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TOTAL SYNTHESIS OF DYSIDIOLIDE, *ENT*-DYSIDIOLIDE, AND LUFFARIELLOLIDE

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RÉSUMÉ

Dans la première partie de cette thèse nous décrirons notre synthèse énantiosélective et convergente de la dysidiolide (1) qui est un produit naturel isolé d'une éponge marine provenant des Caraïbes. Ce sesterterpénoïde possède des propriétés antitumorales grâce à sa capacité d'inhiber la phosphatase Cdc25A récemment découverte. L'inhibition de cette enzyme qui joue un rôle clé dans la division cellulaire pourrait potentiellement ouvrir la voie vers de nouveaux traitements contre le cancer. La complexité moléculaire de la dysidiolide combinée avec son activité biologique remarquable font de ce produit naturel une cible remarquable. Notre première synthèse de la dysidiolide (séquence linéaire la plus longue = 15 étapes, rendement global = 5.3%), fut publiée à peu près en même temps que celles de E.J. Corey et de S.J. Danishefsky, permettant alors de déterminer la configuration absolue de la dysidiolide comme étant tel que décrit pour *ent*-1. Les étapes clées comportent (i) une réaction de Diels-Alder entre un diènophile doublement activé et un diène semicyclique chiral, et (ii) l'utilisation de notre méthode récemment développée permettant de construire des γ-hydroxybutènolides par oxyfonctionnalisation de 2-siloxyfuranes, comme dernière étape de la synthèse. Plus récemment, une version améliorée de notre synthèse initiale a été effectuée en utilisant une réaction de Diels-Alder avec un nouveau diènophile chiral pour construire efficacement le squelette de la dysidiolide de façon complètement régio, endo/exo et diastereofacialement sélèctive. Le rendement global pour cette nouvelle synthèse de *ent*-1 est de 9.8% (séquence linéaire la plus longue = 15 étapes, comme avant), soit presque le double du rendement obtenu lors de la première synthèse.

Dans la deuxième partie de cette thèse, nous décrirons notre synthèse totale hautement convergente de la luffariellolide (2), produit naturel marin anti-inflammatoire. L'étape clée de cette synthèse comporte un couplage croisé sp^3-sp^3 en présence de tétrachlorocuprate de lithium entre un chlorure allylique et un nouveau organométallique siloxyfurane qui promet être un réactif très utile pour la synthèse régiosélective de β-homoallylbutènolides ayant un hydrogène ou un hydroxyl à la position γ. Les études effectuées suggèrent que cette nouvelle méthodologie sera applicable à la synthèse de plusieurs autres produits naturels d'importance biomédicale.

ABSTRACT

In the first part of this thesis we describe an enantioselective, convergent total synthesis of the Caribbean sponge constituent dysidiolide (1), a novel antitumor sesterterpenoid that is the first reported natural product to inhibit the recently discovered phosphatase Cdc25A. Blocking this enzyme, which plays a key role in the cell cycle, may provide a new means for the treatment of cancer. This formidable combination of novel structure and highly sought biological properties makes dysidiolide an eminent target for synthesis. Our first route to dysidiolide (longest linear sequence = 15 steps, overall yield = 5.3%), published at about the same time as the syntheses of E.J. Corey and S.J. Danishefsky, allowed the absolute configuration of the natural product to be established as depicted in *ent*-11. Key steps include (i) a Diels-Alder reaction of a doubly activated dienophile with a chiral semicyclic diene, and (ii) the utilisation of newly developed methodology from our group for regiocontrolled construction of the γ-hydroxybutenolide moiety, based on oxyfunctionalization of a 2-siloxyfuran ring, as the last step of the synthesis. More recently, a substantially improved version of our initial route was developed using a novel chiral difunctionalized dienophile for the Diels-Alder reaction which builds the dysidiolide skeleton with complete regio, endo/exo and diastereofacial selectivity. The overall yield of our new synthesis of dysidiolide is 9.8% (longest linear sequence = 15 steps, as before), a nearly twofold improvement over our previous approach.

In the second part of this thesis we detail a highly convergent total synthesis of the anti-inflammatory marine natural product luffariellolide (2). The key step involves lithium tetrachlorocuprate mediated sp^3-sp^3 cross-coupling between an allylic chloride and a new siloxyfuran reagent which promises to be a versatile building block for the regiocontrolled assemblage of β-homoallylbutenolides bearing a hydrogen or a hydroxyl group at the γ-position. Preliminary studies suggest that this methodology should be applicable to many other natural products of biomedical importance.

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SCHEMES

ABBREVIATIONS & SYMBOLS

A	Angstrom
AA	Arachidonic acid
Ac	Acetyl
aq.	Aqueous
$[\alpha]_D$	Specific rotation
Bn	Benzyl
bp	Boiling point
Bu	Butyl
Bz	Benzoyl
c	Concentration (for optical rotation)
Cak	Cyclin activated kinase
CBS	Corey, Bakshi, Shibata (protocol for the enantioselective reduction of carbonyls)
Cdc	Cell division cycle
Cdi	Cyclin dependant inhibitor
Cdk	Cyclin dependant kinase
COX	Cyclooxygenase
Cyc	Cyclin
DCC	Dicyclohexylcarbodiimide
DMAP	4-(Dimethylamino)pyridine
DME	1,2-(Dimethoxy)ethane
DMF	Dimethylformamide
DNA	Deoxyribonucleic acid
Et	Ethyl
ee	Enantiomeric excess
G	Gap phase
g	Gaseous

h	Hour
HETE	Hydroxyeicosatetraenoic acid
HMPA	Hexamethylphosphoramide
HPLC	High pressure liquid chromatography
HRMS	High resolution mass spectrometry
Hz	Hertz
IC_{50}	Concentration at which 50% inhibition occurs
IR	Infrared
LA	Lewis acid
Lit.	Literature
M	Mitosis phase
Me	Methyl
mp	Melting point
MPF	Mitotic promoting factor
m/z	Mass/charge (mass spectrometry)
Ms	Mesyl
MS	Mass spectroscopy
NMO	N-methylmorpholine oxyde
NMR ^{13}C	Nuclear magnetic resonnance (for carbon)
NMR ^{1}H	Nuclear magnetic resonnance (for proton)
$^{1}O_{2}$	Singlet oxygen
P	Phosphate
PG	Prostaglandin
Ph	Phenyl
PL	Phospholipase
PON	Phosphoramidate
i-Pr	*iso*-Propyl
psi	Pounds per square inch
Py	Pyridine
R_{f}	Retention factor
rt	Room temperature

SAR	Structure-activity relationship
S	Synthesis phase
T	Threonine
TBAF	Tetrabutylammonium fluoride
TBS	*tert*-Butyl dimethylsilyl
t-Bu	*tert*-Butyl
Tf	Triflate
THF	Tetrahydrofuran
TIPS	Tri-*iso*-propylsilyl
TLC	Thin layer chromatography
TMS	Trimethylsilyl
TPAP	Tetrapropylammonium perruthenate
TPS	*tert*-Butyl diphenylsilyl
Ts	Tosyl
Tx	Thromboxane
Y	Tyrosine

For NMR:

br.	Broad signal
δ	Chemical displacement (from TMS as 0 ppm)
d	Doublet
dd	Double doublet
dt	Double triplet
Hz	Hertz
J	Coupling constant in Hertz
m	Multiplet
q	Quadruplet
s	Singulet
t	Triplet

INTRODUCTION

The total synthesis of natural products is one of the most important and challenging fields of today's science. Traditionally, total synthesis has served as the ultimate proof of structure,[1] and in many cases, as the sole reliable means to elucidate relative and/or absolute configuration. Since most natural products are only available in tiny amounts from the natural source, economical access to such compounds can only be ensured by chemical synthesis. The pharmacological evaluation and development of a bioactive natural product is therefore precluded unless a satisfactory synthesis can be devised. Furthermore, the synthetic pathway can be fine-tuned to produce libraries of designed analogues that may provide clues to cellular targets essential to intracellular signalling pathways (e.g. cell cycle inhibition) that may in turn lead to clinical advances of considerable importance. In other words, advances in the field of natural product synthesis are inextricably linked to advances in life sciences, such as molecular biology and medicine among others.

To an organic chemist there is no greater challenge than attempting to reproduce nature's molecular architecture, and no greater satisfaction than to succeed in accomplishing this task. For the medicinal chemist, however, the game has not ended once the natural molecule has been synthesized in the laboratory. In fact, this is usually the starting point of a medicinal chemistry project. The newly developed synthetic technology allows us to go much further and produce a vast number of analogues, always modifying their structure, atom by atom if needs be, so that optimum activity and bioavailability can be achieved with a strict minimum of toxicity.

This is the challenge that is met by today's pharmaceutical industries and to a lesser extent by chemists in academia. Usually, the natural product serves only as the lead compound, and the final product, crafted after several years of research, can be substantially different - often simpler and easier to make than the prototypical natural product - while possessing a superior pharmacological profile that makes it a worthwhile drug candidate. And so it is often with this concept in mind that chemists turn to the formidable undertaking of synthesizing one of nature's millions of molecules.

But with a rapidly growing number of potential targets, the greatest of challenge to the synthetic chemist is to have at his or her disposition methods that allow efficient access to the many different types of substructures and functionalities present in these molecules. Even though much progress has been accomplished toward this goal during the last few decades, many shortcomings still exist in view of the remarkable diversity and complexity present in the natural world. When considering Professor Paul Wender's definition of the ideal synthesis - one step and 100% yield - it is fairly clear that as a science (as well as art) organic synthesis is still at its infancy.

Consequently, there is a great need to develop new and more efficient methods in terms of yield, selectivity, and brevity, that would be economically viable and applicable to the synthesis of structurally complex molecules. Especially important nowadays is the development of methods that are also environmentally friendly or 'green' and therefore suitable for industrial scale synthesis. Much still remains to be accomplished in all of these areas.

Both dysidiolide (1)[2] and luffariellolide (2)[3], whose syntheses are described in this thesis, possess a highly oxygenated heterocyclic unit, namely the γ-hydroxybutenolide. This unit appears to be at least in part responsible for their biological properties, such as antitumor and anti-inflammatory activities, which are believed to arise through inhibition of phosphatase Cdc25A and phosholipase A$_2$ (PLA$_2$) respectively.
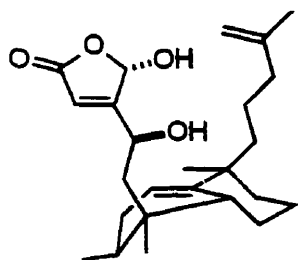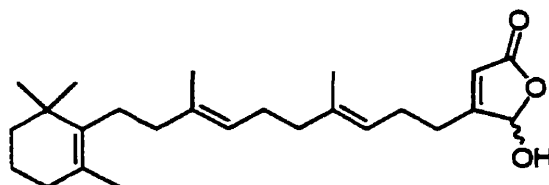
dysidiolide (1)   luffariellolide (2)

Many other natural products possessing interesting biological properties, such as manoalide (3),[4] and thorectolide (4),[5] also contain a γ-hydroxybutenolide unit. Manoalide is a potent anti-inflammatory agent that has undergone phase II clinical trials for the treatment of psoriasis[6] while thorectolide is an inhibitor of HIV-1 nucleocapside and integrase.[5]

manoalide (3)   thorectolide (4)
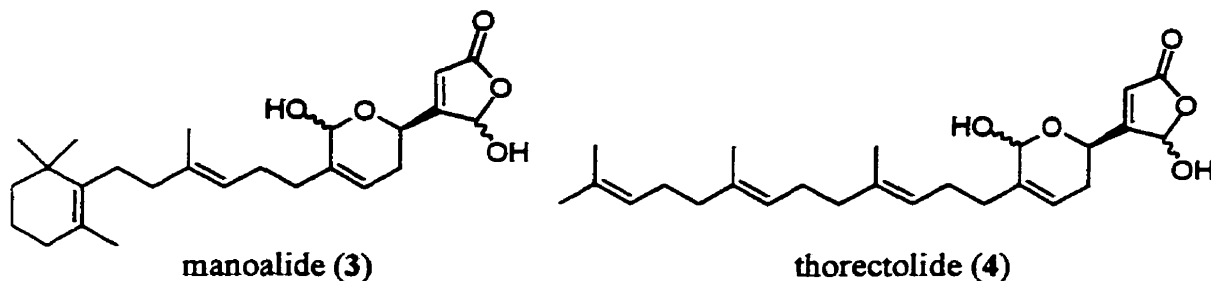
It should be pointed out that the γ-hydroxybutenolide ring (5) is in tautomerism with its acyclic isomer 6 (Fig. 1). Consequently, this heterocycle may be viewed as a masked form of two highly reactive functionalities (e.g. aldehyde and carboxylic acid groups) that may participate in chemical reactions once bound to an enzyme, thereby causing inhibition through covalent bond formation. From the medicinal chemist's standpoint, the γ-hydroxybutenolide may be viewed as a non-classical bioisostere of the phosphate group.

5   6

Fig. 1 Tautomerism of γ-hydroxybutenolides with their acyclic isomers

Few methods for the preparation of γ-hydroxybutenolides are described in the literature.[7] The most commonly employed for their synthesis makes use of singlet oxygen ($^1O_2$).[8] One of these methods, reported by Faulkner in 1988, involves the photooxygenation of monosubstituted furans 7 in the presence of polymer-bound rose bengal as sensitizer. The resulting ozonides 8 are deprotonated *in situ* by a sterically hindered base and transformed into 9 with high regioselectivity (cf. 9 vs 10). The minor regiomer 10 is rarely observed when Hünig's base is used as the base.
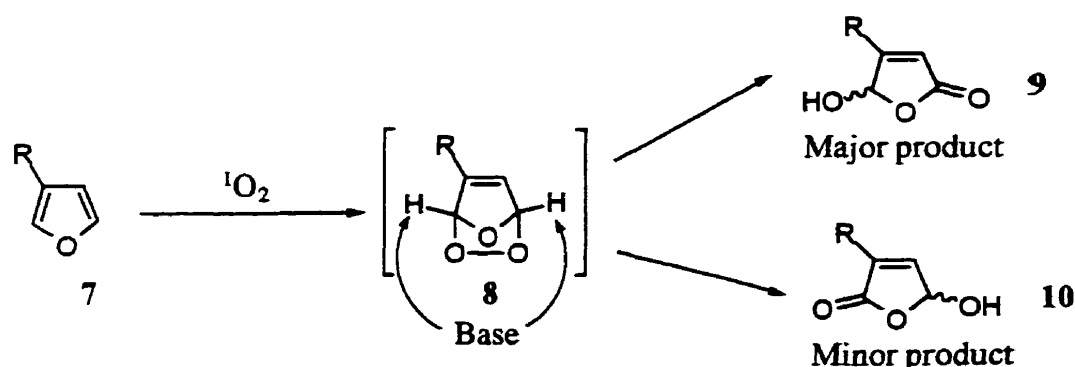
Unfortunately, this method often leads to modest yields and is only useful for small scale synthesis.[9] Another limitation is the fact that this methodology only allows the synthesis of γ-hydroxybutenolides with a substituent at the β position (cf. 9).

A more general method for the preparation of such γ-hydroxybutenolides (12) involves the photooxygenation of trialkylsilylsubstituted furans such as 11.[10] The advantage of this over Faulkner's method is that γ-hydroxybutenolides with diverse substitution patterns can be prepared with complete regiocontrol due to the directing effect of the trialkylsilyl group. Nonetheless, this method is limited by the several steps needed to prepare the required substrates (11), and as with all photooxygenation reactions, the difficulty to upscale.

11 → $^1O_2$ → **12** Only product
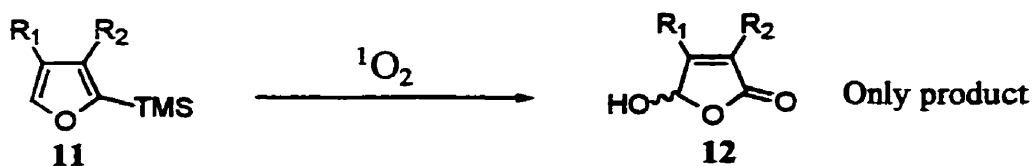
It is with these shortcomings in mind that our group decided to develop a more general and efficient method for the synthesis of γ-hydroxybutenolides. The new method recently reported from our group[7] makes use of a wide variety of mono- and disubstituted 2-trialkylsilyloxyfurans **13** as substrates and these are readily available by silylation of the corresponding butenolides. Oxidation of **13** to the corresponding silyl esters **14** is achieved with high efficiency by the action of dimethyldioxirane (**15**). Hydrolysis of **14** provides the corresponding γ-hydroxybutenolides **16** in consistently high to excellent yields.[7] It is worthy of note that isolation of product **14** is not necessary and that hydrolysis can be conducted directly in the same reaction vessel to yield the desired γ-hydroxybutenolides (**16**) in one-pot fashion.

13 → (CH_2Cl_2, -78°C, **15**) → **14** → H_3O^+ → **16**
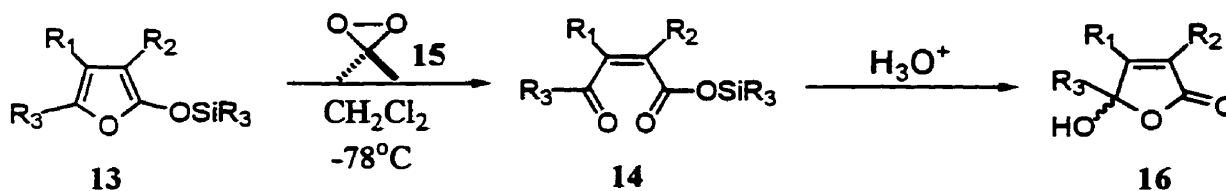
Unlike $^1O_2$, the concentration of dioxirane **15** can be measured by iodometric titration[11] and precise quantities of **15** can be conveniently used to ensure selective oxidation of the electron rich siloxyfuran moiety in the presence of other sensitive functional groups such as double bonds. Even in the presence of a trisubstituted double bond, the siloxyfuran moiety of **17** is selectively oxidized to its corresponding γ-hydroxybutenolide **18** without epoxidation of the double bond.[7] By virtue of the ready availability of a wide range of siloxyfurans, high regio and chemoselectivity and excellent yields, this method is especially attractive for the synthesis of complex natural products.
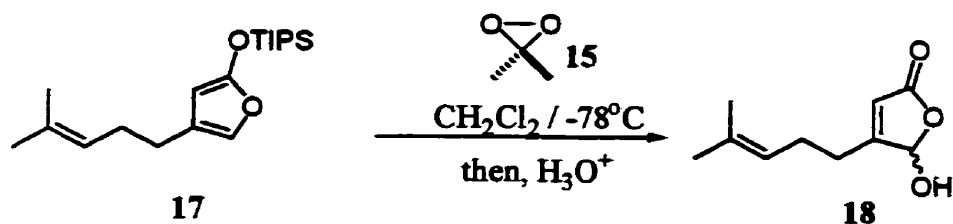
As yet, the mechanism of oxidation has not been investigated. It is however possible to consider three distinct pathways leading to silyl ester **25** (Fig. II). First, an ionic pathway[12] involving nucleophilic attack at the C-5 position of siloxyfuran **19** would produce zwitterion **20** which would collapse to silyl ester **25** either directly or through epoxide **22**.
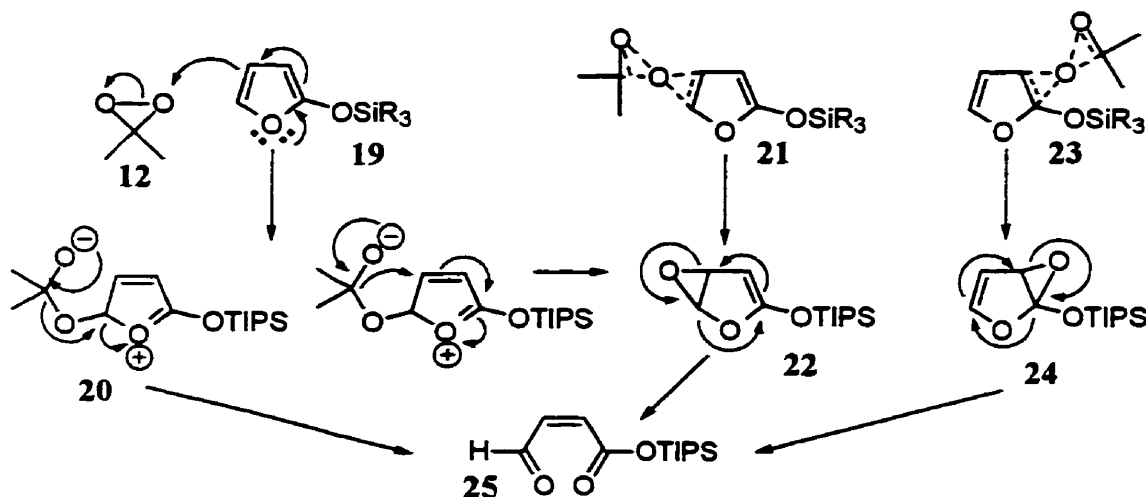
Fig. 2 Mechanistic options for the oxidation of siloxyfurans.

Alternatively, epoxidation of the siloxyfuran at either of the two double bonds through a 'butterfly' mechanism[13] and subsequent rearrangement of these unstable epoxides (**22** or **24**) would produce the same silyl ester **25**. The scope of this new methodology has been extended by the development of new siloxyfuran reagents and applications as key building blocks in the syntheses of dysidiolide (**1**) and luffariellolide (**2**).

RESULTS AND DISCUSSION

CHAPTER 1

BIOLOGICAL BACKGROUND

1.1 Introduction

In order to better appreciate the interest and impact of the synthesis of dysidiolide (1) described in chapter 2, it is essential to understand the unique biological background of this natural product. Indeed it is only after dysidiolide's novel biological profile is understood that one can appreciate the current chemical and biomedical interest in this molecule as well as the need for further work on this exciting new therapeutic lead.
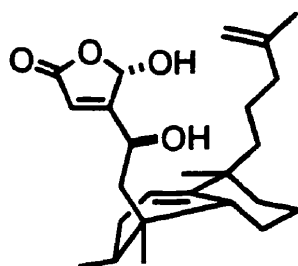
dysidiolide (1)

A crucial development of relevance to dysidiolide was the characterization of a new protein phosphatase named Cdc25A which was shown to play a key role in the promotion of cellular proliferation.[14] Because of the pivotal role of this enzyme in the cell cycle, researchers immediately embarqued on the development of new biological assays to find potential inhibitors of Cdc25A. As was mentionned in the general introduction, natural products are a rich and invaluable source of novel biologically active compounds, and not suprisingly, the search for selective Cdc25A inhibitors began with the assay of natural product extracts.

The isolation of dysidiolide (1) from the Caribbean marine sponge *Dysidea etheria* was reported by Gunasekera at the end of 1996.[2] This secondary metabolite was found to be the first natural product to inhibit phosphatase Cdc25A (IC_{50} = values of 9.4 μM).[2] What is equally interesting is that dysidiolide also inhibits growth of A-549 human lung carcinoma and P388 murine leukemia cell lines at similar concentrations (IC_{50} = values of 4.7 and 1.5 mM respectively).[2] However, because only limited amounts of dysidiolide (a few milligrams) were obtained from the marine sponge (0.05% from wet weight), further indepth studies were precluded unless a chemical synthesis could be devised.

Prior our discussion of our total synthesis of dysidiolide, we will begin by a brief introduction of cancer and the cell cycle. This is necessary in order to understand and appreciate the role of Cdc25A as a key enzyme and as dysidiolide's biological target.

1.2 Cancer

Cancer is one of the most serious afflictions that threatens the lives of millions of individuals across the world. It has been reported that 6.6 million people worldwide have died from cancer in the year 1997 alone[15] and that numbers are increasing. In Canada, 1 in 2.4 men will be diagnosed with some form of cancer during their lifetime and slightly less, 1 in 2.9, for women (Table I).[16]

It is interesting to note that lung cancer is the leading cause of cancer related deaths despite the fact that breast and prostate cancer are more common. This makes the patient with lung cancer more likely to die from the disease than would a patient with any other type of cancer.[16]

Cancer in Canada

Type	Men	Type	Women
All	1 in 2.4	All	1 in 2.9
Prostate	1 in 8	Breast	1 in 9
Lung	1 in 11	Lung	1 in 21

Table I

The pharmaceutical industry offers many different therapeutic regimens for fighting this deadly disease (Table II).[17] The first category comprises antimetabolites which are molecules that are structurally similar to normal cellular components.[17] These are often nucleotide analogues or antagonists (purine or pyrimidine) while antibiotics usually function by complexing DNA and disrupting its normal functions.

Alkylating agents on the other hand are reactive chemicals that alkylate the DNA's nucleotides and disrupt their normal functions.[17] The toxicity of the alkylating agents is substantial and often causes serious side effects, even in low dosage. Mechlorethamine (nitrogen mustard) is an example of such an alkylating agent which was even used in chemical warfare during World War 1 because of its high toxicity.

Anticancer drugs

Antimetabolites	- structurally related to normal cellular components - S-Phase specific
Antibiotics	- disruption of DNA functions - cell cycle nonspecific
Alkylating Agents	- cytotoxic alkylation of DNA - most are cell cycle nonspecific
Mitotic Spindle Poisons	- disruption of the microtubule system - M-Phase specific
Hormones	- control of proliferation of cells - do not cause cytotoxic lesions
Others	- interferons that affect cell proliferation and modulate immunological responses

Table II

Other types of anticancer drugs involve mitotic spindle poisons such as Vinca alkaloids (ex. Vincristine, Vinblastine, etc...) and the more popular Taxol (Paclitaxel).[17] These drugs function by disrupting the cytosqueleton of cells by either causing the depolymerization or the termination of assembly of the cell's microtubules. Both mechanisms result in disfunctional cells that cannot proliferate normally. There are also other types of drugs that antagonize hormone-stimulated processes or that affect cell mobility and proliferation. Even though many therapies exist to fight cancer, no cure has been found thus far. It is therefore most important to explore new avenues that might lead to a potentially novel and viable therapy.

In addition to taxol (26), marketed by Bristol-Meyers Squibb (annual sales = US$ 813 millions) for the treatment of metastatic ovarian cancer,[18] several other promising antitumor natural products have been recently described in the literature, such as epothilone A and B (27),[19] eleutherobin (28),[20] and dysidiolide (1).[2] These natural products are excellent leads for the development of new anticancer drugs.

paclitaxel (Taxol) (**26**)

epothilone A, B
(R=H,Me) (**27**)

dysidiolide (**1**)

eleutherobin (**28**)
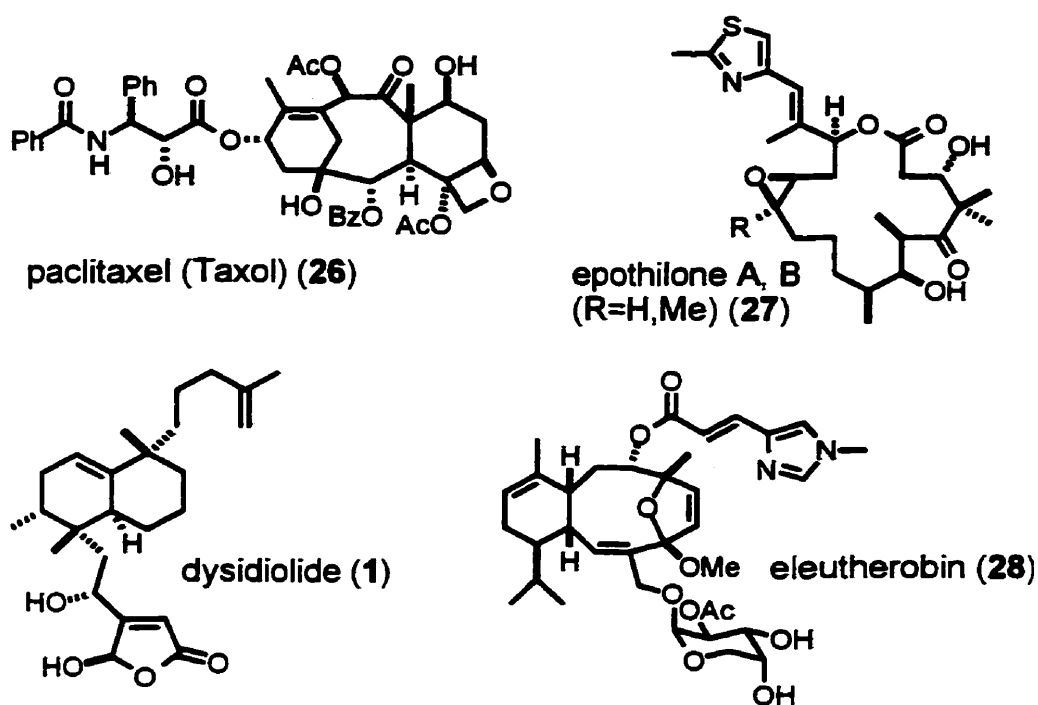
It is important to note that, apart from dysidiolide (**1**), compounds **26**, **27**, and **28** have similar mechanisms of action, that is, they all exert their activity at the level of the disruption of the microtubules. The mechanism of action of dysidiolide (**1**) is novel and completely different, and will therefore be discussed in more detail..

1.3   Cell division

Before being able to understand how dysidiolide inhibits Cdc25A and interferes with cellular proliferation, one must consider some key events in the cell cycle, the biological cascade that leads to cell division. As the cell divides to create two identical daughter cells, it must go through a multitude of biological processes that assure that each of the two new entities possess complete and identical genetic information that is free from error. Simply by observing the status of the genetic information of the cell, we are able to divide the cell cycle in certain obvious phases (Fig. 3).

The cell division cycle
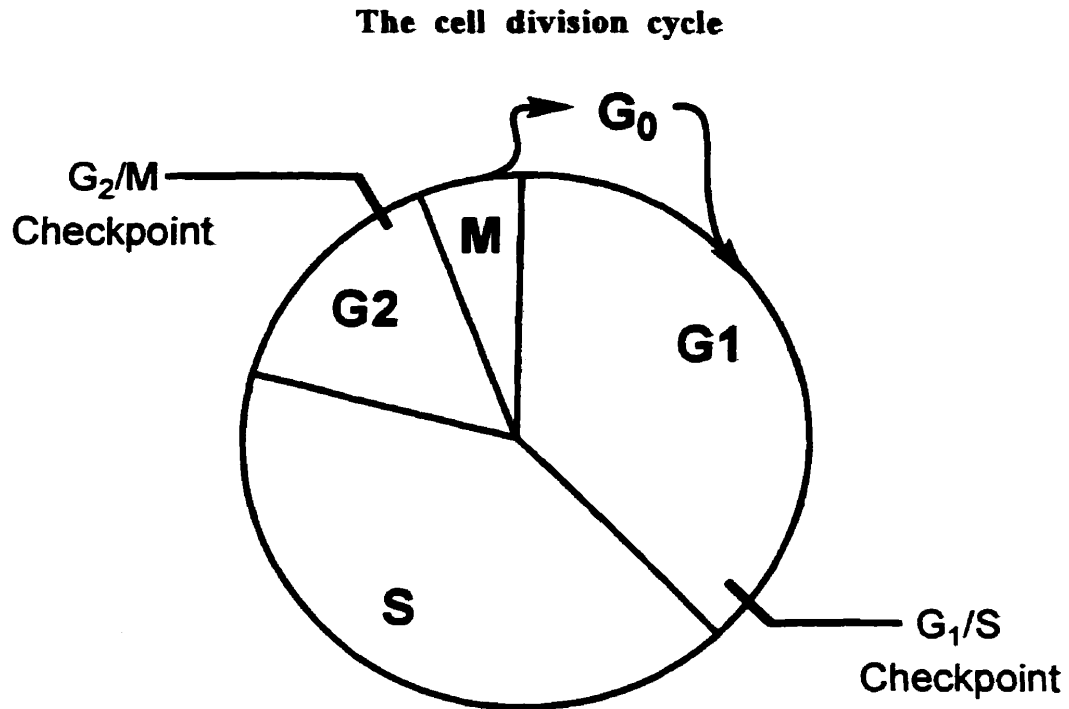
Fig. 3

The different abbreviations are defined as following: G is for 'Gap', S is for 'Synthesis', and M is for 'Mitosis'. G_0 is the indefinite period of time in which the cell in not engaged in cellular division, this is also called the quiescent or resting state. Cells in this phase are either resting or accomplishing another activity that doesn't implicate cellular proliferation (such as synthesizing/secreting hormones, enzymes, etc). The cell can be brought out of this state by adequate mitogenic stimulation (e.g. growth factors, differentiation inducers, etc) and can also return to this state by negative mitogenic stimulation (e.g. antiproliferative cytokins, mitogen antagonists, etc) or improper growth conditions.

G_1 is the gap period in which the cell is engaged in the cellular division cycle, but has not yet commenced the replication of its DNA. At this stage, the cell can be brought back at any time into the resting state G_0 by any of the conditions enumerated above.

In the S phase, the cell has now begun the replication of its DNA and is in the process of preparing two identical DNA copies to be handed down to each of the two daughter cells. In the G_2 phase, the two DNA copies are completed and the cell is getting ready to engage in the final process which involves the division of the mother cell into two identical daughter cells and is called the M phase.

In the cell, biochemical mechanisms are in place to ensure that these phases are not interchangeable. For example, if the cell could bypass the S phase and go directly from G_1 to G_2, it would enter M phase and begin dividing into two daughter cells but with only one DNA copy. This would result in apoptosis (meaning programmed cellular death) or the formation of abnormal cells with increased potential for neoplasia (transformation of normal cells to cancerous cells). On the other hand, if the cell in G_2 could cross back into G_1 and go through the S process once again, it would acquire four DNA copies instead of two. This would again cause entry into M phase with an abnormal genetic status and ultimately lead to apoptosis or risk of neoplasia.

In order to insure that such abberations do not occur, the cell has in place several checkpoints[22] which allow irreversible foward progress from phase to phase only once all of the biochemical prerequisites have been met. Several such checkpoints have been identified and the list continues to grow year after year as new discoveries are made. Examples of checkpoints include the G_1/S transition checkpoint, the DNA damage checkpoint, the G_2/M transition checkpoint, the spindle assembly checkpoint and the chromosome segregation checkpoint.[23]

We will mostly focus on the G_1/S and G_2/M transition checkpoints as these involve Cdc25 protein phosphatases which are especially relevant to our discussion. Another important fact is that the G_1/S transition checkpoint is the most critical checkpoint on which oncogenes and tumor suppressors often converge, mimicking persitent mitogenic stimulation.[24] It is interesting to note that the timing, entry, and irreversible crossing of both of these transition checkpoints are governed by similar biochemical processes (Scheme 1). This control is exerted by similar but biochemically distinct molecules that all work in a similar fashion.
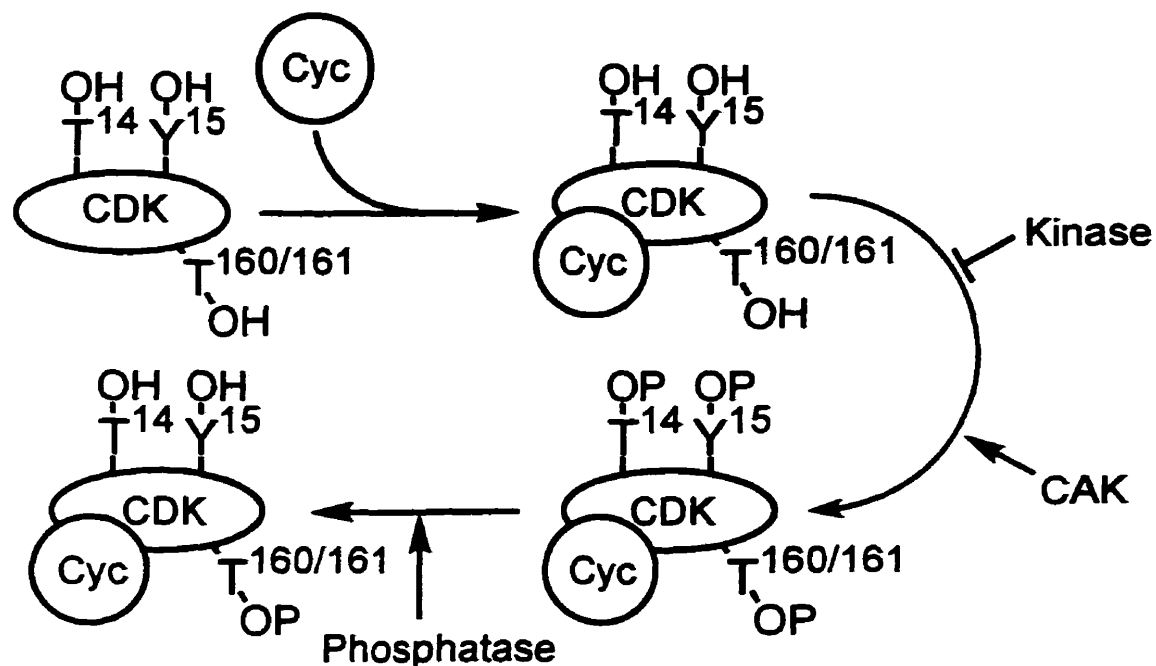
Scheme 1

In all cases we find a catalytic domain called a Cdk (Cyclin dependant kinsase) which is positively regulated by binding with a particular cyclin (regulatory unit) once it reaches a certain threshold level in the cell.[25] This Cdk can also be negatively regulated by binding with a Cdi (Cyclin dependant inhibitor).

