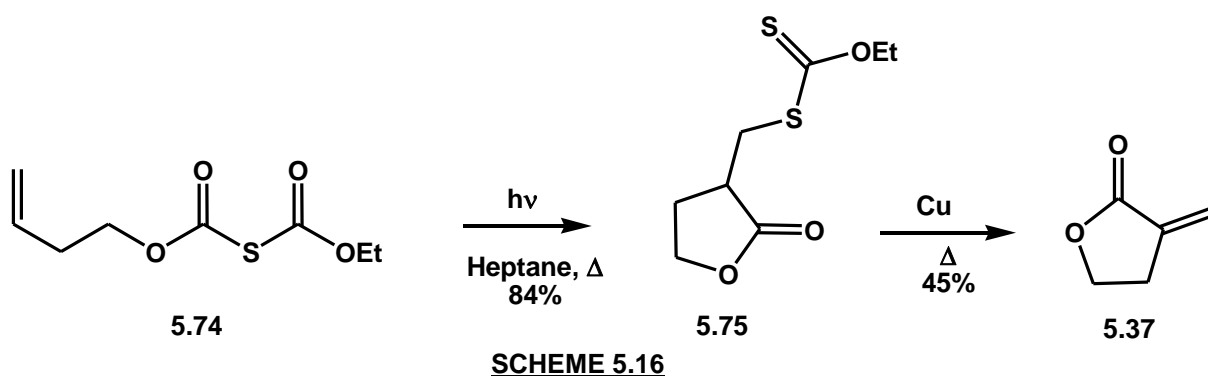
SCHEME 5.16

In 1992, Bachi and Bosch reported a synthetic approach to α-methylene-γ-butyrolactones **5.78** by a radical-induced cyclization of selenocarbonates **5.77** (Scheme 5.**17**).[139] Homopropargylic alcohol **5.76** was treated with phosgene to form the corresponding chloroformate, and this was trapped with phenylselenol to give selenocarbonate **5.77** in 84% yield over the two steps. Thermolysis of selenocarbonate **5.77** in benzene at 80°C generated the corresponding acyl radical, which underwent cyclization to afford α-arylidene-γ-butyrolactone **5.78** as a mixture of isomers in 90% yield. A range of monocyclic and bicyclic α-

---

[138] J. E. Forbes, S. Z. Zard, *J. Am. Chem. Soc.* **1990**, 112, 2034.

[139] M. D. Bachi, E. Bosch, *J. Org. Chem.* **1992**, 57, 4696.

methylene- and α-alkylidene-γ-butyrolactones were prepared by using this procedure.
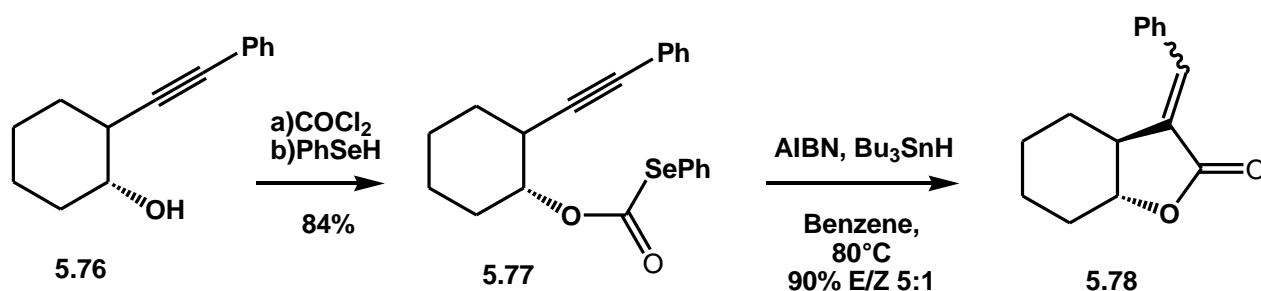
SCHEME 5.17

5.6 THE TELESCOPED INTRAMOLECULAR MICHAEL/OLEFINATION (TIMO) APPROACH TO α-ALKYLIDENE-γ-BUTYROLACTONES.

As previously shown, numerous procedures are available to prepare α-alkylidene-γ-butyrolactones; in particular, the initial construction of the lactone with subsequent methylenation is popular, but the majority of the routes are lengthy and low yielding.[140] As part of growing interest in tandem or telescoped processes,[141] Taylor and co-workers designed a one-pot approach to α-alkylidene-γ-butyrolactones (Scheme **5.18**),[142] In which the HWE approach has been incorporated into an efficient Telescoped intramolecular Michael/olefination (TIMO) sequence.

[140] a) S.-K. Kang,K.-J . Kim,Y .-T Hong, *Angew. Chem. Int. Ed.* **2002**, 41,1584 -1586; b) A. Albrecht, J. Kedzia,J . F. Koszuk,E. Warzycha,T . Janecki, *Tetrahedron Lett.* **2006**, 47,2353 – 2355; c) T. Hayashi,T . Shinbo, M. Shimizu,M. Arisawa,N . Morita,M. Kimura,S . Matsuda, T. Kikuchi, *Tetrahedron Lett*. **1985**, 26, 3699 – 3702. d) J. A. Douglas,B . M. Smallfield, E. J. Burgess, N. B. Perry, R. E. Anderson,M. H. Douglas, V. L. A. Glennie, *Planta Med.* **2004**, 70,166-170;

[141] a) R. J. K. Taylor,M. Reid, J. Foot, S. A. Raw, *Acc. Chem. Res*. **2005**, 38, 851 – 869, and references therein. b) L. F. Tietze,U . Beifuss *Angew. Chem. Int. Ed. Engl.* **1993**, 32,131 – 163; c) L. F. Tietze, *Chem. Rev.* **1996**, 96,115 – 136; d) L. F. Tietze,G . Brasche,K. Gericke, Domino Reactions in Organic Synthesis, Wiley-VCH, Weinheim, **2006**

[142] Edwards, M.G.; Kenworthy, M.N.; Kitson,R.; Scott,M.S.; and Taylor, R. J. K. *Angew. Chem. Int. Ed*. **2008**, 47, 1935 –1937

The key was the use of diethyl phosphonoacetate **5.79**, which, after deprotonation, was expected to undergo an intramolecular Michael addition[143] to give **5.80**. Subsequent proton transfer (anion exchange) generates the more stable phosphonate anion **5.81** and then. addition of an aldehyde, initiates an intermolecular Horner‑Wadsworth‑Emmons (HWE) olefination[144] to yield cyclic dicarbonyl compound **5.82**. In addition, the sequence would be *cis* selective in the formation of tetrahydrobenzofuran-2,5-dione 4 (n=1).
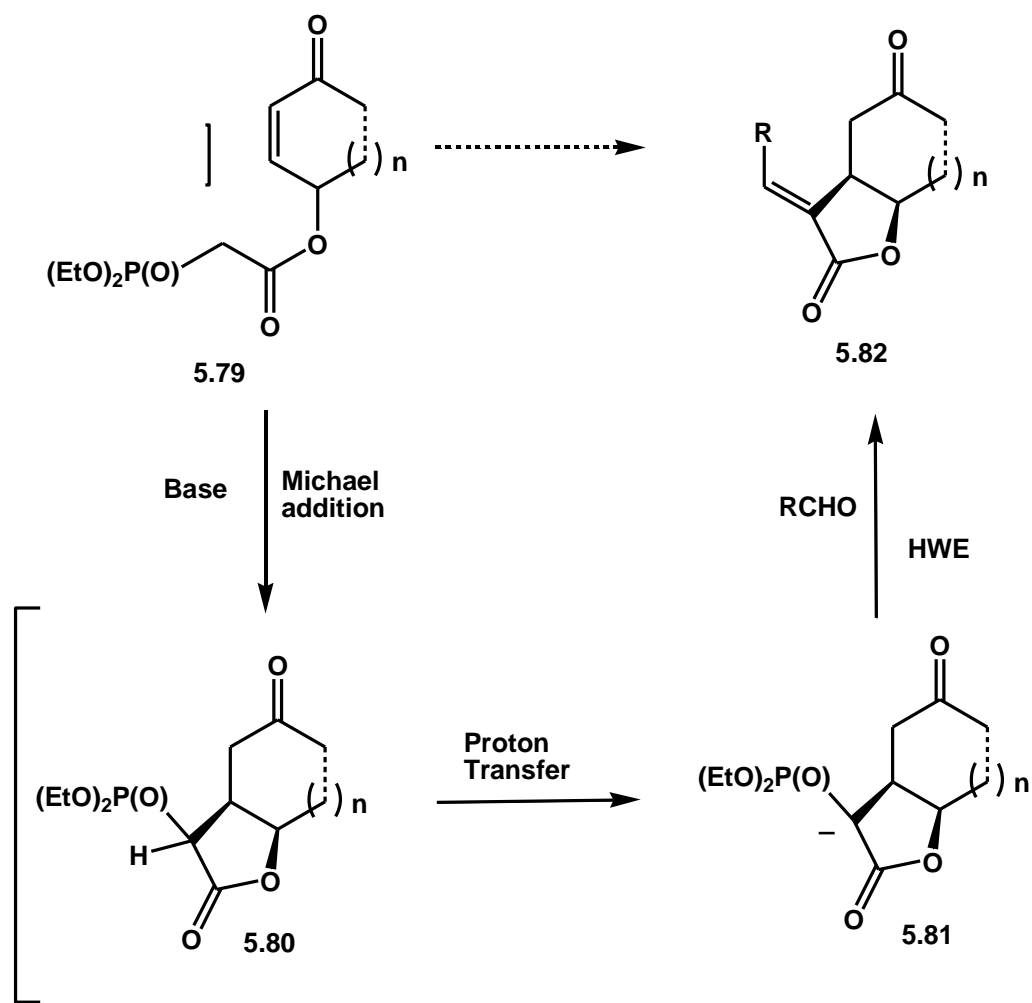
SCHEME 5.18

[143] R. D. Little,M. R. Masjedizadeh,O .Wallquist, J. I. McLoughlin, *Org. React.* **1995**, 47,315‑552. b) M. T. Hechavarria Fonseca B . List, *Angew. Chem.* **2004**, 116,4048‑4050.

[144] a) J. Boutagy,R. Thomas, *Chem. Rev.* **1974**, 74, 87‑99; b) K. C. Nicolaou, M. W. Harter, J. L. Gunzner, A. Nadin, *Liebigs Ann.* **1997**,1283‑1301.

In order to assess the viability of this process ketophosphonate **5.83** was prepared from readily available 4-hydroxy-2-cyclohexenone[145] and commercially available diethyl phosphonoacetic acid by using 2-propanephosphonic acid anhydride (T3P) as the coupling agent. When ketophosphonate **5.83** was treated with KO^{t}Bu in THF, the expected Michael adduct **5.84** was obtained in 50% yield (**Scheme 5.19**). Phosphonate **5.84** was used to optimize the key HWE methylenation in terms of base, solvent, stoichiometry, and formaldehyde source. KO^{t}Bu in THF proved to be the best base/solvent combination and paraformaldehyde was the optimal formaldehyde source. The α methylene lactone product **5.85** was highly base-sensitive, and the use of a substoichiometric quantity of base (0.95 equiv) gave this product in 83% yield.
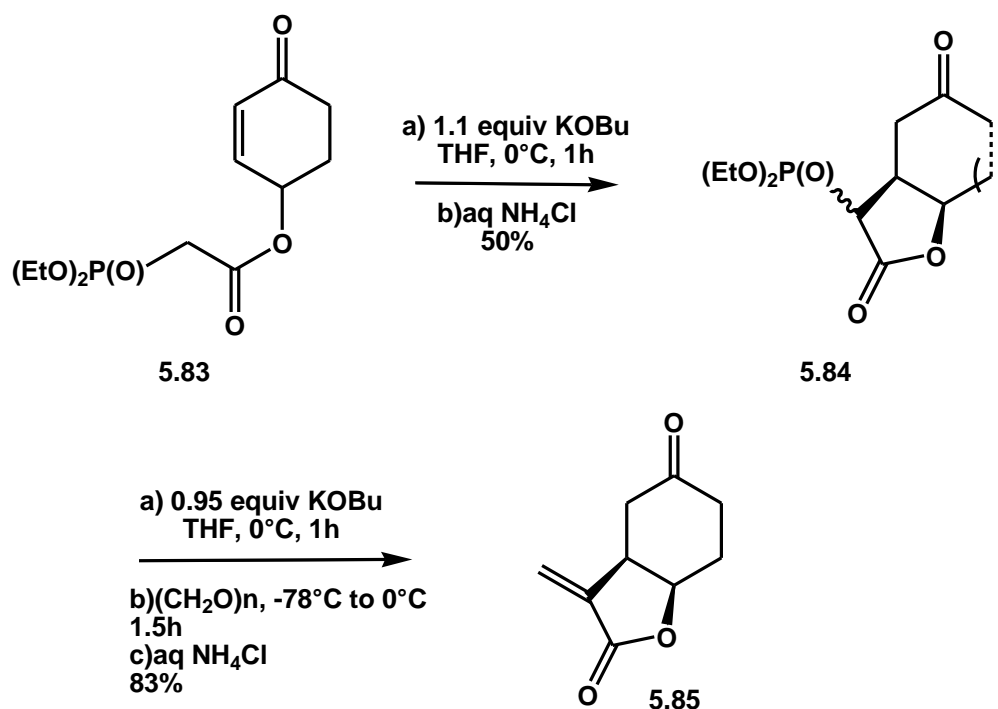
SCHEME 5.19

A more efficient transformation was achieved by performing a sequential one-pot process. Thus, treatment of ketophosphonate **5.83** with KO^{t}Bu (0.95 equiv) in THF, and the addition of paraformaldehyde after 60 minutes, produced the

[145] S. J. Danishefsky, B . Simoneau, *J. Am. Chem. Soc.* **1989**, 111, 2599 – 2604.

expected α-methylene lactone **5.85** in 77% overall yield (**Scheme 5.20**). This result emphasizes the advantages of a sequential one-pot process—it avoids a complicated intermediate workup process and the overall yield is improved (77%, compared to 43% over two steps). This variant was termed a **T**elescoped **I**ntramolecular **M**ichael **A**ddition/HWE **O**lefination (**TIMO**) process. Lactone **5.85** is novel although the corresponding *trans* isomer is known.[146] The *cis* arrangement of **5.85** was confirmed by obtaining an X-ray crystallographic structure.

a) 0.95 equiv KOBu
THF, 0°C, 1h

b)(CH$_2$O)n, -78°C to 0°C
1.5h
c)aq NH$_4$Cl
83%

(EtO)$_2$P(O)

5.83

5.85
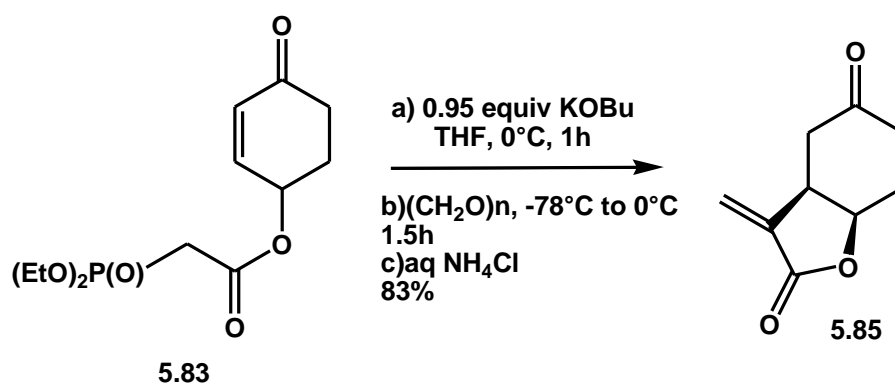
SCHEME 5.20

Thus, the anion of the α-phosphono-γ-lactone **5.87** was generated in situ by an intramolecular Michael/proton-transfer process; subsequent addition of paraformaldehyde then gave the *cis*-fused α-methylene-γ-butyrolactone **5.85** in 77% yield. Aromatic and aliphatic aldehydes could also be employed in this telescoped methodology, thereby generating, for example, the α-alkylidene-γ-butyrolactones **5.88** and **5.89 (Scheme 5.21)**.

[146] R. K. Boeckman,Jr. , M. Ramaiah, *J. Org. Chem*. **1977**, 42,1581 –1586.
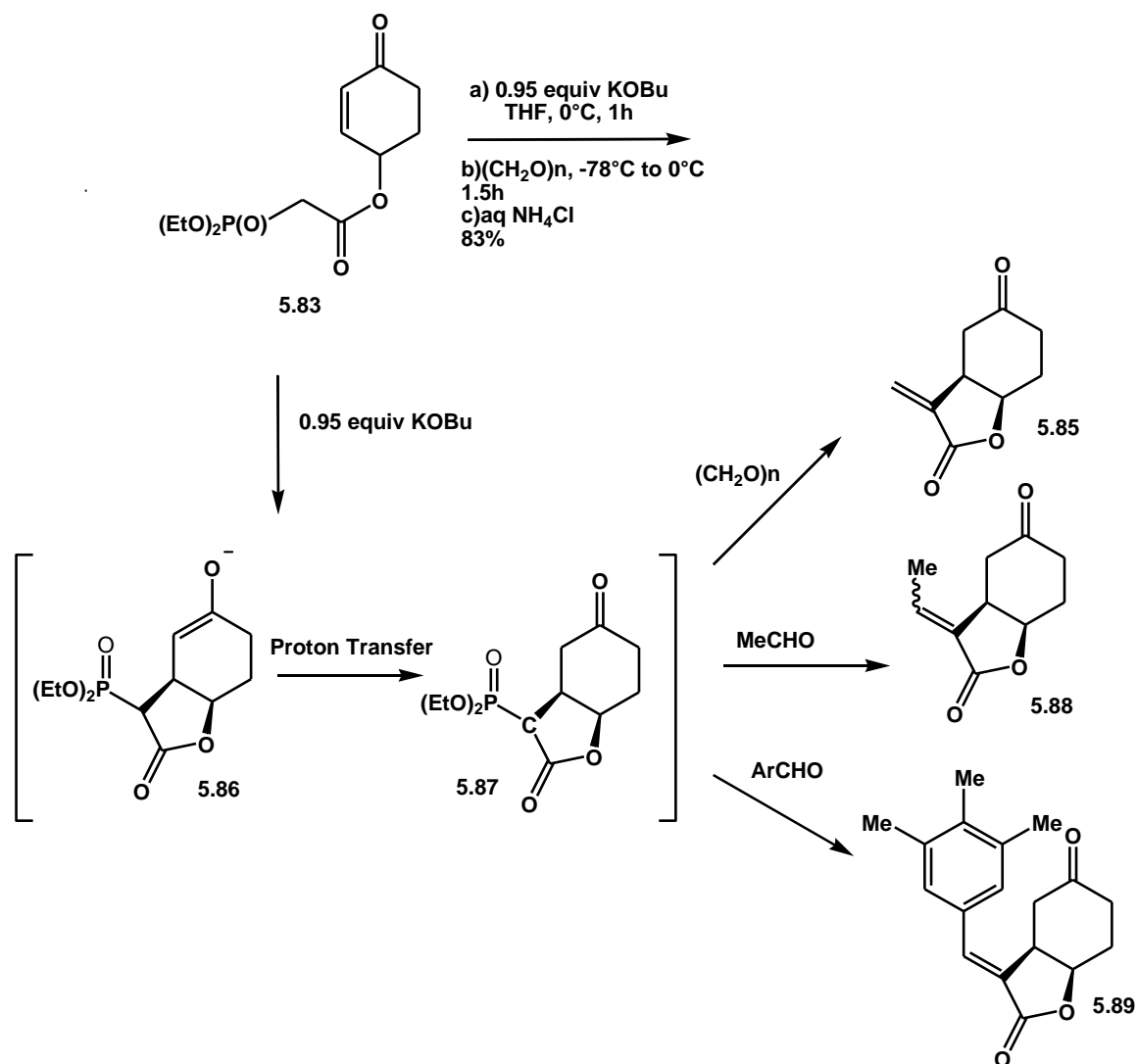
SCHEME 5.21

This TIMO methodology was then utilized as the cornerstone of a concise synthesis of (+)-paeonilactone B (**5.91**; **Scheme 5.22**).[147] It is noteworthy that this telescoped sequence is compatible with an unprotected tertiary alcohol.