Once bound with the corresponding cyclin, two residues of the Cdk, threonine-14 and tyrosine-15, are inactivated by phosphorylation by certain kinases. The inhibitory phosphorylation ensures that the activation of this key enzyme will only take place at the proper time. Subsequently, phosphorylation of the threonine-160 or 161 (depending on the Cdk) is accomplished by a Cak (Cyclin activated kinase) to give the proper three dimensionnal conformation to the enzyme. At this point, the Cdk is ready to begin its catalytic action, the only remaining task is the dephosphorylation of the two residues of its catalytic site.

Once this is accomplished, the Cdk will initiate the phosphorylating cascade that creates the biological response and irreversibly drives foward the cell to the next phase. The key enzyme that catalyses this double dephosphorylation is none other than Cdc25.[26] In the human cells, there exist three different forms of Cdc25, coined Cdc25A-C, which have specific roles at different checkpoints throughout the cell cycle. We will now examine in greater detail the G_1/S transition checkpoint of the cell cycle and the role of Cdc25A (Scheme 2).
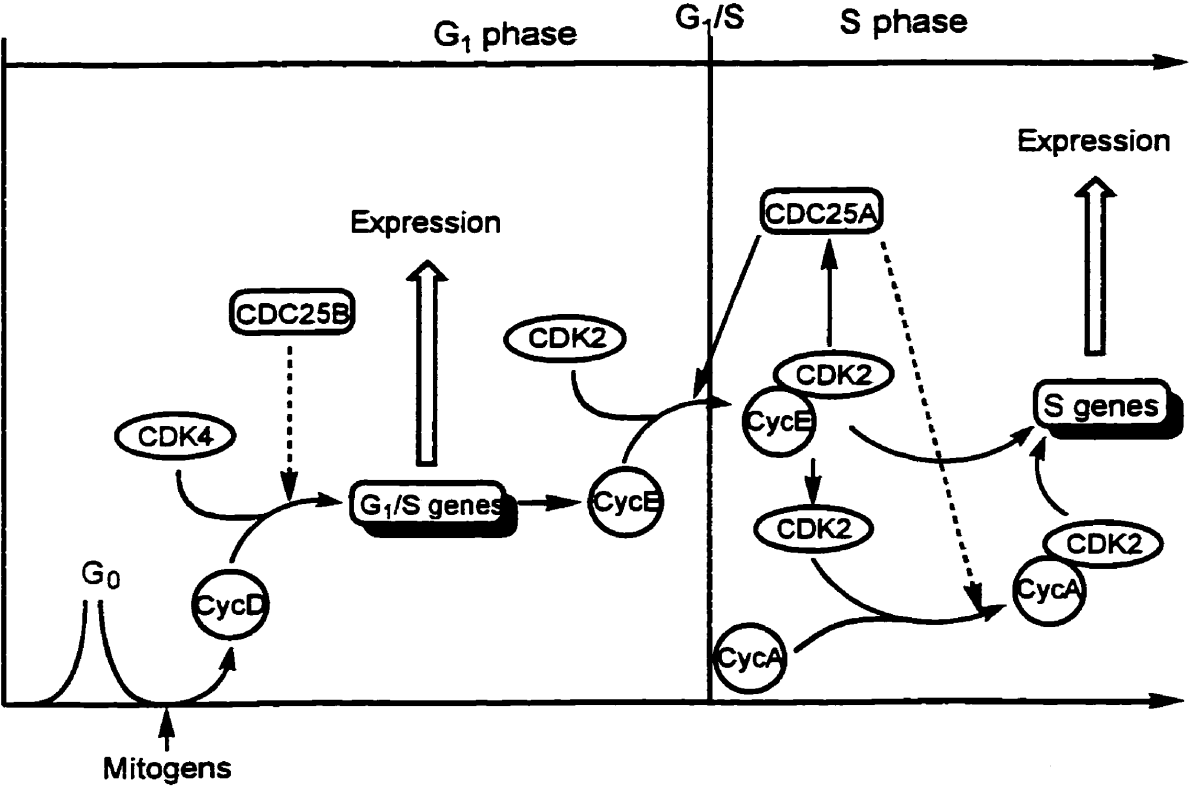
Scheme 2

Shown in Scheme 2 are some key biochemical processes that drive foward the cell from the G_0 to the S phase. Adequate mitogenic stimulation draws the cell out of the G_0 quiescent state and into the G_1 phase. During this G_1 phase, cyclin D is synthesized and, once it reaches its threshold level, it binds with the kinase Cdk4. This Cdk4/CycD complex is then activated by Cdc25B to create a highly active kinase which initiates a phosphorylating cascade thereby producing a biological effect. The presumed end result of this active Cdk4/CycD complex is to cause the expression of various genes necessary for the transit of the cell from the G_1 phase to the S phase. One of the proteins synthesized during this period is Cyclin E which, after attaining its threshold level, binds with the Cdk2 kinase and forms a pre-active complex (Cdk2/CycE) in a similar fashion. This pre-active enzymatic complex, after undergoing double dephosphorylation by the Cdc25A protein phosphatase, becomes highly active and once again initiates a phosphorylation cascade of several relevant proteins which in turn affect the biochemically irreversible transition between the G_1 and S phase.[27]

It is important to mention that anytime prior to this G_1/S transition checkpoint, the cell can be drawn back to the quiescent G_0 state by proper negative mitogenic stimulation. But once the cell crosses the G_1/S transition checkpoint, via the action of Cdc25A, the cell is irreversibly engaged in cellular division. The cell at this stage is refractive to negative stimulation and cannot be turned back.

The biochemical implications of Cdc25A inhibition are tremendous. Failure to activate the Cdk4/CycE complex would result in cellular inability to cross the G_1/S transition checkpoint, and therefore prevent progression toward DNA replication and ultimately, mitosis. This makes the Cdc25A protein phosphatase a key biological target for therapeutical intervention.

Strong evidence that dysidiolide's anticancerous activity is a result of Cdc25A inhibition has been recently demonstrated by Danishefsky and coworkers.[28] These researchers found that certain types of cancerous cells treated with racemic dysidiolide were effectively blocked in the G_1 phase, further enforcing the theory that inhibition of Cdc25A would result in loss of mitogenic properties of the cells.

It is well know that oncogenes and tumor supressors often exert their oncogenic effects at the level of signal transduction, in strategic regulatory pathways, therefore mimicking persistent mitogenic stimulation, perturbing checkpoint control and uncoupling cells from environmental control.[24] It is worthy to note that excessively high levels of Cdc25A and Cdc25B have been observed in head and neck cancers[29] and also more recently in primary non-small cell lung cancer.[30] In such cancers, there is a clear implication of the Cdc25A and/or Cdc25B in the cancerous cells. Consequently, Cdc25A and Cdc25B inhibitors have substantial potential as new drugs against several types of cancers.

Once activated, the Cdk2/CycE complex initiates a phosphorylating cascade and causes the cell to irreversibly enter the S phase. This results in the activation of biologically relevant substrates causing the expression of S phase genes which lead to the replication of the cell's DNA. The Cdk2 eventually dissociates from the cyclin E and binds to the S phase cyclin A to form a new Cdk2/CycA compex. This complex is also believed to be activated by Cdc25A in a similar fashion and would result in the expression of other important genes for the progression through the S phase.

By looking at this critical G_1/S transition checkpoint in greater detail, researchers have considered the possibility that it may function through a positive feedback loop (Scheme 3).[31] Simply stated, a positive feedback loop is a sort of molecular switch which insures that upon activation, the biochemical reaction goes quickly to completion so that there is no 'in between' response.

And so according to this theory, once all of the conditions have been met, Cdc25A causes the double dephosphorylation of a minute amount of the Cdk2/CycE complex which, because of its potent phosphorylating activity, phosphorylates certain residues of Cdc25A, modifying its three dimensionnal conformation and increasing its activity. This activated Cdc25A then rapidly dephosphorylates the remaining amount of the inactive Cdk2/CycE complex and completes the loop.[31]
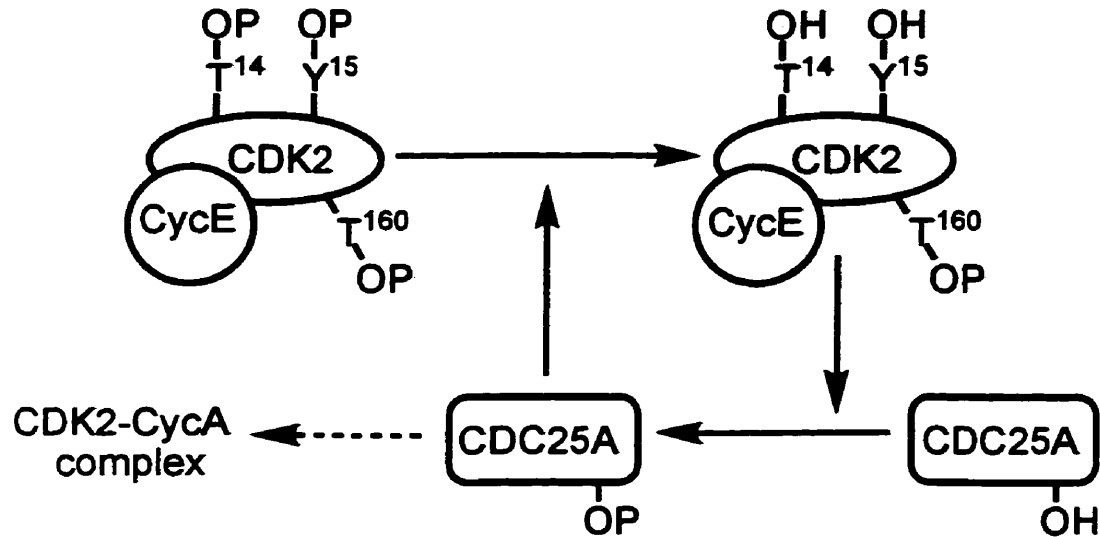
Scheme 3

This process results in an all or nothing response ensuring that, once the cell is ready to engage in the following phase, it does so in a complete and irreversible manner.

The fascinating story of the Cdc25 protein phosphatases however does not end there. The second crucial checkpoint in the cell division cycle is the G_2/M transition checkpoint and this involves the Cdc2/CycB complex (Scheme 4).

Following processes analoguous to those taking place at the G_1/S transition checkpoint, Cyclin B binds to the Cdc2 kinase once it reaches its threshold level to form the Cdc2/CycB complex. This complex is then phosphorylated on two of its catalytic site residues (threonine-14 and tyrosine-15) and activated by phosphorylation of the threonine-161 residue by a Cak. This crucial pre-active Cdc2/CycB complex is called preMPF (pre-mitotic promoting factor).
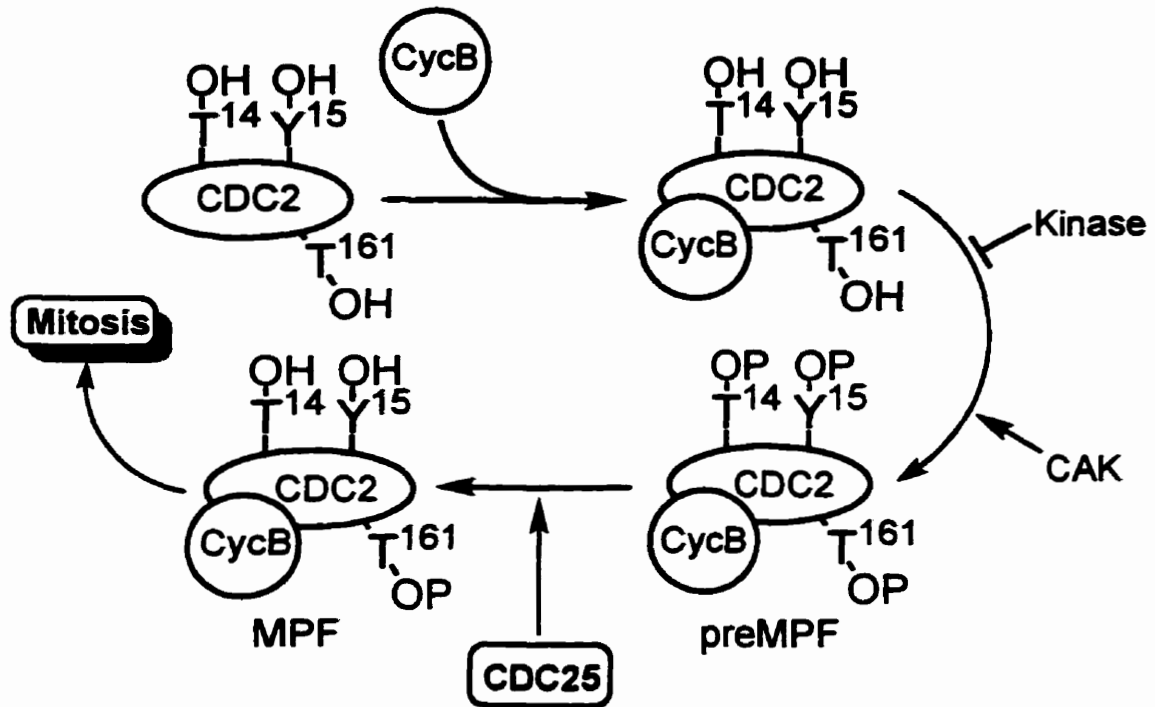
Scheme 4

At this point, the cell is ready to engage in mitosis (the process by which the mother cell divides to form two new identical daughter cells). All that is required for this process to occur is the activation of preMPF into its active form MPF. Once again, this is accomplished by a Cdc25 protein phosphatase, Cdc25C, which catalyses the dephosphorylation of the two phosphorylated residues of the catalytic site of the preMPF complex.

Once activated, MPF initiates a protein phosphorylating cascade which results in the expression of various genes governing mitosis, causing biological processes such as nuclear envelope disassembly, chromosome condensation, construction of the mitotic spindles, etc...[21]

By looking more closely at the activation of the Cdc2/CycB complex (preMPF) by the protein phosphatase Cdc25, researchers have discovered that another positive feedback loop is clearly involved (Scheme 5).[32]
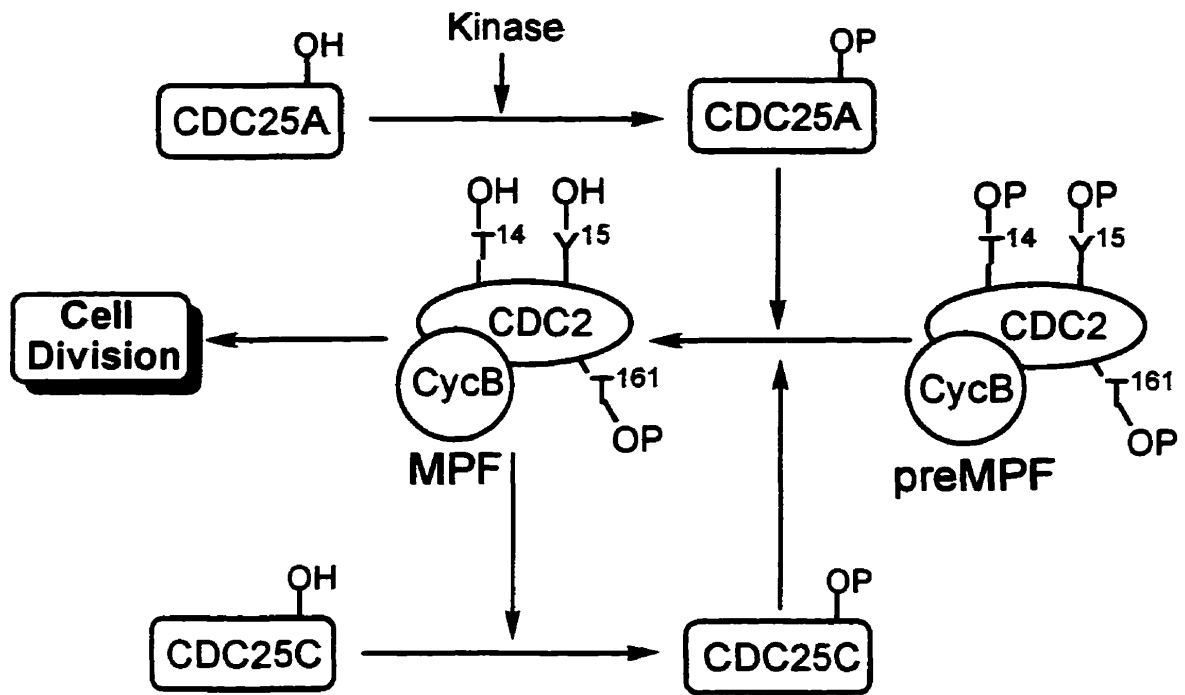
Scheme 5

Following the same explanations given for the G_1/S positive feedback loop, the Cdc2/CycB complex functions as a similar molecular switch. Cdc25C is the protein phosphatase that is involved in the activation of preMPF to MPF once all of the required conditions have been met. Questions regarding the activation of this positive feedback loop still remain,[33] and some researchers propose that Cdc25A might be the protein phosphatase that initiates the loop while Cdc25C is the one that completes it. However, further research is required before a clear picture can emerge.

Reasons to support this theory include the observation that Cdc25A remains highly active throughout the S, G_2 and M phases.[34] The fact that Cdc25A remains active during this period however does not constitute proof that it plays a role in the activation of preMPF. This study also pointed out that Cdc25A can efficiently dephosphorylate preMPF to MPF *in vitro*.[34]

Since Cdc25A has the capacity to acitvate preMPF and has also been shown to remain active during this period of the cell cycle, the proposed theory remains plausible. If this proves to be the case, it would mean that Cdc25A is an enzyme with a crucial role in not one, but two strategic transition checkpoints of the cell cycle.

1.4 Therapeutic potential of Cdc25 inhibitors

Researchers have recently discovered that the G_2/M transition checkpoint is an inhibitory target of other important checkpoints in the cell.[35] For example, cells with unreplicated or damaged DNA insure that they will not engage in mitosis prematurely (e.g. before the situation is corrected) by selectively inhibiting the Cdc25C protein phosphatase.[36]

This inhibition control of Cdc25C prevents the activation of preMPF to MPF and therefore arrests all progression towards mitosis.[37] This naturally occuring pathway underlines well the strategic importance of the Cdc25 protein phosphatases.

One can imagine how a similar strategy could be employed for the development of drugs that would fight proliferative diseases such as cancer by mimicking what the cell does on its own when unreplicated or damaged DNA are present in the cell. The search for Cdc25 protein phosphatase inhibitors is thus of great interest.

Whether or not Cdc25A has a role in the G_2/M transition checkpoint will hopefully be cleared by ongoing research. On the other hand, there is ample evidence that Cdc25A plays a critical role in the irreversible promotion of cell division via the G_1/S transition checkpoint. The important role of Cdc25A in this crucial, strategically located checkpoint makes this enzyme an attractive biological target for new drugs against cancer and other cellular proliferation disorders. Cdc25 protein phosphatases offer brand new molecular targets and will hopefully yield in due time potent inhibitors useful for therapy now that dysidiolide has opened the door.

The German pharmaceutical company BASF Pharma has already invested 48 million dollars to Mitotix (MA) in the search for new drugs that would inhibit Cdc25 phosphatases.[38] Also, the recent crystallisation and structural elucidation of the catalytic domain of Cdc25A will be of invaluable help to those interested in the discovery of new Cdc25A inhibitors.[39] Exciting new developments in this area are awaited with great interest.

It is fair to conclude that the discovery of the first selective Cdc25A inhibitor dysidiolide has provided an excellent biological probe for studying the role of Cdc25A in the cell cycle, and at the same time, a brand new lead in the search for a cure for cancer and other proliferative diseases.

CHAPTER 2

TOTAL SYNTHESIS OF DYSIDIOLIDE

2.1 Introduction

Isolated by Gunesekera and colleagues from the Caribbean marine sponge *Dysidea etheria*, dysidiolide (1) is a novel sesterterpene γ-hydroxybutenolide with the distinction of being the first reported natural product to inhibit protein phosphatase Cdc25A.[2] It also displays antitumor activity that may be a consequence of Cdc25A inhibition (for a detailed discussion see Chapter 1). In light of the broad signals observed in the NMR spectra of dysidiolide, possibly as a result of lactol epimerization or restricted rotation of the lactol side chain, structural elucidation by spectroscopic methods proved particularly difficult. Ultimately, its gross structure and relative - but not absolute - configuration were established by single-crystal X-ray diffraction (1 or *ent*-1). The unprecedented skeleton of dysidiolide, where the two side chains are only about 4 A apart, is believed to arise by an unusual cyclisation of a C_{25} isoprenoid.[2]

Dysidiolide's structure is a true testimony to the extraordinary molecular architecture that can be found in nature. The formidable combination of unique structural features and highly sought biological properties make dysidiolide an eminent target for synthesis.
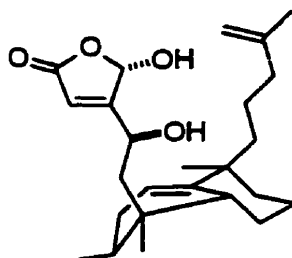
dysidiolide (1)

An enantioselective total synthesis of dysidiolide would not only establish its absolute configuration but would also provide substantial quantities of the natural product for pharmacological evaluation and further pave the way to the synthesis of analogues of improved therapeutic performance.

In designing a synthesis for this molecule one must address several stereochemical issues. The most challenging problem is the construction of the decalin core with the correct stereochemistry of the four chiral centers, two of which are quarternary.

Also challenging is the assemblage of the γ-hydroxybutenolide side-chain together with the C4-stereocenter. Our goal was to devise a synthetic route that would require as few steps as possible and still be highly stereo- and enantioselective. In addition, we opted for maximun flexiblity thereby allowing the generation of analogue libraries from suitably functionalized intermediates.

2.2   Retrosynthetic analysis

Since the absolute configuration of dysidiolide was not known at the outset of our work, we decided to synthesize the enantiomer shown in the article describing the isolation and structure of the natural product (cf. 1).[2]   Our plan, retrosynthetically presented in Scheme 7, was to install the γ-hydroxybutenolide moiety at a late stage in the synthesis. This would be accomplished by using the methodology recently developed in our group which involves the dimethyldioxirane oxidation of a silyloxyfuran, followed by hydrolysis to the corresponding γ-hydroxybutenolide.[7] The question remained as to how the siloxyfuran moiety could be installed onto the carbon squeleton. A convergent approach would involve preparation of an organometallic reagent such as 29 and adding it directly to the aldehyde intermediate 30. In this manner, we would introduce the siloxyfuran precursor required for the last step, and at the same time, install the alcohol group at C-4.

Retrosynthetic analysis
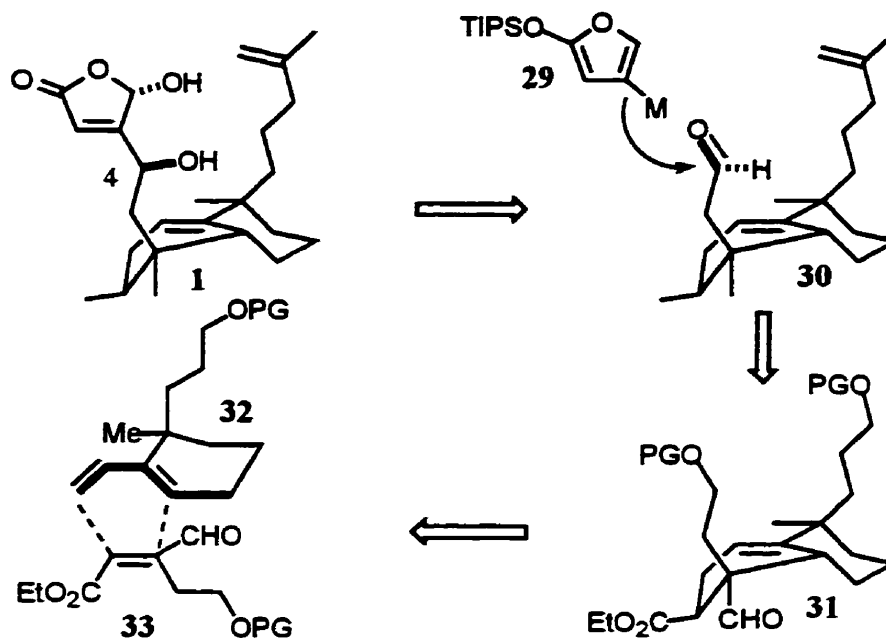
Scheme 6

The question which remained was that of the stereoselectivity of the addition. Examination of Dreiding models revealed that the chiral environment surrounding the aldehyde, in particular the steric bulk of the other side-chain, would force the carbonyl into a conformation in which addition from the *re*-face of the carbonyl would be favored (as shown in Scheme 6). This expectation was further supported by molecular mechanics calculations using CHARMm forcefield quanta simulating software, which suggested that the conformer shown (30) is lower in energy by at least 1,1 kcal/mol over those susceptible to undergo *si*-face attack.

Aldehyde 30 would derive from compound 31 which would in turn be produced by a Diels-Alder reaction of chiral diene 32 with the doubly activated dienophile 33. The attractiveness of this approach stems from the fact that all the chirality of dysidiolide derives from the single quarternary chiral center of the diene 32.

A literature survey revealed that Diels-Alder reactions with doubly activated, unsymmetrically substituted dienophiles are feasible under Lewis acid mediation and proceed with excellent *endo/exo* selectivity.

In pioneering studies, Valenta and co-workers have shown that Diels-Alder reaction of diene **34** with dienophile **35** in the presence of tin tetrachloride provided a single adduct **36**,[40] suggesting that it is the aldehyde and not the ester group of **35** that adopts the *endo* orientation with respect to the diene. The regioselectivity observed is also in accord with our expectation regarding the outcome of the reaction of **32** with **33** (Scheme 6).
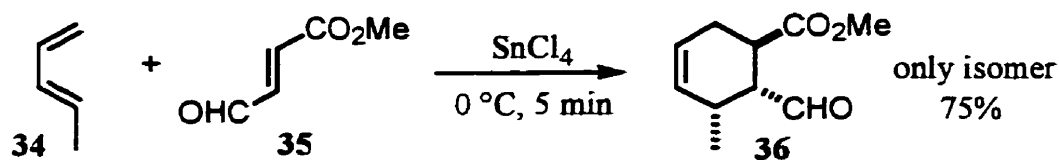
In another example, the Diels-Alder reaction of bifunctionnal dienophile **38** with semi-cyclic diene **37**, carried out at 160 °C in the absence of a Lewis acid, proceeded with complete *endo* selectivity with respect to the aldehyde group to afford the regioisomers **39** and **40** in 67 and 11% yield, respectively.[41] Presumably, the regioselectivity would improve under Lewis acid catalysis.
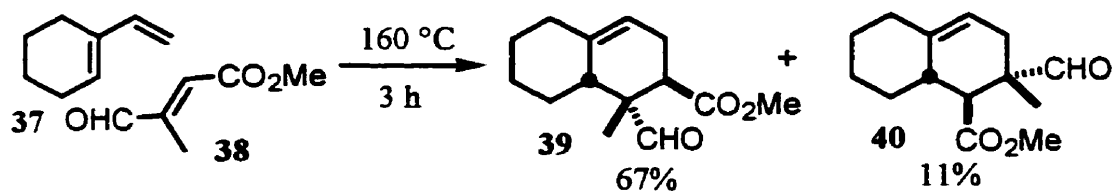
As for the diastereofacial selectivity, it was predicted that allylic 1,2-strain ($A^{1,2}$-strain) between the vinylic proton and the larger substituent in the the pseudo-equatorial position would disfavor conformer (A) thereby favoring (B) where the larger substituent occupies the pseudo- axial position (Fig. 4).[42] Nonetheless, the difference in bulk between the two substituents is not that great so that a fair amount of conformer A may be present along with B.

The larger axial substituent in A would partially shield one of the two faces of the diene and would therefore result in diastereofacial selectivity. Molecular mechanics calculations on diene 32 showed an energy difference of approximately 0.8 kcal/mol in favor of conformer A versus B. Some diastereofacial selectivity was therefore anticipated.

Conformational analysis of diene 32
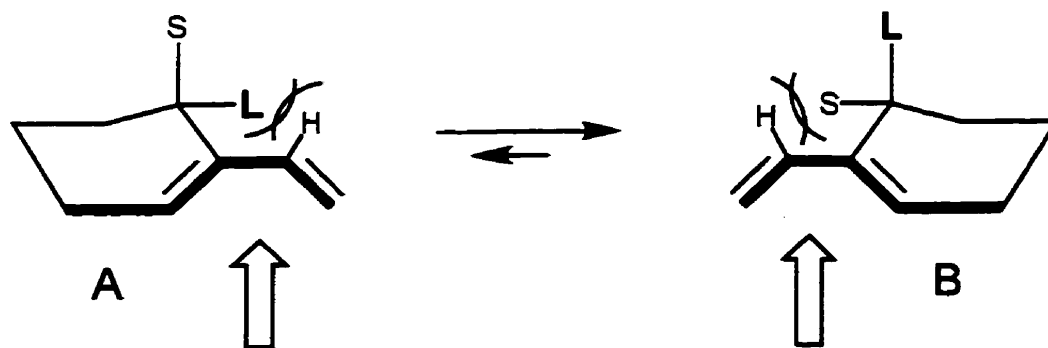
Fig. 4

Having dealt with all stereochemical issues in the plan, we could now turn to the synthesis of dysidiolide by designing short routes to chiral diene 32 and dienophile 33.

2.3   Preparation of chiral diene 50

A systematic search of the literature revealed that d'Angelo's protocol was the most efficient method to install enantioselectively the quarternary center in chiral diene 32.[43] In fact, it is by this method that commercially available ketone 45 and its enantiomer are prepared.

Because ketone 45 is fairly expensive, we decided to prepare this compound on a 20-30 gram scale from racemic 2-methylcyclohexanone (41). The first step involved refluxing together 41 and the desired enantiomer of chiral methylbenzyl amine (42) in toluene overnight in a Dean-Stark apparatus. Crude chiral enamine 43 was obtained following distillation of most of the toluene.[43]
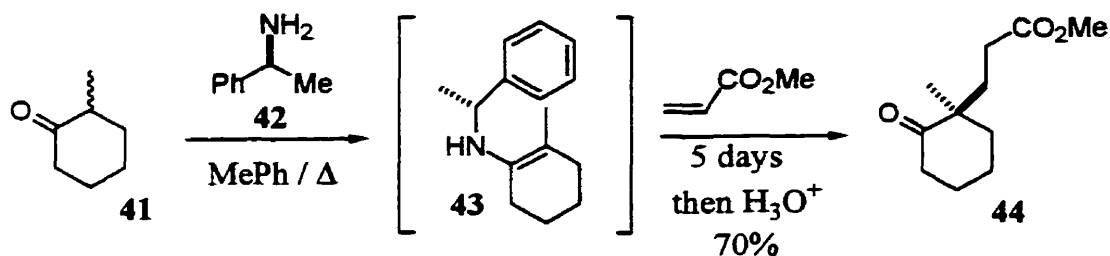
Dropwise addition of methyl acrylate to neat 43 and stirring for five days at room temperature accomplished diastereoselective Michael addition to yield chiral ketone 44 in 70% yield (approximately 90% ee) after hydrolysis and distillation under reduced pressure.

Reduction of both the ketone and ester functionalities of 44 was accomplished by using an excess of lithium aluminium hydride.[44] In this manner, diol 45 was obtained as a mixture of diastereoisomers in quantitative yield. There was no need to separate these isomers since both of them were ultimately transformed into the same compound (ketone 47).
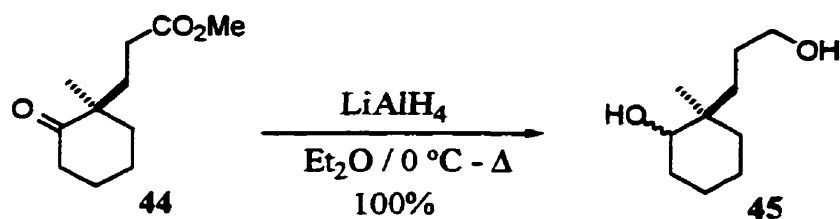
The protecting group chosen for the primary alcohol was the benzyl group because of its robust nature but its ready cleavage under a variety of conditions. Using one equivalent of sodium hydride and a slight excess of benzyl bromide, selective protection of the primary alcohol group of diol **45** afforded the desired monoprotected compound **46** in 76% yield.
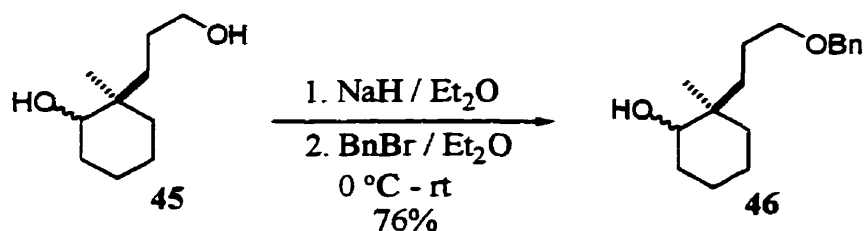
This diastereoisomeric mixture **46** was oxidized using Ley's TPAP oxidation protocol[45] to afford the desired chiral ketone **47** in quantitative yield. It is worth mentioning that when this protocol is used for large scale reactions, an ice bath must be at hand because of the exothermic nature of such oxidations.
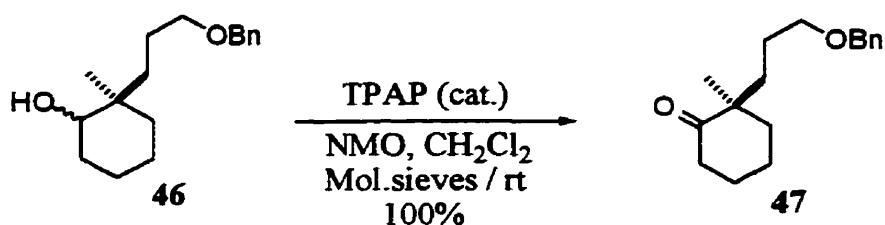
The final steps for the synthesis of chiral diene **50** involved the addition of a vinyl cerium reagent **48**[46] to ketone **47** which resulted in an inconsequential mixture of diastereoisomeric tertiary alcohols **49** which was dehydrated by refluxing in a Dean-Stark apparatus in the presence of excess $CuSO_4$ to afford diene **50** in 85% yield.
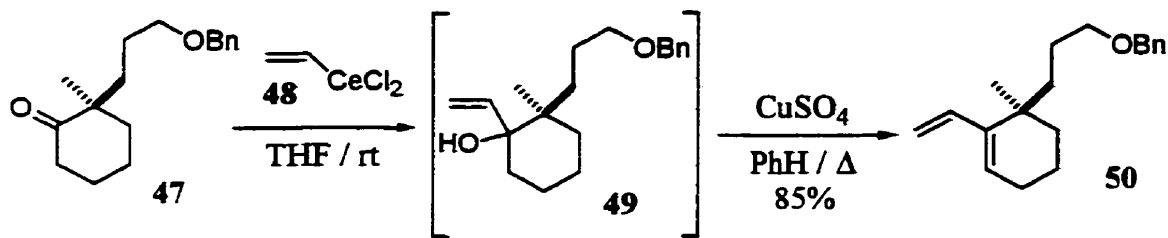
The preparation of the tertiary alcohol **49** was efficient when the organocerium reagent **48** was used. By using a highly oxophilic organocerium reagent, generated in situ from vinyl magnesium bromide and dry $CeCl_3$,[46] addition is greatly favored over deprotonation. However, good results could also be obtained by adding the ketone **47** dropwise to a solution of vinyl magnesium bromide at room temperature (inverse addition protocol).

The overall yield for this five step synthesis of chiral diene **50** from commercially available chiral ketone **45** is approximately 65%.

2.4 Preparation of dienophile 56

The synthesis of difunctional dienophile **56** began with commercially available homopropargylic alcohol **51** which was protected as its tri-*iso*-propylsilyl ether to afford the known compound **52** in 95% yield.[47]

HO⟍⟍⟍≡ **51**   → TIPSCl / imid./ DMF 95%   → TIPSO⟍⟍⟍≡ **52**
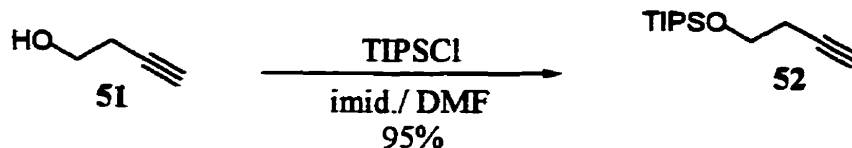
Alkyne **52** was then deprotonated with EtMgBr and added to ethyl chloroformate to produce the propargylic ethyl ester **53** in 76% yield.[48]

TIPSO⟍⟍⟍≡ **52**   1. EtMgBr / THF → 2. ClCO$_2$Et / THF -20°C - 3°C / 18h 76%   TIPSO⟍⟍⟍≡CO$_2$Et **53**
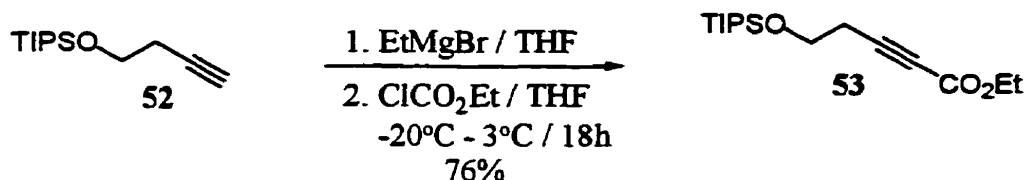
At this point, the crucial step of the synthetic pathway to the desired dienophile **56** was carried out. This involved conjugate addition of a masked aldehyde functional group (cf. vinyl group) to this propargylic ester **53**. Obtention of an isomeric mixture of adducts would render this route useless since it could not provide **56** in an isomerically pure form following the unmasking of the carbonyl function. A completely selective process was therefore necessary.

The 1,4-addition of an *in situ* generated vinyl copper reagent (**54**) to propargylic ester **53** was successful when performed in ether under mild conditions (-78 °C)[49] but resulted in an inseparable 2:1 mixture of isomers in favor of the desired *trans* isomer (**56**). Unfortunately, variation of the reaction conditions only led to a very small change of this modest selectivity. Eventually, the use of THF instead of ether as solvent was found to have a dramatic effect on selectivity.[50] With this sole modification, complete selectivity was observed in favor of the desired isomer **55** (as judged by proton and carbon NMR).

TIPSO~~~CO2Et **53** → (=)₂ CuLi **54** / THF / -78°C then MeOH → TIPSO~~~ EtO2C **55** → O₃ / MeOH/CH₂Cl₂ (1:1) / -78°C 83% (2 steps) → TIPSO~~~CHO EtO2C **56**
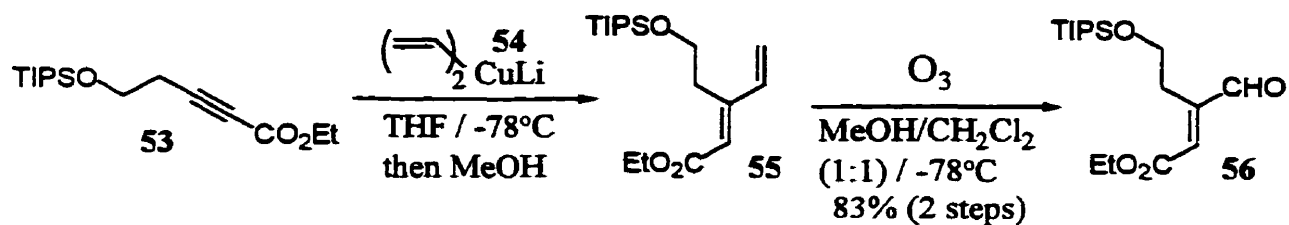
Vinyl copper reagent **54** in dry THF at -78 °C was thus added in a 1,4-fashion to propargylic ester **53** to afford isomerically pure diene **55**. The cuprate reagent **54** was prepared by adding commercially available vinyl lithium (in THF) dropwise to a THF suspension of copper (I) iodide at -35 °C. Alternatively, vinyl lithium could be prepared *in situ* by adding four equivalents of phenyllithium to tetravinyl tin.[51]

Immediately following the conjugate addition, diene **55** was submitted to mild ozonolysis at -78 °C.[52] This ozonolysis accomplished selective oxidative cleavage of the terminal double bond of **55** to unmask the desired aldehyde function thereby providing dienophile **56** in 70-83% yield (2 steps).

It is important to mention that strict control of the reaction temperature (-78 °C) was crucial to the success of the process since higher temperatures resulted in a low yield of **56**, presumably due to oxidative cleavage of the internal double bond of the diene.

This synthetic route to difunctional dienophile **56** is very practical both in terms of brevity and efficiency (four steps, 60% overall yield). The sequence could be carried out on a relatively large scale (10 g) which allowed the preparation of substantial quantities of the requisite dienophile **56**.

2.5   Diels-Alder reaction of chiral diene 50 with dienophile 56

With chiral diene **50** and dienophile **56** in hand, we could now turn to the crucial Diels-Alder reaction. Dropwise addition of the anhydrous Lewis acid $EtAlCl_2$ to a solution of chiral diene **50** and dienophile **56** in CH_2Cl_2 at -78 °C resulted in smooth formation of a 2.3:1 mixture of diastereofacial isomers **57** and **58** (76% yield).
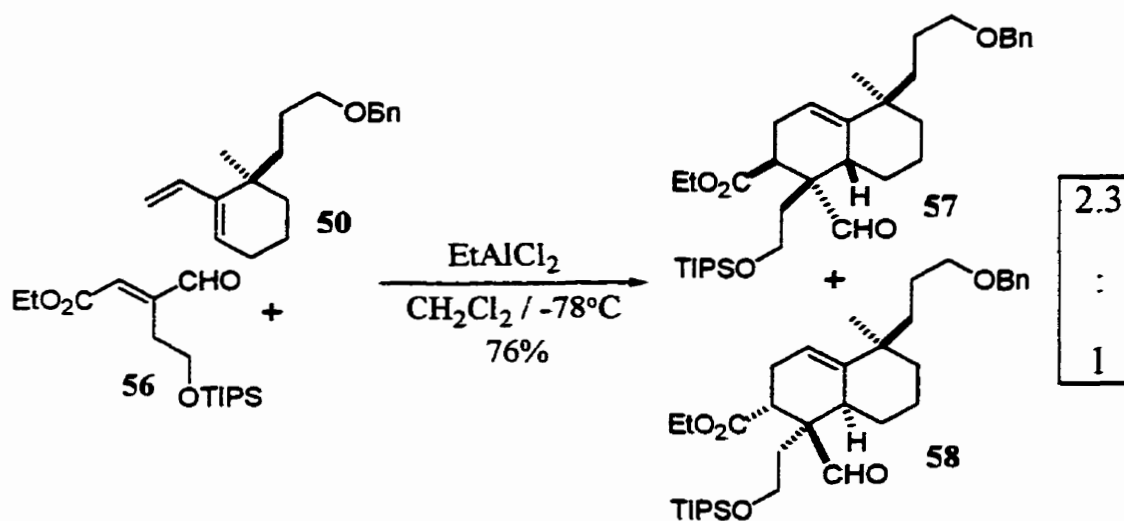
It is important to mention that the degree of selectivity of this reaction is considerable since only two of the eight possible isomeric products have been detected. What remained to be determined was whether these two isomers were in fact facial isomers, and if this was the case, whether the major isomer arises by cycloaddition from the expected less hindered face of diene **50**.

Since these two isomers **57** (major) and **58** (minor) were difficult to separate by flash chromatography, they were directly submitted to Super-Hydride reduction to afford the corresponding diols **59** (major) and **60** (minor) in 43 and 18% yields respectively (2 steps).
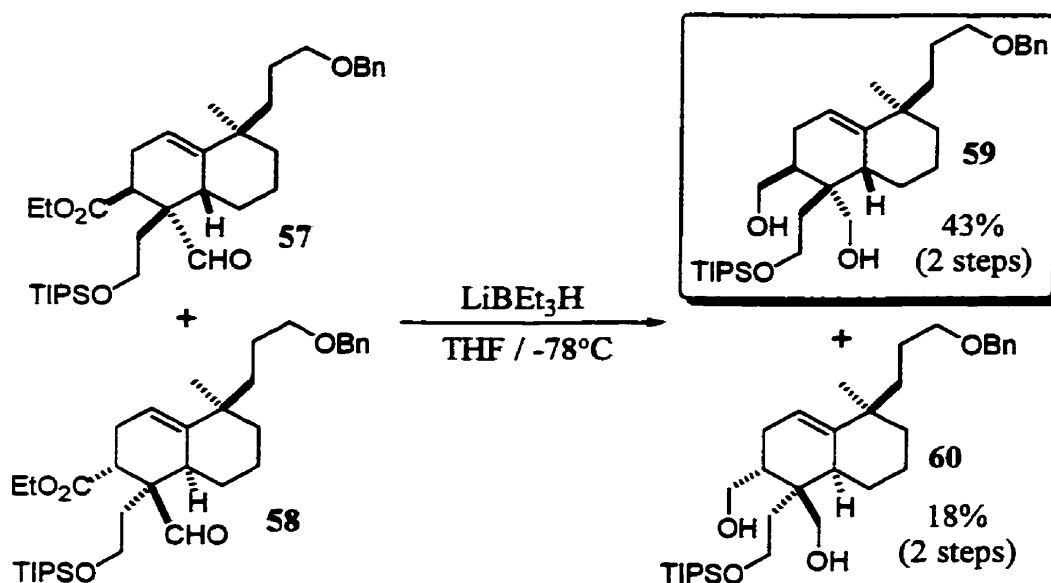
These two isomeric diols (**59** and **60**) were then separately submitted to NOE experiments to dertermine their identities.

Stereochemical assignments of diols 59 and 60
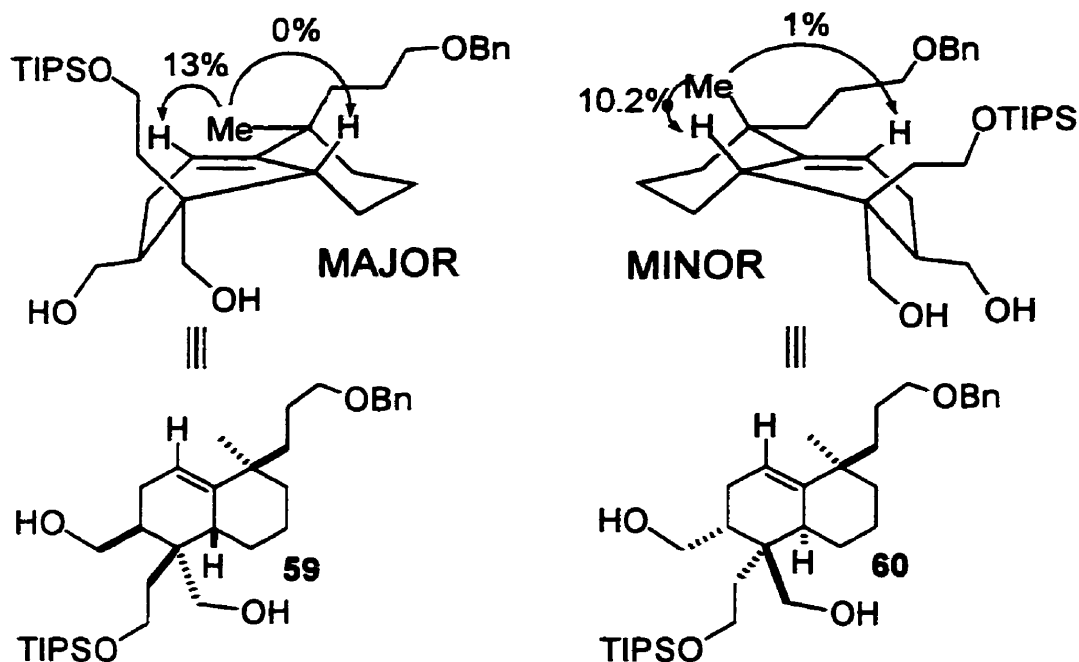
Fig. 5

No NOE effect was observed between the NMR signal of the angular proton and the equatorial methyl group of isomer **59** whereas a relatively strong NOE effect (10.2%) was observed between the same angular proton and the axial methyl group the minor isomer **60**. On this basis, structure **59** was attributed to the major isomer. With the proper major isomer **59** now available, we could continue with the total synthesis of dysidiolide.

2.6 Functionalization to intermediate 67

The next crucial step entailed deoxygenation of the two alcohol groups present in **59** in order to install the requisite methyl substituents. The ideal means for accomplishing this task proved to be Ireland's phoshoramidate method.[53]

This protocol involves transformation of diol **59** to the corresponding diphosphoramidate **61**. This was best accomplished using MeLi as base followed by treatment at room temperature with an excess of N,N,N',N'-tetramethyldiamido-phosphorochloridate (PONCl).[53] The yield of the desired diphosphoramidate **61** was 58% after purification by flash chromatography.
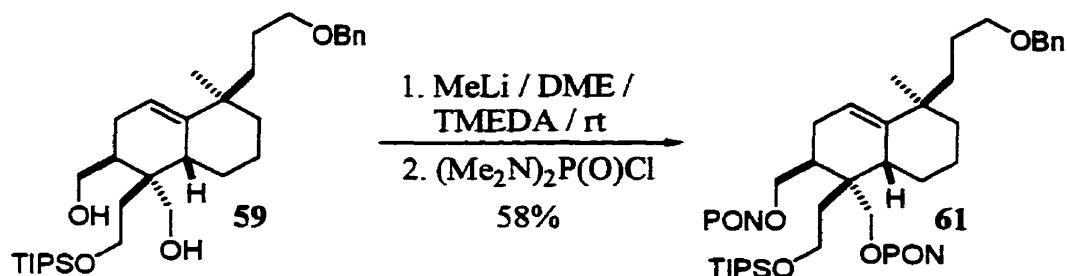
The next step consisted of treatment of **61** with lithium in ethylamine.[53] These strong reducing conditions (Benkeser reduction) not only accomplished double deoxygenation but also unmasked the benzyl-protected alcohol to give **62** in 70% yield.

This reaction proved to be most efficient since it accomplished three synthetic transformations in a single operation. Furthermore, it allows the overall route to be shortened by one step.
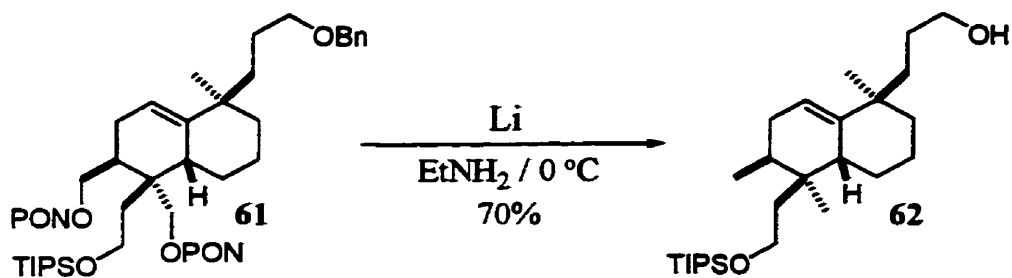
The primary alcohol **62** was then smoothly transformed to the corresponding tosylate **63** in 98% yield.
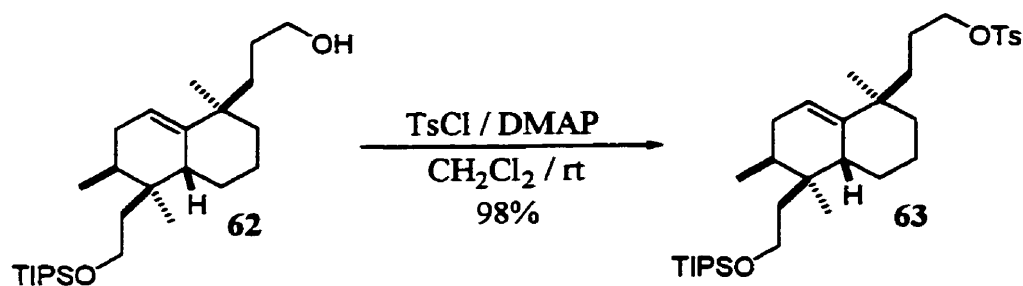
This tosylate **63** was then treated with an excess of *iso*-propenyl magnesium bromide (**64**) in the presence of a catalytic amount of copper (I) iodide to furnish the sp²-sp³ cross-coupling product **65** in 90% yield.[54]
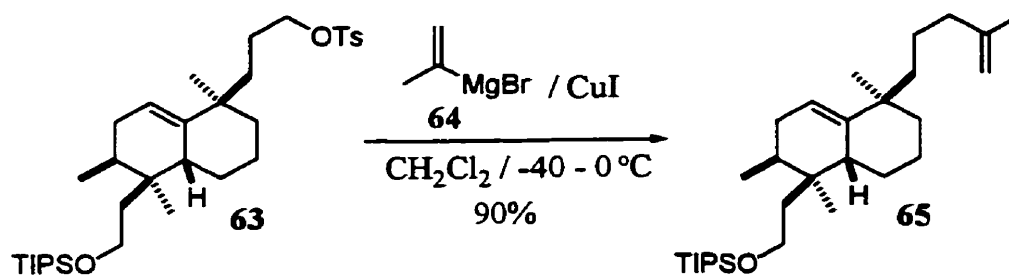
Compound **65** now possesses the dysidiolide unsaturated side-chain. Removal of the TIPS protecting group was accomplished by treatment with TBAF to afford primary alcohol **66** in quantitative yield.
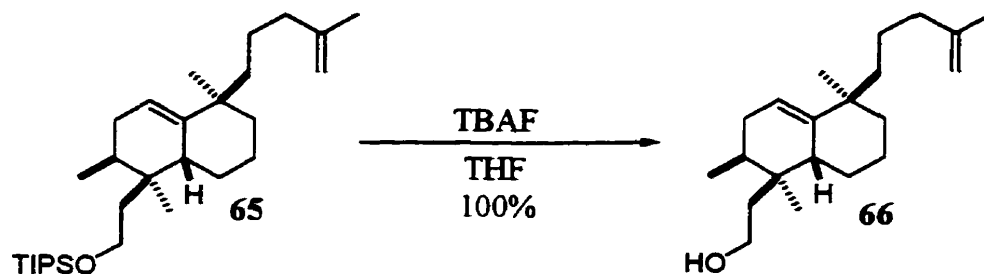
The so obtained primary alcohol **66** was then oxidized to the corresponding aldehyde **67** in 95% yield following Ley's catalytic TPAP-oxidation protocol.[45]
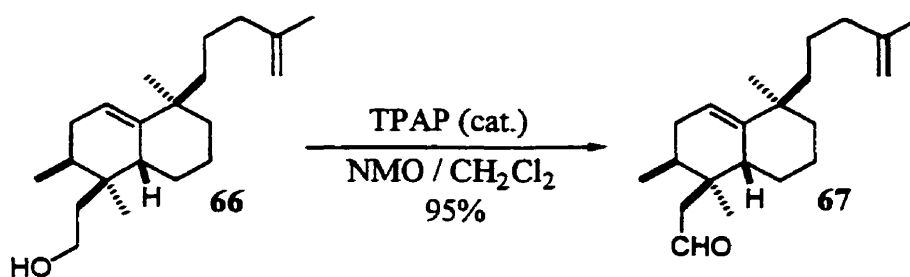
The stage was now set for the end-game of the synthesis of dysidiolide. The final critical step involves the addition of an appropriate siloxyfuran reagent to aldehyde **67**, simultaneously setting in place the siloxyfuran moiety for the ensuing step, and at the same time, creating the requisite secondary alcohol group.

2.7 Preparation of siloxyfuran reagent 72

Preparation of the new siloxyfuran reagent **72** began with commercially available β–tetronic acid (**68**). Using Jas' procedure, **68** was transformed into bromofuranone **69** in 83% yield.[55]
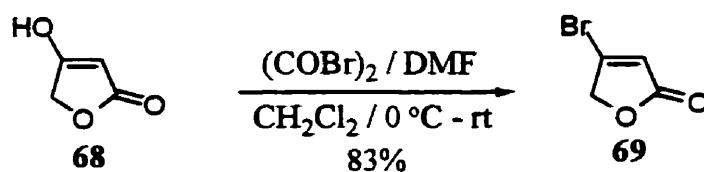
The corresponding siloxyfuran was prepared by treatment of **69** with Et$_3$N and TIPSOTf according to the standard protocol[7] to afford known bromosiloxyfuran **70**[56] in 95% yield.
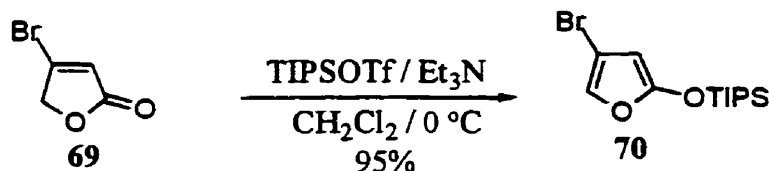
This bromosiloxyfuran **70** was then treated with a slight excess of *n*-BuLi at -78 °C in dry ether to accomplish bromine-lithium exchange.[56,57] The resulting organolithium reagent **71** was transmetallated to the corresponding organotitanium reagent **72**.
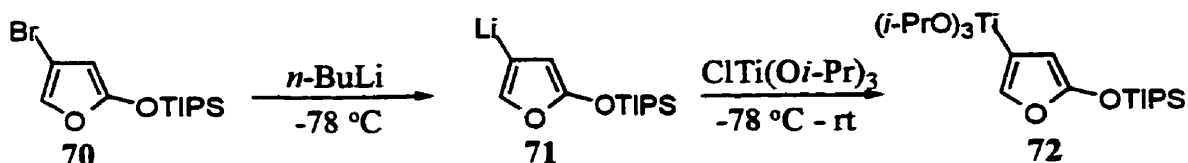
This transmetallation to organotitanium **72** was necessary since its lithium counterpart **71** was presumed to be unstable in the light of previous experience with 3-furyllithium.[57] In this manner, the organotitanium reagent **72** could be prepared well in advance and kept at room temperature until needed for the next step.

2.8   Completion of the synthesis of dysidiolide

Treatment of aldehyde **67** with a solution of siloxyfuryltitanium reagent **72** in dry ether at -78 °C provided a 2.2:1 mixture of the two addition products **73** and **74**, as determined by HPLC analysis of the crude reaction product. These two adducts were separated by flash chromatography to afford diastereoisomerically pure alcohols **73** (major) and **74** (minor) in 58 and 14% yield, respectively.
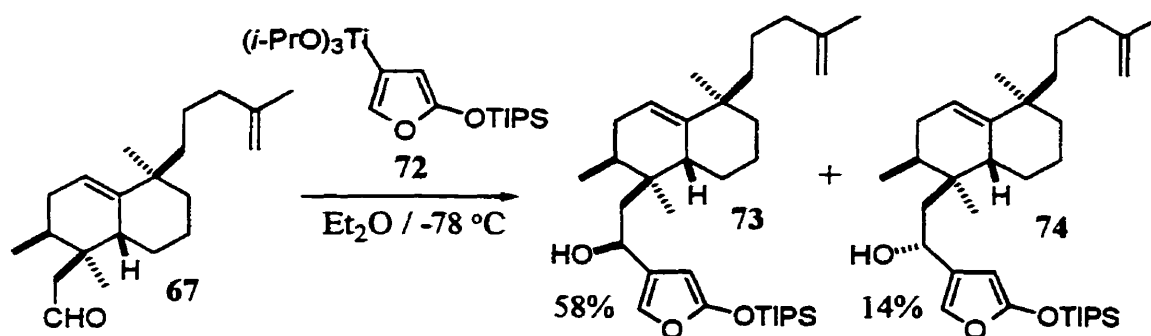
The only reliable way to establish the identity of these two isomers (**73** and **74**) was to transform each of them individually to the corresponding γ-hydroxybutenolide and compare the spectral data of the latter with those of natural dysidiolide.
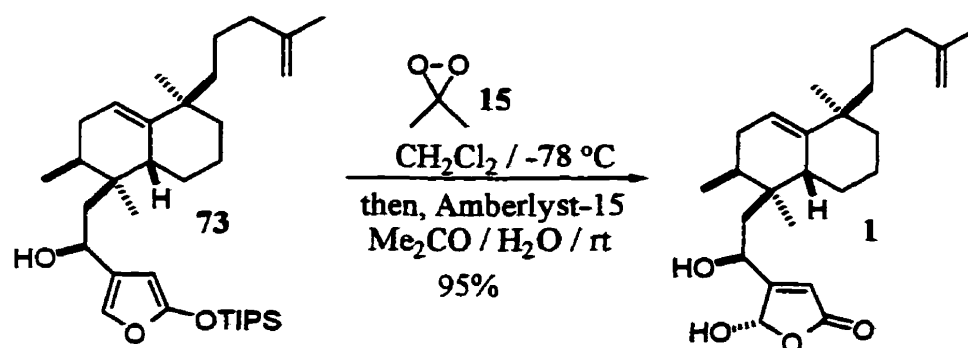
The major diastereoisomer **73** was treated with dimethyldioxirane **(15)** and the intermediate silyl ester (not shown) was hydrolysed in accord with the procedure developed in our group[7] for making γ-hydroxybutenolides. The so obtained product was a white crystalline compound (95% yield) with similar physical appearance to natural dysidiolide, a sample of which was kindly provided to us by Dr. Sarah Gunesekera of Harbor Branch Oceanographic Institution in Fort Pierce, Florida.

On the other hand, oxidation of the minor diastereoisomer **74** afforded 4-epi-**1** as an oily compound (97%) whose spectral and analytical data (HPLC) were different from those of dysidiolide.
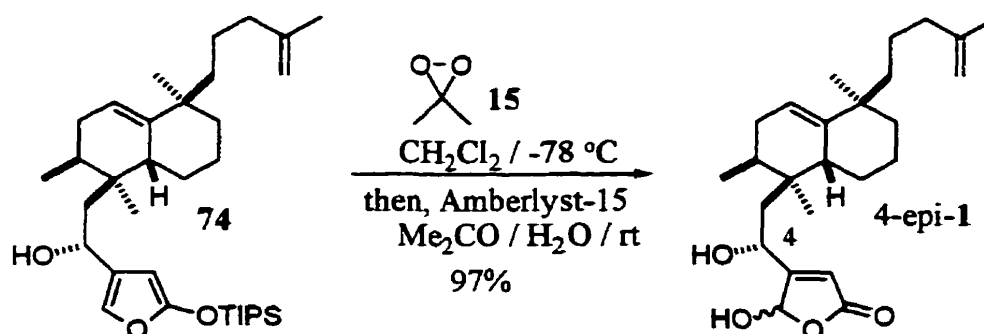
Further examination revealed that the major product **1** was identical with a sample of natural dysidiolide by TLC, HPLC, IR, MS, ^{1}H and ^{13}C NMR. However, the sign of the optical rotation of **1** was opposite to that of the natural product. Therefore, the absolute configuration of natural dysidiolide could be assigned as depicted in *ent*-**1**.
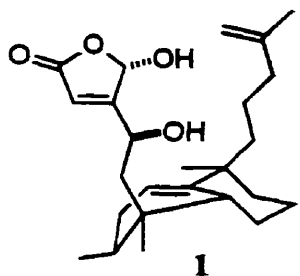
(+)-dysidiolide (synthetic)
mp 181-184 °C
$[\alpha]^{22}_D$ +10.5°
(*c* 0.5, MeOH/CH$_2$Cl$_2$, 1:1)
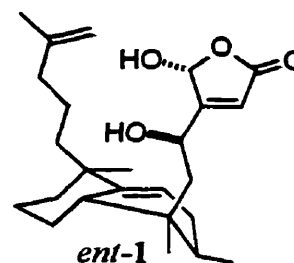
(-)-dysidiolide (natural)
mp 187-188 °C
$[\alpha]^{22}_D$ -11.1°
(*c* 0.6, MeOH/CH$_2$Cl$_2$, 1:1)

Our total synthesis[58,59] has therefore unambiguously established dysidiolide's absolute configuration. The route is convergent and reasonably efficient (longest linear sequence = 15 steps; overall yield of 5.3%). Furthermore, the antipode of natural dysidiolide is available for the first time for biological evaluation.

The Diels-Alder reaction between chiral diene **50** and dienophile **56** has proved to be very useful for the preparation of the key intermediate **59**. However, the facial selectivity observed for this reaction was modest and it would be most desirable to improve this selectivity. We thus decided to develop a second generation synthesis that would address this issue. Since the absolute configuration of dysidiolide was now known, we opted for the preparation of the natural dysidiolide enantiomer (*ent*-**1**).

2.9 Preparation of chiral diene *ent*-50

The requisite chiral diene *ent*-45 was synthesized following the same sequence used to prepare chiral diene 55, but starting from chiral ketone *ent*-44 which was also commercially available. The entire sequence along with yields is shown below (Scheme 7).
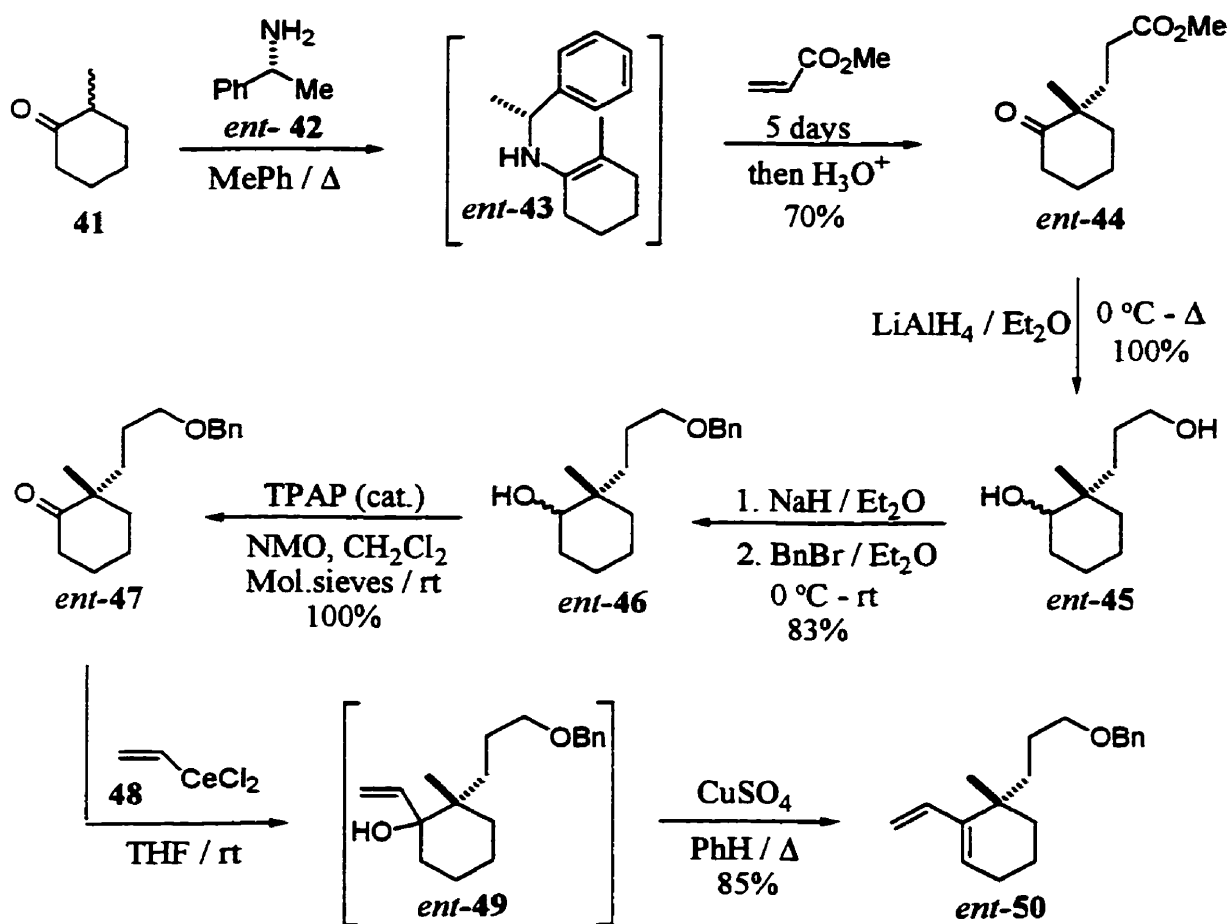
Scheme 7