The TIMO-type processes has been recently investigated by Taylor and co-workers also for total synthesis of yomogin **5.92**, a natural occurring eudesmane sesquiterpene from *Artemisia princeps,* a plant used for a long time in traditional oriental medicine for treating infections, asthma and circulatory problems, as well as interesting anticancer, anti inflammatory and anti allergy properties.[148]

---

[147] M. G. Edwards, M. N. Kenworthy, R. R. A. Kitson, A. Perry, M. S. Scott, A. C. Whitwood, R. J. K. Taylor, *Eur. J. Org. Chem.* **2008**, 4769.

[148] Kitson,R.; McAllister,G.; Taylor R. J. K. Tetrahedron Lett. 2011, 52, 561-564 and references therein.

5.90   KOBu (CH₂O)n   (+)-Paeonilactone B (5.91)

yomogin (5.92)
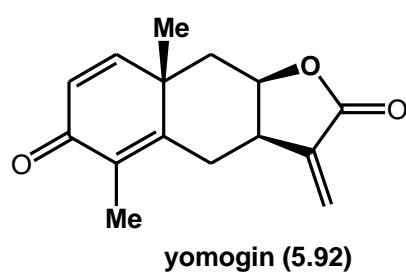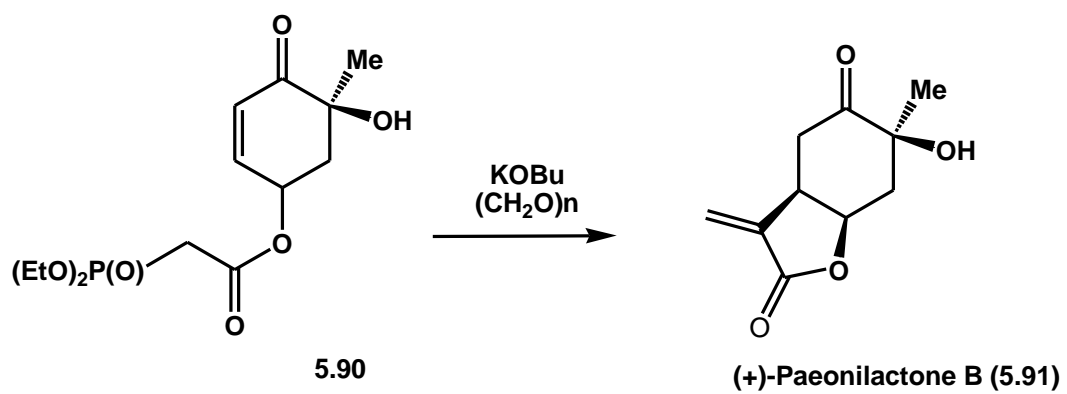
SCHEME 5.22

CHAPTER 6

Towards synthesis of α-alkylidene-γ-butyrolactones :

the Rh(II) carbenoids approach

6.1 C-H INSERTION WITH RHODIUM CARBENOIDS

Novel reactions that can selectively functionalize carbon–hydrogen bonds are of intense interest to the chemical community because they offer new strategic approaches for synthesis. A very promising 'carbon–hydrogen functionalization' method involves the insertion of metal carbenes and nitrenes into C–H bonds.

The reaction of rhodium (II) stabilized carbenoids has been well developed in recent years principally by the group of Huw Davies and Michael Doyle, and used in a wide range of impressive C-H insertion and cyclopropanation reaction. Rhodium, alongside palladium and platinum, is one of the three dominant metals used in modern transition metal catalysis.[i149]

Rhodium is expensive; around 5 times the price of platinum yet, despite this, extensive effort has gone into the development of rhodium complexes as catalysts. This is not only because of the high turnover numbers that are often possible, but also that rhodium complexes are able to perform a huge number of diverse and often unique transformations that have become invaluable to the modern synthetic chemist.

Here several facets of these kinds of C–H functionalization reactions are discussed and provided a perspective on how this methodology has affected the synthesis of complex natural products and potential pharmaceutical agents.

6.1.1 *Metal carbenoid C–H functionalization versus the 'traditional' C–H activation.*

The many opportunities associated with C–H functionalization has made this field an active area of research. Organometallic chemists have focused much attention on developing 'C–H activation' strategies, whereby a highly reactive metal complex inserts into a C–H bond, activating the system for subsequent transformations.[150]

---

[149] M. P. Doyle, *J. Org. Chem.* **2006**, *71*, 9253–9260

[150] a)Waltz, K. M. & Hartwig, J. F. *Science* **1997** 277, 211–213. b)Saaby, S., Bayon, P., Aburel, P. S. Jorgensen, K. A. *J. Org. Chem.* **2002,**67, 4352–4361. C). Bergman, R. G. Nature **2007,** 446, 391–393.

Here, however, we highlight another approach, in which a divalent carbon (carbene)[151], inserts into a C–H bond.

In a traditional C–H activation manifold, the highly reactive metal complex (M=metal, L=ligand) inserts into a C–H bond. Regeneration of the active metal complex to form the C–H activation product has proved difficult. In contrast, C–H functionalization via a metal carbenoid approach typically uses a high-energy diazo compound and loss of nitrogen provides the driving force for the energetically unfavourable formation of the carbenoid.

The highly reactive carbenoid species then inserts into a C–H bond to form the C–H activation product and liberates the metal catalyst for another cycle. (Scheme **6.1**)
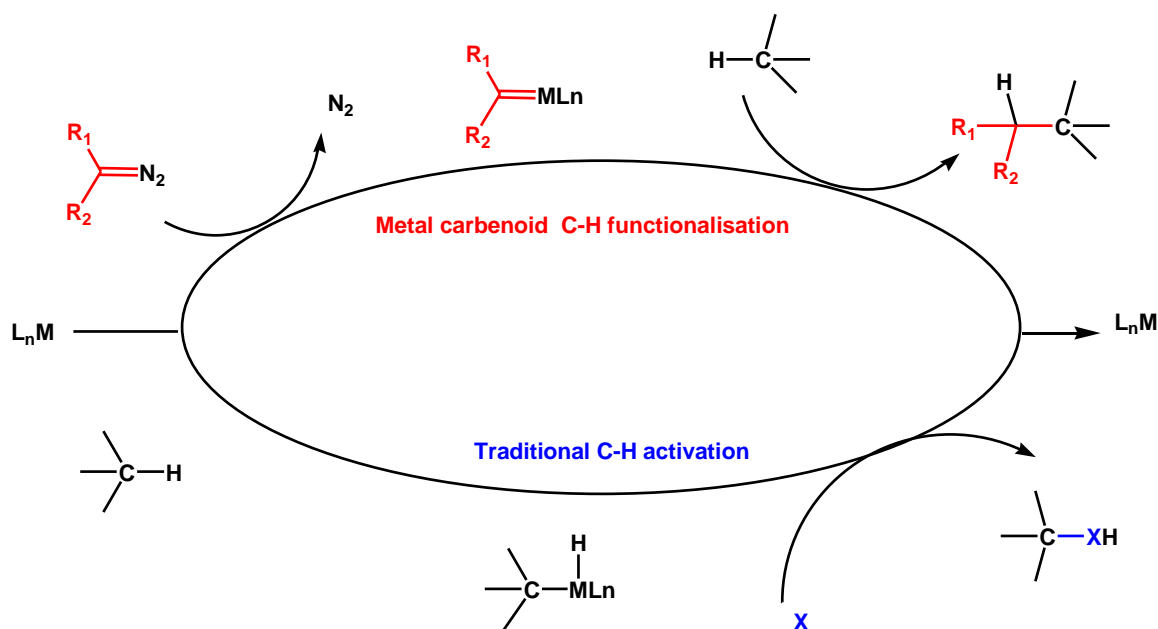
SCHEME 6.1

Metal carbenoid C–H functionalization versus the 'traditional' C–H activation.

[151] a)Davies, H. M. L. & Beckwith, R. E. J. *Chem. Rev*. **2003**, 103, 2861–2903. b) Davies, H. M. L. Angew. Chem. Int. Edn Engl **2006**, 45, 6422–6425.

This alternative approach offers many advantages over the metal-induced C–H insertion because the reactions exhibit high turnover numbers and can lead to high levels of selectivity, both in terms of regioselectivity and stereoselectivity

6.1.2 Carbenoid Formation From Diazo Compounds And C-H Insertion Mechanism

The Rh(II)-catalyzed reactions of α-diazo carbonyl compounds have been well-established as powerful approaches to generate Rh(II) carbene species, which may subsequently undergo diverse synthetically useful transformations, such as cyclopropanation, ylide generation, and X-H (X = C, O,S, N, etc.) insertion.[152] Herein is described the mechanism of transition metal promoted decomposition of diazo compounds. Lewis acidic transition metal complexes, like Rh(II) complexes, are effective catalysts for diazo decomposition. (Scheme **6.2**) Activity of transition metal complexes depends on coordinative unsaturation at metal center, which allows them to react as "electrophiles" for diazo compound:[153]
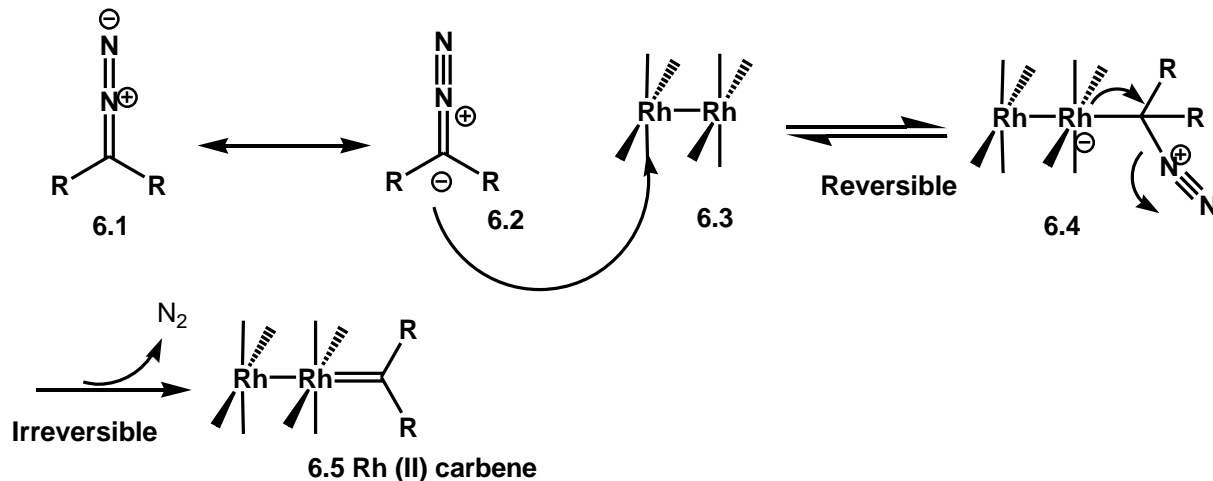
SCHEME 6.2

[152] a)Doyle, M. P.; McKervey, M. A.; Ye, T. *Modern Catalytic Methods for Organic Synthesis with Diazo Compounds*; Wiley-Interscience: New York, **1998.** b) Ye, T.; McKervey, M. A. *Chem. Rev.* **1994**, *94*, 1091

[153] Wong,F.; Wang, J.; C. Hengge,A.; Wu, W**.** *Org. Lett*. **2007**, 9 , 1663.

The proposed mechanism for C-H insertion is there explained: carbenoid's empty p-orbital overlaps with the σ-orbital of the C-H bond and C-C and C-H bond formation with carbenoid carbon proceeds as ligated metal dissociates (**6.8**) or a with transition state in which there is a more pronounced interaction/transfer of hydrogen to rhodium (**6.9**), followed by a reductive elimination.[154](Scheme **6.3**)
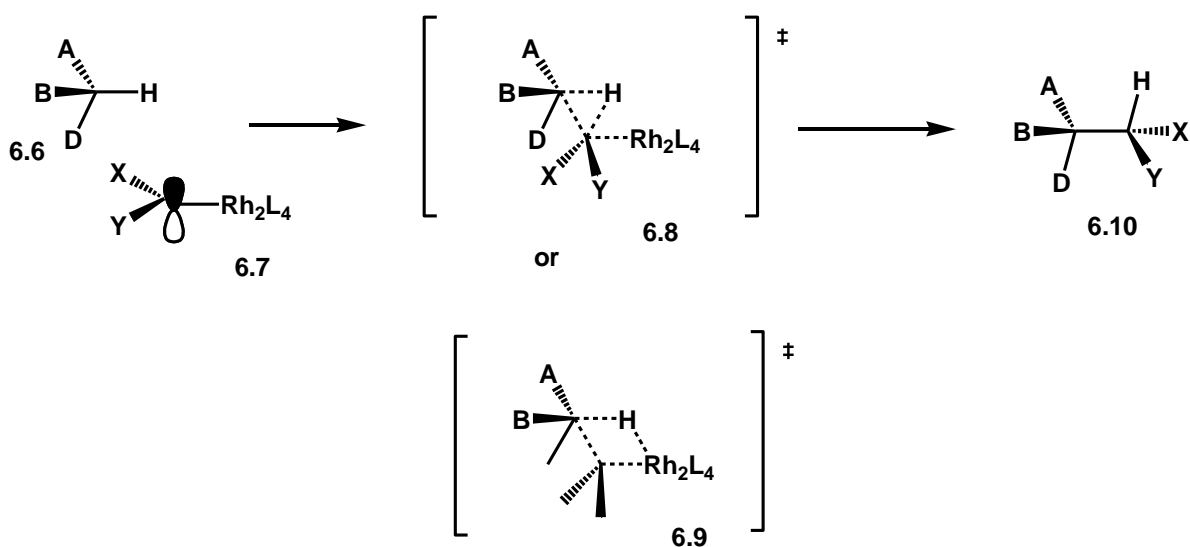
SCHEME 6.3

---

[154] Taber et al *JACS* **1996**, *118*, 547

6.1.3 *Rh(II) Catalysts*

It has known that carbene C-H insertions are highly non-selective while carbenoid C-H insertions are much more selective because of the reduced reactivity of carbenoids themselves.

Rh carbenoids (generated from Rh(II) dimer complexes) have become the most common catalysts for C-H insertion reactions because of their selectivity and the ease with which ligands are modified.

The structure of the simplest dirhodium(II) catalyst, dirhodium(II) tetraacetate **A**,(**Figure 6.1**) was reported in 1962 as having a unique paddlewheel structure, in which two bound rhodium atoms are bridged by four acetate ligands, leaving a vacant axial coordination site on each of the rhodium atoms.[155] The presence of these two sites is key to the catalyst's reactivity, as stabilised carbenoid or nitrenoid species may be formed by reaction with a suitable precursor.[156]

Structure **B** is dirhodium(II)-octaenoate (**Figure 6.1**).
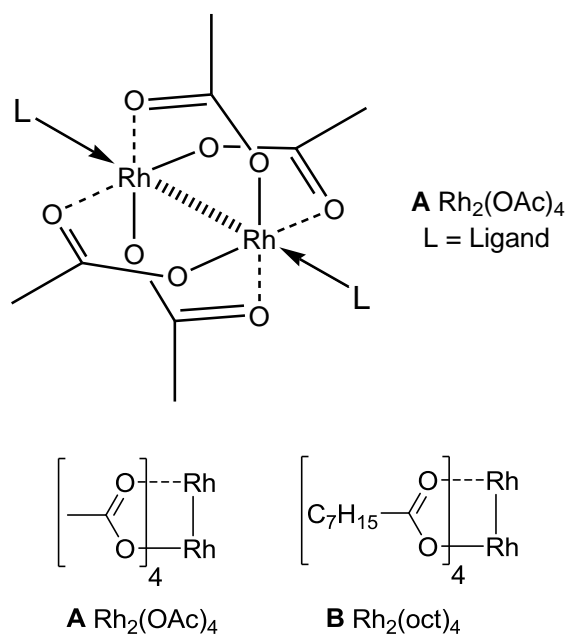
FIGURE 6.1 *Commonly used Rh (II) CarboxylateCatalysts*

[155] Porai-Koshits, M. A.;. Antschishkina, A. S.; *Dokl. Chem,* **1962**, *146*, 902.

[156] Cotton, F. A. DeBoer, B. G.; LaPrade, M. D.; Pipal, Ucko, J. R. D. A. *J. Am. Chem. Soc.*, **1970**, *92*, 2926.

Dirhodium(II) tetraacetate **A** has D4h symmetry with 4 bridging acetate ligands and 1 vacant coordinate site per metal atom presents an octahedral geometry resembling a circular wall with an electron rich circumference and electron deficient core.

In general, Dirhodium (II) carboxylates complexes are air stable and easy to work with. Electron-withdrawing capabilities of carboxylate ligands affect properties of the catalyst.

The first example of such a process was introduced by Teyssié in 1973, who reported the homogeneous rhodium(II)-catalysed formal insertion of ethyl diazoacetate into the hydroxylic bond of alcohols, water and weak acids via a rhodium carbenoid (**Scheme 6.4**).[157] Yields are good and the reaction is general for a range of substrates. The process compared favourably to analogous copper-catalysed,[158] photolytic[159] and thermal[160] variants of that time, which all suffered from a lack of generality and competing C–H insertion pathways.
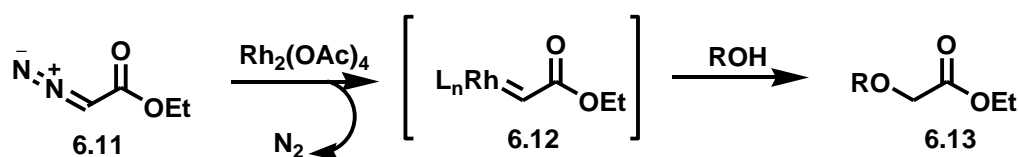
SCHEME 6.4

Variants of dirhodium(II) catalysts capable of performing asymmetric transformations were soon developed, following the discovery that $Rh_2(OAc)_4$ undergoes ligand exchange with a range of bidentate ligands, most commonly chiral carboxylates or carboxamidates (**Figure 6.2**).[161] There have been a number of contributions to this area, although by far the most significant have been from the groups of Doyle [162][principally dirhodium(II) carboxamidates] and

[157] R. Paulissen, H. Reimlinger, E. Hayez, A. J. Hubert. Ph. Teyssié, *Tetrahedron Lett.*, **1973**, *24*, 2233.

[158] T. Saegusa, Y. Ito, S, Kobayashi, K. Hirota, T. Shimizu, *J. Org. Chem.*, **1968**, *33*, 544.

[159] Hesse, S. Majmurdar, *Chem. Ber.*, **1960**, *93*, 1129.

[160] a)T. Do minh, O. P. Strausz, H. E. Gunning, *J. Am. Chem. Soc.*, **1969**, *91*, 1261.
b)H. Chaimovitch, R. J. Vaughan, F. H. Westeimer, *J. Am. Chem. Soc.*, **1968**, *90*, 4088.

[161] M. Q. Ahsam, I. Bernal, J. L. Bear *Inorg. Chem.* **1986**, *25*, 260–265

[162] a) M. P. Doyle, W. R. Winchester, J. A. A. Hoorn, V. Lynch, S. H. Simonsen, R. Ghosh, *J. Am. Chem. Soc,* **1993**, *115*, 9968–9978. b)M.P. Doyle, Q.-L. Zhuo, A. L. Dyatkin, D. A. Rupper, *Tetrahedron. Lett.* **1995**, *36*, 7579–7582 **.** c) M. P. Doyle, A. V. Kalinin, *J. Org. Chem.* **1996**, *61*, 2179–2184.