2.10 Preparation of chiral dienophile 84

Early attempts to find an asymmetric version of the Diels-Alder reaction were unsuccessful. In the presence of various chiral Lewis acids (such as menthol derived 74) there was no reaction, even under forcing conditions (up to rt for several days).
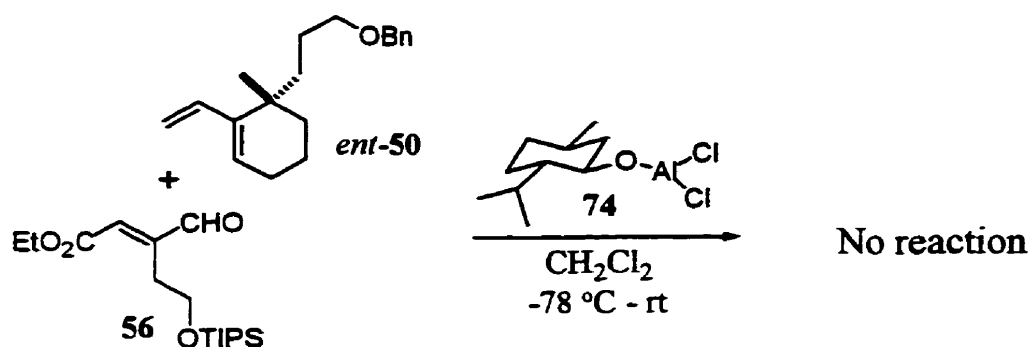
Modification of the ethyl ester of 56 to its chiral menthol derivative 75 did not lead to an improved ratio of diastereofacial isomers.
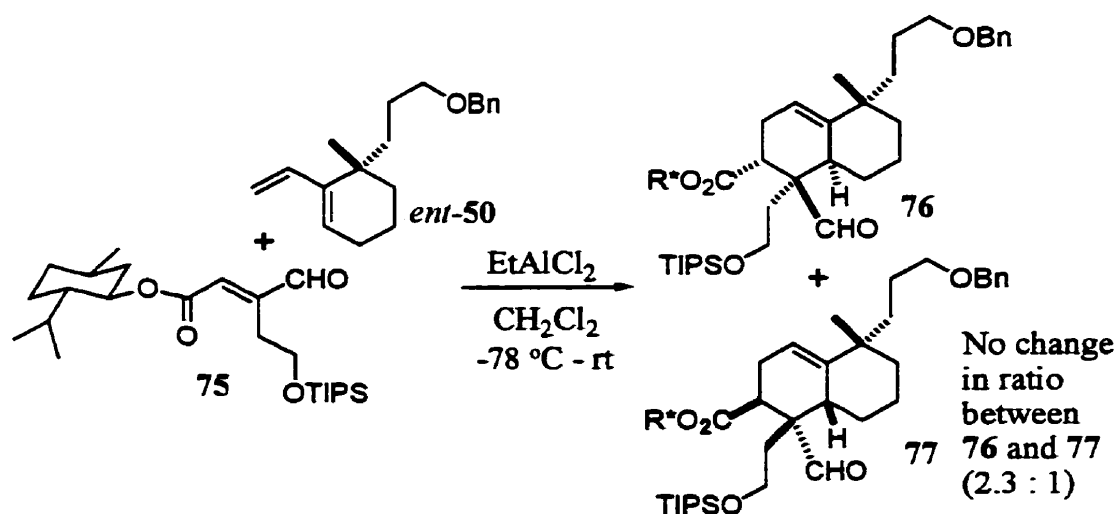
Chiral dienophile **78** was prepared but did not react with diene *ent*-**50** even under forcing conditions (up to rt for several days).
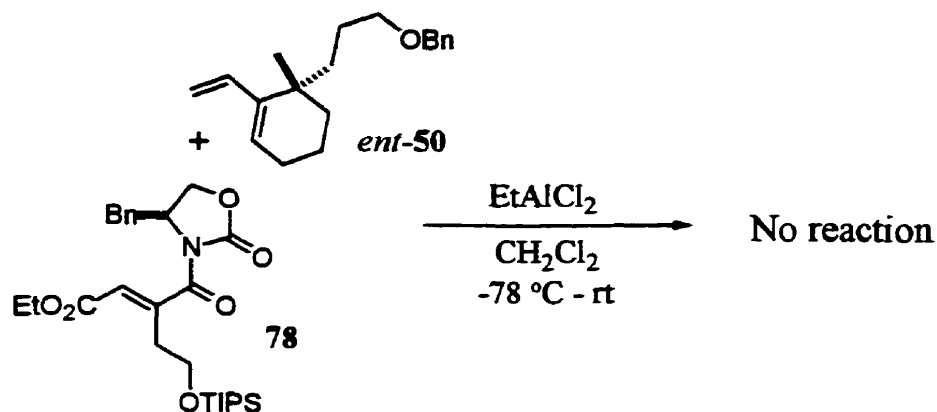
Finally an excellent chiral dienoplile (**79**) was developed by replacing the ethyl residue of the ester by a phenylmenthyl group. Simple chiral dienophiles containing a phenylmenthyl ester moiety were first developed by Corey.[60] It was expected that π-stacking of the phenyl with the double bond would block one face of the dienophile.[61,62]
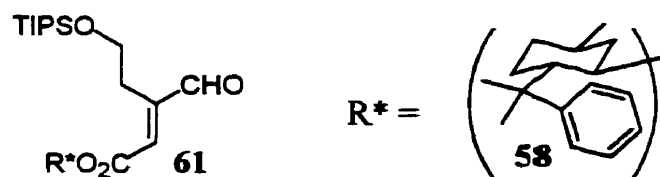
The preparation of dienophile **84** began with the same TIPS protected alkyne **52** which, after deprotonation with EtMgBr, was trapped with dry carbon dioxide (gas) to afford the corresponding propargylic carboxylic acid **81** in 79% yield.[63]
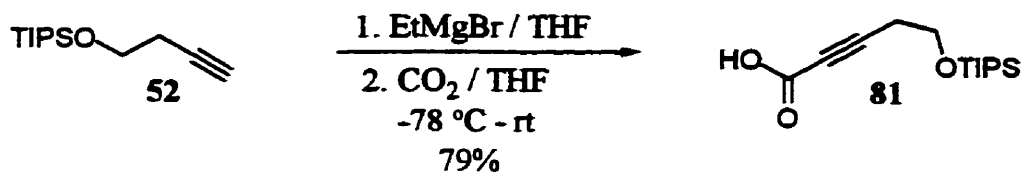
This carboxylic acid **81** was then condensed with commercially available (-)-phenylmenthol (**80**) in the presence of DCC to give chiral ester **82** in 83% yield.[64]
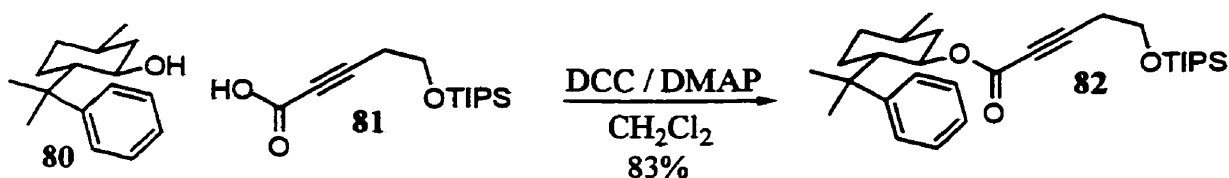
Michael addition of vinyl copper reagent **54** to this new chiral ester **82** afforded chiral diene **83** in quantitative yield. Because of this diene's relative instability, it was immediately submitted to the next step.
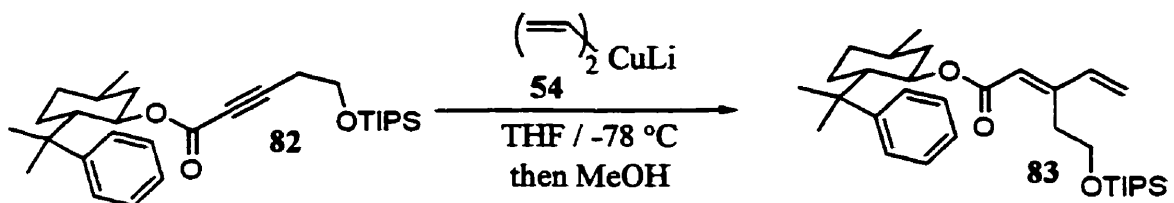
The terminal double bond of **83** was then selectively cleaved with ozone under mild conditions (-78°C) to deliver chiral dienophile **84** in 68% yield (2 steps).
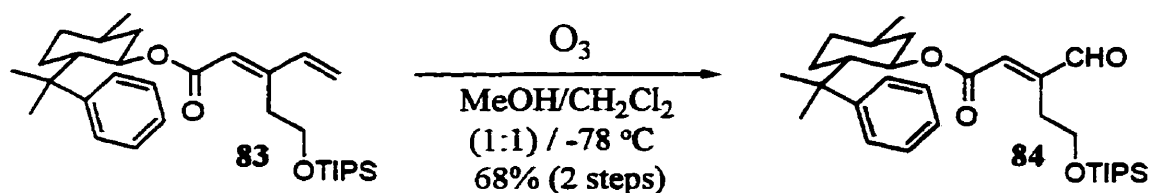
It is again important to mention that strict control of the temparature (-78 °C) is crucial to the success of this reaction. Higher temperatures resulted in pronounced drop in yield of **84**, presumably due to the oxidative cleavage of the internal double and/or the phenyl group.

2.11 Diels-Alder reaction of chiral diene *ent*-50 and chiral dienophile 84

Following the same protocol as for the previous Diels-Alder reaction, chiral diene *ent*-50 and chiral phenylmenthyl dienophile **84** provided a single Diels-Alder adduct (**85**) in 73% yield.
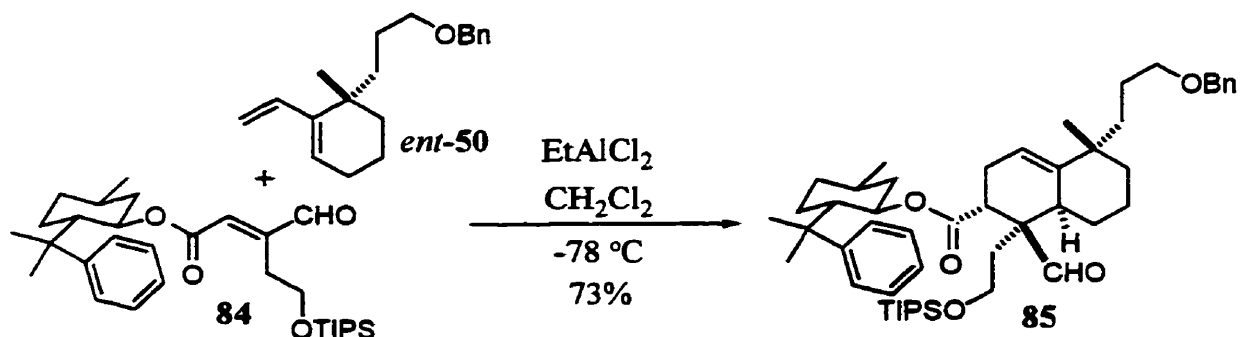
An insignificantly small signal on the crude NMR spectra revealed the possible presence of another isomer in less than 3% yield. This small isomeric amount can simply be the result of the Diels-Alder reaction with the other enantiomer of the diene *ent*-50 since the ee of the starting material was about 90%.

Slight modification of our Lewis acid using Danishefsky's protocol,[65] resulted in a modest increase in the isolated yield of adduct 85. In this protocol THF is added to the reaction mixture to decrease the Lewis acid acidity of the catalyst and thus to avoid polymerization of the diene.[65]
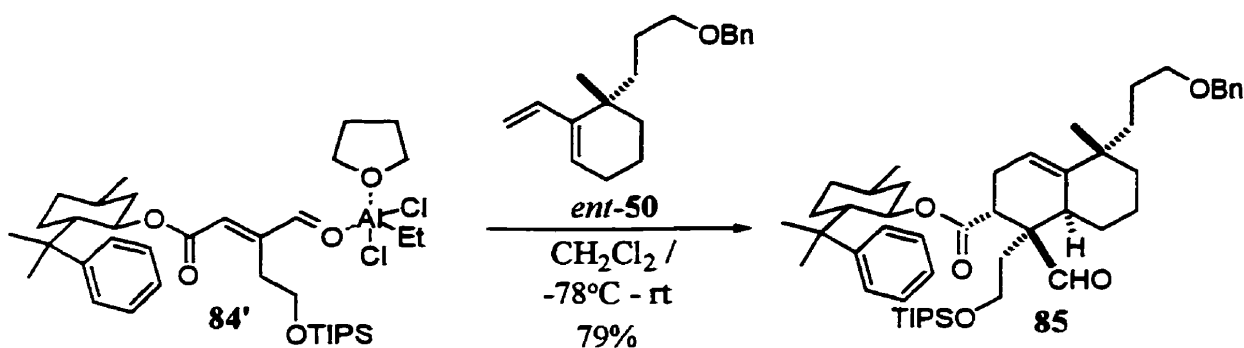
The procedure consisted of premixing chiral dienophile 85 with an equimolar amount of dry THF and then treating the mixture with an excess of EtAlCl$_2$ at -78 °C. To this mixture (84') the chiral diene *ent*-50 (in dry CH$_2$Cl$_2$) was added dropwise and the reaction mixture was allowed to warm to room temperature.

This reaction was cleaner than before (as judged by TLC) and led to an increase in the yield of adduct 85 from 73 to 79%.

Reduction of the adduct **85** with Super-Hydride afforded the desired diol *ent*-**59** in 88% yield. In addition, the phenylmenthol auxilliary **80** was recovered in 97% yield.
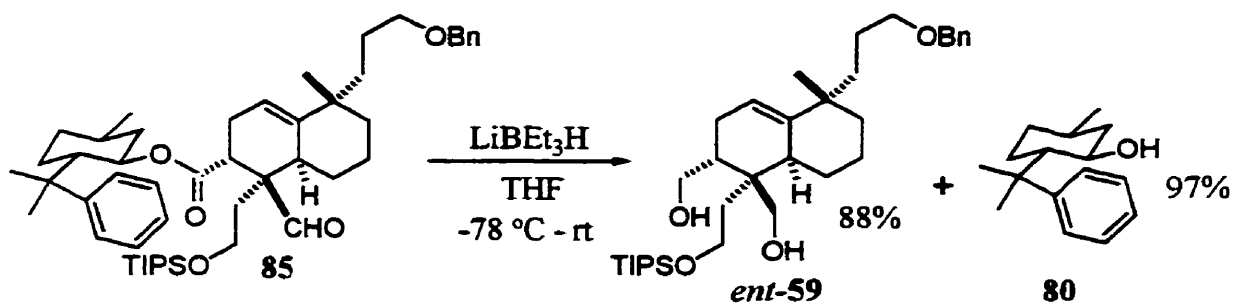
The yield of this improved Diels-Alder reaction combined with reduction was 69% as opposed to 43% previously. This represents a considerable improvement in the synthesis of the key intermediate *ent*-**59** which is now produced in a completely stereoselective fashion.

2.12 Functionalization to intermediate *ent*-67

Advanced intermediate *ent*-**67** was synthesized using the same sequence followed to prepare compound **67** but started from diol *ent*-**59**. The entire sequence along with yields is shown below (Scheme 8).
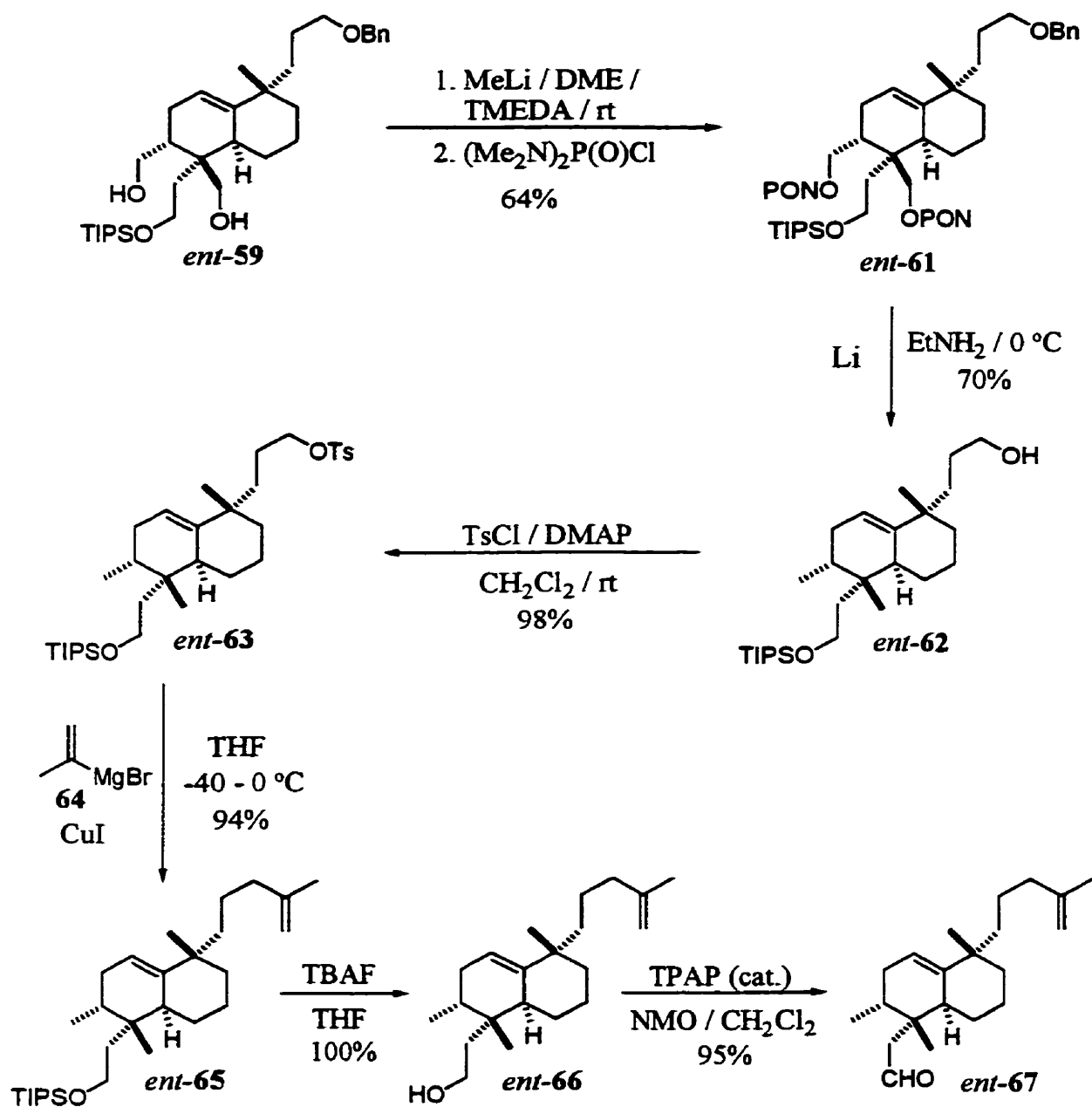
Scheme 8

2.13 Completion of the second generation synthesis of dysidiolide

The total synthesis of natural dysidiolide (*ent-1*) was now nearing completion. The last crucial step remaining was the addition of siloxyfuran reagent 72 to relay aldehyde *ent-67*. Attempts were also made at this stage to increase the selectivity of this addition. Several trials using a chiral ligand such as 86 (or its enantiomer) did not alter the stereoselectivity of the reaction.
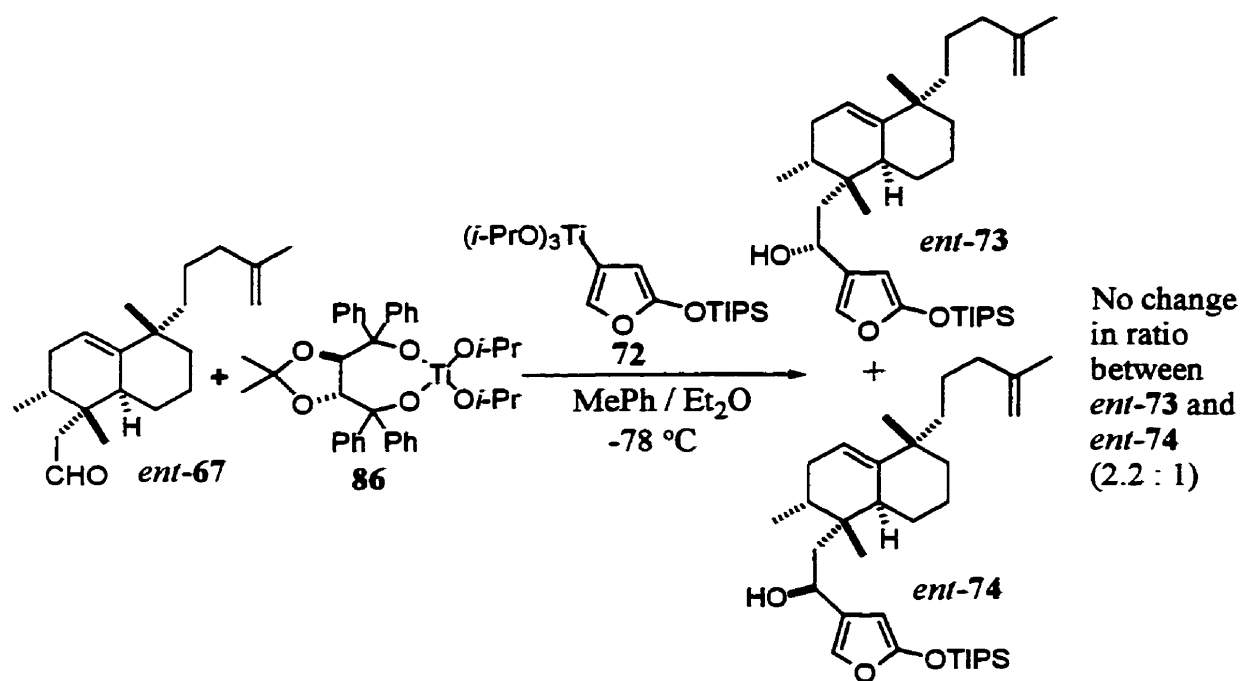
Our last attempt was to take advantage of the chiral environment surrounding the aldehyde group of *ent-67*. It was hoped that complexation of the aldehyde group to a bulky Lewis acid would give a complex such as *ent-67'* and could influence the prefered conformation and therefore alter the ratio of C4-epimeric products.

For this purpose, the desired Lewis acid was prepared *in situ* according to a literature procedure,[66] cooled to -78 °C, and the aldehyde was added dropwise at this temperature to afford complex *ent*-67'. The titanium reagent 72 was then added dropwise until no more aldehyde could be detected by TLC. Examination of the crude reaction mixture by HPLC revealed a slighly improved ratio (3.65 : 1) in favor of the desired adduct *ent*-73.
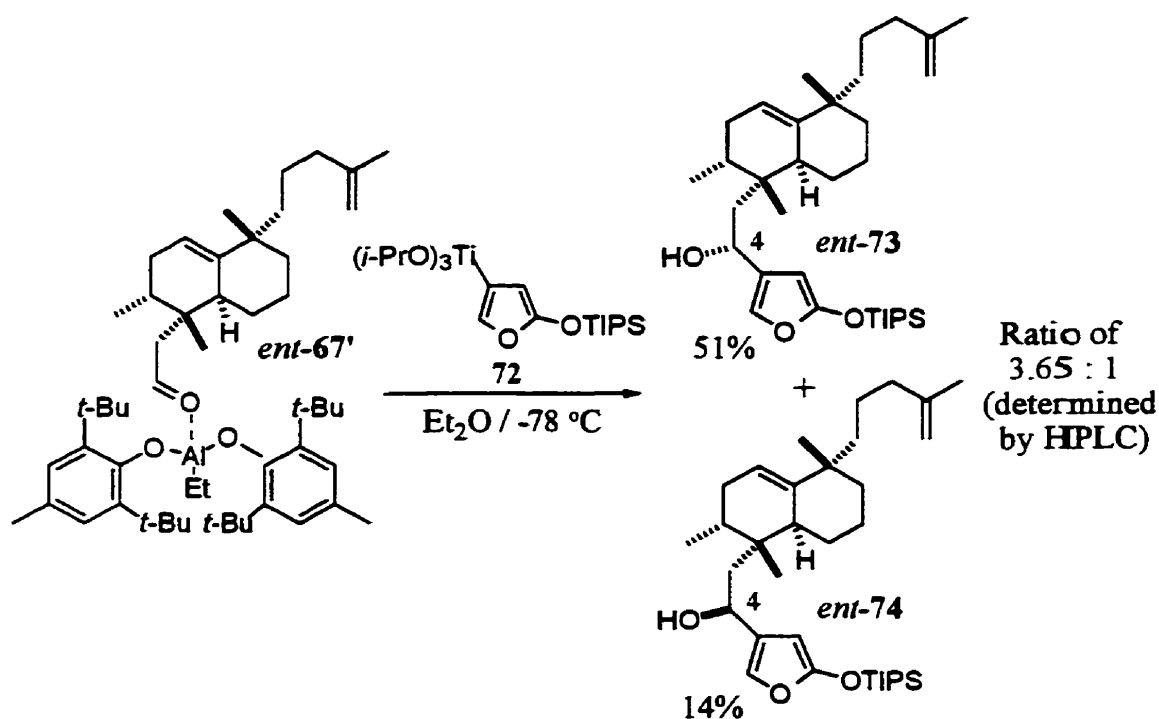
The slightly lower yield of the major adduct (*ent*-73) - from 58 to 51% - is due to the fact that a longer silica gel column was required to separate the decomposed Lewis acid from the reaction mixture, and this resulted in a some decomposition of the siloxyfurans *ent*-73 and *ent*-74.

The siloxyfuran *ent-73* was then oxidized following the protocol well established by our new methodology[7] to afford dysidiolide (*ent-1*) in 95% yield after hydrolysis. The synthetic compound was identical in all respects with an authentic sample of the natural product.
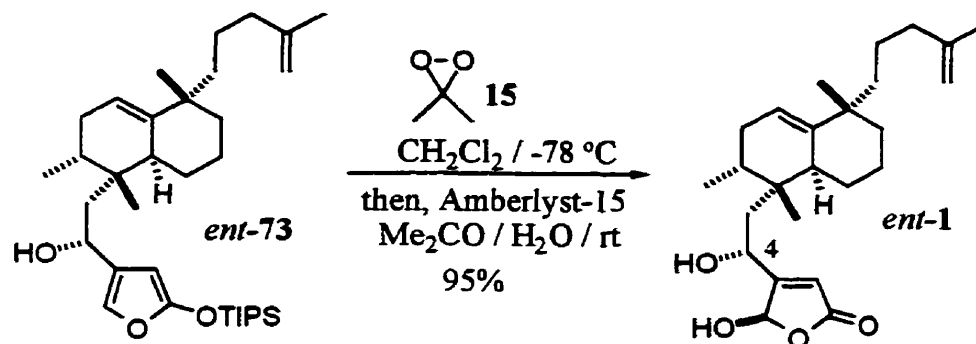
For the purpose of biological screening, siloxyfuran *ent-74* was also oxidized and hydrolysed to afford 4-epi-dysidiolide (4-epi-*ent-1*).
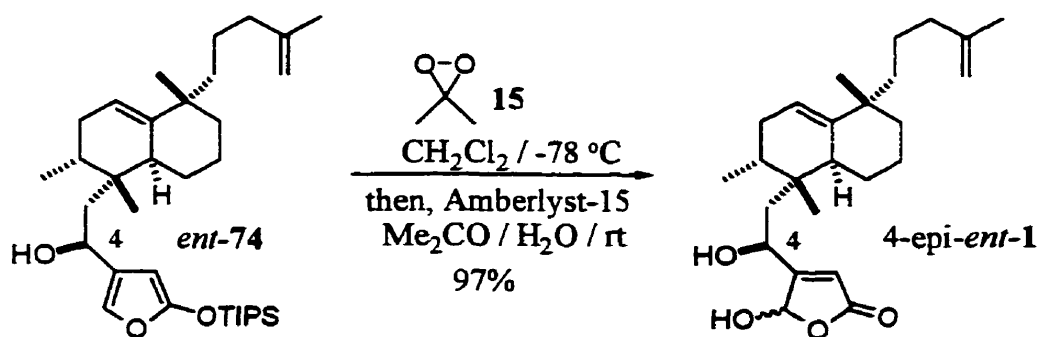
This second generation synthesis thus afforded natural dysidiolide (*ent-1*) in a similar number of steps (longest linear sequence = 15 steps) with an improved overall yield of 9.8% (5.3% for the first generation synthesis).

2.14 Corey and Danishefsky's total syntheses of dysidiolide

A little less than two weeks prior to the publication of our enantioselective synthesis of (+)-dysidiolide (1),[58] E.J. Corey's group from Harvard University published an enantioselective synthesis of (-)-dysidiolide (*ent*-1) (scheme 9).[67]

The Corey synthesis of *ent*-1 is considerably longer since they opted for a completely linear strategy. Their enantioselective synthesis begins with commercially available diketone 87 and requires 28 steps to reach its target.[67]
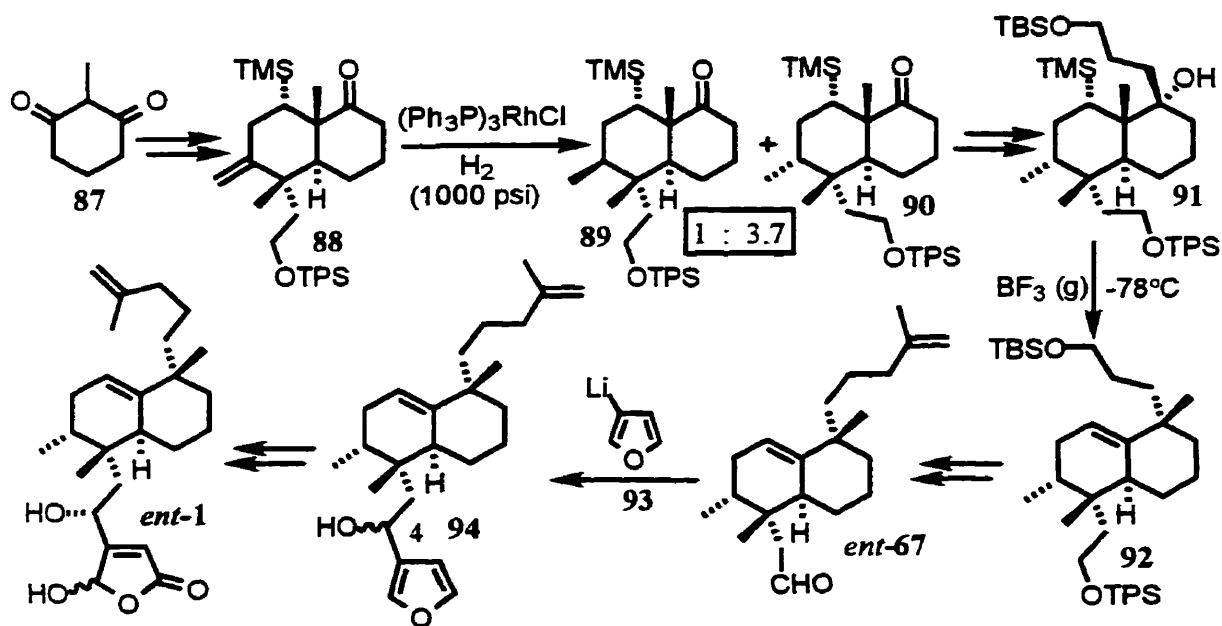
Scheme 9

One problematic step in their synthesis is the hydrogenation of the exocyclic double bond of intermediate **88**. Because the steric environment of the two faces of this double bond are not substantially different, the hydrogenation to the corresponding methyl group gives a 3.7:1 mixture of the two possible diastereoisomers **89** and **90**. Unfortunately, this result could not be improved in their synthesis[67] and so the decalin core of dysidiolide is not constructed in a completely selective fashion. In other words, this synthesis is very long and not entirely stereoselective.

An interesting step in this synthesis is what they call a biomimetic cationic rearrangement of tertiary alcohol **91** to the rearranged decalin structure **92**. Under the action of gaseous BF_3 the tertiary alcohol **91** is converted into a tertiary carbocation. The elimination of the TMS group then facilitates the migration of the adjacent methyl group to the carbocation. This reaction thus completes the assemblage of the decalin core in an elegant fashion.

Addition of 3-lithiofuran (**93**) to aldehyde intermediate *ent*-**67** then yielded a 1:1 mixture of separable C4-epimers (**94**). The wrong isomer was then oxidized to the corresponding ketone and then diastereoselectively reduced to the correct epimer (selectivity = 10:1) using Corey's CBS catalyst. The installation of the γ-hydroxybutenolide ring was accomplished by using singlet oxygen (photooxygenation).

Danishefsky's group also reported a total synthesis of (±)-dysidiolide[28] (Scheme 10) about a month after our enantioselective synthesis. As in our case, Danishefsky used a Diels-Alder strategy to construct the decalin core of dysidiolide. Although Danishefsky's synthesis is convergent (longest linear sequence = 14 steps), it is not enantioselective and therefore provides dysidiolide as a racemic mixture.[28]

Racemic diene (±)-**95** was made from ketone **41** while dienophile **97** was prepared from dioxolane **96**. Danishefsky's construction of the decalin core makes use of a dioxolenium (Gassman) type activated dienophile (**97**). Along with the desired adduct (±)-**98** they isolated another isomer which has not been characterized. This exact selectivity observed in their Diels-Alder reaction has not been disclosed.[28]
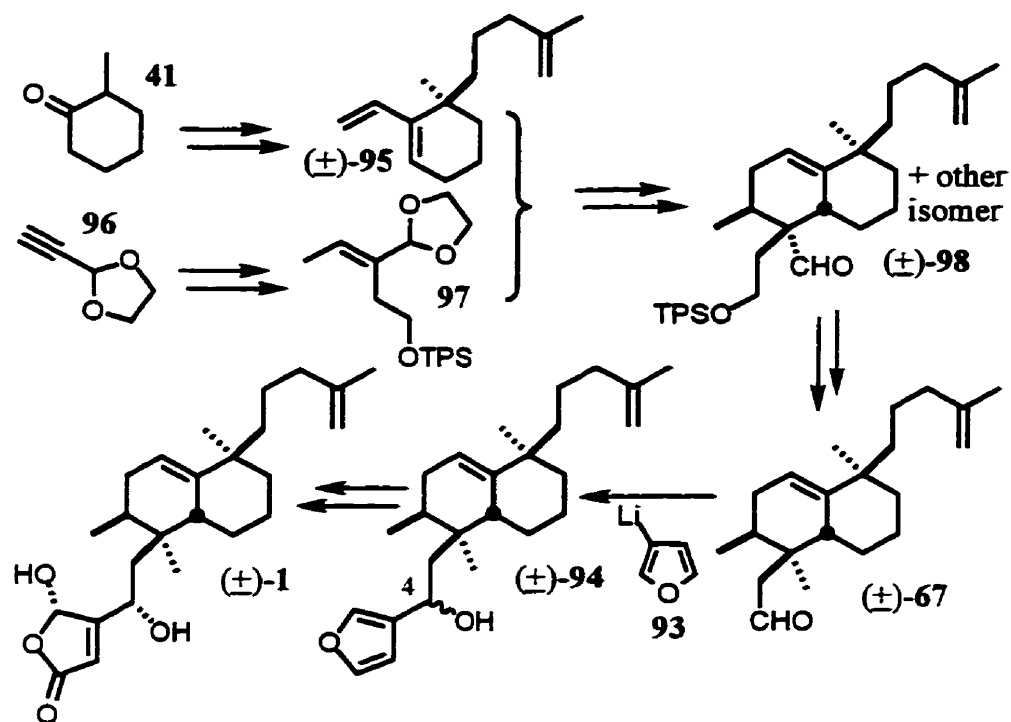
Scheme 10

After Wolff-Kishner reduction of (±)-98 to key intermediate (±)-67 after several other steps, Danishefsky, like Corey, added 3-lithiofuran (93) to the aldehyde intermediate (±)-94 which led to a 1.65:1 ratio in favor of the wrong C4-epimer.

It is interesting to note that for the same reaction Corey reported a 1:1 mixture of C4-epimers. Danishefsky repaired the relative stereochemistry of the wrong epimer by means of the Mitsunobu reaction. Again, Corey claims that the Mitsunobu reaction does not work![67] As in Corey's synthesis, the installation of the γ-hydroxybutenolide moiety was accomplished by photooxygenation with singlet oxygen.[28]

All three syntheses have succeeded in their primary goal which was to find a route that would enable us to synthesize this fascinating natural product in the laboratory. Both our synthesis and Corey's synthesis were successful in establishing the absolute configuration of natural dysidiolide as *ent*-1.

It is worthy of mention that our second generation synthesis is the only one that builds the dysidiolide core in a fully stereocontrolled manner.

CHAPTER 3

INFLAMMATORY PROCESSES

3.1 Introduction

Diseases involving inflammation (such as psoriasis, arthritis, gout, etc) are not usually life threatening but are often very painful and debilitating.[68] Although existing therapeutic agents can provide relief in many cases there is still a need for new and more effective treatments with fewer side effects.

Inflammatory processes are part of normal cellular functions which respond to various physiological or pathological stimuli. To biological organisms, these cellular processes are absolutely essential since they mediate important functions such as immunological responses.

Inflammation is a general term which encompasses various biological processes that are initiated and modulated by a myriad of distinct biological molecules (ex. hormones, prostaglandins, leukotrienes, HETEs, etc). Many of these biomolecules are also linked in other processes such as cellular growth and differentiation, and each of these natural compounds possesses a distinct role and induces specific responses according to the biochemical pathway they target.

In this chapter we will briefly review some of the biological pathways that are relevant to the process of inflammation. By understanding these processes and their role in various disorders, one can develop new drug therapies by targeting key enzymes involved in such biochemical pathways.

3.2 Arachidonic acid (AA) and subsequent enzymatic cascades

Many biologically important compounds involved in the inflammatory process are not stored within the cell, and all derive from a single precursor molecule; arachidonic acid (AA, 99) (Scheme 11).[69] Thus, each different molecule is synthesized via a specific pathway, but all of these molecules are derived from AA. The release (ex. during an inflammation reaction) of any of these bioactive compounds in the living organism is then immediately preceeded by the biosynthesis of the compound in question.[70]

Scheme 11
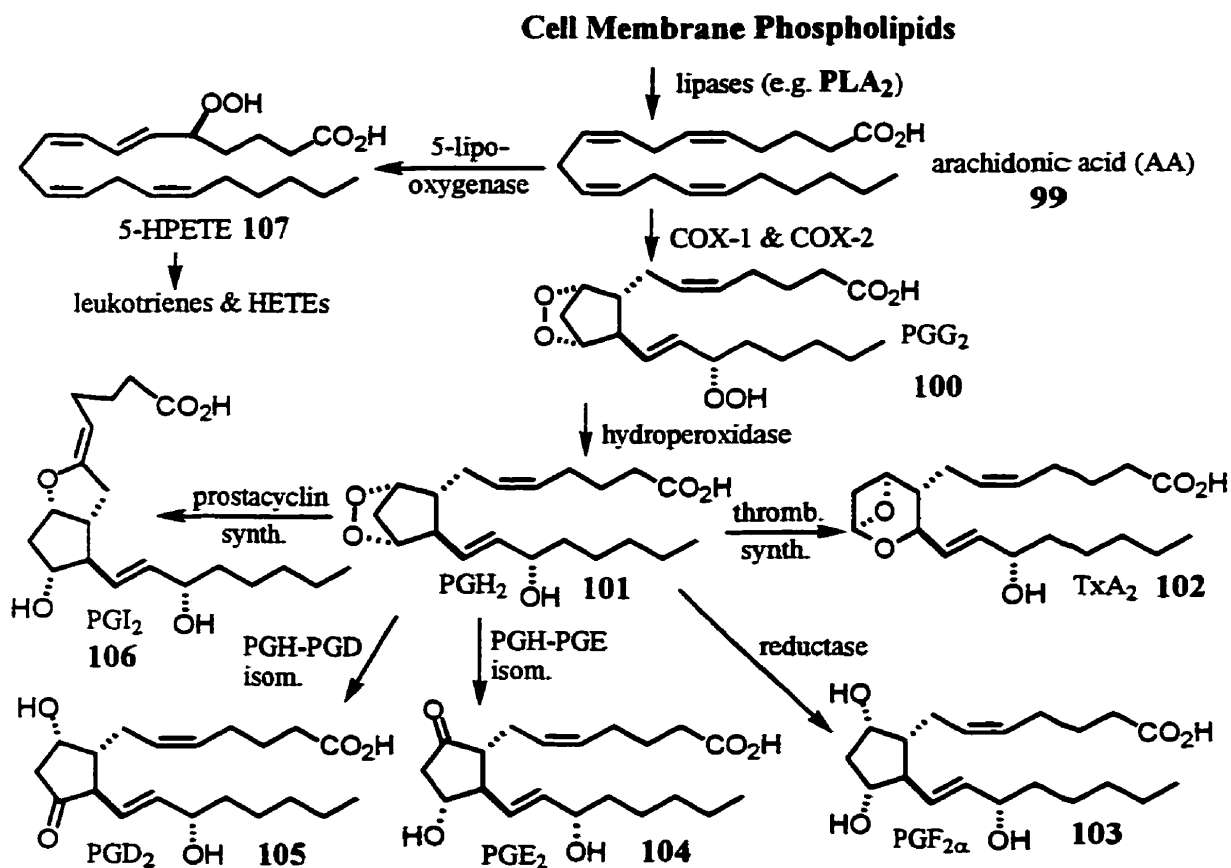
Allylic peroxide PGG_2 (**100**) is synthesized from AA (**99**) through the action of the enzymes COX-1 or COX-2 (cyclooxygenase 1 & 2). PGG_2 can then be reduced by a hydroperoxidase to the corresponding allylic alcohol **101** (PGH_2) and this compound can be transformed to several other substances depending on the enzymatic pathway followed.

Via the action of the thromboxane synthetase, PGH_2 is transformed into thromboxane **102** (TxA_2). Prostanoids **103** ($PGF_{2\alpha}$), **104** (PGE_2), **105** (PGD_2) are also synthesized from **99** through the action of a reductase, a PGH-PGE isomerase or a PGH-PGD isomerase, respectively. The prostacyclin **106** (PGI_2) is also synthesized from the same compound **101** by the prostacyclin synthetase.

Through a different biosynthetic pathway, AA (**99**) is transformed into 5-HPETE (**107**) by the action of the 5-lipooxygenase. This allylic hydroperoxide **107** is the key precursor of a large number of other important bioactive substances called leukotrienes and HETEs.

Following what we have just discussed, one can easily understand the vital role of arachidonic acid (**99**) in this important biochemical pathway. AA truly acts as the chief building block in the synthesis of many biologically important molecules.

One important observation is that cellular levels of AA are extremely low, so low that they cannot account for the biosynthesis of any of the subsequent molecules.[69] It has been discovered that arachidonic acid in cells is present in an esterified form, directly attached to the cell membrane phospholipids.

The release of AA from these phospholipids is therefore the first rate limiting step in the biosynthesis of any of these molecules. [70]

This release of AA from the cell membrane phospholipids is accomplished by a specific enzyme, called phospholipase A_2 (PLA_2).[69] Many PLA_2 enzymes exist and vary in terms of specific function, localization, regulation, etc...[71] However, in all cases, PLA_2 performs the hydrolytic cleavage of a molecule of AA from the cell membrane phospholipids, which is the initial and rate-limiting step in the biosynthesis of all the pro-inflammatory compounds mentioned earlier.[70] This strategic position of this enzyme in the biological pathway makes PLA_2 an eminent therapeutic target.

It has been rightfully stated that, when dealing with a particular inflammatory disease, it is best to select as inhibitory targets enzymes that function late in the biosynthetic pathway rather than early. The rationale behind this statement is that inhibition late in the biosynthetic pathway will avoid inhibiting the synthesis of other important biologically active molecules that are beneficial and not involved in the pathology. This approach is essential when dealing with pathologies involving molecules or enzymes late in the enzymatic cascade, but some pathologies (inflammatory and auto-immune diseases) have been associated with abnormally high concentrations of arachidonic acid and elevated levels of PLA_2 activity[72]. In these cases, selective PLA_2 inhibitors have excellent potential as new drugs.

Also worthy of mention is the fact that, as yet, not all the roles and functions of PLA_2 are understood. It has been very recently proposed that PLA_2 might be involved in the signaling pathway of apoptosis of human umbilical endothelial cells (HUVEC), and that the metabolism of AA might play an important role in this particular apoptotic signal-tranduction system.[73]

Even more intriguing was the recent discovery that PLA_2 activity and AA levels are increased in human colorectal cancers, therefore causing an increase in prostaglandin synthesis and tumor promotion.[74]

As one can see, much research still needs to be carried out. Nonetheless, recent evidence suggests PLA_2 inhibitors have outstanding potential as novel therapeutic agents.

3.3 Non steroidal anti-inflammatory drugs (NSAIDs)

Non steroidal anti-inflammatory drugs (NSAIDs) are drugs that reduce the pain and swelling caused by inflammation diseases. Unfortunately, these NSAIDs can produce side effects in patients who use them, but the side effects caused by these drugs are usually milder than those caused by corticosteroids, which are also used for the treatment of inflammation diseases.[75]

Luffariellolide (2) is a sesterterpene that was isolated from the Palauan sponge *Luffariella* sp. and whose structure was reported in 1987.[3] This natural product is an inhibitor of the important PLA_2 enzyme.
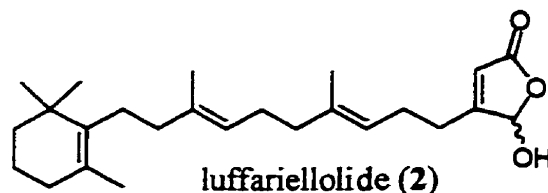
luffariellolide (2)

It is worthy of note that, not only did luffariellolide inhibit hydrolysis of phosphatidyl choline by bee venom PLA_2 ($IC_{50} = 0.23$mM) *in vitro*, but this marine natural product also inhibited induced inflammation *in vivo* in mice treated with phorbol myristate acetate (PMA).[3,76]

Luffariellolide (2) is slightly less potent than manoalide (3), another marine natural product that inhibits PLA_2 and has undergone phase II clinical trials for the treatment of psoriasis.[6] However, luffariellolide is distinguished from manoalide in that it is a reversible inhibitor of PLA_2,[3] and therefore an important new lead since reversible inhibitors are preferred over irreversible ones.[77]
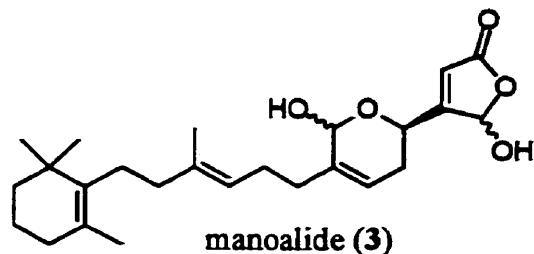
manoalide (3)

As mentionned in the general introduction, the presence of a highly oxygenated γ-hydroxybutenolide in molecules often confers interesting biological properties. In fact, it has been shown that the biological activity of manoalide is due, at least in part, to the γ-hydroxybutenolide moiety.

Luffariellolide and manoalide's similar mechanisms of action have been studied in some detail (Fig. 6). It has been shown that a lysine residue of PLA$_2$ reacts with the aldehyde group of the luffariellolide acyclic isomer 108.[78] A condensation reaction takes place between both functional groups to form a Shiff's base (109) that can then close to the corresponding aminobutenolide 110.[78] This process is reversible and explains the reversible inhibition of PLA$_2$ by luffariellolide.[3]

Manoalide on the other hand possesses two masked aldehyde functional groups, one originating from the γ-hydroxybutenolide moiety and the other from the lactol functional group. These two aldehyde groups can react in a similar fashion to afford the double adduct 111. The irreversible nature of the inhibition of PLA$_2$ by manoalide may reflect the greater stability of complex 111.

Several mechanistic studies on the inhibition of PLA$_2$ by manoalide have been published.[78] These studies are also pertinent to the luffariellolide mechanism of biological action since both compounds react similarly with PLA$_2$.

Results obtained to date indicate that the lysine residues of PLA$_2$ that react with manoalide are not part of the catalytic site.[79] The condensation reaction of manoalide with PLA$_2$ seems to be multiple in the sense that several manoalide molecules are covalently bound to PLA$_2$.

Inhibition of PLA$_2$ with luffariellolide and manoalide
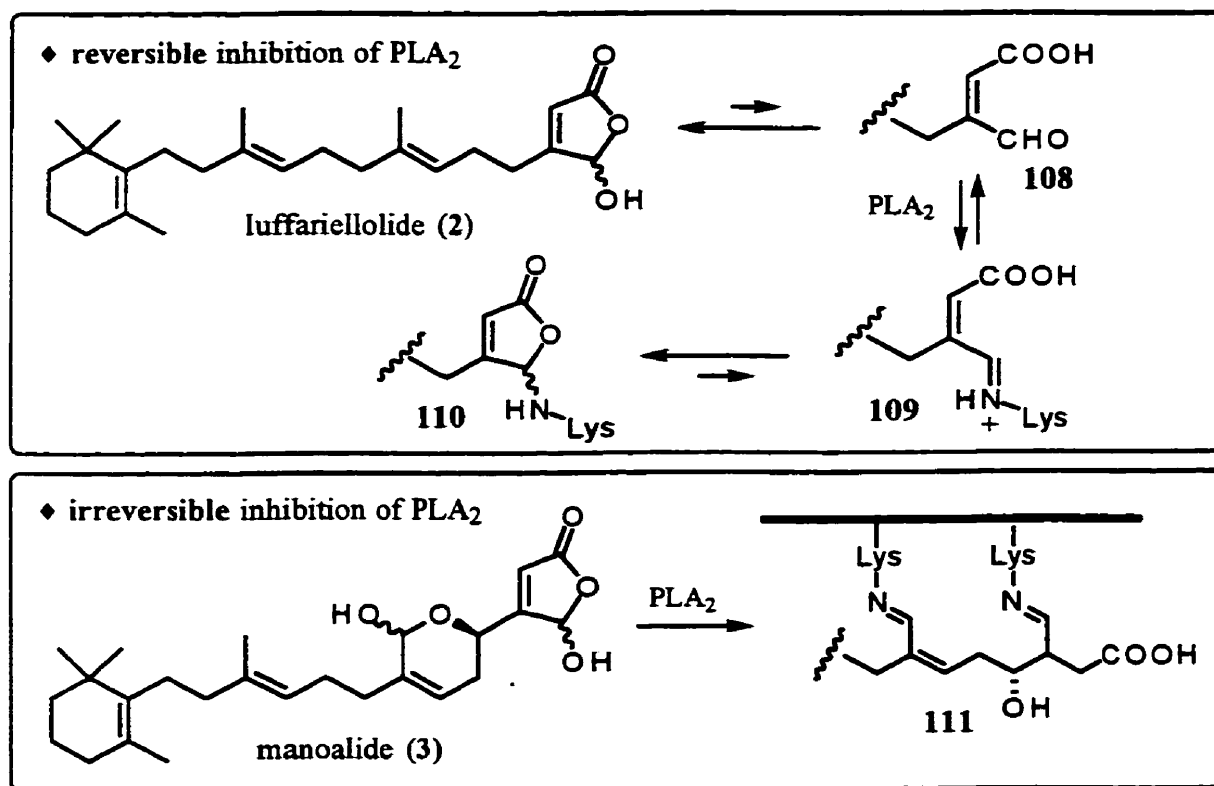
Fig. 6

It has been proposed that this binding might (i) alter the natural conformation of PLA$_2$ and disrupt its normal functions, (ii) block the entry of substrates to PLA$_2$'s catalytic site by virtue of manoalide's large lipophilic portion, or (iii) prevent PLA$_2$ activity by lodging the lipophilic moiety of manoalide into the hydrophobic region of the catalytic site.[80] It is believed that luffariellolide's biological activity would be the result of similar interactions; the only difference being that these would be reversible.[78]

In view of the key role of PLA$_2$ in the biosynthesis of a wide array of bioactive molecules, its inhibition by natural products such as luffariellolide (2) and manoalide (3) provides an interesting therapeutical avenue to modulate the activity of this crucial enzyme when pathology or deregulation occurs.

CHAPTER 4

NEW METHODOLOGY FOR CONSTRUCTING β-HOMOALLYL-SUBSTITUTED BUTENOLIDES AND γ-HYDROXYBUTENOLIDES. TOTAL SYNTHESIS OF LUFFARIELLOLIDE

4.1 Introduction

Although luffariellolide (2) is a fairly simple molecule, it still represents a challenge to the synthetic chemist, especially when brevity and efficiency are the main preoccupations. Structurally, luffariellolide is characterized by the β-homoallyl-γ-hydroxybutenolide unit which is also encountered in many other natural products,[4] nearly all of them of marine origin. To date, no synthesis of luffariellolide has been reported in the literature. Nor does there exist an ideal means for making β-homoallylbutenolides bearing a hydroxyl or hydrogen at the γ-position. The latter lactones are also found in nature, as exemplified by the antifeedent marine natural product lintenone (112).[81]

luffarielolide (2)   lintenone (112)
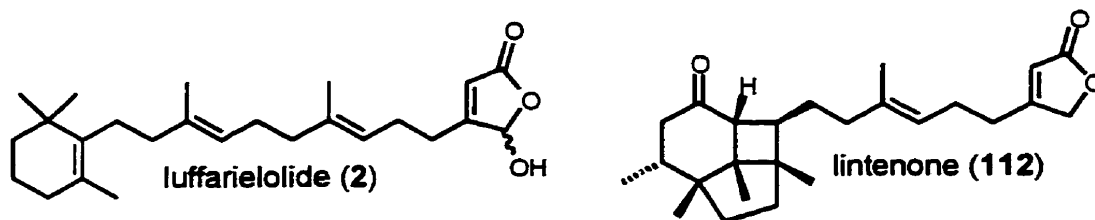
The persisting difficulty in constructing β-homoallyl-γ-hydroxybutenolides is illustrated by a recently published total synthesis of dictyodendrillin-B (**113**) (Scheme 12), a simpler analogue of luffariellolide isolated from the marine sponge *Dictyodendrilla*.[82]

Unlike luffariellolide, dictyodendrillin-B contains a very simple lipophylic side-chain (geranyl) that is found in many commercially available compounds (e.g. geranyl chloride, bromide, etc). Yet, the longest linear route involves seven steps while two parallel sequences were required to reach dictyodendrillin-B from a separable mixture of isomeric key intermediates (**119, 120 and 121, 122**).
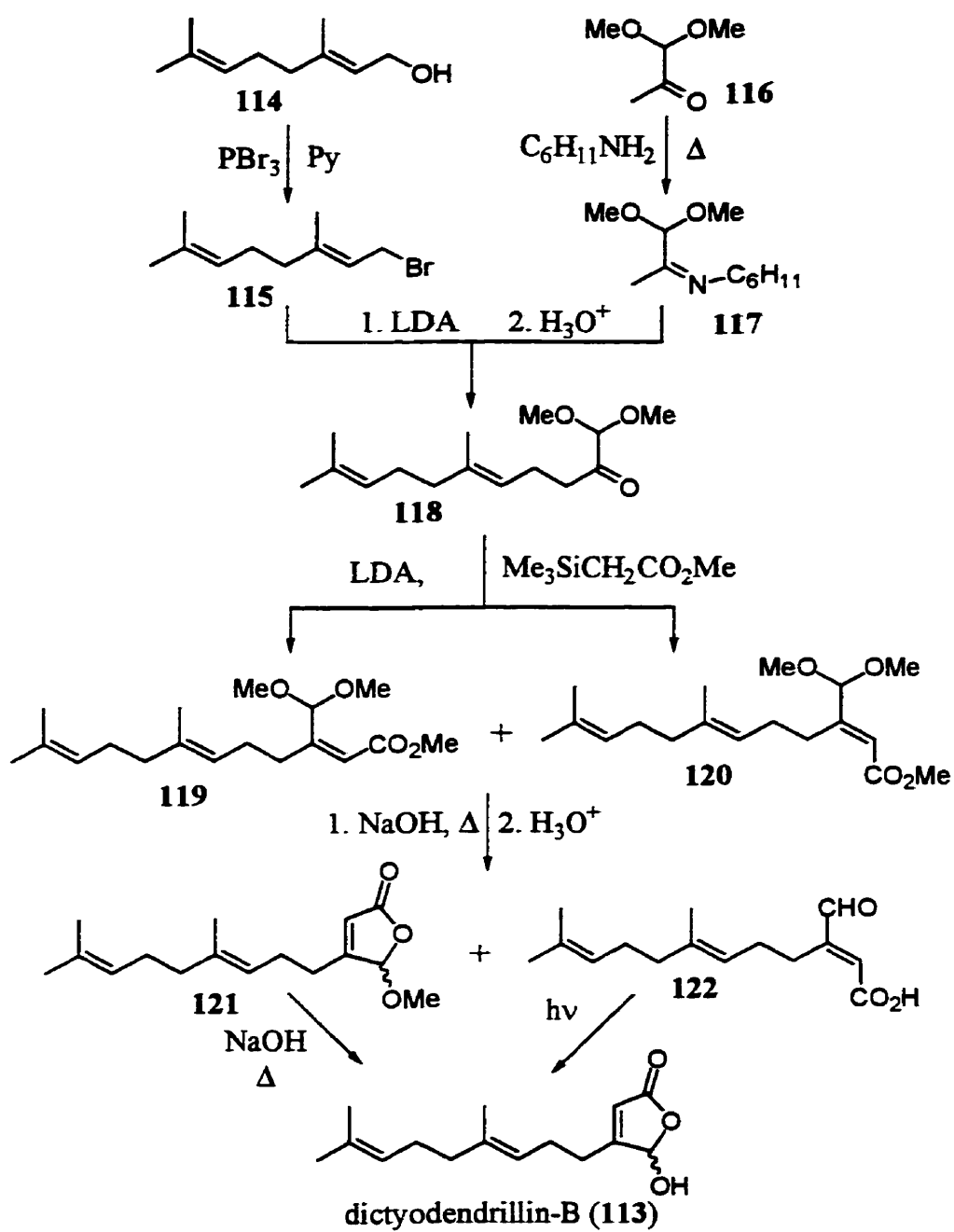
Scheme 12

Among existing methods for making non-hydroxylated β-homoallylbutenolides, the most general is Weiler's protocol.[83] This methodology was applied in the synthesis of the marine natural product mokupalide (123) (Scheme 13)[83] and involves the coupling of sulphone 125 with allylic bromide 124 to afford 126, with subsequent removal of the sulphone group as illustrated by the end-game of the synthesis of mokupalide. Although this method is nearly 20-years old, it is still the most attractive because it is convergent and makes use of readily available allylic bromides as substrates.
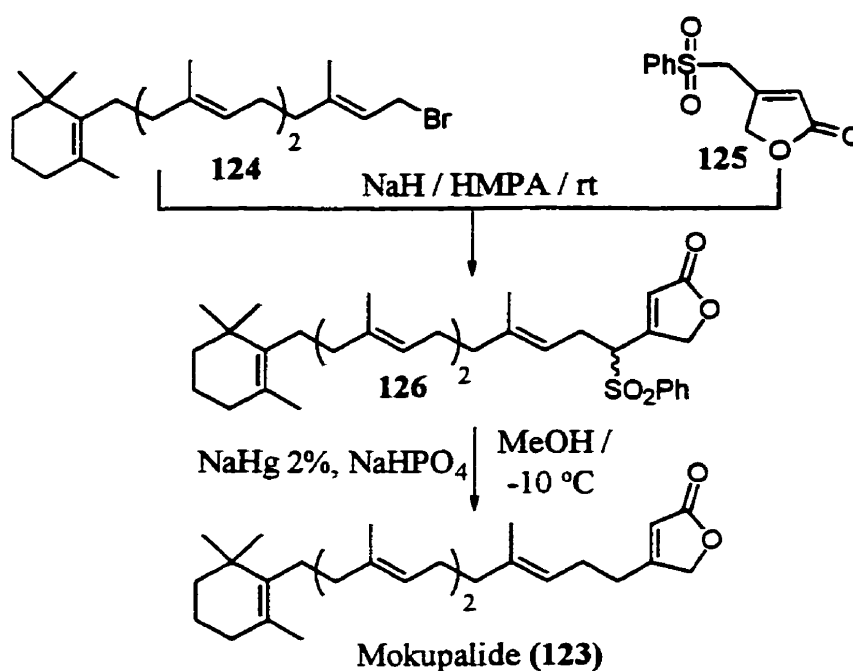
Scheme 13

Our group has been interested for several years in the development of a unified general method for the synthesis of β-homoallyl-γ-hydroxybutenolides and β-homoallylbutenolides, as well as in applying such methology to the first synthesis of luffariellolide.[84,85]

The longstanding plan, retrosynthetically presented in Scheme 14, has been to produce either **127** or **128** at will from the same precursor **129** which should be accessible by sp^3-sp^3 cross-coupling of an allylic halide with a new siloxyfuran building block **131**.
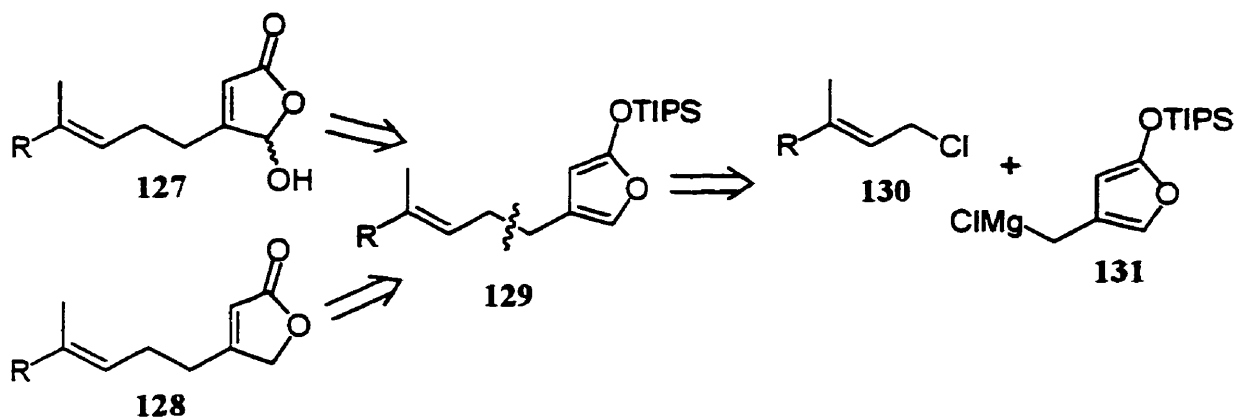
Scheme 14

Tanis had reported related sp^3-sp^3 cross-coupling reactions between β-furylmethyl Grignard reagent (**133**) and allylic chlorides[86] as exemplified by the reaction of geranyl chloride (**132**) with **133**. Nonetheless, previous attempts by François Maltais of this group to reproduce the literature preparation of **134** proved unsuccessful.[85]
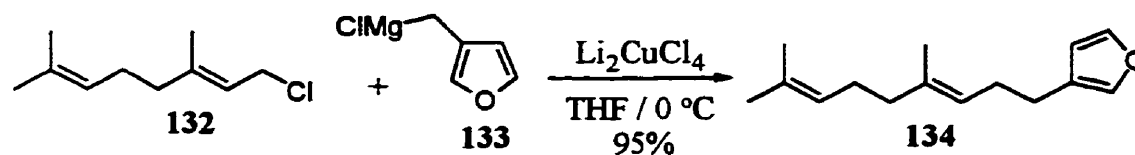
Efforts to couple siloxyfuran **131** with geranyl chloride are also described in the Ph.D. thesis of Maltais, but again, the desired cross-coupling could not be achieved.[85] The problem was suspected to be the formation of the Grignard reagents (**133** and **131**).[85]

Although the original plan failed to materialize, a synthesis of luffariellolide was eventually achieved by using Weiler's method.[85,83] The requisite allylic bromide (**139**) was prepared by conventional methods from the known sulfone **135**[87] and allylic bromide **136**[88] (Scheme 15).

n-BuLi / THF / HMPA / -78 °C 81%

Li / EtNH$_2$ Et$_2$O / -78 °C
76%
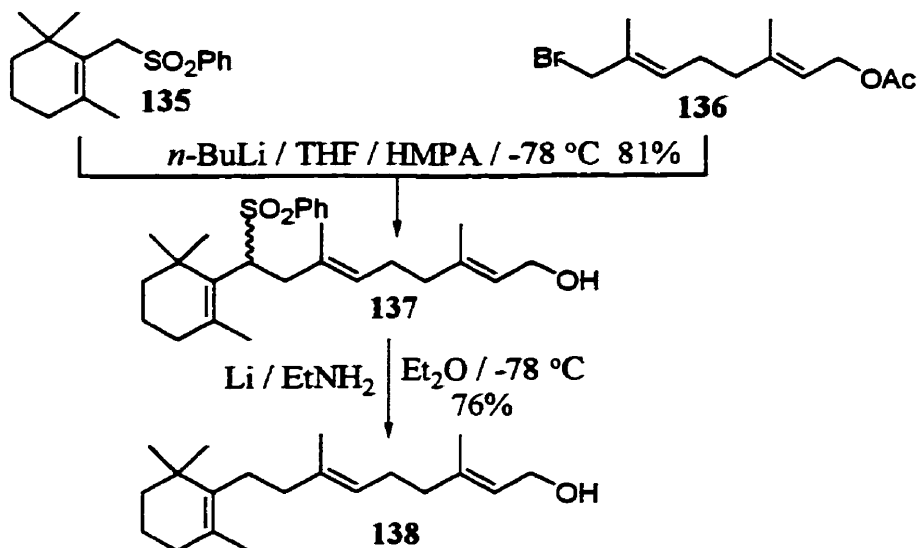
Scheme 15

Weiler coupling of bromide **139** with sulphone **125** followed by desulphonylation delivered homoallylbutenolide **141**, albeit in modest yield (Scheme 16). This butenolide was subsequently silylated (**142**) and oxyfunctionalized to give luffariellolide by straightforward application of the Boukouvalas-Lachance method.[7,84]
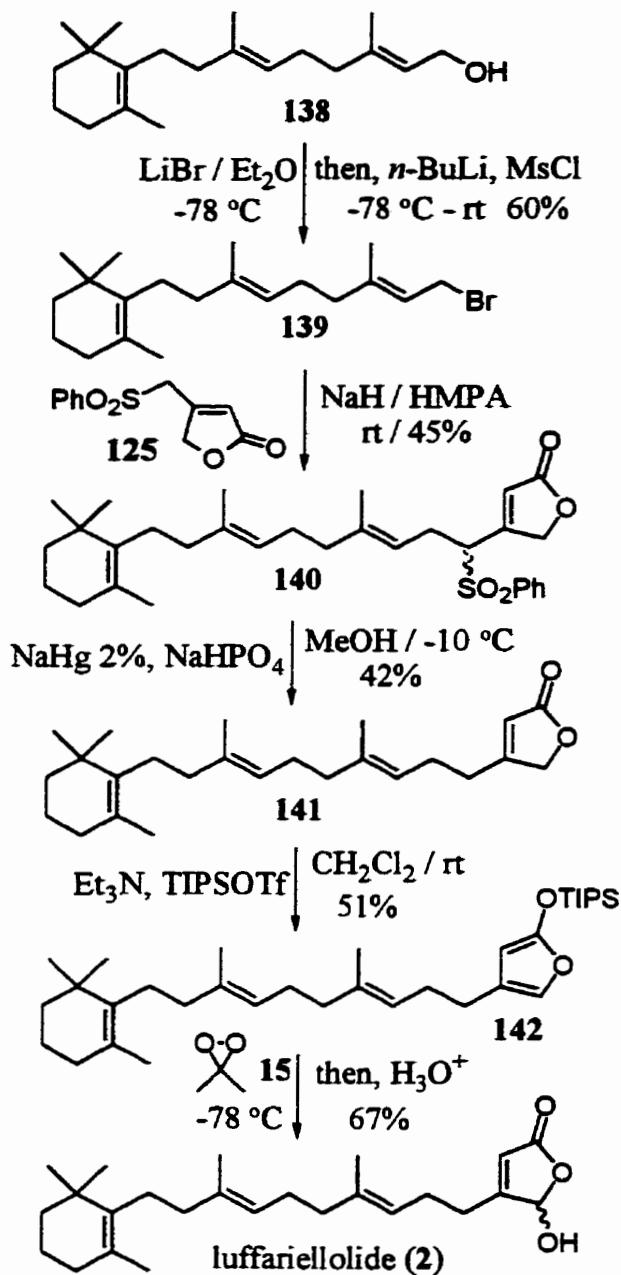
Scheme 16

Unfortunately, this synthesis was not as direct and convergent as originally planned (cf. Scheme 14). The yield of luffariellolide from allylic alcohol **138** is 3.9% (5 steps).[85]

4.2 Successful sp³-sp³ cross-coupling between siloxyfurylmethyl magnesium chloride (131) and allylic chlorides

Meanwhile, several articles - not only by Tanis[86] but also by other authors,[89] as well as Steeves Potvin (M.Sc. memoire,[90] Prof. Canonne's group, Université Laval) describe successful applications of Tanis-type coupling in organic synthesis. We therefore decided to re-examine the feasibility of our original plan (Scheme 14) with a view to applying this technology to the synthesis of luffariellolide.

This meant that a new organometallic siloxyfuran 131 (Scheme 14) would have to be synthesized in order to attempt this crucial cross-coupling reaction with an allylic halide. The requisite precursor 149 had previously been prepared by Nicolas Lachance in this laboratory.[84] The route begins with commercially available 1,3-dihydroxyacetone dimer 143 which was transformed in 3 steps to allylic alcohol 147[91] (Scheme 17) and subsequently to the corresonding allylic chloride 148 using Lalonde's procedure.[92]

Scheme 17

Next, lactone **148** was transformed to the corresponding siloxyfuran **149** in 74% yield.[84] At this point, the crucial formation of the corresponding Grignard reagent **131** was investigated under a variety of conditions and methods for activating magnesium turnings.

It soon became apparent that proper activation of the magnesium turnings was the key to success. Activation of the metal was accomplished by washing successively with aq. 10% HCl, water, acetone and ether. The activated magnesium turnings were then dried in a dessicator under vacuum using anhydrous phosphorus pentoxide as drying agent. The magnesium turnings were heated using a flame with rapid agitation under an atmosphere of dry nitrogen. Once cooled, the magnesium metal was then further activated by adding 1,2-dibromoethane and dry THF. The resulting suspension was heated to reflux with stirring for approximately 15 minutes. During this time, evolution of a gas (ethylene) from the surface of the metal was observed. The activation of magnesium was confirmed by the appearance of a black surface on the metal turnings. The THF was then cannuled out, replaced with dry THF, and the reaction mixture brought to 0 °C. The allylic chloride **149** was then added neat in one portion and the reaction mixture stirrred for one hour, during which time all of the allylic chloride was consumed as judged by TLC.

To the *in situ* generated reagent **131**, allylic chloride **150** was added neat and in one portion, followed immediately by a catalytic amount (5 mol %) of Kochi's salt (Li_2CuCl_4).[86] After 15 minutes, extractive work-up provided siloxyfuran **151** which was submitted to the next step without purification.

Hydrolysis of **151** cleanly afforded butenolide **152** in 84% yield after purification on silica gel. On the other hand, dimethyldioxirane oxidation and subsequent hydrolysis in one-pot fashion[3] afforded the γ-hydroxybutenolide **153** in 75% yield (2 steps) after chromatography. Therefore, sp^3-sp^3 cross-coupling proceeded with at least 84% efficiency.

The success of this copper salt catalyzed cross-coupling reaction opens the door to the total synthesis of luffariellolide (**2**) and many other bioactive natural products containing a butenolide or γ-hydroxybutenolide unit.

4.3   Efficient synthesis of luffariellolide

Alcohol **138**, an intermediate of the initial synthesis of luffariellolide, was transformed to the corresponding allylic chloride **154** in 77% yield using Stork's protocol.[93]
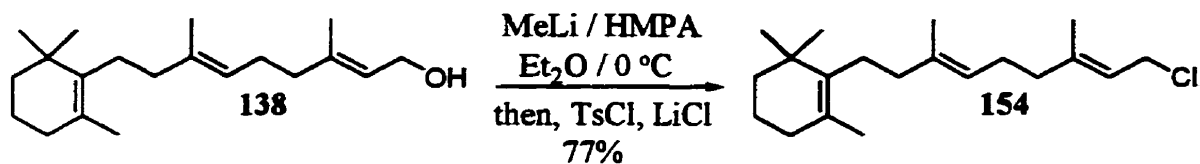
With allylic chloride **154** in hand, the stage was now set for the crucial sp^3-sp^3 cross-coupling with our new organometallic building block **131**.

Thus, generation of Grignard **131** from allylic chloride **149** followed by reaction with chloride **154** in the presence of a catalytic amount of Kochi's salt afforded the desired siloxyfuran **142**.
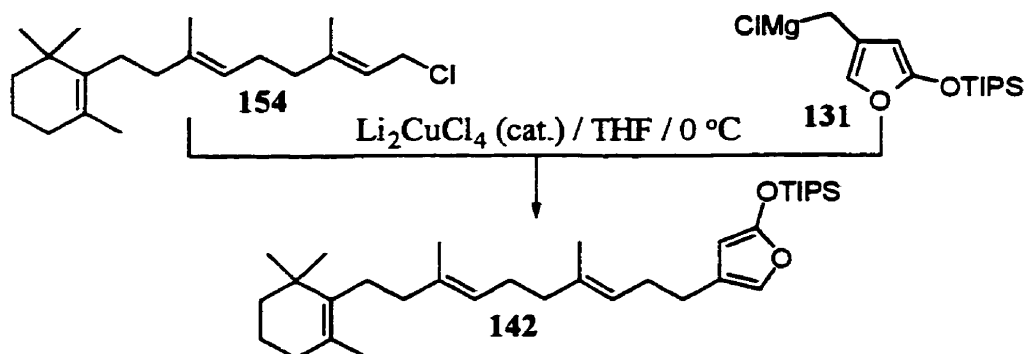
This siloxyfuran was directly submitted to dimethyldioxirane oxidation at -78 °C to afford, after hydrolysis, luffariellolide in 74% yield (2 steps).

142

15

CH$_2$Cl$_2$ / -78 °C

then, Amberlyst-15
Me$_2$CO / H$_2$O / rt

74% (2 steps)
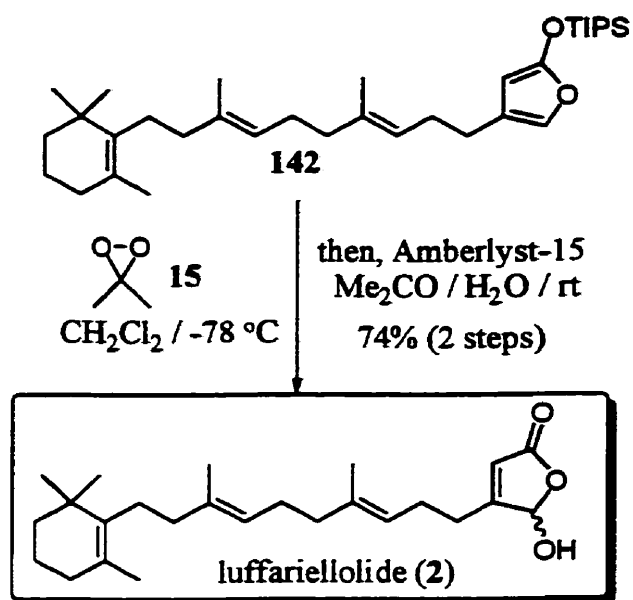
luffariellolide (2)

The so obtained material was identical with the natural product in all respects (IR, ^{1}H & ^{13}C NMR). The present route to luffariellolide is not only shorter but far more efficient than the previous approach outlined in Schemes 14 and 15. It involves only three steps from alcohol **138** (instead of 6 steps) and delivers the natural product in 57% yield, as compared to 3.9% previously.