Davies[163] [principally dirhodium(II) carboxylates] that have resulted in the discovery of numerous highly enantioselective C–H insertion processes.[164]

Commonly used chiral Rh(II) catalysts

C Rh$_2${(*S*)DOSP}$_4$
(Ar = *p*-C$_{12}$H$_{25}$C$_6$H$_4$)

D Rh$_2${(*S*)TBSP}$_4$
(Ar = *t*-BuC$_6$H$_4$)

E Rh$_2${(*S*)MEOX}$_4$
(X = O)

F Rh$_2${(*S*)MEPY}$_4$
(X = CH$_2$)

G Rh$_2${(*S*)-TCPTTL}$_4$

H Rh$_2${4-(*S*)MPPIM}$_4$
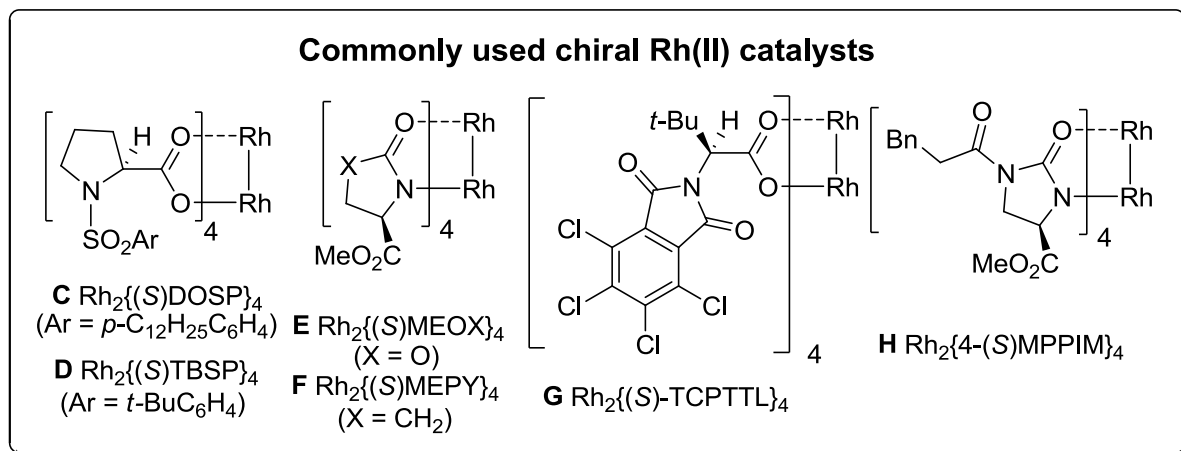
FIGURE 6.2

General Characteristics of a Good Rh Complex for C-H Insertion-

Proficient C-H insertion requires an appropriate level of electrophilic character at the metal center:

• *If the metal center is too electrophilic catalyst displays poor selectivity because of high reactivity, and it is susceptible to undesired competing reactions:*
• *If the metal center is not electrophilic enough catalyst is not reactive enough to insert C-H bond;*

Electron-withdrawing groups on metal or adjacent to carbenoid carbon increase the electrophilicity of the carbenoid intermediate and the best metal complexes bind to the carbenoid carbon through strong σ–donation and weak π-back

[163] a) H. M. L. Davies, P. R. Bruzinski, D. H. Lake, N. Kong, M. J. Fall, *J. Am. Chem. Soc.* **1996**, *118*, 6897–6907. b)H . M. L. Davies, *Eur. J. Org. Chem.* **1999**, 2459–2469.

[164] a)A. Padwa, D. J. Austin, A. T. Price, M. A. Semones, M. P. Doyle, A. Van Oeveren, L. J. Westrum, M. N. Protopopova, T. W. Clayton, *J. Am. Chem. Soc*, **1993**, *115*, 8669–8680. b)M. P. Doyle, W. Hu, M. Valenzuela, *J. Org. Chem.* **2002**, *67*, 2954–2959. c)H. M. L. Davies, T. Hansen. M. R. Churchill. *J. Am. Chem. Soc.* **2000**, *122*, 3063–3070. d)H. M. L. Davies, E. G. Antoulinakis, *Org. Lett.* **2000,** *2*, 4153–4156.

donation, which stabilizes the carbenoid carbon somewhat but still ensures electrophilicity

6.1.4 Trends in Selectivity for Intramolecular C-H Insertion

C-H insertion occurs preferably at a carbon that can stabilize positive charge (electronic effects):

• tertiary carbon > secondary carbon > primary carbon (because of the availability of the electron density in the C-H bond) (**Scheme 6.5**)
*JACS **1986**, 108, 7686.*

C-H insertion Favourited at tertiary carbon
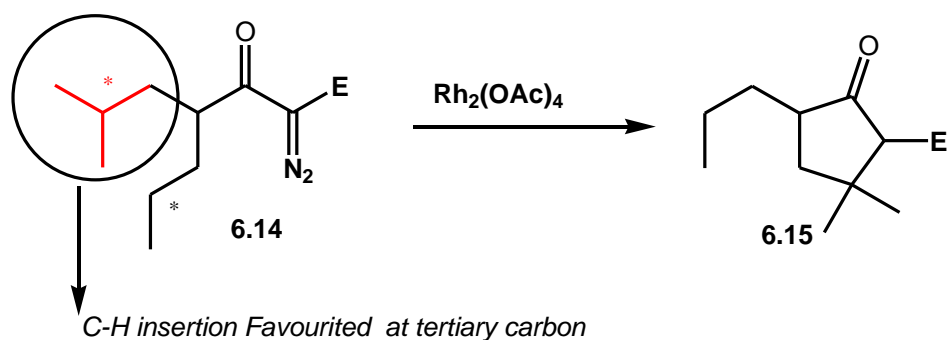
SCHEME 6.5

• alkoxy groups activate adjacent C-H bonds (**Scheme 6.6**)
*(Tetrahedron **1991**, 47, 1765):*

C-H insertion occurs by formation of six member ring
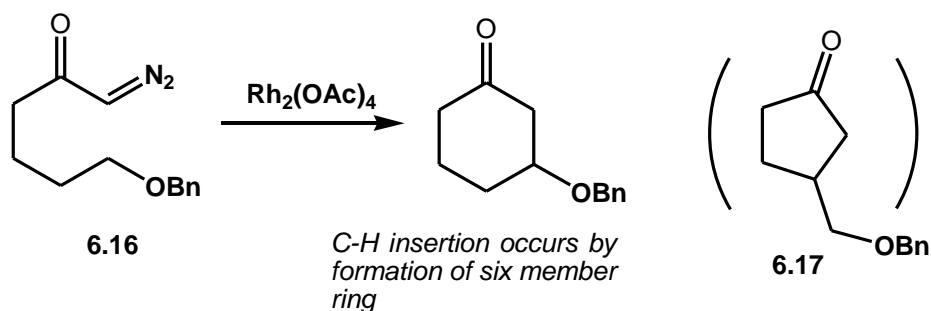
SCHEME 6.6

- electron-withdrawing groups (eg. CO_2Me) inhibit adjacent C-H bonds (Scheme 6.7)(*TL* 1988, *29*, 2283):

6.18 Rh₂(OAc)₄ **6.19**

6.20 Rh₂(OAc)₄ **6.21** COOMe *Not observed*
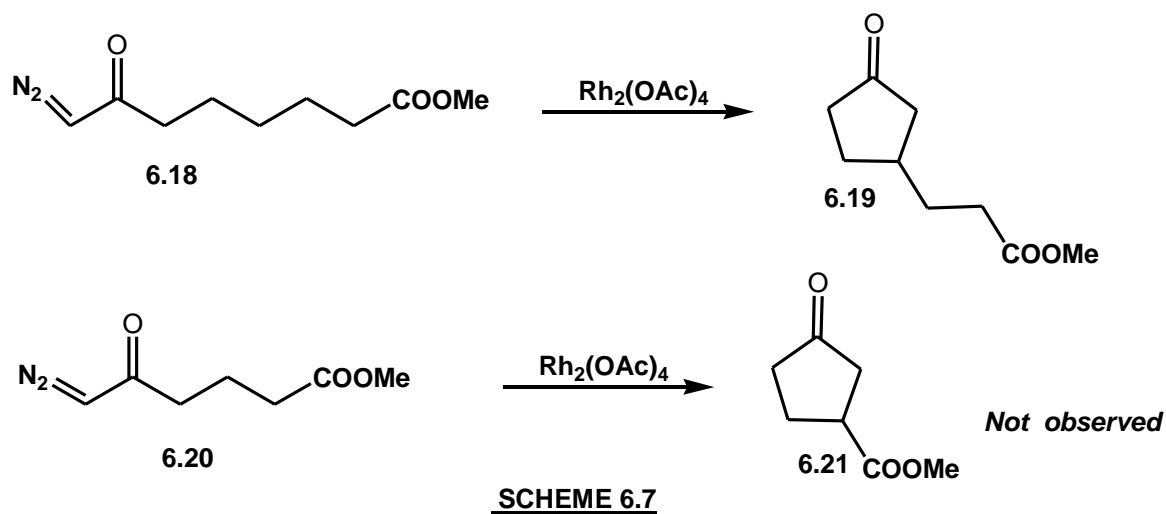
SCHEME 6.7

Sometimes electronic effects are outweighed by *steric* or *conformational* factors

- Fomation of 5 membered rings is more favourite than 6 membered rings: (**Scheme 6.8**)

6.22 Rh₂(OAc)₄ **6.23** **6.24 *Not observed***

SCHEME 6.8
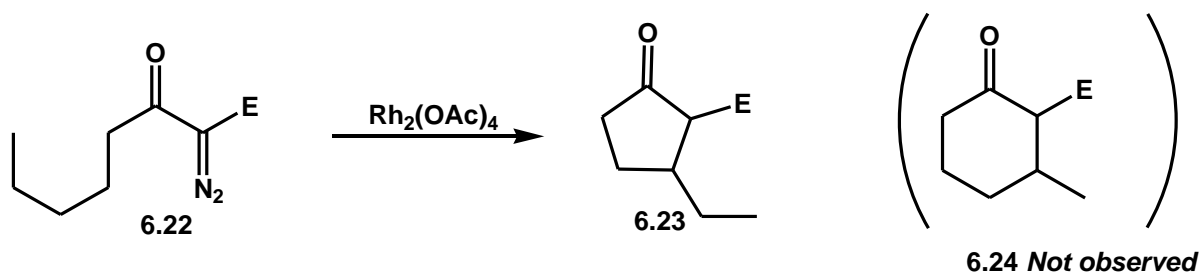
6.2 CONTROLLING FACTORS OF CARBENOID REACTIVITY.

6.2.1 Diastereoselectivity in Intramolecular C-H Insertion- An understanding of the mechanism 4,5 for Rh-mediated intramolecular C-H insertion begins with the recognition that these α-diazo carbonyl derivatives can also be seen as the stabilized ylides, such as **6.24** (**Scheme 6.9**).[165] The catalytic Rh(II) carboxylate **A** is Lewis acidic, with vacant coordination sites at the apical positions. Complexation of the electron density at the diazo carbon with an open Rh coordination site would give **6.25**. Back donation of electron density from the proximal Rh to the carbene carbon with concomitant loss of N2 would then give the intermediate Rh carbene complex **6.26**. The mechanism by which this intermediate Rh carbine complex **6.26** reacts can be more easily understood if it is written as the inverted ylide **6.27**. This species would clearly be electrophilic at carbon. The initial complex of the electrondeficient carbon with the electron density in the target C-H could be depicted (**6.28**) as a three-center, two-electron bond. It has been hypothesized that this complexation would be rapid and reversible, and that for bond formation to proceed, a somewhat different transition state (**6.30**), in which the C-Rh bond is aligned with the target C-H bond, would be required.

As the C-H insertion reaction proceeded, the electron pair in the C-H bond would drop down to form the new C-C bond, and at the same time the electron pair in the C-Rh bond would slide over to form the new C-H bond. This would give the product (**6.30**) and release the initial Rh species **A**, completing the catalytic cycle.

[165] Taber, D.; Yu,K.; Rheingold, A. *JACS* **1996**, 118, 547
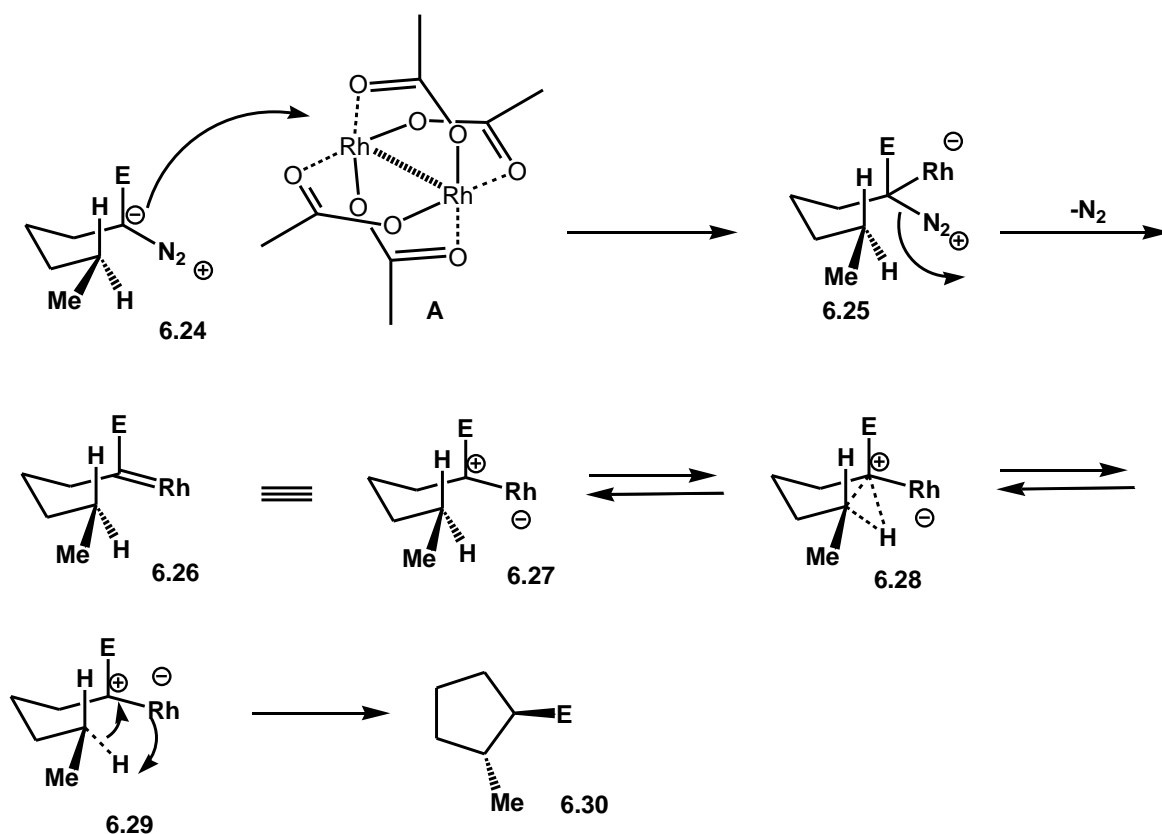
SCHEME 6.9

It has been used this hypothetical transition state (**6.29**) to predict the stereochemical course of the intramolecular Rh mediated C-H insertion reaction and have found it to be effectively predictive.

The actual product from a cyclization will be determined as the intermediate carbene *commits* to a particular diastereomeric transition state. If these diastereomeric transition states are indeed in full thermal equilibrium, then computational modeling of the diastereomeric transition states (**6.29**) could allow to predict which would be favored, and thus which diastereomeric product would be formed.
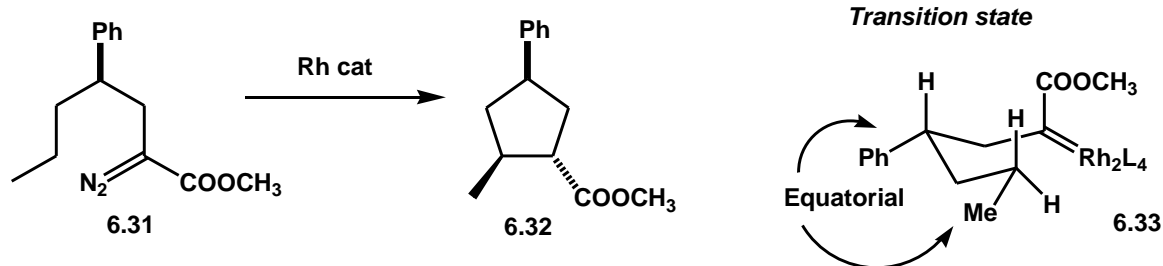
SCHEME 6.10

The vast majority of intermolecular chemoselective rhodium carbenoid reactions use a so-called donor/acceptor system, in which the carbenoid is flanked by an electron withdrawing group (usually a carbonyl) and an electron donating group (usually a vinyl, or aryl group). The electron donating group moderates the reactivity of the carbenoid (*i.e*, by reducing its electrophilicity), thus minimising carbene dimerisation pathways and increasing chemoselectivity in intermolecular reactions. It also renders the system more sterically demanding, meaning that less hindered sites may undergo reaction preferentially. (**Figure 6.3**)
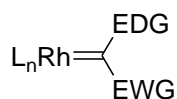
FIGURE 6.3

6.3 RHODIUM (II) CHEMISTRY IN SYNTHESIS OF α-ALKYLIDENE-γ BUTYROLACTONES

The aim of this project is to develop a new straightforward method in synthesis of α-alkylidene-γ-butyrolactones involving the Rh (II) chemistry.

This new route in synthesis should follow on well from a recent work in the Taylor's group on tandem approach to α-Alkyliden-γ-Butrylactones. Performing the chemistry in such a way has notable advantages, especially for the development of tandem processes. This new methodology is focused on a key C-C bond forming step that requires only a catalitic amount of a transition metal (for example. Dirhodium (II) complexes). The only byproduct developed from reaction mixture is nitrogen gas, in addition the activation of C-H bonds simplifies also the synthesis of starting materials.

Thus, the reaction of a suitable alcohol **6.34** with a suitable coupling partner **6.35**, the resulting diazo species should undergo a facile C-H insertion reaction to afford butyrolactone **6.38**. Treatment of **6.38** with base and an aldehyde (or ketone) should then form the α-Alkylidene functionality in **6.39** via HWE (Horner Wadsworth-Emmons) olefination.(**Scheme 6.11**)
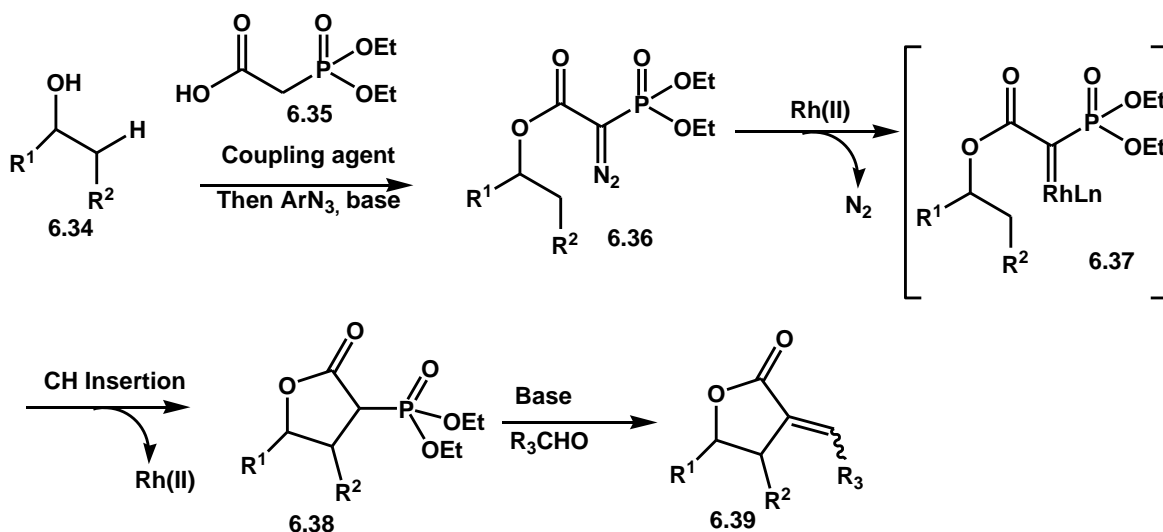
SCHEME 6.11

Given that catalytic Rh(II) is the only byproduct of the C-H insertion , this suggests that the process is suitable for the development of a 1-pot protocol. Furthermore similar insertion reactions can be performed in the presence of a weak base, potentially facilitating an even simpler tandem process.

6.4 <u>INITIAL METHODOLOGY: A STUDY OF C-H INSERTION.</u>

The major of rhodium (II) stabilized carbenoids are tipically highly elctrophilic and, accordingly, insert more readily into electron rich C-H bonds and, in intramolecular variants, will usually undergo insertion to give 5-membered ring lactone products. The model substrates have been chosen as they posses electron rich CH bond and should be easily synthesized from simple starting materials.

6.4.1 *Preparation Of Diazo Phosphonate-*
Herein preparation of diazo phosponates to perform racemic cyclisations, starting from simple alcohol is described.
The phosphonates prepared have been used as crude in the following reactions. The first model compound has a phenyl group. Phosohonate **6.40** has been prepared starting from alcohol **6.40** and ethyl phosphonacetate **6.35** using T3P (Propane Phosphonic Acid Anhydride) as coupling agent. Phosponate **6.41** has been transformed into corresponding diazocompound **6.42** by reaction with 4-acetamidebenzensulfonylazide **B**. This reaction afforded **6.42** in good yield. (**Scheme 6.12**)

.
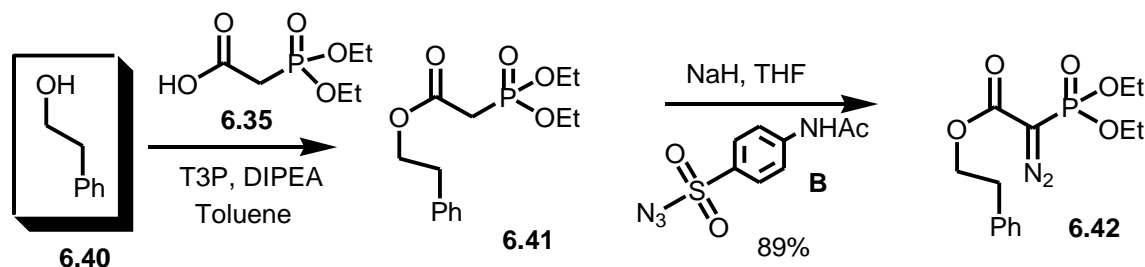
SCHEME 6.12

Then, the same reaction has been carried out simply using alcohols (as in the case of 2- methyl 1- propan-1-ol **6.43**; **Scheme 6.13**) with different electron-rich C-H bonds and, in any case, formation of diazocompounds occur without difficulties

SCHEME 6.13
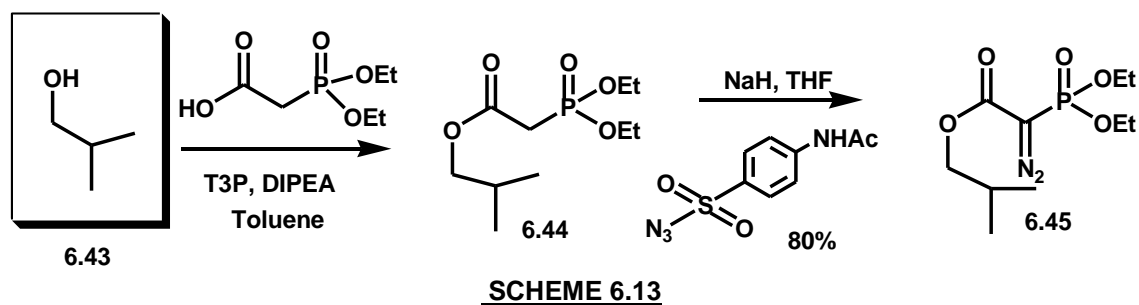
In order to investigate both chemoselectivity and the diastereoselectivity of the Rh(II) catalysed C-H insertion also in the case of formation of polycyclic lactones, some substrate with both cyclohexane and cyclohexyl moieties have been prepared. (**Scheme 6.14**)

Starting from cycloheanol **6.46** diazophosphonate **6.48** has been prepared in good yield.

It has two possible C-H bond that can be involved in C-H insertion process. (**Figure 6.4**)
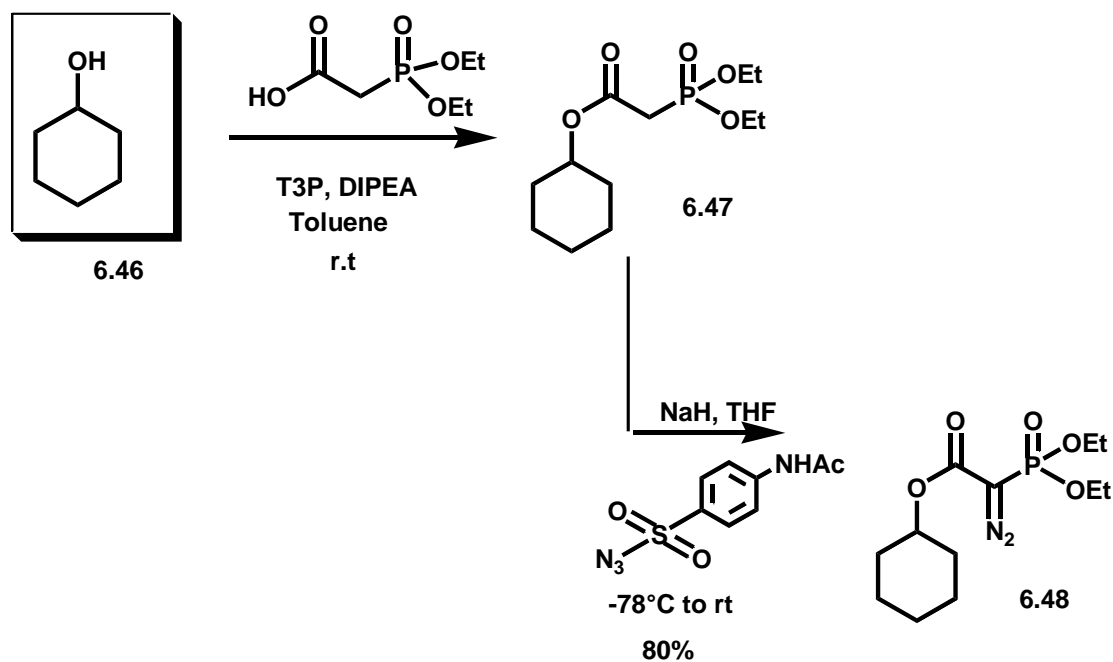
SCHEME 6.14
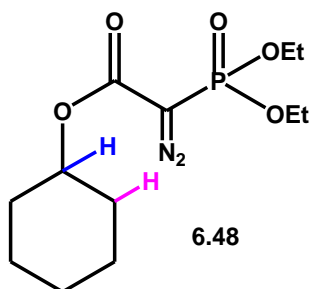
FIGURE 6.4 *Two C-H bonds potentially involved in Rh(II) catalysed cyclisation*

Diazophosphonate **6.51** has been easily prepared starting from alcohol **6.49.** (**Scheme 6.15**).

As in the previous case (**Figure 6.4**), we have many possible C-H bonds that can be involved in C-H insertion process. (**Figure 6.5**)
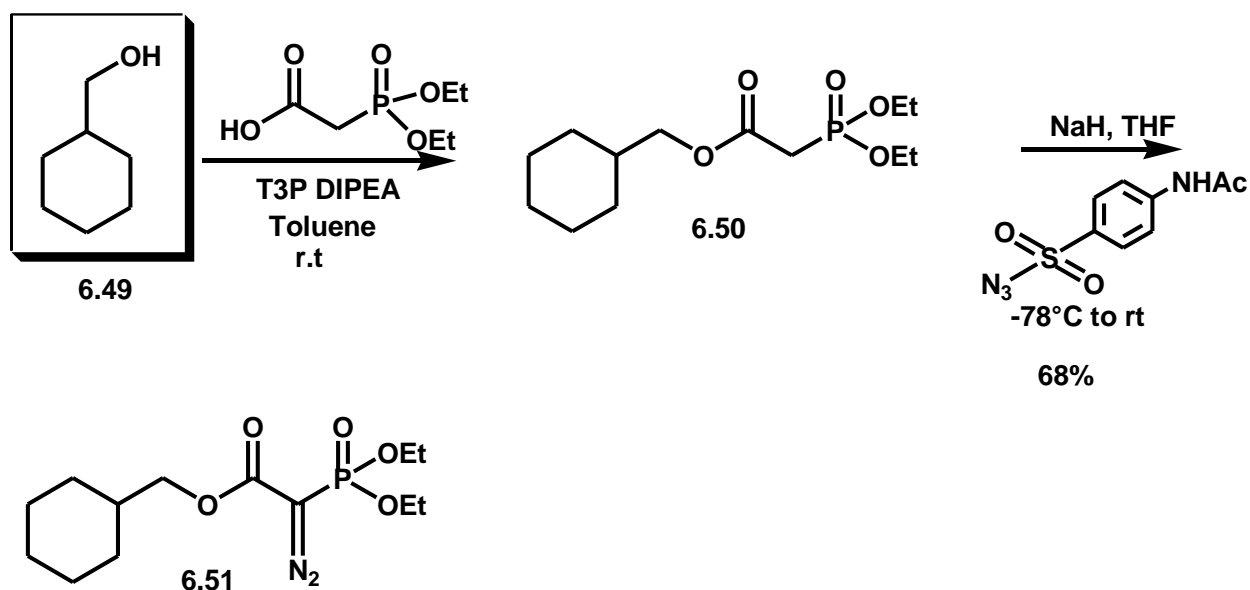
SCHEME 6.15
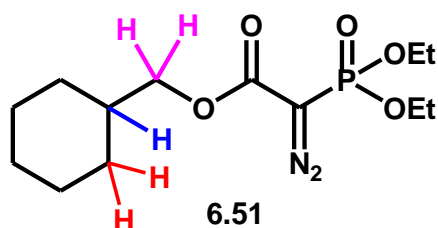
FIGURE 6.5 *C-H bonds potentially involved in Rh(II) catalysed cyclisation*

Our attention has then turned to *trans* fused bicyclic systems as starting material, in order to prepare tricyclic lactones and study the diastereoselectivity of the C-H insertion.

We started from trans decalone, and, performing a stereoselective reduction with NaBH₄, we obtained the two diastereoisomers **6.53** and **6.56** with the predominant formation of **6.53** (OH equatorial).

Phosphonates (**6.54**, **6.57**) and then diazophosphonates (**6.55, 6.58**) of both diastereisomers, were prepared in good yields. (**Scheme 6.16**)
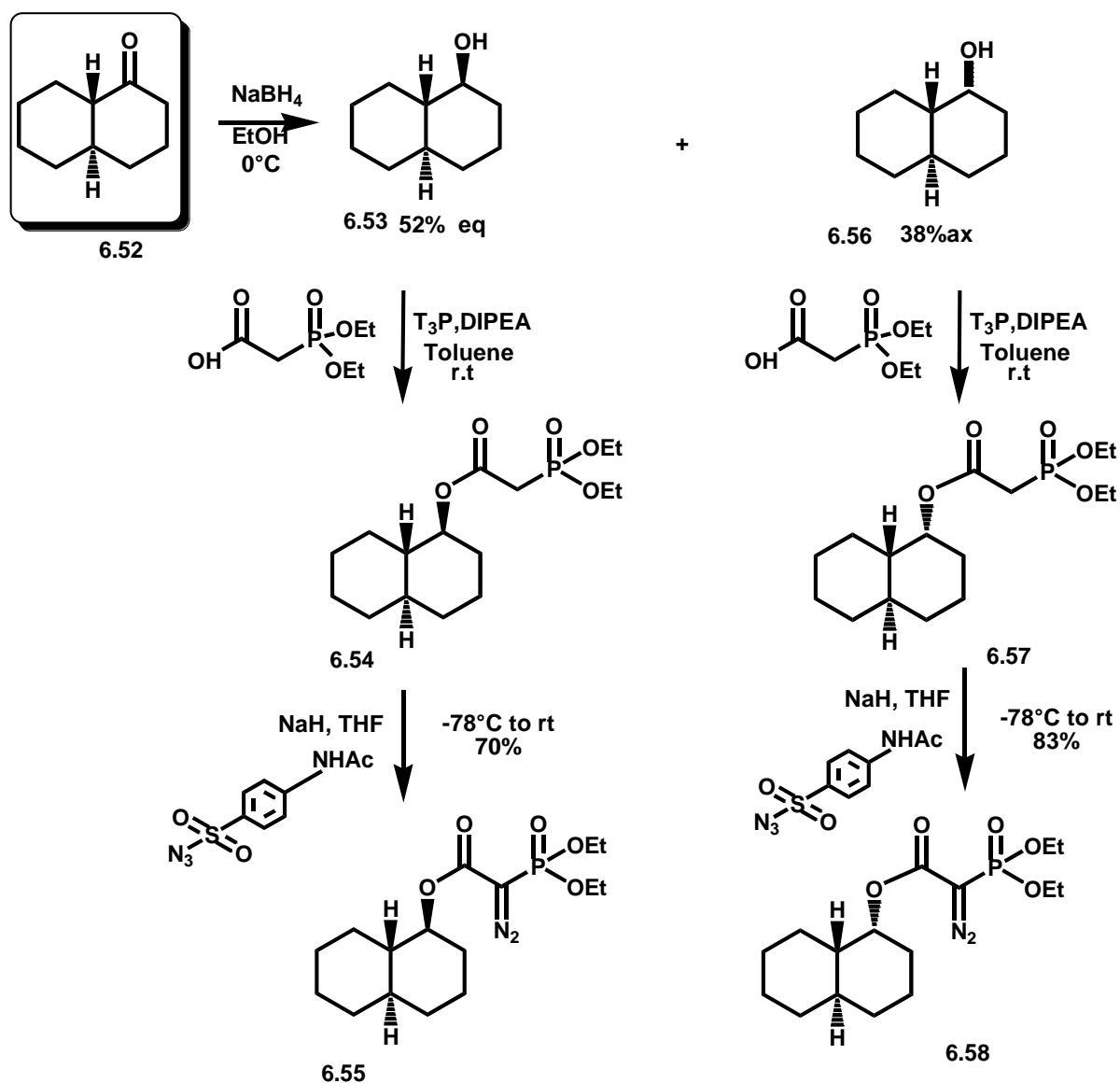
SCHEME 6.16

Once prepared alkyl diazophosphonates derivative, our attention has been focused on preparation of substrates containing an heteroatom close to the C-H bond involved in Rh(II) cyclisation.

Our purpose is to study the electronic effect of the heteroatom located close to the reaction centre and the chance of competing O-H (or N-H) insertion instead of C-H insertion, with potentially formation of interesting enol ether products. (**Scheme 6.17**)
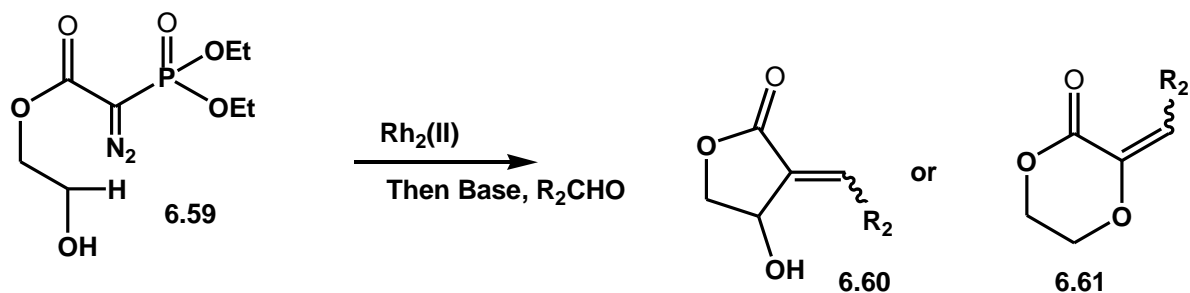
SCHEME 6.17

Thus, It has been reported that C-H insertion adjiacent to a silyl ether the initial C-H insertion product formed was not isolated and undergoes to β elimination directly to afford **6.64**.(**Scheme 6.64**) [166]
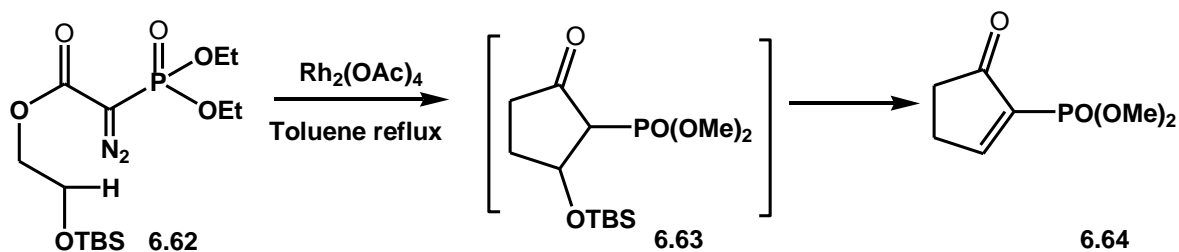
SCHEME 6.18

To investigate these reactions we prepared a number of diazophosphonates with an heteroatom in their framework.

The first diazophosphonate was prepared starting from 2-ethoxyetanol **6.65** (**Scheme 6.19**). Phosphonated compound was successfully obtained but preparation of diazophosphonate **6.67** didn't give the expected product using NaH as base, leading to a decomposition of the starting phosphonate.

Best results have been obtained using LiHMDS as base, affording diazophosphonate **6.67** with a satisfactory yield.

---

[166] Dayoub, W.; Diab, Y.; Doutheau, A.; *Tetrahedron Lett*. **2002**, 4131.
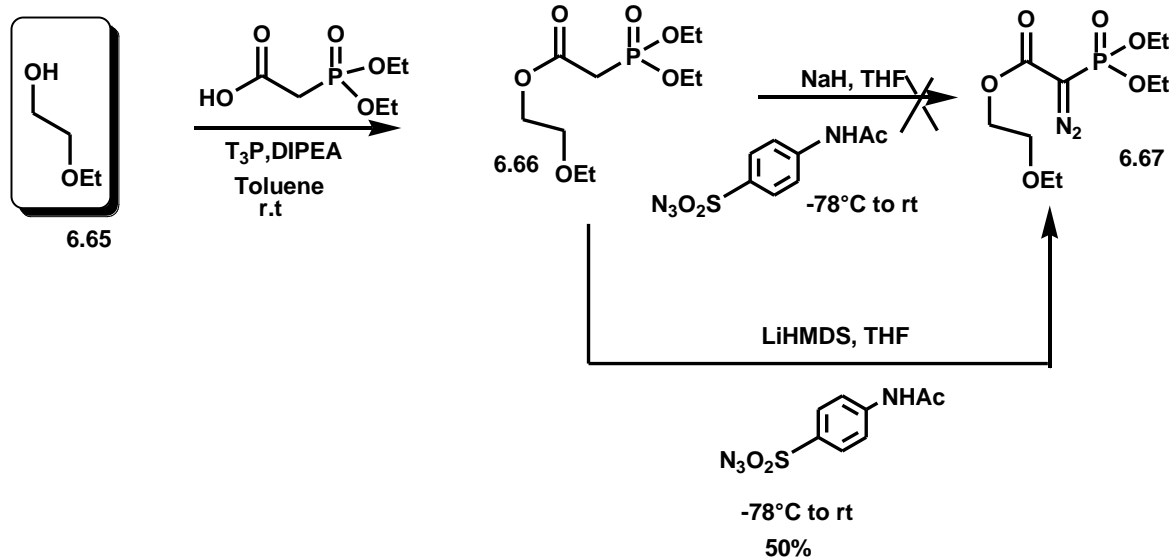
SCHEME 6.19

However, in the case of Diacetone D-Glucose (DAG **6.68**), phosphonate compound **6.69** has been successfully prepared but, the use of NaH as base provided an hydrolysis of phosphonate itself affording the starting material **6.68** again.