CONCLUSION

The novel biological activity of dysidiolide and its therapeutical potential have been discussed. Our first generation enantioselective synthesis of 1 established the absolute configuration of natural dysidiolide as *ent*-1 (Scheme 18).[58] The route is convergent and the overall yield of 1 is 5.3% (15 steps).[58] Although this synthesis was highly competitive with those of E.J. Corey[67] and S.J. Danishefsky,[28] we have developed an even more efficient second-generation synthesis of natural dysidiolide.
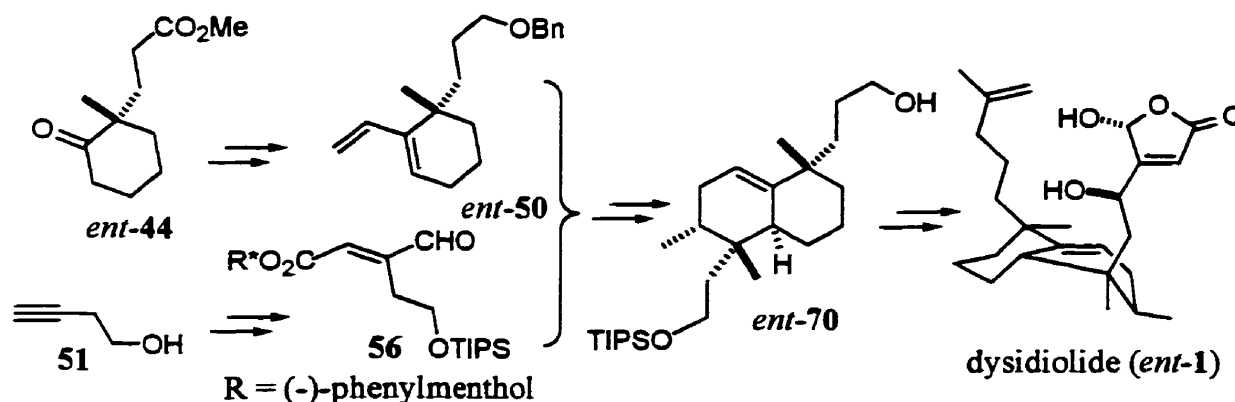
Scheme 18

This new synthesis allows for the first time the fully stereocontrolled construction of the dysidiolide core. It is also highly efficient by any standards delivering dysidiolide in 15 steps and 9.8% overall yield from commercial chemicals. Moreover, the key intermediate *ent*-62 -which contains two differentiated alcohol groups- provides an excellent scaffold for the generation of a wide variety of novel dysidiolide analogs for SAR (Structure-Activity Relationship) studies.

After a brief review on inflammation, we have described a convergent and efficient total synthesis of luffariellolide (2) (Scheme 19), an *in vivo* potent anti-infammatory agent and reversible inhibitor of phosholipase A_2 (PLA$_2$). The yield of luffariellolide from the readily accessible alcohol **138** is 57% (3 steps) thereby a 15-fold improvement over a previous synthesis from the same alcohol (3.9% yield, 5 steps).[85]
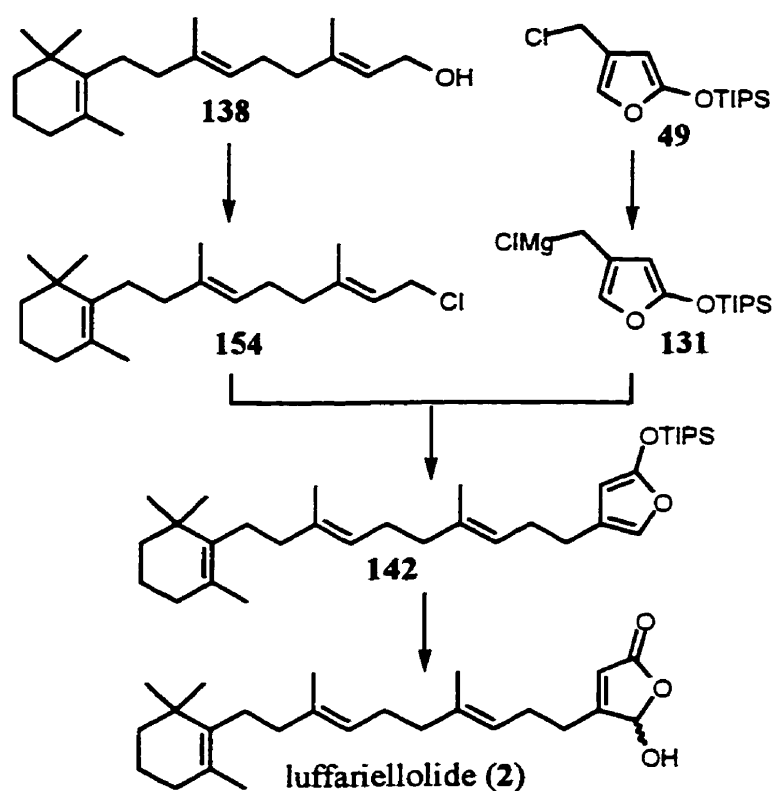
Scheme 19

This improved synthesis also serves to demonstrate the power of custom-designed methodology for constructing β-homoallyl-γ-hydroxybutenolides and β-homoallyl-butenolides from the same siloxyfuran building block via sp^3-sp^3 cross-coupling (Scheme 20).
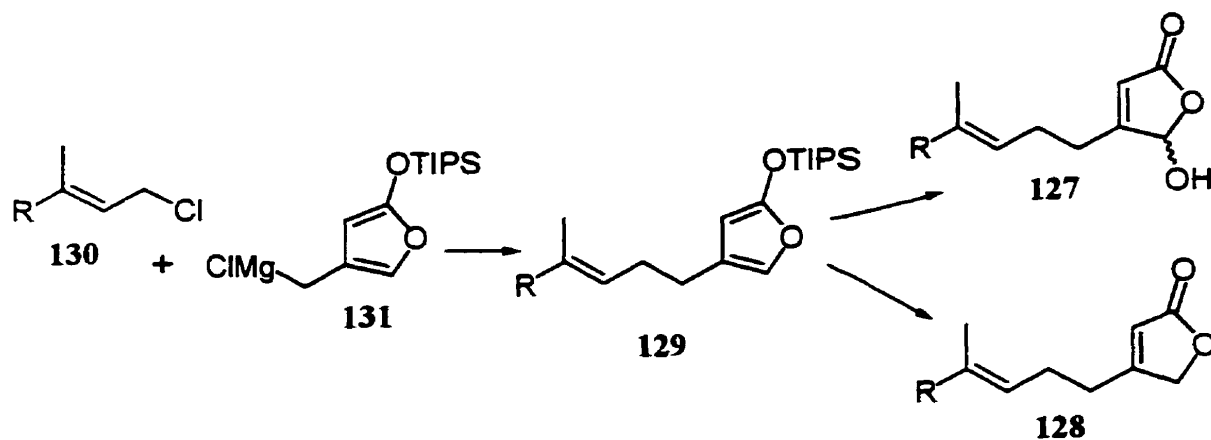
Scheme 20

This methodology should readily provide access to many bioactive natural products and analogues thereof. New applications are under study in our group.

EXPERIMENTAL PART

General Remarks

The following procedures were used unless otherwise noted. Oxygen- and moisture-sensitive reactions were carried out in flame-dried glassware sealed under a positive pressure of dry nitrogen. Moisture-sensitive liquids and solutions as well as anhydrous solvents were transfered by syringe or cannula through rubber septa.

Unless noted otherwise, all commercial reagents, except solvents, were used as received. THF was freshly distilled under argon from sodium-potassium amalgam using benzophenone as an indicator. Dry dichloromethane was distilled from CaH_2 under argon. Anhydrous Et_2O and DME were distilled from $LiAlH_4$ under argon.

NMR spectra were recorded on a Bruker AC-300 spectrometer (^1H at 300 MHz, ^{13}C at 75 MHz) or a Varian Unity-500 (^1H at 500 MHz, ^{13}C at 125 MHz). IR spectra were recorded on a PE-781 spectrometer. High resolution mass spectroscopy (HRMS) was performed by Mr. Gaston Boulay at the Mass Spectroscopy Laboratory, University of Sherbrooke. Elemental analyses were carried out at the Department of Chemistry, University of Montreal. TLC was performed on Merck Kiesegel 60 plates. Flash chromatography was performed on Kiesegel 60 (230-400 mesh) silica gel using redistilled solvents as eluents. HPLC analyses were performed on a Waters system equipped with a 600E solvent delivery system, a 996 photodiode array detector and a UV detector. A Nova-Pak® C_{18} column (3.9 × 150 mm, 4 μm) was employed.

CHAPTER 1

TOTAL SYNTHESIS OF DYSIOLIDE

1.1 Preparation of chiral diene *ent*-50

Methyl 3-(1-methyl-2-oxocyclohexyl)propionate *ent*-44
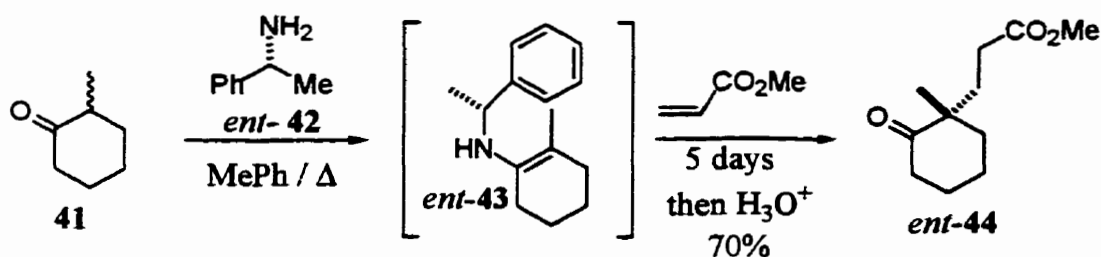
To a solution of (R)-(+)-α-methylbenzylamine (*ent*-42; 24.50 g, 202.17 mmol) in toluene (200 ml) was added 2-methylcyclohexanone (41; 22.67 g, 202.17 mmol) in a Dean-Stark water separation apparatus and the reaction mixture was refluxed overnight under a nitrogen atmosphere. After most of the toluene was distilled out, methyl acrylate (21.80 mL, 242.60 mmol) was added and the mixture was stirred at rt for five days. Hydrolysis of the resulting imine was done by addition of AcOH/H$_2$O (50 mL, 10:1) and stirring for 2 hours. Water was added, followed by solid NaCl, and the mixture was extracted with hexanes (3 x 120 mL). The combined extracts were washed with aq. 10% HCl (60 mL), aq. sat. Na$_2$CO$_3$ (60 mL), brine (60 mL), dried (MgSO$_4$), and concentrated *in vacuo*. Distillation of the resulting oil under reduced pressure (147-152 °C/3.1 mm Hg) afforded *ent*-44 as a colorless oil (28.20 g, 70%). TLC R$_f$ = 0.23 (EtOAc/hexanes, 1/9); [α]$^{26}_D$ -34.9° (*c* = 3.07, EtOH); IR (film) υ$_{max}$ 2928s, 2859m, 1735s, 1699s, 1433m, 1375m, 1299m, 1194s, 1169s, 1120m, 1094m, 983m cm^{-1}; ^{1}H NMR (300 MHz, CDCl$_3$): δ 3.55 (s, 3H), 2.30-1.46 (m, 12H), 0.96 (d, J = 1.9 Hz, 3H); ^{13}C NMR (75 MHz, CDCl$_3$): δ 214.5, 173.7, 51.4, 47.6, 39.0, 38.4, 32.3, 28.7, 27.2, 22.2, 20.8 ppm.

Enantiomer **44** was prepared in a similar manner from **41** and **42** and exhibited the same spectral data as *ent*-44, and [α]$^{22}_D$ +34.0° (*c* = 3.00, EtOH). The spectral data were in accord with those reported in the literature.[43]

2-(3-Hydroxypropyl)-2-methylcyclohexanol *ent*-45
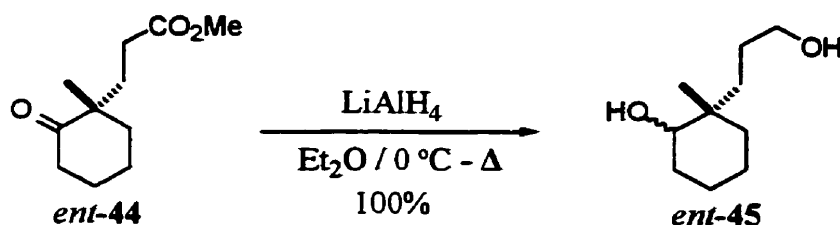
To a suspension of LiAlH$_4$ (2.30 g, 60.60 mmol) in dry Et$_2$O (150 mL) at 0 °C under a nitrogen atmosphere was added dropwise a solution of ester *ent*-44 (6.00 g, 30.30 mmol) in dry Et$_2$O (50 mL). The reaction mixture was warmed to rt and refluxed for one hour. The unreacted LiAlH$_4$ was carefully quenched with water at 0 °C and the salts were filtered off, suspended in methylene chloride (100 mL) and stirred for two hours. This process was repeated four times and the combined extracts were concentrated *in vacuo*. The residue was purified by flash chromatography over silica gel (EtOAc/hexanes, 8:2) to afford diol *ent*-45 (5.21 g, 100%); TLC R$_f$ = 0.41 (EtOAc/hexanes, 1:1); IR (film) υ_{max} 3300br, 2920s, 2855s, 1449m, 1376m, 1143w, 1054br, 982w, 896w cm^{-1}; ^{1}H NMR (300 MHz, CDCl$_3$): δ 3.81 (s, 1H), 3.52 (s, 2H), 3.30 (d, J = 11.2 Hz, 1H), 3.14 (s, 1H), 1.63-0.88 (m, 12H), 0.84 (s, 3H); ^{13}C NMR (75 MHz, CDCl$_3$): δ 75.7, 74.9, 63.2, 63.1, 37.5, 36.8, 35.1, 34.2, 30.1, 29.7, 29.3, 25.9, 25.8, 24.6, 23.6, 22.8, 21.1, 21.0, 17.1 ppm.

Enantiomer 45 was prepared in a similar manner from 44 and exhibited the same spectral data as *ent*-45. The spectral data were in accord with those reported in the literature.[44]

Benzyl monoprotected diol *ent*-46
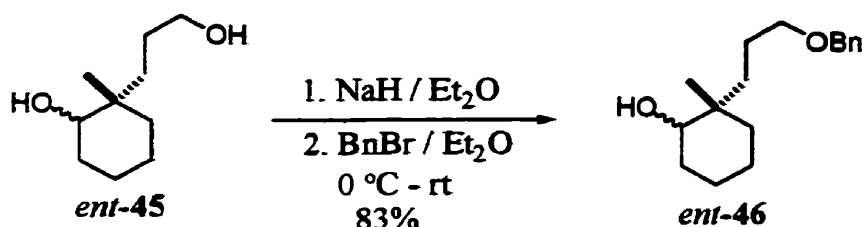
To a stirred suspension of sodium hydride (60% in mineral oil, 0.64 g, 25.30 mmol) in dry Et_2O (70 mL) was added dropwise a solution of diol *ent*-45 (4.35 g, 25.30 mmol) in dry Et_2O (20 mL) at 0 °C under a nitrogen atmosphere. After stirring for two hours at rt, a solution of benzyl bromide (3.2 mL, 26.57 mmol) in THF (30 mL) was added dropwise at 0 °C and the reaction mixture was stirred at rt for another 16 hours. Water (50 mL) was added to dissolve the precipitate and the mixture was diluted with ether (150 mL). The organic layer was separated and the aqueous phase extracted with ether (2 × 50 mL). The combined extracts were washed with brine (2 × 20 mL), dried (MgSO$_4$) and concentrated *in vacuo*. The residue was purified by flash chromatography on silica gel (EtOAc/hexanes, 95:5, 9:1 then 8:2) to furnish alcohol *ent*-46 (mixture of diastereoisomers; 5.55 g, 83%) as a colorless oil: TLC R_f = 0.38 (EtOAc-hexanes, 2:8); IR (film) υ_{max} 3410br, 2928s, 2857s, 1449m, 1356w, 1093br, 731m, 694m cm^{-1}; ^1H NMR (300 MHz, CDCl$_3$): δ 7.30 (m, 5H), 4.51 (4.50) (s, 2H), 3.41 (m, 3H), 1.82-0.97 (m, 13H), 0.93 (0.87) (s, 3H).

Enantiomer 46 was prepared in a similar manner from 45 and exhibited the same spectral data as *ent*-46, and HRMS (EI, 70 eV) m/z 262.1938 [M$^+$; Calcd for $C_{17}H_{26}O_2$, 262.1933].

Ketone *ent*-47
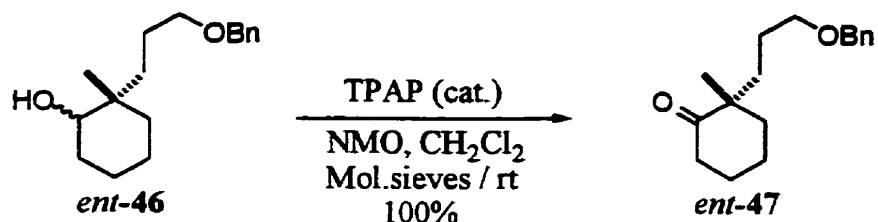
Tetrapropylammonium perruthenate (0.15 g, 0.43 mmol) was added in one portion to a stirred mixture of diol *ent*-46 (3.70 g, 14.10 mmol), 4-methylmorpholine-4-oxide (2.48 g, 21.17 mmol), and 4 Å molecular sieves (7.0 g, 0.5g/mmol) in dry CH_2Cl_2 (60 mL) under nitrogen atmosphere at rt. The reaction mixture was stirred for 1.5 hour at this temperature and filtered through a silica gel column (CH_2Cl_2 100%). Evaporation of the solvent afforded ketone *ent*-47 (3.66 g, 100%) as a colorless oil: TLC R_f= 0.58 (EtOAc/hexanes, 2:8); $[\alpha]^{25}_D$ -41.8° (c = 1.01, $CHCl_3$); IR (film): υ_{max} 2924s, 2850m, 1700s, 1447m, 1360w, 1094s, 739m, 692m cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.30 (m, 5H), 4.48 (s, 2H), 3.44 (t, J = 6.1 Hz, 2H), 2.36 (m, 2H), 1.89-1.22 (m, 10H), 1.05 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 215.8, 138.5, 128.3, 127.6, 127.5, 72.8, 70.6, 48.3, 39.3, 38.7, 34.0, 27.5, 24.2, 22.5, 21.0 ppm.

Enantiomer **47** was prepared in a similar manner from **46** and exhibited the same spectral data as *ent*-47, and $[\alpha]^{22}_D$ +39.0° (c = 3.0, $CHCl_3$); HRMS (CI, NH_3) m/z 260.1771 [M$^+$; Calcd for $C_{17}H_{24}O_2$, 260.1776]. Anal. Calcd for $C_{17}H_{24}O_2$: C, 78.42; H, 9.29. Found: C, 78.31; H, 9.31.

Diene *ent*-50
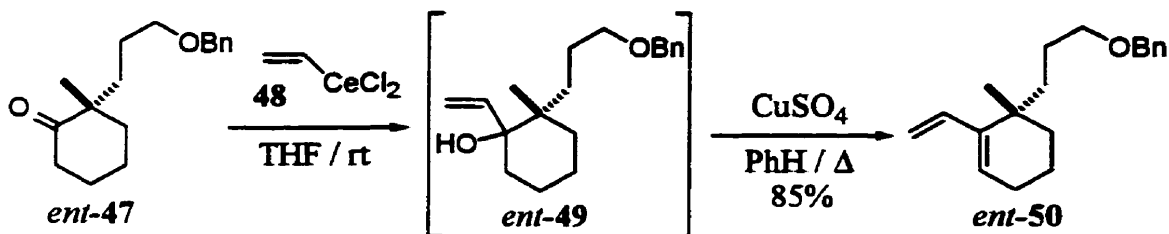
To a suspension of anhydrous CeCl₃ [obtained from CeCl₃·7H₂O (10.26 g, 27.54 mmol) by heating first at 75 °C/3 mm Hg for 4 h and then at 135-140 °C/3 mm Hg for 3 h] in THF (80 mL) was added a solution of ketone *ent*-47 (6.80 g, 26.12 mmol) in dry THF (20 mL), followed by a solution of vinylmagnesium bromide (1 M, 35.0 mL, 35.0 mmol) under a nitrogen atmosphere at rt. The reaction mixture was stirred at rt for 18 hours and diluted with ether (150 mL). Water (50 mL) was added dropwise and the mixture was stirred for another for two hours. The precipitate was filtered off and washed with ether (2 × 50 mL). The filtrate was evaporated *in vacuo* to give a yellowish residue. The residue containing alcolol *ent*-49 was dissolved in benzene (150 mL) and CuSO₄·5H₂O (9.81 g, 39.29 mmoL) was added. The resulting mixture was heated at reflux fror 10 h (Dean-Stark trap), allowed to cool to room temperature and filtered through Celite. Evaporation of the solvent gave a yellow oil which was purified by flash chromatography over silica gel (EtOAc/hexanes, 3:97, then 5:95) to afford diene *ent*-50 (6.01 g, 85%) as a colorless oil: TLC R_f = 0.49 (EtOAc/hexanes, 1:9); $[\alpha]^{26}_D$ +14.3° (*c* = 1.93, CHCl₃); IR (film): υ_{max} 3015m, 2928s, 2852s, 1620w, 1602w, 1492w, 1450m, 1358m, 1200m, 1098s, 1024w, 984w, 900m, 730m, 692m cm⁻¹; ¹H NMR (300 MHz, CDCl₃): δ 7.32 (m, 5H), 6.28 (dd, J = 11.0, 16.9 Hz, 1H), 5.86 (t, J = 4.0 Hz, 1H), 5.28 (dd, J = 1.8, 16.9 Hz, 1H), 4.91 (dd, J = 1.8, 11.0 Hz, 1H), 4.50 (s, 2H), 3.43 (t, J = 6.3 Hz, 2H), 2.04 (d, J = 4.0 Hz, 2H), 1.72-1.17 (m, 8H), 1.06 (s, 3H); ¹³C NMR (75 MHz, CDCl₃): δ 143.6, 138.7, 137.1, 128.3, 127.6, 127.4, 124.4, 112.8, 72.8, 71.2, 36.7, 36.0, 35.0, 26.9, 26.1, 24.5, 19.0 ppm.

Enantiomer 50 was prepared in a similar manner from 47 and exhibited the same spectral data as *ent*-50, and $[\alpha]^{22}_D$ -19.7° (*c* = 1.10, CHCl₃); HRMS (EI, 70 eV) m/z 270.1988 [M⁺; Calcd for C₁₉H₂₆O, 270.1984]. Anal. Calcd for C₁₉H₂₆O: C, 84.39; H, 9.69. Found: C, 84.40; H, 9.94.

1.2   Preparation of dienophile 56

Alkyne 52

HO–CH₂CH₂–C≡CH **51**   →[TIPSCl / imid./ DMF / 95%]→   TIPSO–CH₂CH₂–C≡CH **52**
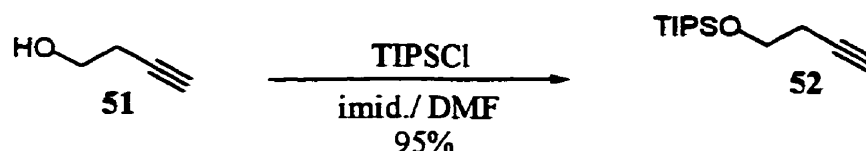
To a solution of 3-butyn-1-ol **51** (10.00 g, 142.67 mmol) in dry DMF (250mL) was added imidazole (11.17 g, 164.07 mmol), TIPSCl (32.00 mL, 149.80 mmol) dropwise at rt, and the reaction mixture was stirred overnight under a nitrogen atmosphere. Water (5 mL) was added and the reaction mixture was poured into ice-cold water (400 mL). The product was extracted with Et₂O (3 x 150mL) and the combined extracts were successively washed with aq. 10% HCl (15 mL), aq. sat. NaHCO₃ (25 mL), brine (50 mL), dried (MgSO₄), and concentrated *in vacuo*. Distillation of the resulting crude yellowish oil under reduced pressure (100-102 °C / 2.8 mm Hg) afforded **52** (30.615 g, 95%) as a colorless oil. TLC R$_f$ = 0.72 (EtOAc/hexanes, 1:9); IR (film): υ_{max} 3302m, 2932s, 2859s, 1460m, 1382w, 1109s, 878m, 628m cm⁻¹; ¹H NMR (300 MHz, CDCl₃): δ 3.81 (t, J = 7.3 Hz, 2H), 2.42 (dt, J = 2.7, 7.3 Hz, 2H), 1.94 (t, J = 2.7 Hz, 1H), 1.06 (m, 21H); ¹³C NMR (75 MHz, CDCl₃): δ 81.4, 69.3, 62.0, 22.9, 17.9, 12.0 ppm.

The spectral data were in accord with those reported in the literature.[47]

Ester 53
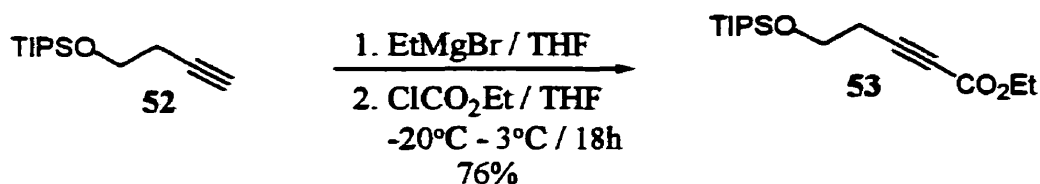
To a stirred solution of ethylmagnesium bromide (3.0 M, 15.8 mL, 47.43 mmol) was added dropwise a solution of alkyne **52** (10.71 g, 47.30 mmol) in dry THF (50 mL) at rt under a nitrogen atmosphere, and stirring was continued for another 2.5 hours. This solution was then added dropwise to a solution of ethyl chloroformate (5.70 g, 52.48 mmol) in dry THF (50 mL) at -20 °C. The mixture was stirred at -15 °C for 30 minutes, warmed to 3 °C and kept at this temperature overnight. The precipitate was filtered off and washed with ice-cold ether (3 × 20 mL). The combined filtrates were diluted with ether (150 mL), washed with brine (5 × 15 mL), dried (MgSO$_4$) and concentrated *in vacuo*. The residue was purified by flash chromatography over silica gel (EtOAc/hexanes, 2:98) to afford ester **53** (10.61 g, 76%) as a colorless oil: TLC R$_f$ = 0.41 (EtOAc/hexanes, 1:9); IR (film): υ$_{max}$ 2918s, 2860s, 2240s, 1712s, 1460s, 1384m, 1367m, 1244s, 1110s, 1069s, 1012m, 980s, 750m, 676m cm^{-1}; ^{1}H NMR (300 MHz, CDCl$_3$): δ 4.14 (q, J = 7.3 Hz, 2H), 3.80 (t, J = 7.1 Hz, 2H), 2.50 (t, J = 7.1 Hz, 2H), 1.22 (t, J = 7.3 Hz, 3H), 1.07 (m, 21H); ^{13}C NMR (75 MHz, CDCl$_3$): δ 153.5, 86.1, 74.0, 61.6, 60.9, 23.0, 17.8, 13.9, 11.8 ppm; Anal. Calcd for C$_{16}$H$_{30}$O$_3$Si: C, 64.38; H, 10.13. Found: C, 64.29; H, 10.38.

Diene 55
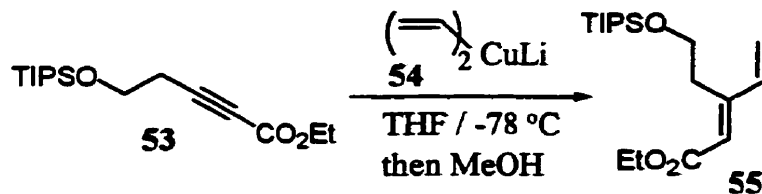
To a solution of tetravinyltin (3.4 mL, 4.24 g, 18.7 mmol) in anhydrous THF (25 mL) was added rapidly a cyclohexane-ether (7:3) solution of phenyllithium (1.8 M, 41.5 mL, 74.7 mmol) under a nitrogen atmosphere at rt . Vigorous stirring was continued for 30 minutes and a precipitate formed. The dark solution was filtered through a cannula fitted with cotton, transfered to a dry flask, and the precipitate was rinced with anhydrous THF (5 mL). The dark vinyllithium solution was added dropwise to a suspension of recrystallized copper (I) iodide (7.02 g, 36.86 mmol) in dry THF (70 mL) at -30 °C. The resulting mixture was stirred for 15 minutes at this temperature and then cooled to - 78 °C and stirred for another 10 minutes. To this mixture (containing vinyl copper reagent **54**) was added dropwise a precooled (-78 °C) solution of ester **53** (5.00 g, 16.76 mmol) in dry THF (30 mL). The reaction mixture was stirred for 2 hours at -78 °C, quenched by adding dropwise a precooled (-78 °C) solution of methanol (3 mL) in dry THF (15 mL), and warmed to rt. The solvent was removed *in vacuo* and ether (200 mL) was added to precipitate the salts which were subsequently filtered off through a pad of Florisil and washed with ether (200 mL). Evaporation of the solvent *in vacuo* followed by flash chromatography of the resulting oil over silica gel (EtOAc/hexanes, 2:98) gave diene **55** (5.46 g, 100%) as a colorless oil (contaminated with a small amount of tetraphenyl tin which was completely removed in the next step). TLC $R_f = 0.39$ (EtOAc/hexanes, 1:9); IR (film): υ_{max} 2933s, 2859s, 1713s, 1600s, 1461m, 1372w, 1248m, 1191m, 1156s, 1097s, 917m, 878m cm^{-1}; ^1H NMR (300 MHz, CDCl$_3$): δ 6.36 (dd, J = 10.8, 17.4 Hz, 1H), 5.82 (s, 1H), 5.73 (d, J = 17.4 Hz, 1H), 5.38 (d, J = 10.8 Hz, 1H), 4.17 (q, J = 7.1 Hz, 2H), 3.83 (t, J = 6.9 Hz, 2H), 3.09 (t, J = 6.9 Hz, 2H), 1.28 (t, J = 7.1 Hz, 3H), 1.06 (m, 21H); ^{13}C NMR (75 MHz, CDCl$_3$): δ 166.3, 153.5, 139.3, 120.6, 119.6, 62.9, 59.7, 30.8, 18.0, 14.2, 11.9 ppm. Anal. Calcd for $C_{18}H_{34}O_3Si$: C, 66.21; H, 10.50. Found: C, 66.16; H, 10.74.

Dienophile 56
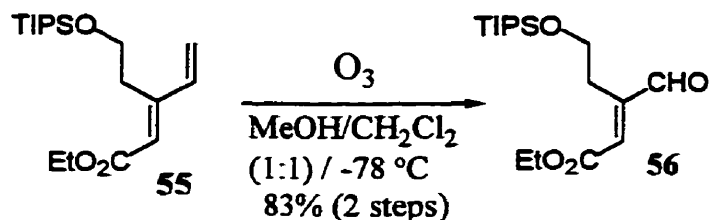
Through a solution of diene **55** (5.46 g, 16.73 mmoL) in CH_2Cl_2/MeOH (150 mL, 1:1) was bubbled ozone at -78 °C until all starting material was consumed according to TLC (requiring 4-10 hours). Methyl sulfide (2 mL) was added and the reaction mixture was warmed to rt. The solvent was removed *in vacuo* and the crude product was purified by flash chromatography over silica gel (EtOAc/hexanes, 2:98) to provide the desired dienophile **56** (3.84 g, 70%) as a colorless oil: TLC R_f= 0.33 (EtOAc/hexanes, 1:9); IR (film): υ_{max} 2938s, 2863s, 2710w, 1726s, 1698s, 1462m, 1247m, 1191s, 1099s, 1058m, 674m cm^{-1}; ^1H NMR (300 MHz, CDCl$_3$): δ 9.52 (s, 1H), 6.51 (s, 1H), 4.23 (q, J = 7.1 Hz, 2H), 3.77 (t, J = 6.6 Hz, 2H), 2.99 (t, J = 6.6 Hz, 2H), 1.30 (t, J = 7.1 Hz, 3H), 1.06 (m, 21H); ^{13}C NMR (75 MHz, CDCl$_3$): δ 194.1, 165.1, 151.4, 136.0, 61.7, 61.0, 28.3, 17.9, 14.0, 11.8 ppm; Anal. Calcd for $C_{17}H_{32}O_4Si$: C, 62.15; H, 9.82. Found: C, 62.20; H, 9.96.

1.3 Preparation of chiral dienophile 84

Carboxylic acid 81
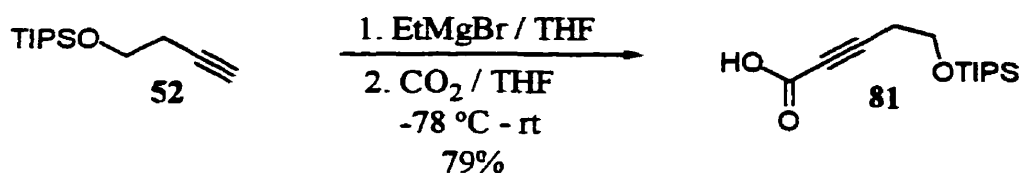
To a solution of ethylmagnesium bromide (3.0M, 6.20 mL, 18.58 mmol) in dry THF (50 mL) was added alkyne **52** (4.00 g, 17.70 mmol) in dry THF (50 mL) under nitrogen atmosphere at rt over a period of approximately 15 minutes. The reaction mixture was stirred for 1.5 hour at this temperature and cooled to -78 °C. Dry CO_2 (g) was then bubbled through the reaction mixture. The mixture was warmed to rt and stirred overnight under a CO_2 atmosphere, diluted with Et_2O (100 mL), and then water (80 mL) was added and the aqueous phase was acidified with aq. 10% HCl. The product was extracted with Et_2O (3 x 80 mL) and the combined extracts were washed with brine (50 mL), dried ($MgSO_4$), and concentrated *in vacuo*. The residue was purified by flash chromatography over silica gel (EtOAc/hexanes, 2:8, then 4:6) to afford carboxylic acid **81** (3.98 g, 79%) as a colorless oil: TLC $R_f = 0.16$ (EtOAc/hexanes, 2:8 + 2 drops of CH_3CO_2H); IR (film): υ_{max} 3070br, 2924s, 2230s, 1694s, 1459m, 1408m, 1385m, 1260m, 109m, 1068w, 1011w, 992w, 878m cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 10.68 (s, 1H), 3.87 (t, J = 6.9 Hz, 2H), 2.59 (t, J = 6.9 Hz, 2H), 1.08 (21H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 157.8, 89.2, 73.4, 60.7, 23.1, 17.4, 11.8 ppm. HRMS (CI, NH_3) m/z 271.1725 [M^++1; Calcd for $C_{14}H_{27}O_3Si$, 271.1729].

Chiral ester 82
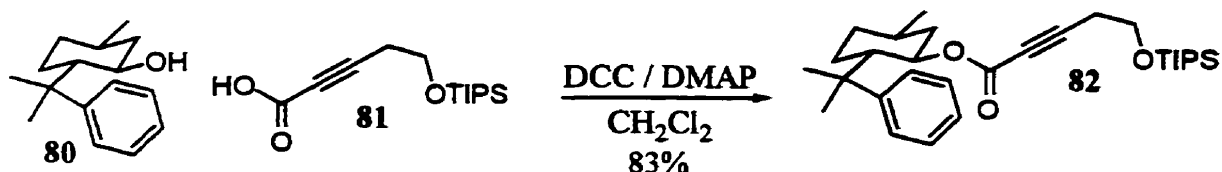
To a solution of carboxylic acid **81** (300 mg, 1.11 mmol) and commercially available (-)-8-phenylmenthol **80** (516 mg, 2.22 mmol) in dry CH_2Cl_2 (5 mL) was added at 0 °C DMAP (27 mg, 0.222 mmol), followed by DCC (458 mg, 2.22 mmol) in two portions under a nitrogen atmosphere. The ice bath was removed and the reaction mixture was stirred at rt for another 6 hours and then quenched with water (2 mL), diluted with Et_2O, and extracted with ether (3 x 80 mL). The combined extracts were successively washed with aq. 10% HCl (10 mL), sat. aq. $NaHCO_3$ (15 mL), brine (20 mL), dried ($MgSO_4$), and concentrated *in vacuo*. The residue was purified by flash chromatography over silica gel (EtOAc/hexanes, 2/98, then 5/95 and 1/9) to afford ester **82** (446 mg, 83%) as a white yellowish oil, and chiral alcohol **80** (186 mg). Ester **82** exhibited the following data: $[\alpha]^{21}_D$ +7.9° (c = 2.01, $CHCl_3$); TLC R_f = 0.63 (EtOAc/hexanes, 2:8); IR (film): ν_{max} 2915s, 2240s, 1704s, 1601w, 1460m, 1389m, 1247s, 1108s, 1068s, 880m cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 7.30-7.11 (m, 5H), 4.87 (dt, J = 4.4, 10.7 Hz, 1H), 3.83 (t, J = 7.2 Hz, 2H), 2.51 (t, J = 7.2 Hz, 2H), 2.02-1.89 (m, 2H), 1.62-0.82 (m, 36H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 152.9, 150.6, 127.9, 125.4, 125.0, 85.7, 76.1, 74.2, 60.9, 50.4, 41.3, 39.8, 34.3, 31.2, 27.0, 26.7, 26.0, 23.0, 21.6, 17.8, 11.8 ppm. HRMS (CI, NH_3) m/z 485.3441 [M^++1; Calcd for $C_{30}H_{49}O_3Si$, 485.3453].