Otherwise the use of LiHMDS as base afforded the wanted product **6.70** in good yield.(**Scheme 6.20**)
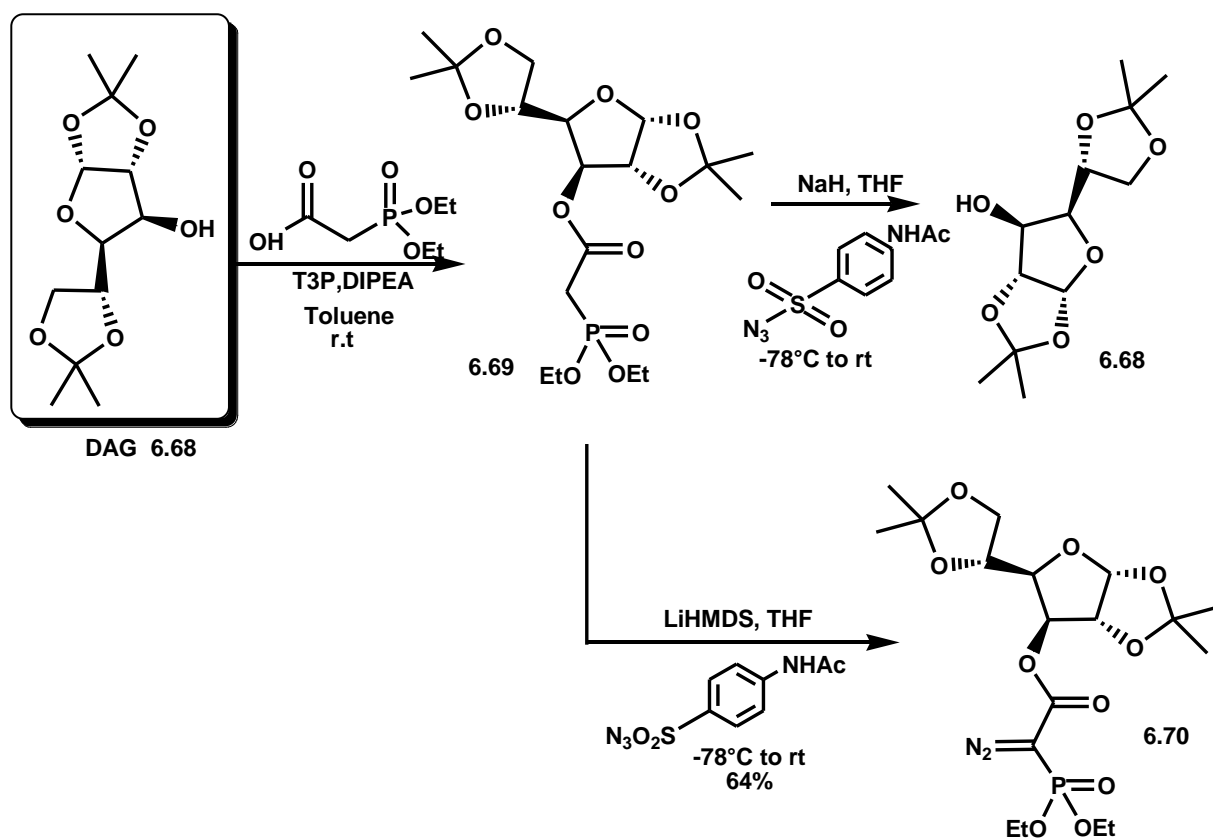
SCHEME 6.20

In order to investigate the selectivity of Rh(II) C-H insertions in heterocyclic systems,(**Figure 6.6**) compound **6.74** has been prepared starting from **6.71**.

First of all, an hydroboration–oxidation has been achieved on **6.71,** the resulting alcohol has been coupled with diethyl chlorophosphate affording phosphonate **6.73**. In this case diazophosphonate **6.74** has been obtained, even if in not too high yield, by using of NaH as base. (**Scheme 6.21**)

SCHEME 6.21

6.74
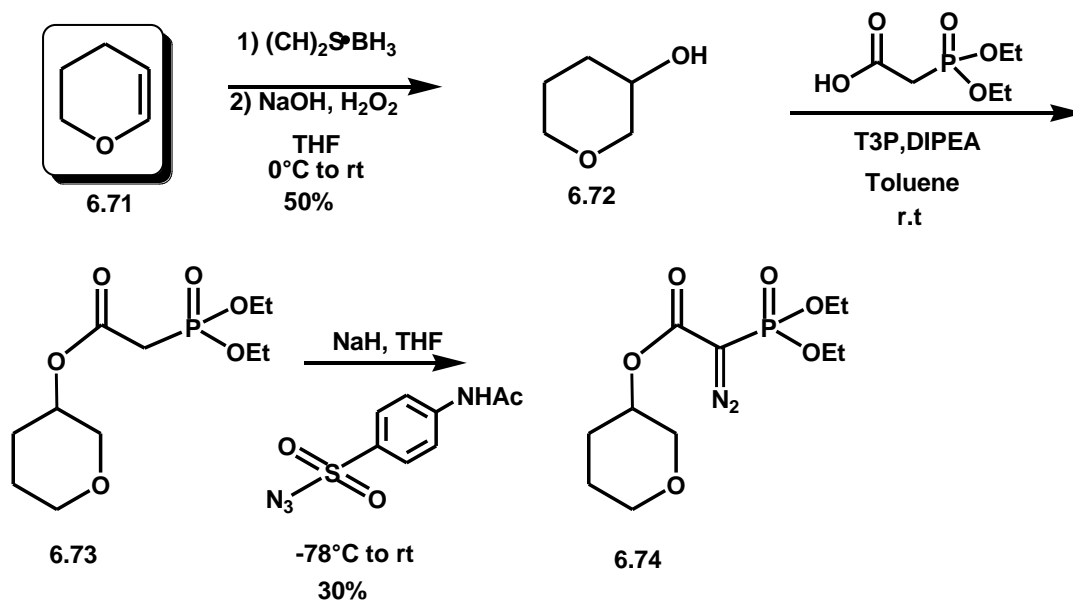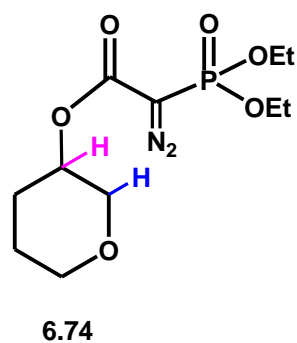
FIGURE 6.6 *C-H bonds potentially involved in Rh(II) catalysed cyclisation*

Starting from L-valinol **6.75**, a diazophosphonate **6.78** containing an amino group has been prepared. The aminogroup in **6.75** was protected as Boc (*tert* butyl carbamate) derivative using the anhydride Boc$_2$O.

Phosphonate **6.77** has been obtained in very good yield from protected aminoacid, a 30% of diazophosphonate **6.78** has been obtained by use of NaH as base.(**Scheme 6.22**)
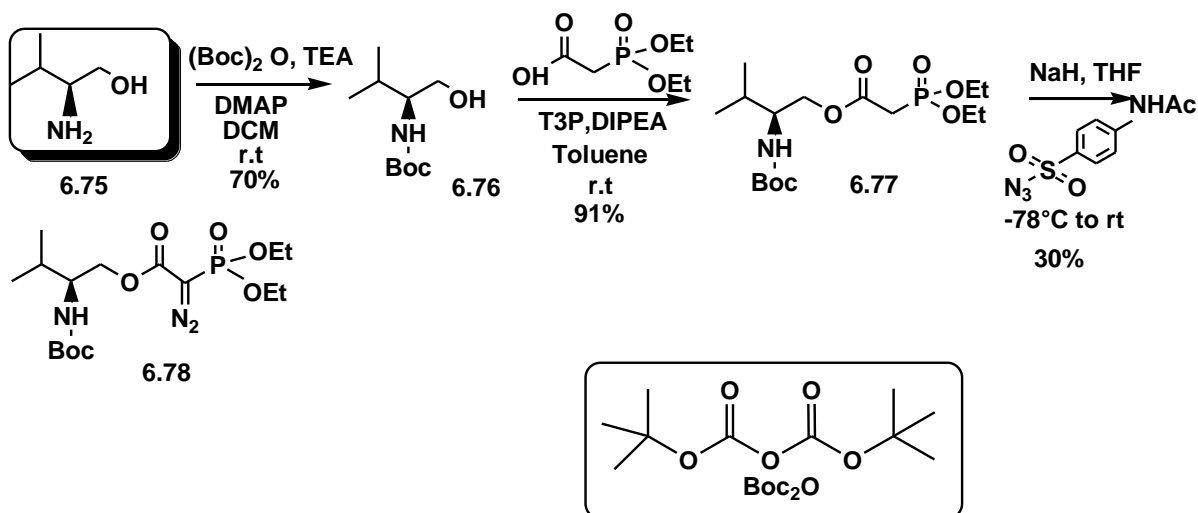
SCHEME 6.22

6.4.2 Studies towards Rh(II) catalysed Cyclisations.

Once prepared a wide range of diazocompounds, our attention has been focused on studies towards Rh(II) cyclisation in synthesis of alkyliden butyrolactones. We used $Rh(II)_2(Oct)_4$ as Dirhodium catalyst, because carboxylate derivative are air stable and easy to work with.

Cyclisation performed on compound **6.42** gave the expected five membered lactone **6.79** in good yield and high diastereoselectivity, then the following HWE reaction, using paraformaldehyde, successfully afforded the α-alkylidene motiety in **6.80**.(**Scheme 6.23**)
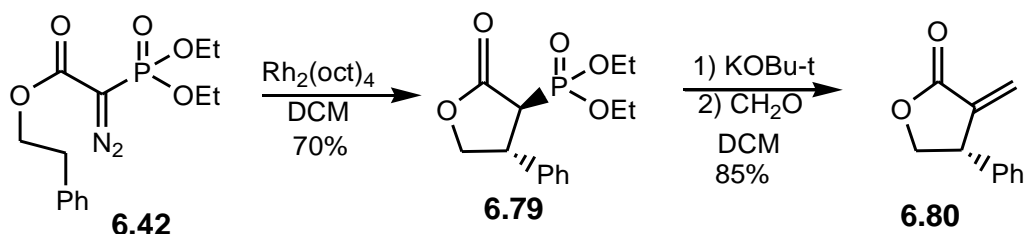
SCHEME 6.23

The same protocol performed on **6.45** gave first the 5 member lactone and the following HWE reaction provided the alkylidene moiety yielding 50% of very volatile lactone **6.82**. (**Scheme 6.24**)
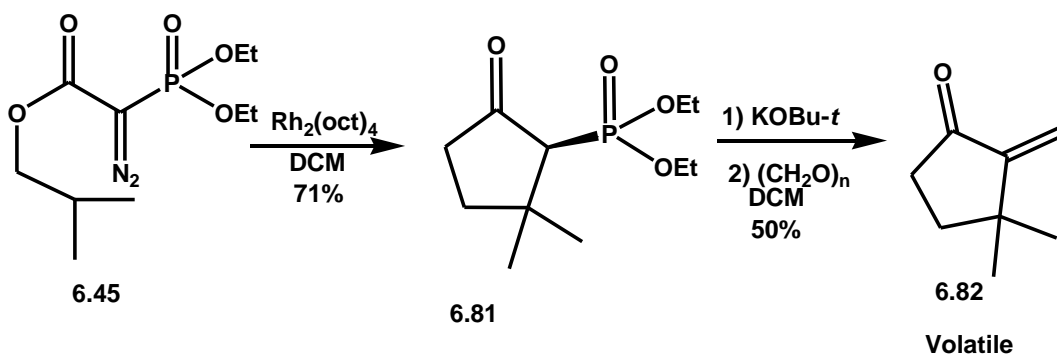
SCHEME 6.24

Cyclisation performed on cyclohexane derivative **6.48** has involved both the two possible electron rich C-H bonds. C-H insertion involving the C-H bond immediately close to the oxygen leads to formation of compound **6.83,** while the two possible five member lactones diastereoisomers **6.84** (major) and **6.85** (minor) are generated from insertion of the further C-H bond involved. Following HWE reactions gave then the alkylidene moieties in **6.86** and **6.87**.(**Scheme 6.25**)
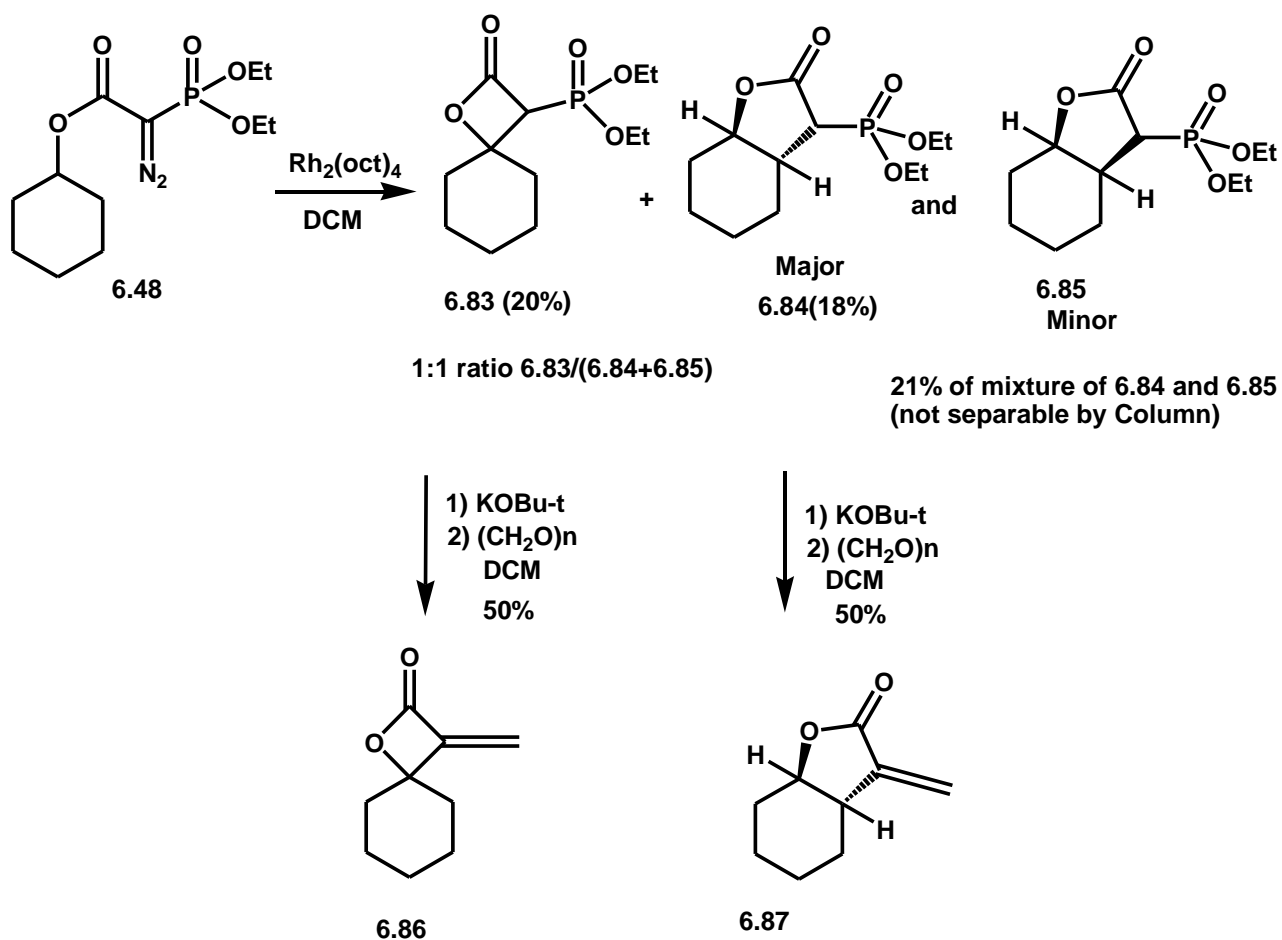
SCHEME 6.25

The same protocol has been applied to cyclohexyl derivative **6.51** giving five and four member lactone (**6.88** and **6.89** respectively), demonstrating that Rh(II) cyclisation can involve both of the two possible C-H bonds, mainly the C-H bond close to the oxigen (**Scheme 6.26**)

SCHEME 6.26

Then we turned to study Rh(II) cyclisation on trans fused decalinic systems previously prepared. With these substrate we tried to attempt the telescoped procedure (one pot) to synthesize the three member lactones. Both **6.55** and **6.56** underwent to Rh(II) catalyzed cyclization and were subsequently treated with base and paraformaldehyde.(**Scheme 6.27**)