Chiral diene 83: Procedure A
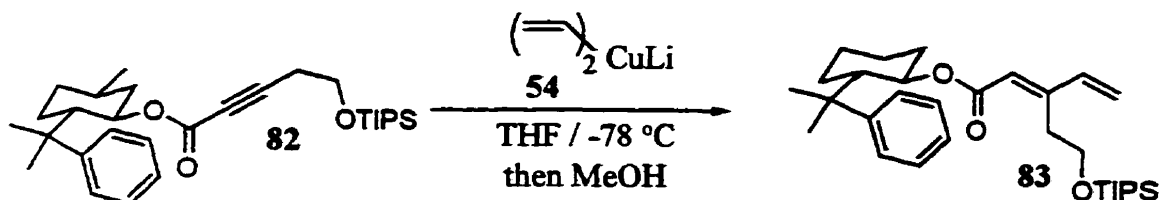
A vinyllithium solution [2.2M in THF, purchased from Organometallics Inc., E. Hampstead, N.H., 15.6 mL, 34.38 mmol] was added dropwise to a suspension of recrystallized copper (I) iodide (3.273 g, 17.19 mmol) in dry THF (60 mL) under a nitrogen atmosphere at -30 °C. The resulting mixture was stirred for 15 minutes at this temperature, cooled to -78 °C, and stirred for an additional 10 minutes. To this mixture (containing vinyl copper reagent 54) was added dropwise a precooled (-78 °C) solution of ester 82 (4.164 g, 8.59 mmol) in dry THF (20 mL). The reaction mixture was stirred for 2 hours at -78 °C and quenched by adding dropwise a precooled (-78 °C) solution of MeOH (2 mL) in dry THF (10 mL) and the reaction mixture was warmed to rt. The solvent was removed *in vacuo* and ether (200 mL) was added to precipitate the salts which were subsequently filtered off through a pad of Florisil and washed with ether (150 mL). Evaporation of the solvent *in vacuo* followed by flash chromatography over silica gel (EtOAc/hexanes, 2:98, then 5:95) produced chiral diene 83 (4.37 g, 98%) as a colorless oil. $[\alpha]^{26}_D$ +21.2° (c = 1.12, CHCl$_3$); TLC R$_f$ = 0.38 (EtOAc/hexanes, 5:95); ^{1}H NMR (300 MHz, CDCl$_3$): δ 7.40-7.00 (m, 5H), 6.23 (dd, J = 17.4, 10.8 Hz, 1H), 5.70 (d, J = 17.4 Hz, 1H), 5.36 (d, J = 10.8 Hz, 1H), 5.13 (s, 1H), 4.86 (dt, J = 4.2, 10.7 Hz, 1H), 3.82 (t, J = 6.8 Hz, 2H), 3.03 (t, J = 6.8 Hz, 2H), 2.10-1.91 (m, 2H), 1.72-0.82 (m, 36H); ^{13}C NMR (75 MHz, CDCl$_3$): δ 165.4, 152.9, 151.5, 139.4, 128.1, 125.3, 124.7, 120.9, 119.2, 73.5, 63.0, 50.6, 41.8, 39.6, 34.5, 31.2, 30.7, 27.4, 26.6, 25.2, 21.7, 18.0, 11.9 ppm.

Chiral diene 83: Procedure B

To a solution of tetravinyltin (810 µL, 4.30 mmol) in dry THF (10 mL) was added at rt a cyclohexane-ether (7:3) solution of phenyllithium (1.8 M, 9.6 mL, 17.2 mmol) under a nitrogen atmosphere. Vigorous stirring was continued for 30 minutes and a precipitate formed. The dark solution was filtered through a cannula fitted with cotton and transfered to a dry flask. The dark vinyllithium solution was added dropwise to a suspension of recrystallized copper (I) iodide (1.638 g, 8.60 mmol) in dry THF (25 mL) at -30 °C. The resulting mixture was stirred for 15 minutes at this temperature, cooled to -78 °C, and stirred for an additional 10 minutes. To this mixture (containing vinyl copper reagent 54) was added dropwise a precooled (-78 °C) solution of ester 82 (2.00 g, 4.127 mmol) in dry THF (10 mL). The reaction mixture was stirred for 1.75 hours at -78 °C, quenched by adding dropwise a precooled (-78 °C) solution of MeOH (2 mL) in dry THF (10 mL), and warmed to rt. The solvent was removed *in vacuo* and ether (100 mL) was added to precipitate the salts which were subsequently filtered off through a pad of Florisil and washed with ether (100 mL). Evaporation of the solvent *in vacuo* followed by purification by flash chromatography over silica gel (EtOAc/hexanes, 2:98, then 5:95) provided diene 83 (2.82 g) as a colorless oil (containing a small amount of tetraphenyl tin which was completely removed in the next step).

Chiral dienophile 84
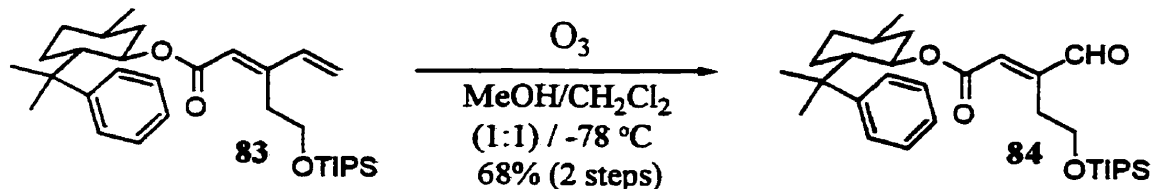
Through a solution of chiral diene **83** (2.11, 4.127 mmoL) in CH_2Cl_2/MeOH (50 mL, 1:1) was bubbled ozone at -78 °C until all the starting material was consumed according to TLC (4 hours). Methyl sulfide (1 mL) was added and the reaction mixture was warmed to rt, concentrated *in vacuo* and the residue was purified by flash chromatography over silica gel (EtOAc/hexanes, 2:98, then 5:95) to provide dienophile **84** (1.44 g, 68%) as a yellowish white oil: $[\alpha]^{26}_D$ +8.2° (*c* = 2.09, CHCl$_3$); TLC R$_f$= 0.36 (EtOAc/hexanes, 1:9); IR (film): υ_{max} 2930s, 2860s, 1701s, 1460m, 1249m, 1197s, 1099s, 880m, 762m cm^{-1}; ^{1}H NMR (300 MHz, CDCl$_3$): δ 9.30 (s, 1H), 7.30-7.06 (m, 5H), 5.58 (s, 1H), 4.90 (dt, J = 4.4, 10.7 Hz, 1H), 3.73 (t, J = 6.7 Hz, 2H), 2.88 (m, 2H), 2.15-0.80 (m, 38 H); ^{13}C NMR (75 MHz, CDCl$_3$): δ 194.1, 164.1, 151.7, 150.6, 136.7, 127.9, 125.2, 124.8, 74.7, 61.8, 50.3, 41.5, 39.4, 34.4, 31.2, 29.0, 28.0, 26.2, 23.3, 21.6, 17.9, 11.8 ppm. HRMS (CI, NH$_3$) m/z 515.3564 [M$^+$+1; Calcd for C$_{31}$H$_{51}$O$_4$Si, 515.3556].

1.4 Diels-Alder reaction and subsequent reduction
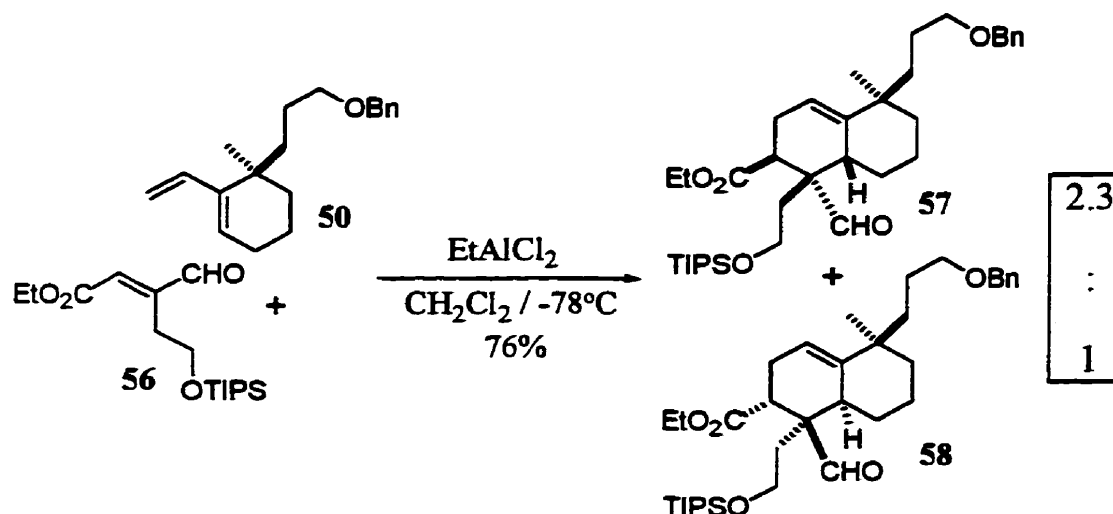
A solution of ethylaluminium dichloride (1.0 M in hexanes, 8.26 mL, 8.26 mmol) was added dropwise to a stirred solution of chiral diene 50 (1.51 g, 5.51 mmol) and dienophile 56 (1.81 g, 5.51 mmol) in CH_2Cl_2 (50 mL) under a nitrogen atmosphere at -78 °C. The reaction mixture was stirred at -78 °C for 20 minutes and poured into ether (200 mL) and sat. aq. $NaHCO_3$ (20 mL). The organic layer was separated, washed with brine (20 mL), dried ($MgSO_4$), and concentrated *in vacuo*. The residue was purified by flash chromatography over silica gel (EtOAc/hexanes, 1:15) to give a colorless oil consisting of diastereoisomers 57 and 58 (ratio = 2.3:1 as determined by ^{1}H-NMR; 2.52g, 76%). These isomers were not easy to separate and therefore were used as a mixture in the next step. For the purpose of characterization, a small amount of the major isomer (57) was separated by chromatography: TLC R_f= 0.33 (EtOAc/hexanes, 1:9); $[\alpha]^{22}_D$ +64.9° (c = 1.3, $CHCl_3$); IR (film): υ_{max} 2932s, 2863s, 1720s, 1455s, 1370s, 1170s, 1094s, 879s, 732s, 675m cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): δ 9.84 (s, 1H), 7.33 (m, 5H), 5.40 (br, 1H), 4.49 (s, 2H), 4.12 (q, J = 7.0 Hz, 2H), 3.75 (t, J = 7.0 Hz, 2H), 3.41 (t, J = 6.1 Hz, 2H), 2.93 (dd, J = 5.5, 11.4 Hz, 1H), 2.48 (m, 2H), 2.24 (m, 2H), 1.95 (m, 1H),1.80-1.50 (m, 7H), 1.30-1.07(m, 9H), 1.03 (d, J = 2.9 Hz, 18H), 0.99 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 205.7, 174.2, 144.1, 138.5, 128.2, 127.4, 127.3, 116.5, 72.8, 70.9, 60.4, 59.5, 51.3, 41.7, 41.4, 39.9, 38.9, 34.1, 33.2, 30.5, 25.9, 25.4, 24.5, 21.8, 17.9, 13.9, 11.8 ppm; HRMS (CI, NH_3) m/z 615.4073 [M^++NH_3; Calcd for $C_{36}H_{61}O_5NSi$, 615.4081]. Anal. Calcd for $C_{36}H_{58}O_5Si$: C, 72.19; H, 9.76. Found: C, 72.18; H, 9.47.

Diols 59 and 60
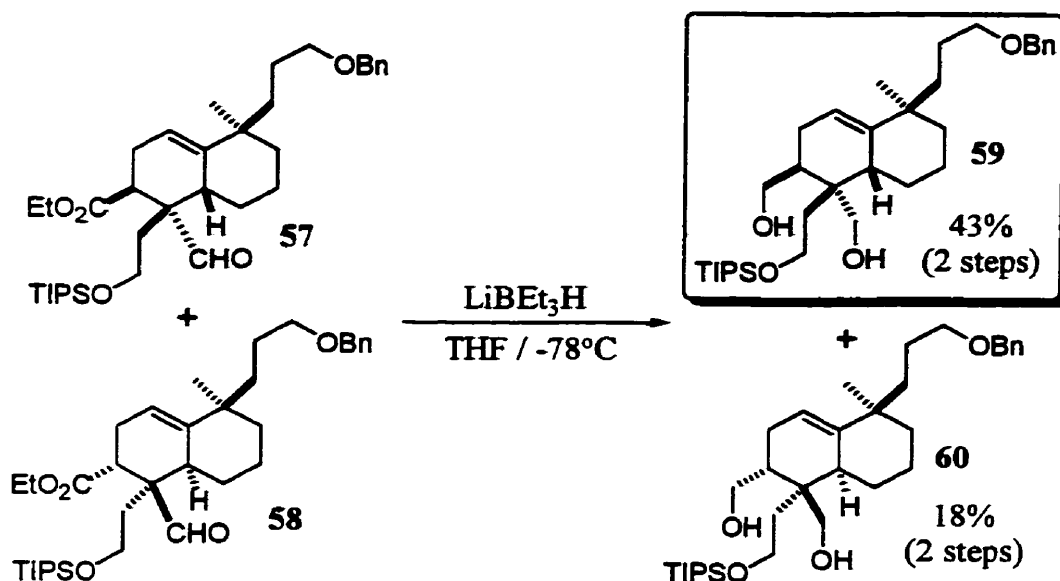
To a solution of the **57/58** mixture (2.80 g, 4.68 mmol) in dry THF (25 mL) cooled at -78 °C was added dropwise a THF solution of LiEt₃BH (1.0 M, 18.80 mL, 18.80 mmol) under a nitrogen atmosphere. The reaction mixture was stirred at -78 °C for 30 minutes, warmed to -30 °C, stirred for one hour, and carefully quenched by addition of water at -30 °C (2 mL). The reaction mixture was warmed to 0 °C and sat. aq. NaHCO₃ (20 mL) was added followed by an aq. 30% hydrogen peroxide solution (8 mL). After stirring for one hour at rt, the reaction mixture was diluted with ether (200 mL), the organic layer separated, and the aqueous layer extracted with ether (2 × 50 mL). The combined extracts were washed with brine (30 mL), dried (MgSO₄), and concentrated *in vacuo*. The residue was purified by flash chromatography on silica gel (EtOAc/hexanes, 1:5) to furnish diol **59** (1.51 g, 43% from **57**) and diol **60** (0.60 g, 18% from **58**).

Major diol 59: Colorless oil; TLC R$_f$ = 0.31 (EtOAc/hexanes, 1:5); [α]^{22}$_D$ +42.8° (c =
2.2, CHCl$_3$); IR (film): υ$_{max}$ 3340s, 2930s, 2860s, 1655w, 1454m, 1353m, 1237w,
1197w, 1090s, 1066s, 1032s, 1008m, 877m,728m, 689m cm^{-1}; ^{1}H NMR (300 MHz,
CDCl$_3$): δ 7.32 (m, 5H), 5.28 (d, J = 2.7 Hz, 1H), 5.01 (br, 1H), 4.78 (br, 1H), 4.45 (s,
2H), 3.93 (t, J = 10.5 Hz, 1H), 3.80-3.60 (m, 4H), 3.40 (m, 3H), 1.80-2.00 (m, 5H),
1.67-1.47 (m, 7H), 1.28-1.09 (m, 7H), 1.14 (d, J = 4.6 Hz, 18 H), 1.04 (s, 3H); ^{13}C
NMR (75 MHz, CDCl$_3$): δ 144.7, 138.4, 128.2, 127.4, 127.3, 117.0, 72.8, 71.1, 67.8,
73.3, 59.1, 42.3, 39.9, 39.8, 39.6, 33.3, 28.7, 26.4, 25.8, 24.8, 22.4, 17.8, 11.7 ppm.;
HRMS (CI, NH$_3$) m/z 559.4178 [M$^+$+1; Calcd for C$_{34}$H$_{59}$O$_4$Si, 559.4182]. Anal. Calcd
for C$_{34}$H$_{58}$O$_4$Si: C, 73.06; H, 10.46. Found: C, 73.32; H, 10.62.

The enantiomer of **59** (*ent-59*) exhibited identical spectral data, and [α]^{22}$_D$ -40.4° (c =
0.82, CHCl$_3$).

Minor diol 60: Colorless oil; TLC R$_f$ = 0.23 (EtOAc/hexanes, 1:5); [α]^{22}$_D$ -21.5° (c =
1.9, CHCl$_3$); IR (film): υ$_{max}$ 3360br, 2930s, 2860s, 1645w, 1450s, 1378m, 1360s,
1240m, 1080s, 878s, 810w, 730s, 680s cm^{-1}; ^{1}H NMR (300 MHz, CDCl$_3$): δ 7.30 (m,
5H), 5.28 (s, 1H), 4.68 (br, 1H), 4.51 (s, 2H), 3.93 (t, J = 10.5 Hz, 1H), 3.79-3.59 (m,
4H), 3.47 (t, J = 6.6 Hz, 2H), 3.32 (t, J = 9.8 Hz, 1H), 2.03 (d, J = 11.2 Hz, 1H),
1.93-1.12 (m, 17H), 1.09 (d, J = 4.9 Hz, 18H), 1.04 (m, 2H), 0.97 (s, 3H); ^{13}C NMR
(75 MHz, CDCl$_3$): δ 146.1, 138.7, 128.3, 127.6, 127.5, 115.0, 72.9, 71.4, 68.2, 63.4,
59.3, 40.0, 39.2, 37.4, 29.4, 26.3, 24.3, 23.0, 17.9, 11.8; HRMS (CI, NH$_3$) m/z
559.4178 [M$^+$+1; Calcd for C$_{34}$H$_{59}$O$_4$Si, 559.4182].

Diels-Alder Adduct 85: Procedure A
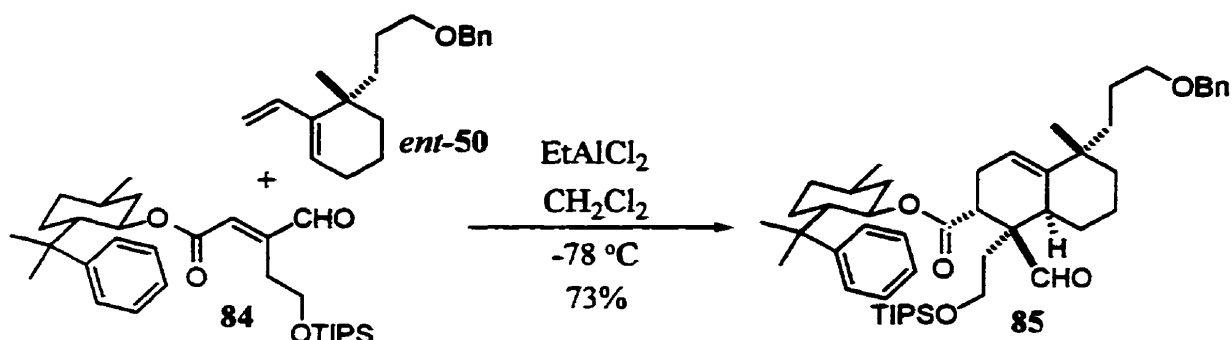
To a solution of chiral dienophile **84** (1.15 g, 2.37 mmol) and chiral diene *ent-50* (775 mg, 2.85 mmol) in dry CH_2Cl_2 (50 mL) cooled to -78 °C was added dropwise a solution of ethylaluminium dichloride (1.0M in hexanes, 2.9 mL, 2.9 mmol) under a nitrogen atmosphere and the mixture was stirred for 15 minutes at -78 °C, then poured into ether (100 mL) and saturated $NaHCO_3$ (20 mL), and extracted with ether (3 x 60 mL). The combined organic extracts were washed with brine (20 mL), dried ($MgSO_4$), and concentrated *in vacuo*. The residue was purified by flash chromatography on silica gel (EtOAc/hexanes, 5:95, then 93:7, then 9:1) to give **85** as a colorless oil (1.304 g, 73%). TLC R_f = 0.31 (EtOAc/hexanes, 1:9); $[\alpha]^{28}_D$ -82.5° (c = 1.00, $CHCl_3$); IR (film): υ_{max} 2930s, 2862s, 1720s, 1456m, 1366m, 1217m, 1092s, 1009m, 883m, 763m, 732m, 698m cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 9.71 (s, 1H), 7.36-7.09 (m, 5H), 5.22 (m, 1H), 4.77 (dt, J = 4.3, 10.7 Hz, 1H), 4.47 (s, 2H), 3.64 (m, 1H), 3.38 (m, 3H), 2.55 (dd, J = 5.7, 11.6 Hz, 1H), 2.22-0.85 (m, 56H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 205.6, 173.0, 151.9, 144.0, 138.5, 128.2, 127.8, 127.5, 127.3, 125.3, 124.8, 116.7, 74.5, 72.7, 70.9, 59.9, 50.3, 50.1, 43.5, 41.7, 41.3, 39.8, 39.5, 39.3, 34.5, 34.3, 33.0, 31.1, 30.5, 28.4, 26.4, 26.0, 24.5, 24.4, 24.1, 21.8, 21.6, 17.9, 11.8 ppm. HRMS (CI, NH_3) m/z 785.5519 [M^++1; Calcd for $C_{50}H_{77}O_5Si$, 785.5540].

Diels-Alder Adduct 85: Procedure B

To a solution of chiral dienophile **84** (216 mg, 0.44 mmol) in dry CH_2Cl_2 (7 mL) cooled at -78 °C was added successively under a nitrogen atmosphere dry THF (35 µL, 0.44 mmol), a solution of ethylaluminium dichloride dropwise (1.0M in hexanes, 630 µL, 0.63 mmol) and a solution of chiral diene *ent*-**50** (100 mg, 0.37 mmol) in dry CH_2Cl_2 (2 mL). The reaction mixture was stirred at -78 °C for 5 minutes, warmed to rt, stirred for another two hours, and poured into ether (50 mL) and sat. aq. $NaHCO_3$ (20 mL). The mixture was extracted with ether (3 x 40 mL) and the combined extracts were washed with brine (20 mL), dried ($MgSO_4$), and concentrated *in vacuo*. The residue was purified by flash chromatography over silica gel (EtOAc/hexanes, 2:98, then 4:96, then 6:94) to give **85** (220 mg, 79%) as a colorless oil.

Diol *ent-59*
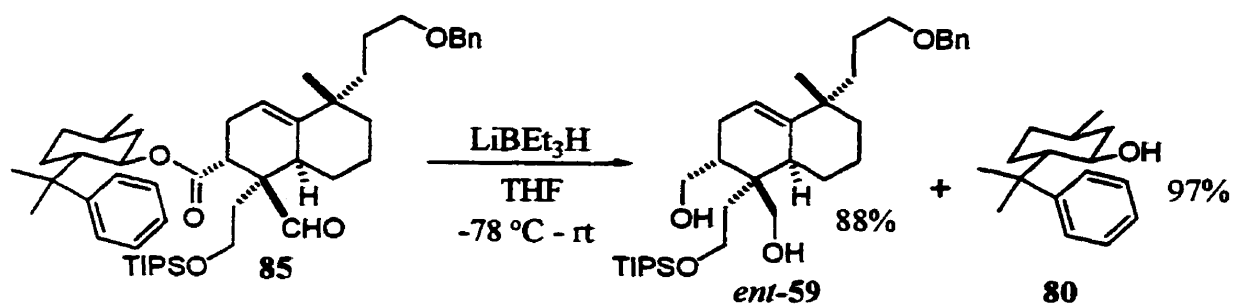
To a solution of Diels-Alder adduct **85** (150 mg, 0.198 mmol) in dry THF (8 mL) cooled at -78 °C was added dropwise a solution of Super-Hydride in THF (1.0M, 790 μL, 0.79 mmol) under a nitrogen atmosphere. The reaction mixture was stirred at -78 °C for 30 minutes, warmed to rt, stirred for another 30 minutes at this temperature, cooled to 0 °C and carefully quenched with water (20 mL). The mixture was extracted with ether (3 × 50 mL) and the combined extracts were washed with brine (20 mL), dried (MgSO₄), and concentrated *in vacuo*. The residue was purified by flash chromatography over silica gel (EtOAc/hexanes, 1:9) to give alcohol **80** (45 mg, 97%) and diol *ent-59* (778 mg, 83%) as a colorless oil.

1.5   Functionalization to intermediate *ent-*67

Diphosphoramidate *ent-*61
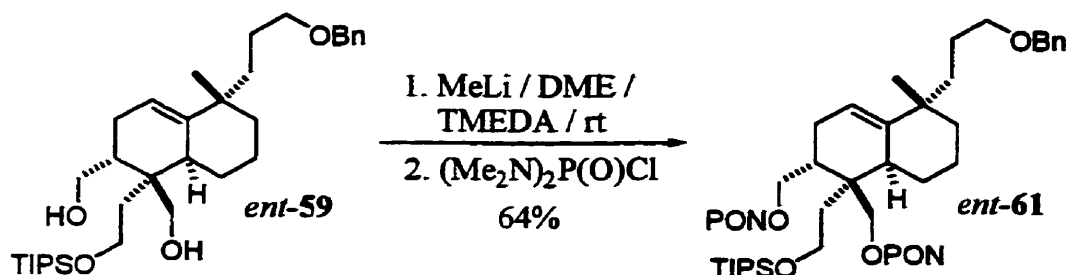
An ether solution of MeLi (1.2M, 3.3 mL, 3.98 mmol) was added dropwise to a stirred solution of diol *ent-*59 (795 mg, 1.42 mmol) in DME-TMEDA (28 mL, 3:1) under a nitrogen atmosphere at 0 °C and after stirring for one hour at rt, $(Me_2N)_2P(O)Cl$ (2.6 mL, 17.0 mmol) was added. The reaction mixture was stirred for another 18 hours at rt, aq. sat. $NaHCO_3$ (30 mL) was added, and stirring was continued for another hour. The reaction mixture was extracted with EtOAc (4 × 100 mL) and the combined extracts were successively washed with an aq. sat. $CuSO_4$ solution (2 × 20 mL), brine (50 mL), dried $(MgSO_4)$, and concentrated *in vacuo*. The residue was purified by flash chromatography over silica gel (MeOH/EtOAc, 1:9) to afford diphosphoramidate *ent-*61 (0.750 mg, 64%) as a pale yellow oil: TLC R_f = 0.23 (MeOH/EtOAc, 1:9); $[\alpha]^{27}_D$ -27.5° (*c* = 2.41, $CHCl_3$); IR (film): υ_{max} 2930s, 2860s, 1630w, 1451m, 1299m, 1202s, 1090m, 1063m, 1024m, 985s, 877m, 749m, 671m cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 7.28 (m, 5H), 5.36 (br, 1H), 4.45 (s, 2H), 4.05-3.70 (m, 6H), 3.38 (m, 2H), 2.63 (d, J = 4.6 Hz, 12H), 2.60 (d, J = 4.8 Hz, 12H), 2.39-1.13 (m, 16H), 1.05 (m, 21H), 0.97 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 144.2, 138.6, 128.2, 127.4, 127.3, 116.3, 72.8, 71.2, 68.3, 65.3, 59.7, 40.6, 39.7, 39.6, 36.5, 36.4, 36.3, 33.8, 29.4, 25.9, 24.5, 22.3, 18.0, 11.9 ppm.

Enantiomer 61 was prepared in a similar manner from 59 and exhibited the same spectral data as *ent-*61, and $[\alpha]^{22}_D$ +26.0° (*c* = 2.3, $CHCl_3$); HRMS (EI, 70 eV) m/z 826.5309 $[M^+;$ Calcd for $C_{42}H_{80}O_6N_4P_2Si$, 826.5322]. Anal. Calcd for $C_{42}H_{80}O_6N_4P_2Si·1.5H_2O$: C, 59.06; H, 9.79; N, 6.56. Found: C, 59.12; H, 10.07; N, 6.57.

Alcohol *ent-62*
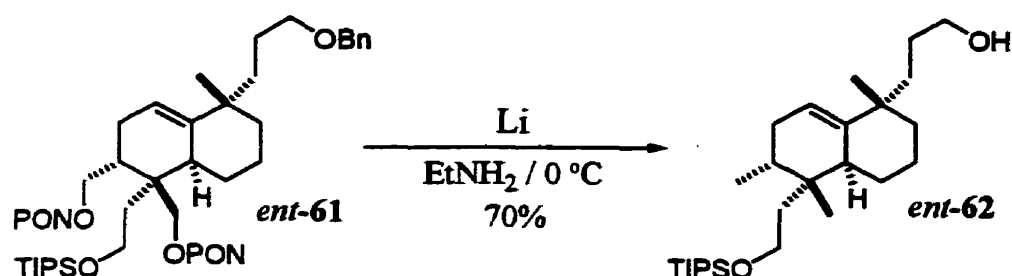
A solution of diphosphoramidate *ent-61* (950 mg, 1.15 mmol) in dry THF/*t*-BuOH (10:1, 8.7 mL) was added over a period of 50 minutes dropwise to a blue solution of lithium wire (175 mg, 25.22 mmol) in anhydrous ethylamine (25 mL) under a nitrogen atmosphere at 0 °C; the rate of addition was controlled so that the color of the solution remained blue. The mixture was stirred for 10 minutes and solid ammonium chloride was carefully added until the blue color disappeared. The reaction mixture was then left standing at rt overnight so that most of the ethylamine evaporated. The residue was then dissolved in water (20 mL) and extracted with ether (3 × 60 mL). The combined extracts were washed with brine (20 mL), dried (MgSO$_4$), and concentrated *in vacuo*. The resulting light yellow oil was purified by flash chromatography over silica gel (EtOAc/hexanes, 1:10) to furnish alcohol *ent-62* (350 mg, 70%) as a colorless oil: TLC R$_f$ = 0.49 (EtOAc/hexanes, 1:10); [α]^{24}$_D$ -52.5° (*c* = 1.58, CHCl$_3$); IR (film): υ$_{max}$ 3340br, 2930s, 2860s, 1670w, 1460m, 1378m, 1247w, 1098s, 1065s, 1014m, 882m, 676m cm^{-1}; ^{1}H NMR (300 MHz, CDCl$_3$): δ 5.30 (t, J = 3.4 Hz, 1H), 3.73 (t, J = 8.1 Hz, 2H), 3.65 (m, 1H), 3.47 (m, 1H), 2.00 (br, 4H), 1.74-1.42 (m, 10H), 1.25-1.07 (m, 5H), 1.05 (d, J =3.1Hz, 18H), 1.04 (m, 3H), 0.97 (s, 3H), 0.80 (d, J = 6.7 Hz, 3H), 0.79 (s, 3H); ^{13}C NMR (75 MHz, CDCl$_3$): δ 117.1, 63.3, 60.4, 42.1, 41.0, 39.7, 35.4, 33.3, 33.0, 31.6, 29.2, 28.2, 26.0, 22.4, 22.1, 18.0 (x2), 14.9, 12.1 ppm.

Enantiomer **62** was prepared in a similar manner from **61** and exhibited the same spectral data as *ent-62*, and [α]^{22}$_D$ +53.4° (*c* = 1.3, CHCl$_3$); HRMS (EI, 70 eV) m/z 436.3730 [M$^+$; Calcd for C$_{27}$H$_{52}$O$_2$Si, 436.3736]; Anal. Calcd for C$_{27}$H$_{52}$O$_2$Si: C, 74.25; H, 12.00. Found: C, 74.47; H, 12.35.

Tosylate *ent*-63
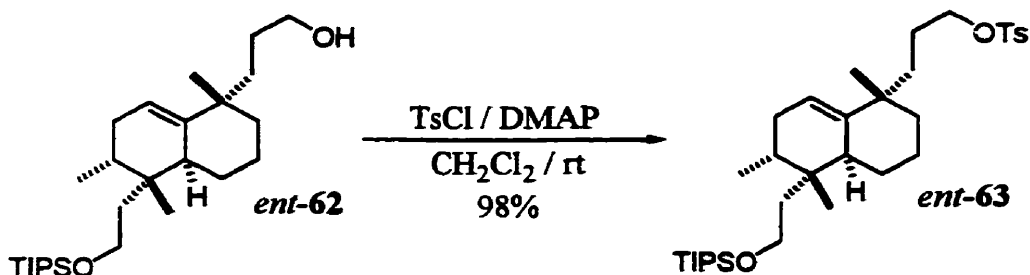
Tosyl chloride (162 mg, 0.85 mmol) was added to a solution of alcohol *ent*-62 (310 mg, 0.71 mmol) and DMAP (130 mg, 1.06 mmol) in CH_2Cl_2 (7 mL) under nitrogen atmosphere at rt and the reaction mixture was stirred at this temperature for 12 hours. The mixture was diluted with ether (50 mL), washed with aq. 10% HCl (5mL), aq. sat. $NaHCO_3$ (5 mL), brine (5mL), and dried ($MgSO_4$). Evaporation of the solvent *in vacuo* afforded a light yellow oil which was purified by flash chromatography over silica gel (EtOAc/hexanes, 1:20) to give tosylate *ent*-63 (412 mg, 98%) as a colorless oil: TLC R_f = 0.69 (EtOAc/hexanes, 1:10); $[\alpha]^{24}_D$ -30.6° (c = 0.90, $CHCl_3$); IR (film): υ_{max} 2930s, 2863s, 1597w, 1457m, 1360m, 1186s, 1174s, 1095s, 917m, 878m, 810m, 658m cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 7.77 (d, J = 8.2 Hz, 2H), 7.33 (d, J = 8.2 Hz, 2H), 5.27 (t, J = 3.5 Hz, 1H), 3.98 (m, 2H), 3.68 (m, 2H), 2.44 (s, 3H), 1.74-1.09 (m, 19H), 0.97 (d, J = 2.3 Hz, 18H), 0.90 (s, 3H), 0.82 (s, 3H), 0.76 (d, J = 6.7 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 144.5, 133.3, 129.7, 127.8, 117.4, 115.7, 71.6, 60.0, 41.1, 39.2, 35.5, 33.1, 25.8, 24.0, 22.3, 21.6, 18.1, 14.9, 12.0 ppm.

Enantiomer 62 was prepared in a similar manner from 63 and exhibited the same spectral data as *ent*-62, and $[\alpha]^{22}_D$ +31.9° (c = 0.60, $CHCl_3$); HRMS (CI, NH_3) m/z 591.3892 [M^++1; Calcd for $C_{34}H_{59}O_4SSi$, 591.3903]; Anal. Calcd for $C_{34}H_{58}O_4SSi$: C, 69.10; H, 9.89. Found: C, 69.37; H, 9.90.