We isolated the two lactones **6.90** and **6.92** and two products, **6.91** and **6.93** in which a new bond oxygen-phosphorus is generated. C-H insertions catalyzed by Rh(II) complexes can be affected by presence of water: in fact, addition of water to the carbenoid intermediate **6.94** can occur instead of C-H insertion, and, after adding of base, a migration of phosphorus on oxygen (**6.96**) directs the reaction to the formation of compound **6.97** (**Scheme 6.28**)
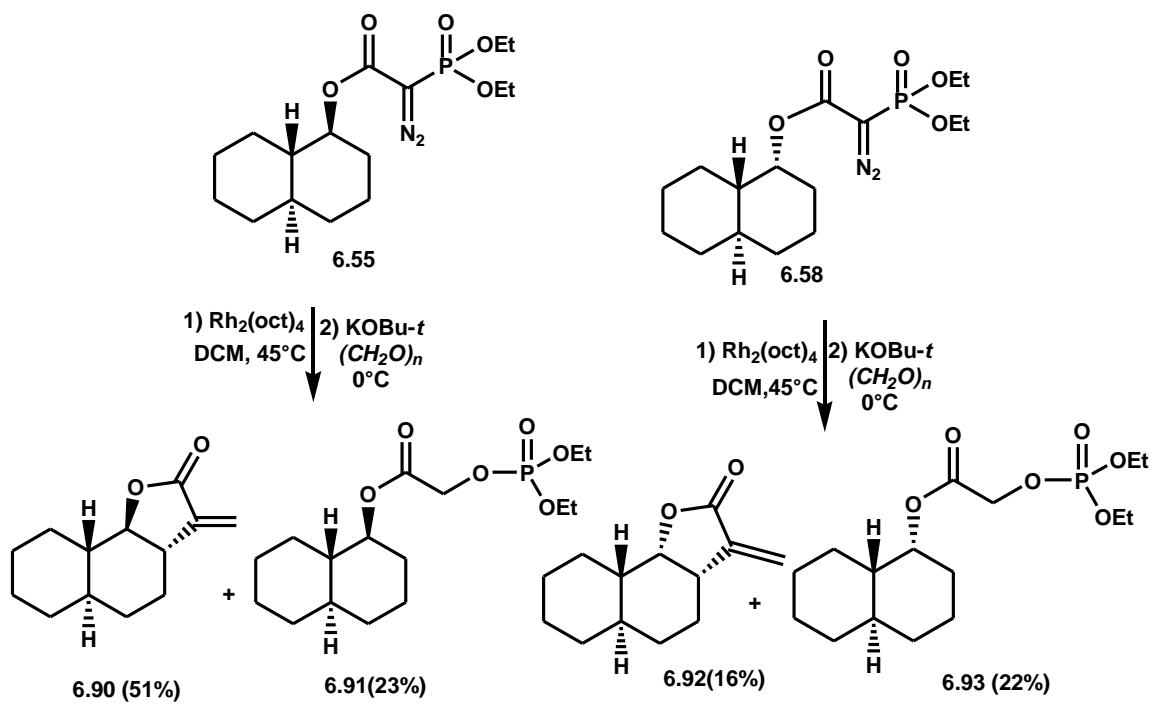
SCHEME 6.27
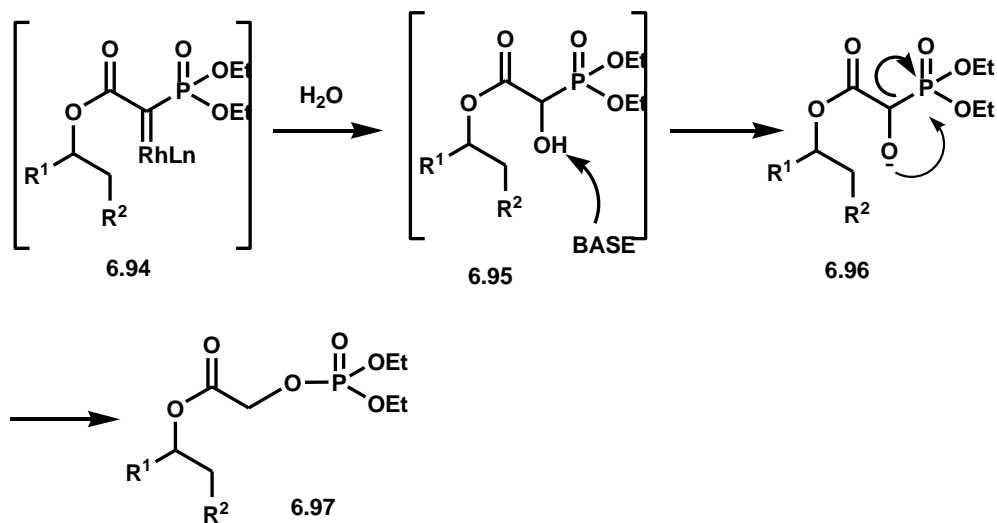
SCHEME 6.28

Then we turned to study C-H insertion in substrate with heteroatoms. Cyclisation performed on **6.67**, performed in DCM at 45°C gave a mixture in which **6.100** seems to be predominant. In order to optimize this process we tried the reaction at room temperature: the reaction proceeded quite slowly and after adding of base, the migration product **6.99** coming from addition of water has been formed. (**Scheme 6.29**)
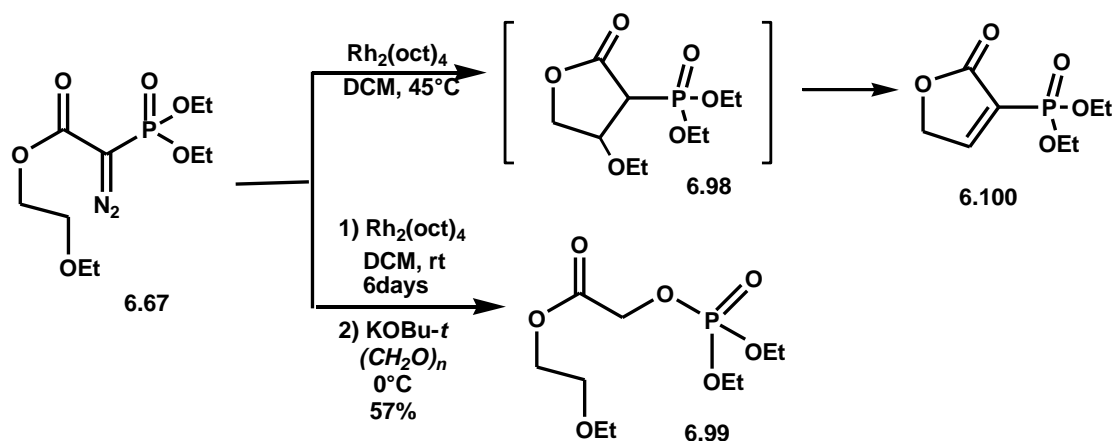
SCHEME 6.29

Finally the C-H insertion performed on **6.70** gave as only product **6.101** in which the cyclisation has been affected from presence of water, while cyclisation performed on 6.64 afforded predominantly **6.102** in which C-H bond involved is close to the oxygen (**Scheme 6.30**).
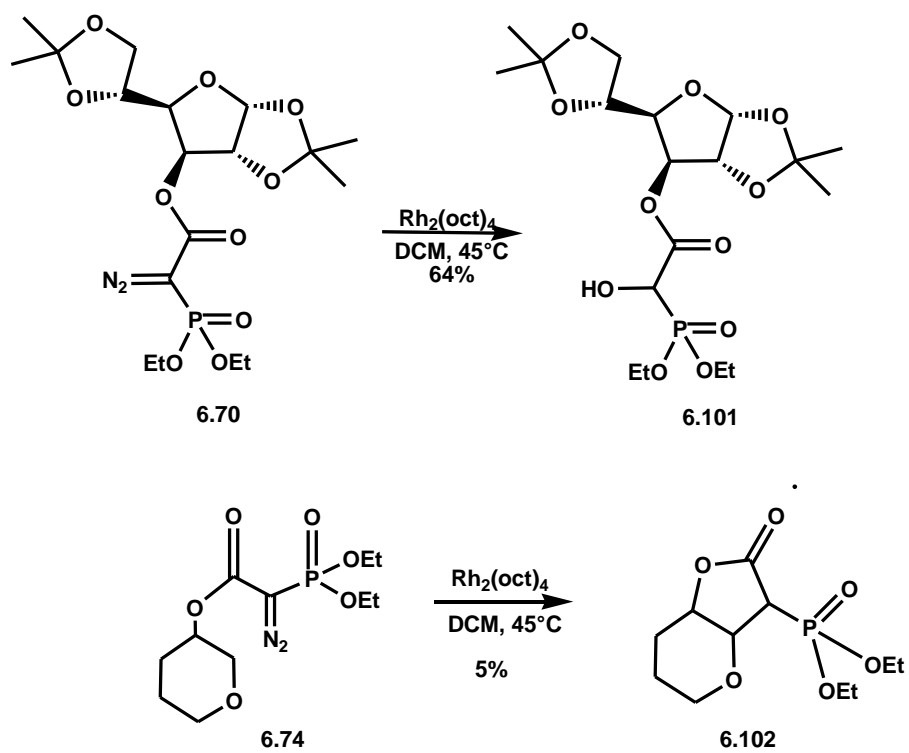
SCHEME 6.30

6.4.3 A First Approach To Enantioselective Synthesis Of α-Alkyliden-γ-Butrylactones

In this section a first approach to enantioselectivesynthesis of α-Alkyliden-γ-butrylactones is described. In the **Figure 6.7** the chiral catalyst has been reported.

C Rh$_2${(*S*)-DOSP}$_4$
(Ar = *p*-C$_{12}$H$_{25}$C$_6$H$_4$)
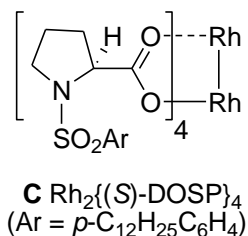
FIGURE 6.7 Rh$_2$[S(DOSP)$_4$] chiral catalyst

The reaction has been performed on a model substrate 6.103 changing solvents and conditions (Table 6.1). In any case the *ee*, extimated by HPLC analysis is low so the enantioselective C-H insertion followed telescoped olefination in synthesis of α-Alkyliden-γ-Butyrolactones still remains an unexplored field.
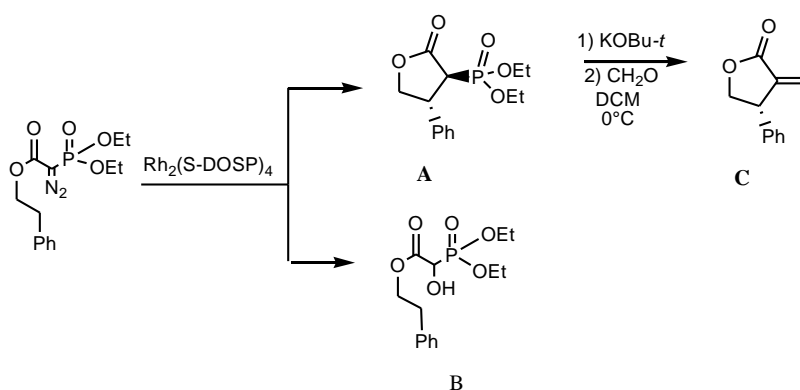
Entry	Solvent	T (°C)	A(%)	B(%)	C (%)	e.e (%)*
1	DCM	45			63*	4
2	DCM	rt	-	80	-	-
3	Hexane	45	7	11	-	-
4	Toluene	45	18	3	60#	6

* one pot protocol   # A to C   *Extimated by HPLC

6.5 – CONCLUSIONS

A wide number of α-Alkyliden-γ-Butyrolactones have been prepared in order to investigate the diastereoselectivity of the Rh(II) catalysed C-H insertion process and how the electronic effect of EDN groups can direct the C-H insertion.

The best substrates have phenyl or alkyl group, with the preference of insertion in C-H bond more electron rich.

6.6 –EXPERIMENTAL

***6.6.1 General Experimental*:** 1H, 13C and NMR spectra were recorded with a JEOL EXC400 spectrometer operating at 400, 100 and 162 MHz, respectively. All spectroscopic data was acquired at 295 K. Chemical shifts are quoted in parts per million (ppm) using the residual solvent peak as an internal standard [^{1}H NMR 7.26 ppm for CHCl3 and 13C NMR 77.0 ppm for CDCl3, 1H NMR 4.84 (s), 3.31 (quintuplet) and 13C NMR 49.05 (septuplet) for D_3COD]. Coupling constants (*J*) are reported in Hz. Multiplicity abbreviations used: s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet) and br. (broad). Signal assignments were accomplished by analysis of COSY, NOESY, HSQC and HMBC experiments where necessary. Infrared spectra were recorded with a Thermo Nicolet IR100 spectrometer using NaCl plates. Low- and high-resolution mass spectra were obtained for all novel compounds. Electrospray ionization (ESI) and chemical ionization (CI, using ammonia gas) were measured with a Micromass Autospec spectrometer. Thin-layer chromatography (TLC) was performed using Merk silica gel 60F254 pre-coated aluminum backed plates. The compounds were visualized using UV light (254 nm) and KMnO4 or anisaldehyde. Flash chromatography was performed at medium pressure using slurry packed Fluka silica gel 35–70 µm, 60 Å with the eluant specified. Petroleum ether is the fraction with b.p. 40–60 °C. Tetrahydrofuran was distilled from sodium–benzophenone ketyl immediately before use. Anhydrous toluene and dichloromethane were obtained from an M Braun SPS solvent purification system. Water refers to deionised water. Except where specified, all reagents were purchased from commercial sources and were used without further purification.

6.6.2 General Procedure

preparation of phosphonates To a solution of alcohol (1=) in toluene (5.2mL/mmol of alcohol) under argon was added sequentially dietihylphosphonacetic acid (1.05=) DIPEA (2.6=) and T3P (1.3=, 50% by mass in THF). The resulting solution was stirred at room temperature and monitored by TLC for the consumption of starting alcohol. Diluted the reaction mixture with water extract with EtOAc (3x) wash sequentially with 10% aq HCl. sat aq NaHCO$_3$ and brine dry over MgSO$_4$ and concentrate in _vacuo_. The resulting compounds were used without any further purification in the following step.

Preparation of diazo phosphonates. A solution of phosphonate (1=) in THF (5 mL/mmoL, THF still) under argon was cooled to -78°C. NaH (1.2 of a 60% in mineral oil) or where indicated LiHMDS solution in THF (1.2=) was added and the resulting suspension was warmed to room temperature and stirred for 10 mins. 4-acetamidobenzenesulfonylazide (1.2=) was added and the mixture stirred at room temperature for 1h. Diluted the reaction mixture with water extract with EtOAc (3x) wash sequentially with sat aq NaHCO$_3$ and brine dry over MgSO$_4$ and concentrate in vacuo. Purification by column chromatography afforded the diazo compounds.

Rh(II) Catalised cyclisation and HWE olefination.
To a solution of diazophosphonate (1=) in DCM (20mL/mmol) in a sealable tube Rh$_2$oct$_4$ (0.05=) was added The resukting green solution was stirred at 45°C for 1h and then cooled to 0°C KOBu-t (0.9=) was the added and the mixture stirred at 0°C for 30 min before adding paraformaldehyde. (10=) and stirring another 30 min at 0°C. Diluted the reaction with DCM wash with water and concentrate in vacuo.<purification by colums chromatography gave the desired α-Alkyliden-γ-Butrylactone.

SPECTROSCOPIC DATA
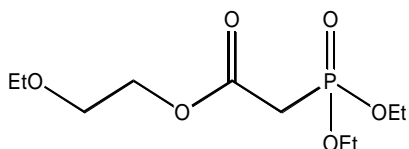
Compound 1. Yellow oil. ^{1}H NMR (400 MHz, CDCl$_3$): δ = 4.29 (t, 2 H, -CH$_2$OC(O)-), 4.15 (quintet, 4 H, 2x -CH$_2$OP(O)-), 3.62 (t, 2 H, -OCH$_2$CH$_2$OC(O)-), 3.50 (q, ^3J = 7.0 Hz, 2 H, -OCH$_2$CH$_3$), 2.98 (d, $^2J_{PH}$ = 21.4 Hz, 2 H), 1.32 (t, ^3J = 6.6 Hz, 6 H, 2x -(O)POCH$_2$CH$_3$), 1.19 (t, 3 H, -OCH$_2$CH$_3$) ppm. ^{13}C NMR (100 MHz, CDCl$_3$): δ = 165.8 (C(O)), 68.0 (-OCH$_2$CH$_2$OC(O)-), 64.6 (-OCH$_2$CH$_3$), 62.7 (-CH$_2$OC(O)-), 62.6 (2x -CH$_2$OP(O)-), 34.2 (d, J$_{CP}$ = 133 Hz, -(O)CCH$_2$P(O)-), 16.3 and 16.2 (2x -(O)POCH$_2$CH$_3$), 15.0 (-OCH$_2$CH$_3$) ppm. MS (ESI) found for (C$_{10}$H$_{21}$O$_6$PH$^+$) 269.1154, found for (C$_{10}$H$_{21}$O$_6$PNa$^+$) 291,0973.
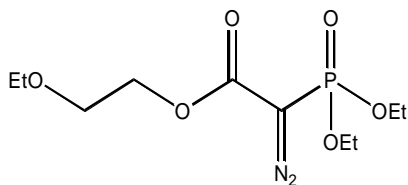
Compound 2. Slightly pink oil. ^{1}H NMR (400 MHz, CDCl$_3$): δ = 4.29 (t, ^3J = 4.6 Hz, 2 H, -CH$_2$OC(O)-), 4.25-4.05 (m, 4 H, 2x -CH$_2$OP(O)-), 3.60 (t, 2 H, -OCH$_2$CH$_2$OC(O)-), 3.48 (q, ^3J = 7.0 Hz, 2 H, -OCH$_2$CH$_3$), 1.31 (t, 6 H, 2x -(O)POCH$_2$CH$_3$), 1.14 (t, 3 H, -OCH$_2$CH$_3$) ppm. ^{13}C NMR (100 MHz, CDCl$_3$): δ = 163.0 (x2) (C(O) and C(N)$_2$), 68.0 (-OCH$_2$CH$_2$OC(O)-), 66.5 (-OCH$_2$CH$_3$), 64.6 (-CH$_2$OC(O)-), 63.6(4) and 63.6(0) (2x -CH$_2$OP(O)-), 16.0(1) and 16.0(0) (2x -(O)POCH$_2$CH$_3$), 15.0 (-OCH$_2$CH$_3$) ppm. MS (ESI) found for (C$_{10}$H$_{19}$N$_2$O$_6$PH$^+$) 295.1053, found for (C$_{10}$H$_{19}$N$_2$O$_6$PNa$^+$) 317,0873.
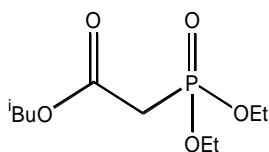
Compound 3. Yellow oil. ^{1}H NMR (400 MHz, CDCl$_3$): δ = 3.90 (quintet, 4 H, 2x -CH$_2$OP(O)-), 3.65 (d, ^3J = 6.6 Hz, 2 H, ^{i}PrCH$_2$O-), 2.72 (d, $^2J_{PH}$ = 21.6 Hz, 2 H), 1.69 (m, 1 H, -CH- of ^{i}Bu), 1.08 (t, ^3J = 7.1 Hz, 6 H, 2x -(O)POCH$_2$CH$_3$), 0.69 (d, ^3J = 6.8 Hz, 6 H, 2x –CH$_3$ of ^{i}Bu) ppm. ^{13}C NMR (100 MHz, CDCl$_3$): δ = 165.2 (C(O)), 70.9 (^{i}PrCH$_2$O-), 62.0 and 61.9 (2x -CH$_2$OP(O)-), 33.7 (d, J$_{CP}$ = 133 Hz, -(O)CCH$_2$P(O)-), 27.1 (-CH- of ^{i}Bu), 18.4 (2x –CH$_3$ of ^{i}Bu), 15.7(5) and 15.7(0) (2x -(O)POCH$_2$CH$_3$), ppm. MS (ESI) found for (C$_{10}$H$_{21}$O$_5$PH$^+$) 253.1199, found for (C$_{10}$H$_{21}$O$_5$PNa$^+$) 275,1019.
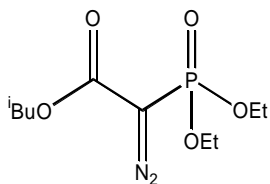
Compound 4. Yellow oil. ^{1}H NMR (400 MHz, CDCl$_3$): δ = 4.15-3.95 (m, 4 H, 2x -CH_2OP(O)-), 3.84 (d, ^3J = 6.4 Hz, 2 H, ^{i}PrCH_2O-), 1.90-1.75 (m, 1 H, -CH- of ^{i}Bu), 1.22 (t, ^3J = 7.2 Hz, 6 H, 2x -(O)POCH$_2$CH_3), 0.81 (d, ^3J = 6.6 Hz, 6 H, 2x –CH_3 of ^{i}Bu) ppm. ^{13}C NMR (100 MHz, CDCl$_3$): δ = 163.2 and 163.1 (C(O) and C(N)$_2$), 71.2 (^{i}PrCH_2O-), 63.2(2) and 63.2(0) (2x -CH$_2$OP(O)-), 27.5 (-CH- of ^{i}Bu), 18.5 (2x –CH_3 of ^{i}Bu), 15.8(3) and 15.8(0) (2x -(O)POCH$_2$CH_3), ppm. MS (ESI) found for (C$_{10}$H$_{19}$N$_2$O$_5$PH$^+$) 279.1104, found for (C$_{10}$H$_{19}$N$_2$O$_5$PNa$^+$) 301,0924.
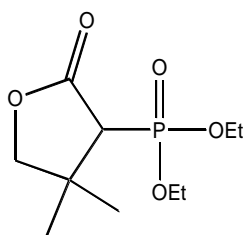
Compound 5. Yellow oil. ^{1}H NMR (400 MHz, CDCl$_3$): δ = 4.33-4.08 (m, 5 H, 2x -CH_2OP(O)- and –CH_aH$_b$OC(O)-), 3.91 (d, ^3J = 8.6 Hz, 1 H, -CH$_a$$H_b$OC(O)-), 2.76 (d, $^2J_{PH}$ = 24.4 Hz, 1 H), 1.43-1.23 (m, 12 H, 2x -(O)POCH$_2$CH_3 and 2x –CH_3) ppm. ^{13}C NMR (100 MHz, CDCl$_3$): δ =172.0 (C(O)), 79.8 (-CH_2OC(O)-), 63.3 and 62.5 (2x -CH$_2$OP(O)-), 50.7 (d, J$_{CP}$ = 143 Hz, CP), 40.2 (quaternary carbon), 27.1 (-CH_3), 22.4 and 16.3 (2x -(O)POCH$_2$CH_3 and -CH_3) ppm. MS (ESI) found for (C$_{10}$H$_{19}$O$_5$PH$^+$) 251.1043, found for (C$_{10}$H$_{19}$O$_5$PNa$^+$) 273,0862.

Compound 6. Pink oil. ^{1}H NMR (400 MHz, CDCl$_3$): δ = 7.40 – 7.20 (m, 5H, aromatic protons), 4.37 (t, 2 H, -CH$_2$OC(O)-), 4.15 (quintet, 4 H, 2x -CH$_2$OP(O)-), 3.10 – 2.90 (m, 4H, -(O)CCH$_2$P(O)- and –CH_2Ph), 1.34 (t, ^3J = 7.2 Hz, 6 H, 2x -(O)POCH$_2$CH_3), ppm. ^{13}C NMR (100 MHz, CDCl$_3$): δ = 165.1 (C(O)), 136.9 (aromatic C), 128.4 and 128.3 (4x aromatic CH), 127.9 (aromatic CH), 65.3 (-CH_2OC(O)-), 62.1 and 62.0 (2x -CH$_2$OP(O)-), 34.3 (-CH_2Ph), 33.7 (d, J$_{CP}$ = 133 Hz, -(O)CCH_2P(O)-), 15.8 and 15.7 (2x -(O)POCH$_2$CH_3), ppm. MS (ESI) found for (C$_{14}$H$_{21}$O$_5$PH$^+$) 301.1199, found for (C$_{14}$H$_{21}$O$_5$PNa$^+$) 323,1019.

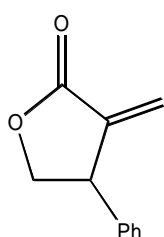**Compound 7.** Yellow oil. ^{1}H NMR (400 MHz, CDCl$_3$): δ = 7.45 – 7.15 (m, 5H, aromatic protons), 4.44 (t, ^3J = 6.9 Hz, 2 H, -CH$_2$OC(O)-), 4.35-4.00 (m, 4 H, 2x -

CH$_2$OP(O)-), 2.99 (t, 2H, –CH_2Ph), 1.34 (t, ^3J = 7.1 Hz, 6 H, 2x -(O)POCH$_2$CH_3), ppm. ^{13}C NMR (100 MHz, CDCl$_3$): δ = 162.9 and 162.8 (C(O) and C(N)$_2$), 137.0 (aromatic C), 128.5 and 128.1 (4x aromatic CH), 126.3 (aromatic CH), 65.5 (-CH$_2$OC(O)-), 63.2(3) and 63.2(0) (2x -CH$_2$OP(O)-), 34.6 (-CH$_2$Ph), 15.7(6) and 15.7(0) (2x -(O)POCH$_2$$CH_3$), ppm. MS (ESI) found for (C$_{14}$H$_{19}$N$_2$O$_5$PH$^+$) 327.1110, found for (C$_{14}$H$_{19}$N$_2$O$_5$PNa$^+$) 349,0929.
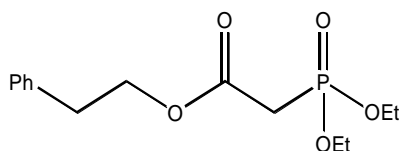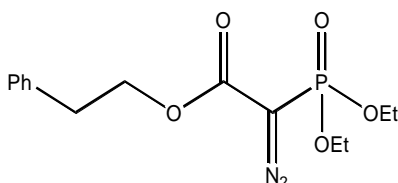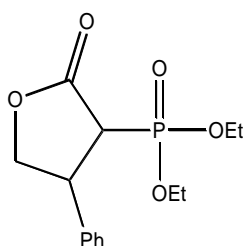
Compound 8. Colourless oil. ^{1}H NMR (400 MHz, CDCl$_3$): δ = 7.45-7.15 (m, 5H, aromatic protons), 4.75 (d, ^3J = 7.9 Hz, 1 H, -CH_aH$_b$OC(O)-), 4.40-4.00 (m, 6 H, 2x -CH$_2$OP(O)-, -CH$_a$$H_b$OC(O)- and -C$H$Ph), 3.15 (dd, $^2J_{PH}$ = 23.8 Hz, ^3J = 6.0 Hz, 1 H, -(O)CCHP(O)-), 1.40-1.15 (m, 6 H, 2x -(O)POCH$_2$CH_3) ppm. ^{13}C NMR (100 MHz, CDCl$_3$): δ =171.5 (C(O)), 140.1 (aromatic C), 129.2, 128.0 and 126.6 (5x aromatic CH), 73.7 (-CH$_2$OC(O)-), 63.8 and 62.9 (2x -CH$_2$OP(O)-), 47.2 (d, J$_{CP}$ = 140 Hz, CP), 43.4 (-CHPh), 16.3 and 16.2 (2x -(O)POCH$_2$$CH_3$) ppm. MS (ESI) found for (C$_{14}$H$_{19}$O$_5$PH$^+$) 299.1043, found for (C$_{14}$H$_{19}$O$_5$PNa$^+$) 321.0862.

Compound 9. White oil. ^{1}H NMR (400 MHz, CDCl$_3$): δ = 7.40 – 7.10 (m, 5H, aromatic protons), 6.32 (d, J = 3.0 Hz, 1H, CH_aH$_b$=C-), 5.41 (d, 1H, CH$_a$$H_b$=C-), 4.75-4.55 (m, ^3J = 5.5 Hz, 1H, -CH_aH$_b$OC(O)-), 4.30-4.10 (m, 2H, -CH$_a$$H_b$OC(O)- and -C$H$Ph), ppm. ^{13}C NMR (100 MHz, CDCl$_3$): δ = 170.0 (C(O)), 139.5 and 138.8 (aromatic C and CH$_2$=C-), 129.2 (aromatic CH), 127.8(5) and 127.8(2) (4x aromatic CH), 124.0 (CH$_2$=C-), 72.7 (-CH$_2$OC(O)-), 45.7 (-CHPh), ppm. MS (ESI) found for (C$_{11}$H$_{10}$O$_2$H$^+$) 175.0759, found for (C$_{11}$H$_{10}$O$_2$Na$^+$) 197,0578.
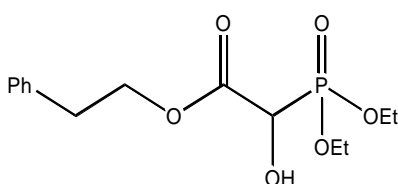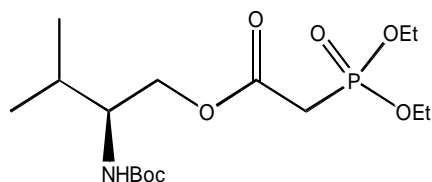
Compound 10. Yellow oil. ^{1}H NMR (400 MHz, CDCl$_3$): δ = 7.35-7.10 (m, 5H, aromatic protons), 4.55-4.35 (m, 3 H, -(O)CCHP(O)- and -CH$_2$OC(O)-), 4.25-4.00 (m, 4 H, 2x -CH$_2$OP(O)-), 3.76 (bs, 1H, -OH), 2.93 (t, ^3J = 7.1 Hz, 2H,
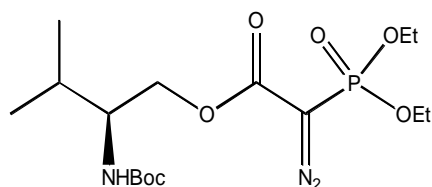
-CH_2Ph), 1.25 (t, ^3J = 7.2 Hz, 6 H, 2x -(O)POCH$_2$CH_3), ppm. ^{13}C NMR (100 MHz, CDCl$_3$): δ = 169.4 (C(O)), 136.9 (aromatic C), 128.8 and 128.5 (4x aromatic CH), 126.7 (aromatic CH), 68.8 (d, J_{CP} = 154 Hz, -(O)CCHP(O)-), 66.9 (-CH$_2$OC(O)-), 63.6 (2x -CH$_2$OP(O)-), 34.8 (-CH$_2$Ph), 16.4 and 16.3 (2x -(O)POCH$_2$CH$_3$), ppm. MS (ESI) found for (C$_{14}$H$_{21}$O$_6$PH$^+$) 317.1143, found for (C$_{14}$H$_{21}$O$_6$PNa$^+$) 339,0961.
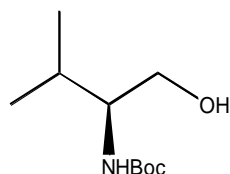
Compound 11. Yellow oil. ^{1}H NMR (400 MHz, CDCl$_3$): δ = 4.70 (bs, 1 H, -NH-), 3.75-3.65 (m, 1 H, -CH_aH$_b$OH-), 3.65-3.55 (m, 1 H, -CH$_a$$H_b$OH-), 3.50 (bs, 1 H, -C$H$NHBoc-), 2.55 (bs, 1 H, -OH), 1.90-1.70 (m, 1 H, -CH- of ^{i}Pr), 1.43 (s, 9 H, 3x -CH$_3$ of Boc), 0.92 (d, ^3J = 7.0 Hz, 6 H, 2x –CH_3 of ^{i}Pr) ppm. ^{13}C NMR (100 MHz, CDCl$_3$): δ = 158.5 (C(O)), 80.0 (-CO^{t}Bu), 64.3 (-CH$_2$OH), 59.0 (-CHNHBoc), 29.3 (-CH- of ^{i}Pr), 28.3 (3x -CH$_3$ of Boc), 19.5 and 18.5 (2x -CH$_3$ of ^{i}Pr), ppm. MS (ESI) found for (C$_{10}$H$_{21}$NO$_3$Na$^+$) 226,1414.

Compound 12. Yellow oil. ^{1}H NMR (400 MHz, CDCl$_3$): δ = 4.94 (d, J_{NH} = 9.8 Hz, 1 H, -NH-), 4.32-4.12 (m, 6 H, -CH$_2$OC(O)- and 2x -CH$_2$OP(O)-), 3.67-3.55 (m, ^3J = 4.9 Hz, ^3J = 4.3 Hz, 1 H, -CHNHBoc-), 3.07-2.87 (m, $^2J_{PH}$ = 21.4 Hz, 2 H), 1.92-1.77 (m, 1 H, -CH- of ^{i}Pr), 1.44 (s, 9 H, 3x -CH$_3$ of Boc), 1.36 (t, ^3J = 6.7 Hz, 6H, 2x -(O)POCH$_2$CH_3), 0.96 (d, ^3J = 6.7 Hz, 6 H, 2x –CH_3 of ^{i}Pr) ppm. ^{13}C NMR (100 MHz, CDCl$_3$): δ = 168.0 (C(O)), 158.5 (-NHC(O)O-), 79.5 (-CO^{t}Bu), 66.0 (-CH$_2$OP(O)- and -CH$_2$OC(O)-), 62.0 (-CH$_2$OP(O)-), 55.0 (-CHNHBoc), 34.8 (d, J_{CP} = 133 Hz, -(O)CCH$_2$P(O)-), 30.0 (-CH- of ^{i}Pr), 28.4 (3x -CH$_3$ of Boc), 20.0 (2x -CH$_3$ of ^{i}Pr), 18.0 (2x -(O)POCH$_2$CH$_3$), ppm. MS (ESI) found for (C$_{16}$H$_{32}$NO$_7$PH$^+$) 382,1989 and for (C$_{16}$H$_{32}$NO$_7$PNa$^+$) 404,1809.
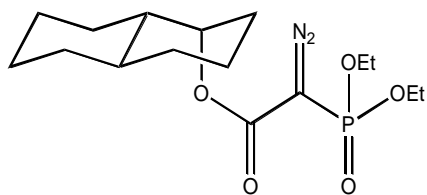
Compound 13. Colourless oil. ^{1}H NMR (400 MHz, CDCl$_3$): δ = 4.83 (d, J_{NH} = 9.8 Hz, 1 H, -NH-), 4.28-4.03 (m, 6 H, -CH$_2$OC(O)- and 2x -CH$_2$OP(O)-), 3.68-3.48 (m, 1 H, -CHNHBoc-), 1.92-1.72 (m, 1 H, -CH- of ^{i}Pr), 1.39 (s, 9 H, 3x -CH$_3$ of Boc), 1.33 (t, ^3J = 6.7 Hz, 6H, 2x -(O)POCH$_2$CH_3), 0.89 (d, ^3J = 6.7 Hz, 6 H, 2x –CH_3 of ^{i}Pr) ppm. ^{13}C NMR (100 MHz, CDCl$_3$): δ = 164,0 (C(O)), 156.5 (-NHC(O)O-), 155.5 (C(N)$_2$), 79.2 (-CO^{t}Bu), 65.8 (-CH$_2$OP(O)- and -CH$_2$OC(O)-), 62.7 (-CH$_2$OP(O)-), 55.0 (-CHNHBoc),
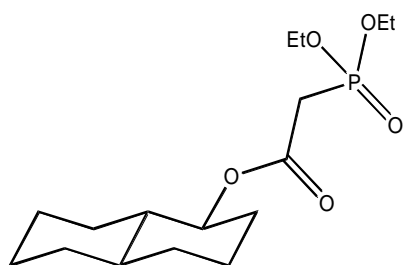
30.0 (-CH- of ^{i}Pr), 28.2 (3x -CH_3 of Boc), 19.4 (2x -CH_3 of ^{i}Pr), 18.4 (2x -(O)POCH$_2$CH$_3$), ppm. MS (ESI) found for ($C_{16}H_{30}N_3O_7PH^+$) 408,1894 and for ($C_{16}H_{30}N_3O_7PNa^+$) 430,1714.
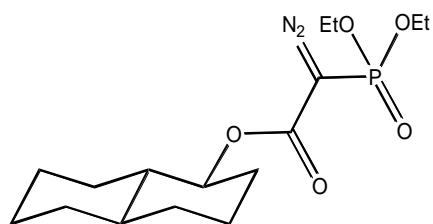
Compound 14. Strong yellow oil. ^{1}H NMR (400 MHz, CDCl$_3$): δ = 4.96 (d, ^3J = 2.4 Hz, 1 H, -CHOC(O)-), 4.27-4.07 (quintet, ^3J = 7.3 Hz, $^3J_{PH}$ = 14.7 Hz, 4 H, 2x -CH$_2$OP(O)-), 3.07-2.87 (m, $^2J_{PH}$ = 21.4 Hz, 2H), 2.02-0.77 (m, 16H), 1.40 (t, 6H, 2x -(O)POCH$_2$CH_3) ppm. ^{13}C NMR (100 MHz, CDCl$_3$): δ = 166.0 (C(O)), 75.0 (-CHOC(O)-), 62.4(8) and 62.4(1) (2x -CH$_2$OP(O)-), 45.7, 36.3, 35.0 (d, J$_{CP}$ = 133 Hz, -(O)CCH$_2$P(O)-), 34.2, 33.4, 30.6, 29.1, 26.4, 26.2, 20.4, 16.3(2) and 16.3(0) (2x -(O)POCH$_2$$CH_3$), ppm. MS (ESI) found for ($C_{16}H_{29}O_5PH^+$) 333,1825 and for ($C_{16}H_{29}O_5PNa^+$) 355,1645.
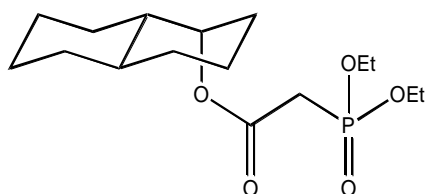
Compound 15. Pink oil. ^{1}H NMR (400 MHz, CDCl$_3$): δ = 5.03 (s, 1 H, -CHOC(O)-), 4.33-4.08 (quintet, ^3J = 7.3 Hz, $^3J_{PH}$ = 14.7 Hz, 4 H, 2x -CH$_2$OP(O)-), 1.94 (d, J = 13.0 Hz, 1H), 1.88-1.33 (m, 10 H), 1.40 (t, 6H, 2x -(O)POCH$_2$CH_3), 1.33-1.05 (m, 3H), 1.05-085 (m, 2H), ppm. ^{13}C NMR (100 MHz, CDCl$_3$): δ = 164.0 (C(O)), 150.0 (C(N)$_2$), 76.0 (-CHOC(O)-), 62.3 (x2) (2x -CH$_2$OP(O)-), 45.8, 36.3, 34.1, 33.4, 30.9, 29.2, 26.3, 26.2, 20.4, 16.1(7) and 16.1(1) (2x -(O)POCH$_2$$CH_3$), ppm. MS (ESI) found for ($C_{16}H_{27}N_2O_5PH^+$) 359,1730 and for ($C_{16}H_{27}N_2O_5PNa^+$) 381,1550.
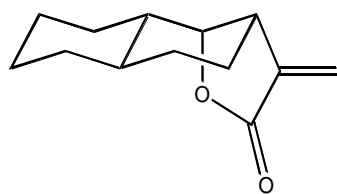
Compound 16. Strong yellow oil. ^{1}H NMR (400 MHz, CDCl$_3$): δ = 4.52 (ddd, J = 4.2 Hz, 1 H, -CHOC(O)-), 4.23-4.08 (quintet, ^3J = 7.2 Hz, 4 H, 2x -CH$_2$OP(O)-), 2.95 (d, $^2J_{PH}$ = 21.6 Hz, 2H), 2.08-0.78 (m, 16H), 1.35 (t, 6H, 2x -(O)POCH$_2$CH_3) ppm. ^{13}C NMR (100 MHz, CDCl$_3$): δ = 166.0 (C(O)), 79.0 (-CHOC(O)-), 62.5(8) and 62.5(5) (2x -CH$_2$OP(O)-), 47.3, 41.2, 34.5 (d, J$_{CP}$ = 133 Hz, -(O)CCH$_2$P(O)-), 33.5, 33.0, 31.9, 28.7, 26.1, 25.8, 23.7, 16.3 and 16.2 (2x -(O)POCH$_2$CH$_3$), ppm. MS (ESI) found for ($C_{16}H_{29}O_5PH^+$) 333,1825 and for ($C_{16}H_{29}O_5PNa^+$) 355,1645.

Compound 17. Yellow oil. ^{1}H NMR (400 MHz, CDCl$_3$): δ = 4.59 (ddd, J = 4.2 Hz, 1 H, -CHOC(O)-), 4.28-4.08 (m, 4 H, 2x -CH$_2$OP(O)-), 2.08-0.83 (m, 16 H), 1.40 (t, 6H, 2x -(O)POCH$_2$CH_3), ppm. ^{13}C NMR (100 MHz, CDCl$_3$): δ = 164.0 (C(O)), 150.0 (C(N)$_2$), 79.0 (-CHOC(O)-), 63.5(2) and 63.5(0) (2x -CH$_2$OP(O)-), 47.5, 41.2, 33.4, 32.9, 32.3, 28.8, 26.1, 25.8, 23.7, 16.1(3) and 16.1(0) (2x -(O)POCH$_2$CH_3), ppm. MS (ESI) found for (C$_{16}$H$_{27}$N$_2$O$_5$PH$^+$) 359,1730 and for (C$_{16}$H$_{27}$N$_2$O$_5$PNa$^+$) 381,1550.

Compound 18. Oil. ^{1}H NMR (400 MHz, CDCl$_3$): δ = 5.03 (d, ^3J = 2.2 Hz, 1 H, -CHOC(O)-), 4.59 (d, J = 10.4 Hz, 2 H, -CH_2OP(O)(OEt)$_2$) 4.28-4.13 (quintet, 4 H, 2x -CH$_2$OP(O)-), 2.03-0.83 (m, 16H), 1.36 (t, ^3J = 7.2 Hz, 6H, 2x -(O)POCH$_2$CH_3) ppm. ^{13}C NMR (100 MHz, CDCl$_3$): δ = 167.6 (C(O)), 75.1 (-CHOC(O)-), 64.3 and 64.2 (2x -P(O)OCH$_2$CH$_3$-), 63.3 (-CH_2OP(O)(OEt)$_2$), 45.7, 36.4, 34.1, 33.3, 30.7, 29.2, 26.4, 26.1, 20.5, 17.9 (x2) (2x -(O)POCH$_2$CH_3), ppm. MS (ESI) found for (C$_{16}$H$_{29}$O$_6$PH$^+$) 349,1754 and for (C$_{16}$H$_{29}$O$_6$PNa$^+$) 371,1569.

Compound 19. Yellow oil. ^{1}H NMR (400 MHz, CDCl$_3$): δ = 6.05 (d, J = 1.1 Hz, 1 H, CH_aH$_b$=C-), 5.50 (d, J = 0.9 Hz, 1 H, CH$_a$$H_b$=C-), 4.26 (d, ^3J = 4.6 Hz, 1 H, -CHOC(O)-), 2.97-2.82 (m, 1H, CH$_2$=C-CH-), 1.87-1.47 (m, 7H), 1.42-0.82 (m, 7H), ppm. ^{13}C NMR (100 MHz, CDCl$_3$): δ = 171.0 (C(O)), 142.5 (CH$_2$=C-), 119.1 (CH$_2$=C-), 80.6 (-CHOC(O)-), 43.9, 40.9 (CH$_2$=C-CH-), 34.9, 33.6, 31.1, 28.8, 28.7, 26.7, 26.0, ppm. MS (ESI) found for (C$_{13}$H$_{18}$O$_2$H$^+$) 207,1380 and for (C$_{13}$H$_{18}$O$_2$Na$^+$) 229,1199.
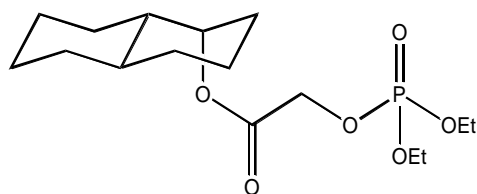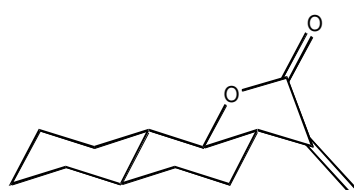
Compound 20. White oil. ^{1}H NMR (400 MHz, CDCl$_3$): δ = 6.06 (d, J = 3.3 Hz, 1 H, CH_aH$_b$=C-), 5.38 (d, J = 3.1 Hz, 1 H, CH$_a$$H_b$=C-), 3.45 (t, ^3J = 10.6 Hz, 1 H, -CHOC(O)-), 2.57-2.42 (m, 1H, CH$_2$=C-CH-), 2.12 (d, J = 13.0 Hz, 2H),

1.87-1.57 (m, 4H), 1.52-0.82 (m, 8H), ppm. ^{13}C NMR (100 MHz, CDCl$_3$): δ = 171.0 (C(O)), 139.9 (CH$_2$=C-), 117.0 (CH$_2$=C-), 86.7 (-CHOC(O)-), 48.6 (CH$_2$=C-CH-), 47.0, 41.6, 32.8, 32.6, 28.9, 26.1, 25.2, 25.1, ppm.
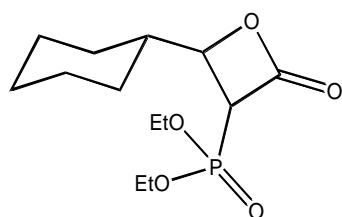
Compound 21. Yellow oil. ^1H NMR (400 MHz, CDCl$_3$): δ = 4.22-4.12 (quintet, 4 H, 2x -CH$_2$OP(O)-), 3.95 (d, J = 6.4 Hz, 2 H, -CH$_2$OC(O)-), 2.97 (d, $^2J_{PH}$ = 21.6 Hz, 2H), 1.82-0.92 (m, 11H), 1.35 (t, ^3J = 7.2 Hz, 6H, 2x -(O)POCH$_2$CH_3) ppm. ^{13}C NMR (100 MHz, CDCl$_3$): δ = 165.7 (C(O)), 70.4 (-CH$_2$OC(O)-), 62.4 (x2) (2x -CH$_2$OP(O)-), 36.8 (-CH-), 34.1 (d, J$_{CP}$ = 133 Hz, -(O)CCH$_2$P(O)-), 29.3 (x2) (2x -CH$_2$-), 26.1 (-CH$_2$-), 25.4 (x2) (2x -CH$_2$-), 16.1 (x2), (2x -(O)POCH$_2$$CH_3$), ppm. MS (ESI) found for (C$_{13}$H$_{25}$O$_5$PH$^+$) 293,1512 and for (C$_{13}$H$_{25}$O$_5$PNa$^+$) 315,1332.
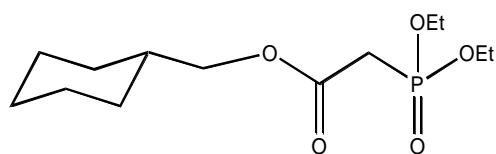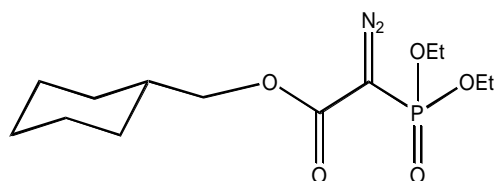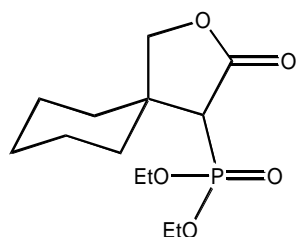
Compound 22. Yellow oil. ^1H NMR (400 MHz, CDCl$_3$): δ = 4.33-4.08 (m, 4 H, 2x -CH$_2$OP(O)-), 4.02 (d, J = 6.4 Hz, 2 H, -CH$_2$OC(O)-), 1.83-0.93 (m, 11H), 1.37 (t, ^3J = 7.2 Hz, 6H, 2x -(O)POCH$_2$CH_3) ppm. ^{13}C NMR (100 MHz, CDCl$_3$): δ = 164.0 (C(O)), 149.0 (C(N)$_2$), 70.7 (-CH$_2$OC(O)-), 63.6 and 63.5 (2x -CH$_2$OP(O)-), 37.2 (-CH-), 29.4 (2x -CH$_2$-), 26.2 (-CH$_2$-), 25.6 (x2) (2x -CH$_2$-), 16.1(5) and 16.1(0) (2x -(O)POCH$_2$$CH_3$), ppm. MS (ESI) found for (C$_{13}$H$_{23}$N$_2$O$_5$PH$^+$) 319,1417 and for (C$_{13}$H$_{23}$N$_2$O$_5$PNa$^+$) 341,1237.
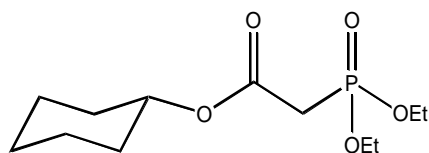
Compound 23. Yellow oil. ^1H NMR (400 MHz, CDCl$_3$): δ = 4.48-4.33 (m, 1H, -CHOC(O)-), 4.31-4.13 (m, 4 H, 2x -CH$_2$OP(O)-), 3.80 (d, $^2J_{PH}$ = 18.3 Hz, 1H), 2.03-0.93 (m, 11H), 1.37 (t, ^3J = 7.2 Hz, 6H, 2x -(O)POCH$_2$CH_3) ppm. $^{13}C$ NMR (100 MHz, CDCl$_3$): δ = 163.0 (C(O)), 76.0 (-CHOC(O)-), 63.4(2) and 63.4(0) (2x -CH$_2$OP(O)-), 52.4 (d, J$_{CP}$ = 144 Hz, -(O)CCHP(O)-), 41.8 (-CH-), 27.9, 27.0, 25.8, 25,2 and 25.0 (5x -CH$_2$-), 16.4 and 16.3 (2x -(O)POCH$_2$$CH_3$), ppm. MS (ESI) found for (C$_{13}$H$_{23}$O$_5$PH$^+$) 291,1356 and for (C$_{13}$H$_{23}$O$_5$PNa$^+$) 313.1175.

Compound 24. Yellow oil. ^{1}H NMR (400 MHz, CDCl$_3$): δ = 4.33 (d, 1H, -CH$_a$H$_b$OC(O)-), 4.28-4.13 (quintet, 4H, 2x -CH$_2$OP(O)-), 4.08 (d, 1H, -CH$_a$H$_b$OC(O)-), 2.76 (d, $^2J_{PH}$ = 24.2 Hz, 1H), 1.98-1.13 (m, 10H), 1.36 (t, ^3J = 7.2 Hz, 6H, 2x -(O)POCH$_2$CH$_3$) ppm. ^{13}C NMR (100 MHz, CDCl$_3$): δ = 172.2 (C(O)), 76.0 (-CH$_2$OC(O)-), 63.4 and 62.5 (2x -CH$_2$OP(O)-), 51.0 (d, J$_{CP}$ = 144 Hz, -(O)CCHP(O)-), 43.8 (quaternary C), 36.1, 30.7, 25.2 (x2) and 22,5 (5x -CH$_2$-), 16.4 and 16.3 (2x -(O)POCH$_2$CH$_3$), ppm. MS (ESI) found for (C$_{13}$H$_{23}$O$_5$PH$^+$) 291,1356 and for (C$_{13}$H$_{23}$O$_5$PNa$^+$) 313.1175.

Compound 25. Yellow oil. ^{1}H NMR (400 MHz, CDCl$_3$): δ = 4.45 (m, 1 H, -CHOC(O)-), 3.82 (quintet, ^3J = 7.2 Hz, 4 H, 2x -CH$_2$OP(O)-), 2.60 (d, $^2J_{PH}$ = 21.6 Hz, 2 H), 1.60-0.90 (m, 10 H, 5x -CH$_2$-), 1.00 (t, 6 H, 2x -(O)POCH$_2$CH$_3$), ppm. ^{13}C NMR (100 MHz, CDCl$_3$): δ = 164.3 (C(O)), 72.9 (-CHOC(O)-), 61.6(8) and 61.6(1) (2x -CH$_2$OP(O)-), 33.8 (d, J$_{CP}$ = 133 Hz, -(O)CCH$_2$P(O)-), 30.6 (x3), 24.5 and 22.7 (-CH$_2$-), 15.5(3) and 15.5(0) (2x -(O)POCH$_2$CH$_3$), ppm. MS (ESI) found for (C$_{12}$H$_{23}$O$_5$PH$^+$) 279.1356, found for (C$_{12}$H$_{23}$O$_5$PNa$^+$) 301.1175.
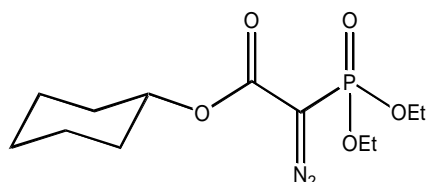
Compound 26. Yellow oil. ^{1}H NMR (400 MHz, CDCl$_3$): δ = 4.68 (m, 1 H, -CHOC(O)-), 4.15-3.90 (m, 4 H, 2x -CH$_2$OP(O)-), 1.75-1.00 (m, 10 H, 5x -CH$_2$-), 1.15 (t, ^3J = 7.2 Hz, 6 H, 2x -(O)POCH$_2$CH$_3$), ppm. ^{13}C NMR (100 MHz, CDCl$_3$): δ = 162.4 (C(O)), 162.3 (C(N)$_2$), 73.6 (-CHOC(O)-), 63.0(3) and 63.0(0) (2x -CH$_2$OP(O)-), 31.0 (x3), 24.7 and 22.8 (-CH$_2$-), 15.6 and 15.5 (2x -(O)POCH$_2$CH$_3$), ppm. MS (ESI) found for (C$_{12}$H$_{21}$N$_2$O$_5$PH$^+$) 305.1261, found for (C$_{12}$H$_{21}$N$_2$O$_5$PNa$^+$) 327.1080.
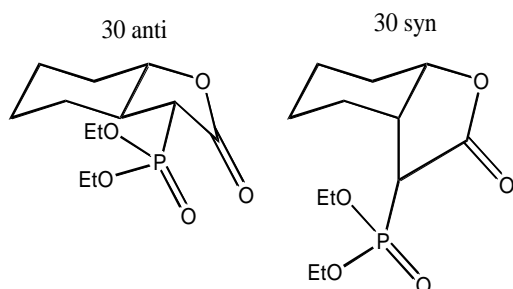
**Compound 30.** Yellow oil. ^{1}H NMR (400 MHz, CDCl$_3$): δ = 4.45-4.10 (m, ^3J = 7.2 Hz, 4H, 2x -CH$_2$OP(O)-), 3.90-3.70 (ddd, ^3J = 3.8 Hz, 1H, -CHOC(O)-), 2.95-2.70 (dd, ^3J = 12.8 Hz, $^2J_{PH}$ = 21.0 Hz, 1H, -(O)CCHP(O)-), 2.45-

30 anti

30 syn

30 anti/ 30 syn ca 4

1.10 (m, 15H, -CH-, -CH$_2$- of Cy and 2x -(O)POCH$_2$CH_3), ppm. ^{1}H NMR significant signals of the minor diastereoisomer: 4.50-4.40 (ddd, 1H, -CHOC(O)-), 3.15-3.70 (dd, 1H, -(O)CCHP(O)-), ppm. ^{13}C NMR (100 MHz, CDCl$_3$): δ = 171.0 (C(O)), 84.0 (-CHOC(O)-), 63.5 and 62.0 (2x -CH$_2$OP(O)-), 46.6 (-CH-), 46.0 (d, J$_{CP}$ = 144 Hz, -(O)CCHP(O)-), 29.9, 28.1, 25.0, 23,9 (4x -CH$_2$-), 16.4 (x2) (2x -(O)POCH$_2$CH_3), ppm. MS (ESI) found for (C$_{12}$H$_{21}$O$_5$PH$^+$) 277.1199, found for (C$_{12}$H$_{21}$O$_5$PNa$^+$) 299.1019.
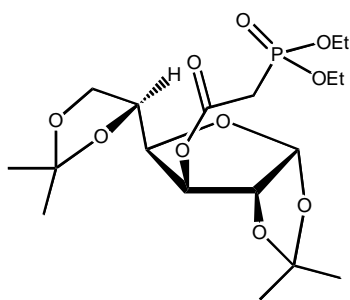
Compound 28. ^{1}H NMR (CDCl$_3$, 400 MHz) δ = 5.85 (d, J$_{1,2}$ = 3.6 Hz, 1H, H-1), 5.26 (d, J$_{3,4}$ = 3.0 Hz, 1H, H-3), 4.53 (d, 1H, H-2), 4.33-3.93 (m, 8H, H-4, H-5, H-6a, H-6b and 2x -CH$_2$OP(O)-), 3.13-2.88 (m, ^{2}J$_{PH}$ = 21.6 Hz, 2H), 1.53 (s, 3H, -CH$_3$), 1.43-1.23 (m, 15H, 3x -CH$_3$ and 2x -(O)POCH$_2$CH_3), ppm. ^{13}C NMR (100 MHz, CDCl$_3$): δ = 164.5 (C(O)), 112.2 and 109.3 (2x –(O)$_2$C(Me)$_2$), 104.9 (C-1), 82.9 (C-2), 79.4, 78.0 (C-3), 72.1, 66.9 (x2) (2x -CH$_2$OP(O)-), 62.7 (C-6), 34.2 (d, J$_{CP}$ = 133 Hz, -(O)CCH$_2$P(O)-), 26.8, 26.6, 26.1 and 25.1 (4x -CH$_3$), 16.3 and 16.2 (2x -(O)POCH$_2$CH_3), ppm. MS (ESI) found for (C$_{18}$H$_{31}$O$_{10}$PH$^+$) 439.1727, found for (C$_{18}$H$_{31}$O$_{10}$PNa$^+$) 461.1550.
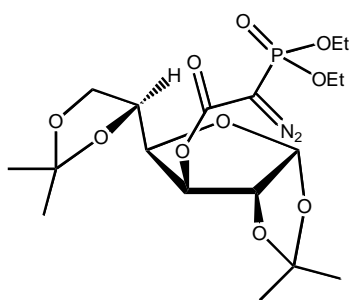
Compound 29. ^{1}H NMR (CDCl$_3$, 400 MHz) δ = 5.82 (d, J$_{1,2}$ = 3.3 Hz, 1H, H-1), 5.28 (d, J$_{3,4}$ = 2.4 Hz, 1H, H-3), 4.53 (d, 1H, H-2), 4.28-3.93 (m, 8H, H-4, H-5, H-6a, H-6b and 2x -CH$_2$OP(O)-), 1.53 (s, 3H, -CH$_3$), 1.43-1.18 (m, 15H, 3x -CH$_3$ and 2x -(O)POCH$_2$CH_3), ppm. ^{13}C NMR (100 MHz, CDCl$_3$): δ = 169.0 (C(O)), 162.0 (C(N)$_2$), 112.4 and 109.4 (2x –(O)$_2$C(Me)$_2$), 104.9 (C-1), 83.1 (C-2), 79.6, 78.0 (C-3), 72.2, 67.2 (x2) (2x -CH$_2$OP(O)-), 63.8 (C-6), 26.9, 26.5, 26.0 and 25.1 (4x -CH$_3$), 16.0(5), 16.0(0) (2x -(O)POCH$_2$CH_3), ppm. MS (ESI) found for (C$_{18}$H$_{29}$N$_2$O$_{10}$PH$^+$) 465.1633, found for (C$_{18}$H$_{29}$N$_2$O$_{10}$PNa$^+$) 487.1452.
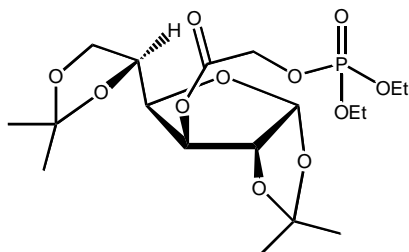
Compound 31. ^{1}H NMR (CDCl$_3$, 400 MHz) δ = 5.86 (d, J$_{1,2}$ = 3.7 Hz, 1H, H-1), 5.34 (d, J$_{3,4}$ = 2.4 Hz, 1H, H-3), 4.71-3.96 (m, 11H), 1.51 (s, 3H, -CH$_3$), 1.46-1.06 (m, 15H, 3x -CH$_3$ and 2x -(O)POCH$_2$CH_3), ppm.

^{13}C NMR (100 MHz, CDCl$_3$): δ = 169.0 (C(O)), 112.4 and 109.5 (2x –(O)$_2$*C*(Me)$_2$), 105.1 (C-1), 82.6 (C-2), 79.4, 77.9 (C-3), 72.2, 67.2 (x2) (2x CH$_3$*C*H$_2$OP(O)-), 64.0 and 63.5 (-*C*H$_2$OP(O)(OEt)$_2$ and C-6), 26.9, 26.7, 26.1 and 25.1 (4x -CH$_3$), 16.3(6), 16.3(1) (2x -(O)POCH$_2$*C*H$_3$), ppm. MS (ESI) found for (C$_{18}$H$_{31}$O$_{11}$PH$^+$) 455.1633, found for (C$_{18}$H$_{31}$O$_{11}$PNa$^+$) 477.1488.

Thanks to   Dott Antonello Pastore

Dott William P.Unsworth

Pofessor Richard Taylor