Silyl Ether *ent-65*
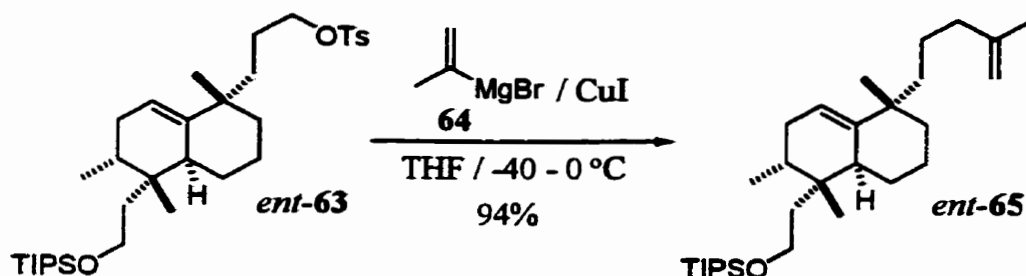
A solution of isopropenylmagnesium bromide (**64**; 0.5 M, 15.6 mL, 7.8 mmoL) was added dropwise to a solution of *ent-***63** (710 mg, 1.20 mmol) and recrystallized copper (I) iodide (185 mg, 0.96 mmol) in dry THF (25 mL) at -40 °C under a nitrogen atmosphere. The reaction mixture was warmed to 0 °C, stirred for 7 hours at this temperature, quenched with MeOH (2 mL) and concentrated *in vacuo*. The residue was diluted with ether (150 mL) and filtered through a pad of Florisil. Evaporation of the solvent followed by purification by flash chromatography over silica gel (hexanes 100%) provided *ent-***65** (518 mg, 94%) as a colorless oil: TLC R_f = 0.26 (hexanes 100%); $[\alpha]^{24}_D$ -29.4° (c = 1.23, CHCl₃); IR (film): υ_{max} 2933s, 2862s, 1645w, 1455m, 1370m, 1092s, 1009w, 991w, 880s, 808w, 675m cm⁻¹; ¹H NMR (300 MHz, CDCl₃): δ 5.31 (t, J = 3.6 Hz, 1H), 4.67 (s, 1H), 4.65 (s, 1H), 3.72 (m, 2H), 1.97-1.86 (m, 3H), 1.76 (br, 1H), 1.69 (s, 3H), 1.63-1.07 (m, 17H), 1.06 (d, J = 2.5 Hz, 18H), 0.97 (s, 3H), 0.87 (s, 3H), 0.82 (d, J = 6.8 Hz, 3H); ¹³C NMR (75 MHz, CDCl₃): δ 146.8, 145.4, 117.0, 109.4, 59.7, 42.0, 41.2, 39.8, 38.7, 37.3, 35.6, 32.7, 31.5, 29.4, 26.1, 22.5, 22.5, 22.4, 17.7, 14.8, 12.3 ppm.

Enantiomer **65** was prepared in a similar manner from **63** and exhibited the same spectral data as *ent-***65**, and $[\alpha]^{22}_D$ +25.0° (c = 1.20, CHCl₃); HRMS (EI, 70 eV) m/z 460.4107 [M⁻; Calcd for C₃₀H₅₆OSi, 460.4100]; Anal. Calcd for C₃₀H₅₆OSi: C, 78.19; H, 12.25. Found: C, 78.57; H, 12.58.

Alcohol *ent-66*
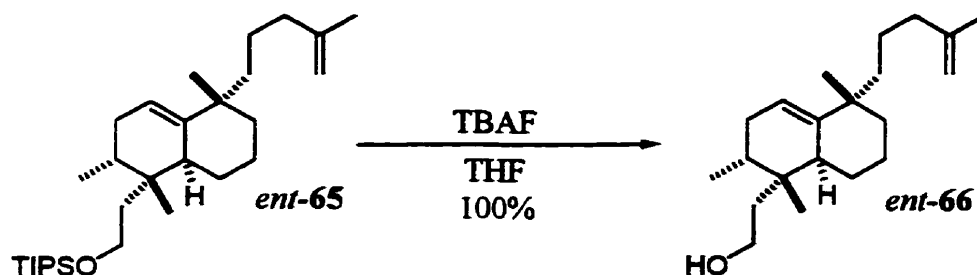
A solution of tetrabutylammonium fluoride (1M, 0.90 mL, 0.90 mmol) was added to a solution of silyl ether *ent-65* (291 mg, 0.63 mmol) in dry THF (5 mL) under a nitrogen atmosphere at rt. The resulting mixture was stirred at rt for 5 hours. After addition of water (1 drop), the mixture was concentrated *in vacuo* and purified by flash chromatography over silica gel (EtOAc/hexanes, 1:5) to afford alcohol *ent-66* (191 mg, 100%) as a colorless oil: TLC R_f= 0.3 (EtOAc/hexanes, 1:5); $[\alpha]^{24}_D$ -65.9° (c = 0.76, CHCl₃); IR (film): υ_{max} 3400br, 3020w, 2930s, 2860m, 1649w, 1443m, 1371m, 1050br, 880m cm⁻¹; ¹H NMR (300 MHz, CDCl₃): δ 5.29 (t, J = 3.5 Hz, 1H), 4.68 (s, 1H), 4.66 (s, 1H), 3.66 (t, J = 7.8 Hz, 2H), 1.98-1.74 (m, 6H), 1.71 (s, 3H), 1.65-1.00 (m, 13H), 0.98 (s, 3H), 0.85 (s, 3H), 0.81 (d, J = 6.7 Hz, 3H); ¹³C NMR (75 MHz, CDCl₃): δ 146.9, 143.2, 117.0, 109.4, 59.8, 42.0, 41.2, 39.9, 38.7, 37.2, 35.6, 32.9, 31.5, 29.4, 26.1, 22.6, 22.5, 14.8 ppm.

Enantiomer **66** was prepared in a similar manner from **65** and exhibited the same spectral data as *ent-66*, and $[\alpha]^{22}_D$ +55.2° (c = 1.6, CHCl₃); HRMS (EI, 70 eV) m/z 304.2772 [M⁺; Calcd for C₂₁H₃₆O, 304.2766]. Anal. Calcd for C₂₁H₃₆O: C, 82.83; H, 11.92. Found: C, 82.72; H, 12.06.

Aldehyde *ent*-67
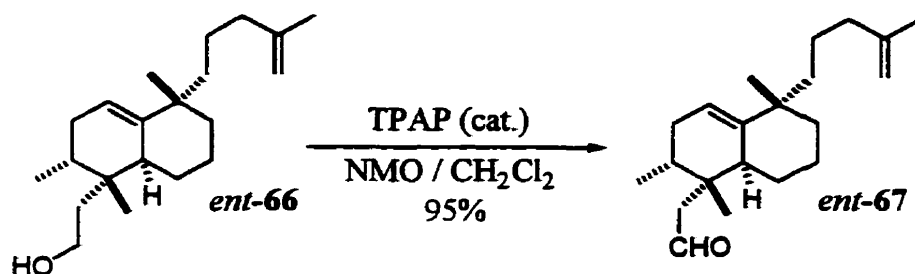
Tetrapropylammonium perruthenate (TPAP; 5 mg, 0.014 mmol, 4 mol%) was added in one portion to a stirred mixture of alcohol *ent*-66 (112 mg, 0.37 mmol), 4-methylmorpholine 4-oxide (65 mg, 0.56 mmol), and powdered 4 Å molecular sieves (250 mg) in dry CH_2Cl_2 (5 mL) under a nitrogen atmosphere at rt. The reaction mixture was stirred at rt for 1.5 hour and filtered through a pad of silica gel (CH_2Cl_2 100%). Evaporation of the solvent *in vacuo* provided aldehyde *ent*-67 (106 mg, 95%) as a colorless oil: TLC R_f= 0.50 (EtOAc/hexanes, 1:10); $[\alpha]^{25}_D$ -71.0° (c = 2.98, $CHCl_3$); IR (film): υ_{max} 2920s, 2715w, 1735s, 1646w, 1445m, 1370m, 1060br, 880m cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): δ 9.88 (t, J = 3.2, 1H), 5.33 (t, J = 2.8 Hz, 1H), 4.66 (s, 1H), 4.64 (s, 1H), 2.32 (dd, J = 3.2, 14.2, 1H), 2.23 (dd, J = 3.2, 14.2 Hz, 1H), 2.08-1.71 (m, 7H), 1.67 (s, 3H),1.66-1.08 (m, 9H), 1.07 (s, 3H), 0.98 (s, 3H), 0.84 (d, J = 6.4 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 204.4, 146.2, 145.0, 117.1, 109.7, 47.1, 42.5, 42.0, 40.0, 38.6, 37.6, 37.0, 33.0, 31.6, 29.4, 26.0, 23.0, 22.5, 22.4, 22.3, 14.8 ppm.

Enantiomer 67 was prepared in a similar manner from 66 and exhibited the same spectral data as *ent*-67, and $[\alpha]^{22}_D$ +75.4° (c = 0.7, $CHCl_3$); HRMS (EI, 70 eV) m/z 258.2347 [M^+-C_2H_4O; Calcd for $C_{19}H_{30}$, 258.2351].

1.6   Preparation of siloxyfuran reagent 72

Bromofuranone 69

$$\text{68} \xrightarrow[\substack{CH_2Cl_2 \ / \ 0 \ ^\circ C \ - \ rt \\ 83\%}]{(COBr)_2 \ / \ DMF} \text{69}$$
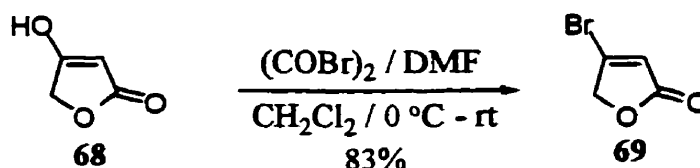
To a solution of tetronic acid (**68**) (8.34 g, 83.33 mmol) and DMF (8.40 mL, 108.33 mmol) in dry CH_2Cl_2 (200 mL) cooled at 0 °C was added dropwise a solution of oxalyl bromide in CH_2Cl_2 (2.0M, 50 mL, 100 mmol) over a period of one hour under a nitrogen atmosphere. The reaction mixture was warmed to rt after three hours and quenched with water (250 mL). The phases were separated and the aqueous phase was extracted with Et_2O (2 x 100 mL). The combined extracts were washed with aq. sat. $NaHCO_3$ (50 mL), brine (50 mL), dried ($MgSO_4$), and concentrated *in vacuo*. Purification of the residue by flash chromatography over silica gel (EtOAc/hexanes, 25:75) afforded **69** (11.2 g, 83%) as a white solid (mp = 78.1 °C). TLC $R_f = 0.24$ (EtOAc/hexanes, 2:8); ^{1}H NMR (300 MHz, $CDCl_3$): δ 6.32 (t, J = 1.8 Hz, 1H), 4.84 (d, J = 1.5 Hz, 2H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 170.7, 146.1, 121.6, 74.8 ppm.

The spectral data were in accord with those reported in the literature.[55]

Bromosilyloxyfuran 70
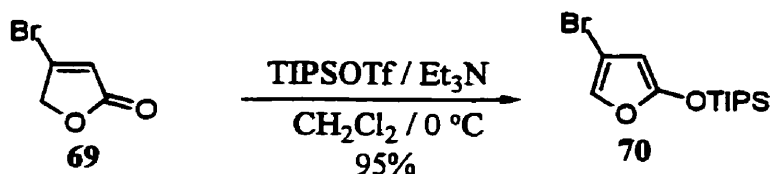
To a solution of bromofuranone **69** (1.50 g, 9.20 mmol) in dry CH_2Cl_2 (25 mL) was added dropwise Et_3N (1.7 mL, 11.96 mmol) under a nitrogen atmosphere at 0 °C. After 15 minutes of stirring, TIPSOTf (2.70 mL , 10.12 mmol) was added dropwise and the reaction mixture stirred at 0 °C for one hour. The reaction was quenched with aq. 1% NaOH (2 mL) and the mixture stirred for another 15 minutes. Water (30 mL) was added and the product was extracted with CH_2Cl_2 (2 x 50 mL). The combined extracts were dried $(MgSO_4)$, and the yellowish residue was immediately purified by flash chromatography over a short column (approximately 2 cm) of silica gel (100% hexanes) to yield **70** (2.79 g, 95%) as a colorless oil. TLC R_f = 0.86 (100% hexanes); IR (film) υ_{max} 3142w, 2940m, 2861s, 1608s, 1533m, 1462m, 1357m, 1268m, 1190m, 1099m, 956m, 918m, 880m, 830m, 748m, 684m cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 6.84 (d, J = 1.2 Hz, 1H), 5.25 (d, J = 1.3 Hz, 1H), 1.25 (m, 3H), 1.02 (d, J = 7.0 Hz, 18H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 156.6, 127.0, 100.3, 87.6, 17.3, 12.0 ppm.

The spectral data were in accord with those reported in the literature.[56]

Siloxyfuran reagent 72
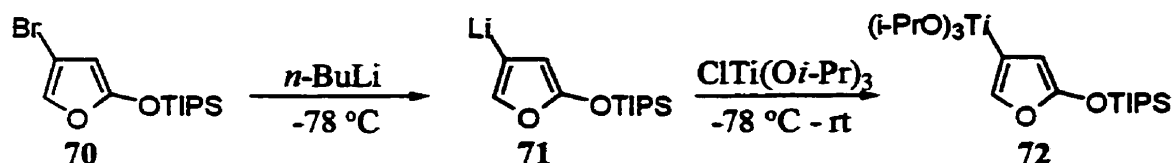
To a solution of bromosiloxyfuran **70** (970 mg, 3.04 mmol) in dry Et$_2$O (6 ml) was added dropwise a solution of *n*-BuLi (2.6M, 1.46 mL, 3.80 mmol) under a nitrogen atmosphere at -78 °C and the reaction mixture was stirred for another 30 minutes at that temperature. The mixture (containing lithiofuran **71**) was added dropwise to a solution of ClTi(O*i*-Pr)$_3$ (1.0M, 3.65 mL, 3.64 mmol) in a dry centrifugation tube at -78 °C. The dark reaction mixture was stirred for 30 minutes at -78 °C, allowed to warm to rt, and stirred for another 30 minutes. The mixture containing siloxyfuran reagent **72** was centrifuged for approximately 15 minutes at high speed to precipitate the salts from the solution after which it was ready for use.

1.7 Completion of the synthesis of dysidiolide

Preparation of siloxyfurans 73 and 74: Procedure A
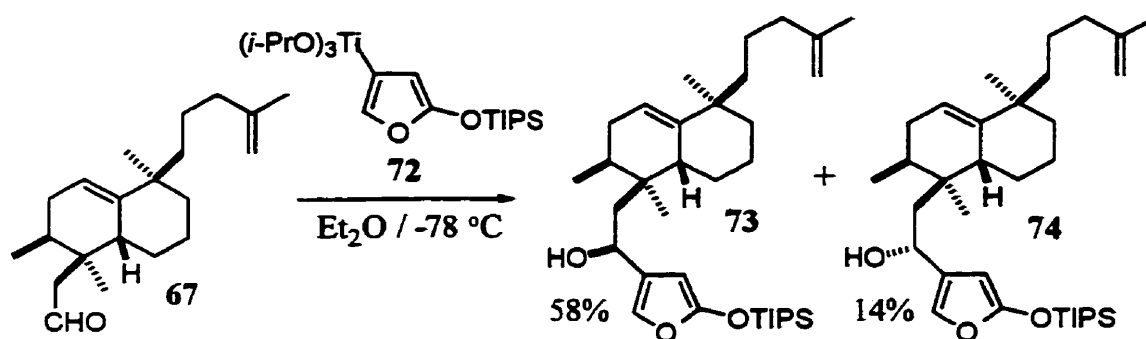
The titanium reagent **72** (*ca.* 0.27 M, 1.35 mL, 0.36 mmol) was added dropwise to a solution of aldehyde **67** (27 mg, 0.089 mmol) in dry Et_2O (1 ml) under a nitrogen atmosphere at -78 °C. The mixture was stirred for 40 minutes at -78 °C, quenched with water (1 ml) at the same temperature, allowed to warm to rt, diluted with water (20 ml), and extracted with Et_2O (5 × 15 ml). The combined extracts were washed with brine (10 ml), dried ($MgSO_4$), and concentrated *in vacuo*. The residue was immediately purified by flash chromatography over a column (8-10 cm) of oven-dried silica gel (Hexanes/EtOAc/Et$_3$N, 96:3:1) to give **73** (28.5 mg, 58%) and **74** (6.9 mg, 14%). These epimers were formed in a ratio of 2.2 : 1 (**73** : **74**), as determined by HPLC of the crude reaction mixture.

Preparation of siloxyfurans *ent*-73 and *ent*-74: Procedure B

To a solution of commercially available 2,6-di-*tert*-butyl-4-methylphenol (263 mg, 1.18 mmol) in dry toluene (3 mL) was added dropwise a solution of trimethylaluminium (2.0M, 300 µL, 0.591 mmol) under a nitrogen atmosphere at rt and the mixture was stirred at that temperature for one hour and cooled to -78 °C. A solution of aldehyde *ent*-67 (59.5 mg, 0.197 mmol) in dry Et_2O (3 mL) was added dropwise at -78 °C. To this mixture, the titanium reagent 72 was added dropwise at the same temperature until no aldehyde could be detected by TLC. The reaction mixture was quenched with water (3 ml) at -78 °C, warmed to rt, diluted with water (20 ml), saturated with solid NaCl, and extracted with Et_2O (4 × 25 ml). The combined extracts were washed with brine (10 ml), dried ($MgSO_4$), and concentrated *in vacuo*. The residue was immediately purified by flash chromatography over a 10 cm column of oven-dried silica gel (hexanes/EtOAc/Et_3N, 98:1:1, then 95:4:1) to furnish epimeric alcohols *ent*-73 (34 mg, 51%) and *ent*-74 (14 mg, 14%). These epimers were formed in a ratio of 3.65 : 1 (*ent*-73 : *ent*-74), as determined by HPLC of the crude reaction mixture.

Major isomer *ent*-73: Colorless oil; TLC $R_f = 0.45$ (EtOAc / hexanes, 1:9), IR (film) ν_{max} 3440s, 2920s, 1722m, 1630m, 1563m, 1451m, 1370m, 1254m, 1075br, 957m, 877m, 834w, 790 w, 675w cm^{-1}; ^{1}H NMR (300 MHz, CDCl$_3$): δ 6.70 (s, 1H), 5.31 (br.s, 1H), 5.15 (s, 1H), 4.69 (m, 1H), 4.67 (s, 1H), 4.61 (s, 1H), 2.00-1.74 (m, 6H), 1.65 (s, 3H), 1.58-1.50 (m, 5H), 1.30-1.16 (m, 7H), 1.08 (d, J = 7.1 Hz, 18H), 1.05 (s, 3H), 1.03-1.00 (m, 4H), 0.95 (s, 3H), 0.87 (d, J = 6.5 Hz, 3H); ^{13}C NMR (75 MHz, CDCl$_3$): δ 157.1, 150.2, 146.1, 132.5, 127.2, 117.1, 109.8, 82.6, 64.6, 41.6, 40.0, 38.5, 36.2, 33.4, 29.7, 26.1, 22.3, 21.9, 17.7, 17.5, 15.0, 12.3, 12.2 ppm.

Enantiomer **73** exhibited the same spectral data as *ent*-**73**, and HRMS (EI, 70 eV) m/z 542.4163 [M$^+$; Calcd for C$_{34}$H$_{58}$O$_3$Si, 542.4155].

Minor isomer *ent*-74: Colorless oil; TLC $R_f = 0.51$ (EtOAc/ hexanes, 1:9); ^{1}H NMR (300 MHz, CDCl$_3$): δ 6.72 (s, 1H), 5.32 (br.s, 1H), 5.14 (s, 1H), 4.66 (m, 1H), 4.65 (s, 2H), 1.95 (t, J = 7.4 Hz, 2H), 1.82-1.78 (m, 4H), 1.68 (s, 3H), 1.59-1.50 (m, 6H), 1.31-1.16 (m, 10H), 1.09 (d, J = 7.1 Hz, 18H), 0.99 (s, 3H), 0.94 (s, 3H), 0.76 (d, J = 6.4 Hz, 3H); ^{13}C NMR (75 MHz, CDCl$_3$): δ 157.1, 146.5, 132.63, 127.3, 117.0, 109.5, 82.6, 65.6, 38.6, 33.0, 26.1, 22.5, 22.4, 17.5, 14.8, 12.2 ppm.

Enantiomer **74** exhibited the same spectral data as *ent*-**74**, and HRMS (EI, 70 eV) m/z 542.4163 [M$^+$; Calcd for C$_{34}$H$_{58}$O$_3$Si, 542.4155].

Dysidiolide (*ent*-1)
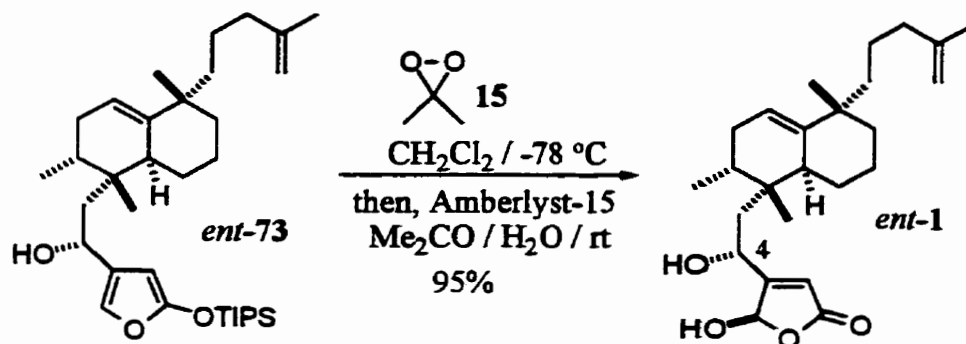
To a solution of *ent*-73 (55 mg, 0.102 mmol) in acetone (5 ml) was added dropwise a solution of dimethyldioxirane (15; 0.05M in acetone, 2.3 mL, 0.115 mmol) under a nitrogen atmosphere at -78 °C. The reaction mixture was stirred for one hour at this temperature and the solvent was evaporated *in vacuo* at -78 °C. The residue was dissolved in acetone (5 ml) and water (0.7 ml) was added followed by Amberlyst-15 (30 mg). The reaction mixture was stirred at rt for 1.5 hour and then the resin was filtered off, rinsed with Me_2CO (20 mL) and the solvent evaporated *in vacuo* to give a pale yellow solid. The crude product was washed with hexanes (2×1.5 ml) to afford pure *ent*-1 (39.5 mg, 95%) as a white solid (m.p. 181-184 °C); TLC R_f = 0.31 (CH_2Cl_2/MeOH, 95:5); HPLC (C_{18} column): t_R = 6.90 min (85% MeOH-15% H_2O, flow rate = 1 mL/min) and 9.13 min (70% CH_3CN-30% H_2O, flow rate =1 mL/min); $[\alpha]^{24}_D$ -10.3° (c = 0.64, MeOH / CH_2Cl_2, 1:1); IR (KBr) υ_{max} 3320br, 2920s, 1735s, 1644w, 1441m, 1266m, 1247m, 1128s, 940s cm^{-1}; ^1H NMR (500 MHz, d_6-DMSO): δ 7.84 (s, 1H), 6.12 (br.s, 1H), 5.94 (br.s., 1H), 5.29 (s, 1H), 5.23 (br.s, 1H), 4.64 (s, 1H), 4.60 (s, 1H), 4.47 (br.s, br.s, 1H), 2.23 (br.s, 1H), 1.98 (m, 1H), 1.90 (br.s, 3H), 1.78 (d, J = 9.9 Hz, 2H), 1.78-1.62 (m, 1H), 1.62 (s, 3H), 1.51-1.49 (m, 5H), 1.23 (m, 2H), 1.12 (br.s, 2H), 1.01 (m, 2H), 0.94 (s, H), 0.85 (m, 2H), 0.82 (d, J = 6.8 Hz, 3H); ^{13}C NMR (125 MHz, d_6-DMSO): δ 170.5 (br), 145.3, 116.1 (br), 115.8 (br), 110.0, 98.1 (br), 64.2 (br), 41.0 (br), 37.9, 36.4 (br), 33.1 (br), 29.7 (br), 25.9, 22.1, 21.7, 21.4 (br), 14.9 ppm.

Enantiomer 1 was prepared in a similar manner from 73 and exhibited the same spectral data as *ent*-1, and $[\alpha]^{22}_D$ +10.5° (c = 0.5, MeOH / CH_2Cl_2, 1:1); MS (CI, NH_3): 403(M^++1, 22), 385 (74), 377 (38), 341 (14), 301 (46), 285 (31), 259 (66), 176 (100), 161 (25), 147 (20), 121 (30), 109 (23), 95 (28); HRMS (CI, NH_3) m/z 403.2854 [M^++1; Calcd for $C_{25}H_{39}O_4$, 403.2848]. The spectral data were in accord with those reported in the literature.[2]

4-Epi-*ent*-dysidiolide (4-epi-*ent*-1)
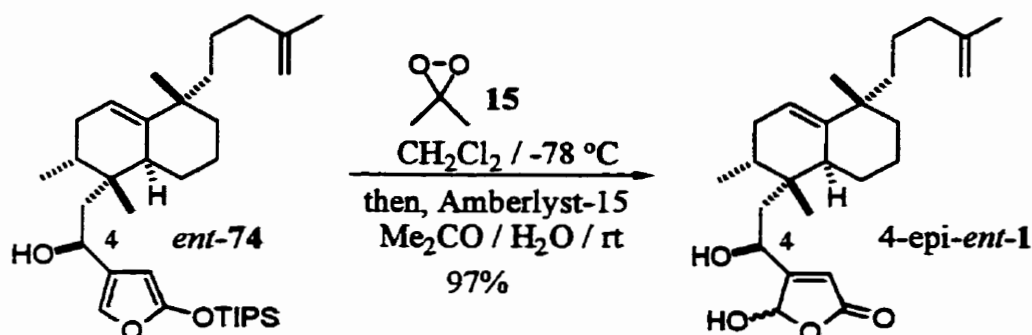
To a solution at -78 °C of *ent*-74 (15.5 mg, 0.0286 mmol) in acetone (3 ml) under nitrogen atmosphere was added dropwise a solution of dimethyldioxirane (15; 0.05M in acetone, 0.63 mL, 0.0315 mmol) and the reaction mixture was stirred for one hour at this temperature and the solvent was evaporated *in vacuo* at this temperature. The crude residue was dissolved in acetone (5 ml), water (0.7 ml) was added, followed by Amberlyst-15 (15 mg), and the reaction mixture was stirred at rt for 1.5 hour. The Amberlyst-15 was then filtered off, rinced with Me_2CO (15 mL) and the solvent evaporated *in vacuo*. The crude product was purfied by flash chromatography over silica gel (CH_2Cl_2/MeOH, 95:5) to afford 4-epi-*ent*-1 as a colorless oil (97%, 11.1 mg): TLC R_f = 0.31 (CH_2Cl_2/MeOH, 95:5); HPLC (C_{18} column): R_t = 7.77 min (85% MeOH-15% H_2O, flow rate = 1 mL/min); $[\alpha]^{24}_D$ -92.4° (*c* = 0.33, MeOH / CH_2Cl_2, 1:1); IR (film): υ_{max} 3370br, 2930s, 1747s, 1645w, 1448m, 1375m, 1332w, 1134m, 1062w, 950m, 884m, 810w, 735w, 699w cm^{-1}; ^{1}H NMR (500 MHz, d_6-DMSO): δ 7.89 (br.s, 1H), 6.14 (s, 1H), 5.83 (br, 1H), 5.32 (s, 1H), 5.26 (s,1H), 4.62 (s, 1H), 4.60 (s, 1H), 4.52 (br.s, 1H), 2.70 (br.s, 1H), 1.99-1.76 (m, 4H), 1.74 (d, J = 10.3 Hz, 2H), 1.63 (s, 3H), 1.54 (br.s, 2H), 1.45 (d, J = 12.7 Hz, 2H), 1.35-0.97 (m, 7H), 0.93 (s, 6H), 0.76 (d, J = 6.6 Hz, 3H): ^{13}C NMR (75 MHz, d_6-DMSO): δ 170.6, 145.9, 116.1 (br), 109.9, 65.2, 38.2, 36.2, 32.7, 26.3, 22.5, 22.0, 14.9 ppm.

Enantiomer 4-epi-1 was prepared in a similar manner from **74** and exhibited the same spectral data as 4-epi-*ent*-1, and $[\alpha]^{22}_D$ +65.0° (*c* = 0.55, MeOH / CH_2Cl_2, 1:1); MS (CI, NH$_3$): 403(M$^+$+1, 22), 385 (58), 377 (15), 341 (5), 301 (46), 285 (22), 259 (55), 176 (100), 161 (16), 149 (15), 121 (26), 109 (13), 95 (12); HRMS (CI, NH$_3$) m/z 403.2854 [M$^+$+1; Calcd for $C_{25}H_{39}O_4$, 403.2848].

CHAPTER 2

LUFFARIELLOLIDE (2)

2.1 Preparation of building block 149

1,3-Diacetoxyacetone 144
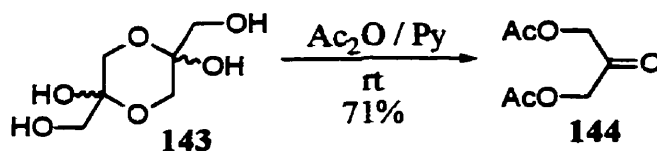
To a solution of commercially available 1,3-dihydroxyacetone dimer **143** (15.0 g, 166.5 mmol) in pyridine (60 mL) was added dropwise excess acetic anhydride (60 mL) with periodic cooling using an ice bath to avoid excessive heating. The reaction mixture was stirred overnight at rt and then diluted with EtOAc (400 mL). The mixture was repeatedly washed with aq. 10% HCl until the aqueous phase remained acidic. The organic layer was then washed twice with water followed by repeated washing with sat. aq. $NaHCO_3$ until the aqueous phase remained basic. The organic layer was then washed with brine (2 x 70 mL), dried ($MgSO_4$), and concentrated *in vacuo*. The residue was then purified by flash chromatography (Et_2O/hexanes, 70/30, then Et_2O 100%) to afford 1,3-diacetoxyacetone **144** (20.51 g, 71%) as an oily solid (mp = 46-48 °C); TLC R_f = 0.20 (Et_2O/hexanes, 1/1); IR (film): υ_{max} 2980m, 2936m, 1745s, 1417s, 1373s, 1235s, 1173s, 1129s, 1052s, 1037s, 984m, 905m, 848m cm⁻¹; ¹H NMR (300 MHz, $CDCl_3$): δ 4.73 (s, 4H), 2.15 (s, 6H).

The spectral data were in accord with those reported in the literature.[91]

Ethyl 3,3-diacetoxymethyl acrylate 146
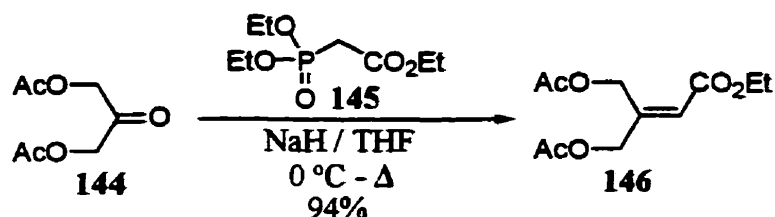
To a suspension of NaH (95%, 3.125 g, 123.7 mmol) in dry THF (100 mL) at 0 °C under a nitrogen atmosphere was added the commercially available Emmons-Horner reagent **145** (24.5 mL, 123.7 mmol) dropwise and the mixture was stirred for one hour at this temperature. 1,3-Acetoxyacetone **144** (20.5 g, 117.8 mmol) in dry THF (100 mL) was then added dropwise to the reagent **115** and the mixture was stirred at 0 °C for one hour, after which it was refluxed for another hour. The reaction mixture was cooled to rt, poured into brine (100 mL), and extracted with ether (5 x 80 mL). The combined ether extracts were then dried (MgSO$_4$), and concentrated *in vacuo*. The resulting crude liquid was distilled under reduced pressure (90-110 °C) to afford the desired ethyl 3,3-diacetoxymethyl acrylate **146** (23.5 g, 94%); TLC R$_f$ = 0.49 (Et$_2$O/hexanes, 1/1); IR (film): ν_{max} 3000m, 1755s, 1670m, 1230s, 1160s, 1045s cm^{-1}; ^{1}H NMR (300 MHz, CDCl$_3$): δ 5.99 (s, 1H), 5.24 (s, 2H), 4.71 (s, 2H), 4.18 (q, J = 7.2 Hz, 2H), 2.12 (s, 3H), 2.07 (s, 3H), 1.28 (t, J = 7.2 Hz, 3H).

The spectral data were in accord with those reported in the literature.[91]

4-(Hydroxymethyl)-2-(5H)-furanone 147
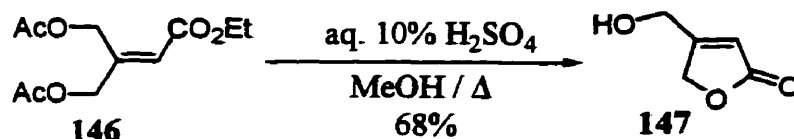
To a solution of ethyl 3,3-diacetoxymethyl acrylate **146** (23.5g, 110.8 mmol) in MeOH (45 mL) was added dropwise aq. 10% H_2SO_4 (45 mL) and the mixture was refluxed for two hours. Solid $NaHCO_3$ was then added until the solution was neutralized. The salts were then filtered off, washed with MeOH, and the solvent concentrated to approximately 10% of its original volume. Water (50 mL) was then added and the product was extracted with EtOAc (5 x 100 mL). The combined extracts were then dried ($MgSO_4$) and concentrated *in vacuo*. The crude product was then purified by flash chromatography over silica gel (EtOAc/CH_2Cl_2, 1/1 then EtOAc 100%) to afford 4-(hydroxymethyl)-2-(5H)-furanone **147** (8.59 g, 68%); mp = 50-52°C; TLC R_f = 0.25 (EtOAc/CH_2Cl_2, 1/1); IR (film): υ_{max} 3380br, 3100w, 1730s, 1640m, 1450m, 1250m, 1140m, 1065m, 1010m, 900m, 850m cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): δ 6.03 (s, 1H), 4.86 (s, 2H), 4.59 (s, 2H), 2.38 (s, 1H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 174.5, 170.9, 114.3, 71.5, 58.6 ppm.

The spectral data were in accord with those reported in the literature.[91]

4-Chloromethyl-2-(5H)-furanone 148
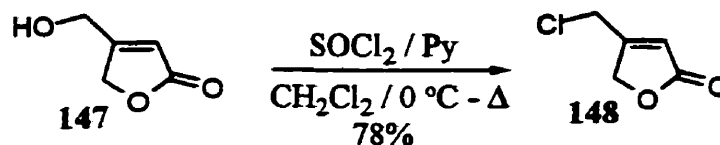
To a solution of 4-(hydroxymethyl)-2-(5H)-furanone **147** (820 mg, 7.19 mmol) in dry CH_2Cl_2 (20 mL) at 0 °C under a nitrogen atmosphere was added pyridine (880 µL, 10.79 mmol) under a nitrogen atmosphere followed by a solution of $SOCl_2$ (1.6 mL, 21.58 mmol) in dry CH_2Cl_2 (20 mL). The temperature was then gradually increased so that the reaction mixture was refluxed for a period of approximately 3 hours until no starting material could be detected by TLC. The solvent was evaporated with a stream of dry nitrogen and the residue was poured into water (20 mL) and CH_2Cl_2 (50 mL) and extracted with CH_2Cl_2 (2 x 50 mL). The combined extracts were then washed with water (2 x 20 mL), dried ($MgSO_4$), and concentrated *in vacuo*. The crude residue was filtered over silica gel (Et_2O 100%) to afford 4-chloromethyl-2-(5H)-furanone **148** as a slightly yellow oil (742 mg, 78%): TLC R_f = 0.80 ($EtOAc/CH_2Cl_2$, 1:1); IR (film): υ_{max} 3098m, 2927m, 1922w, 1750s, 1644s, 1435s, 1350m, 1318s, 1267s, 1233s, 1168s, 1126s, 1030s, 882s, 830m, 735s, 698s, 612m cm^{-1}; ^{1}H NMR (300 MHz, CDCl$_3$): δ 6.04 (d, J = 1.4 Hz, 1H), 4.82 (d, J = 0.9 Hz, 2H) 4.36 (s, 2H); ^{13}C NMR (75 MHz, CDCl$_3$): δ 172.4, 164.0, 118.0, 71.4, 37.7 ppm.

The spectral data were in accord with those reported in the literature.[92]

4-(Chloromethyl)-2-(triisopropylsilyloxy)-furan 149
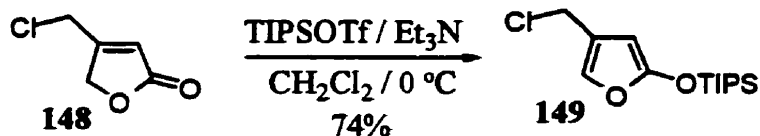
To a solution of 4-chloromethyl-2-(5H)-furanone **148** (526 mg, 3.96 mmol) in dry CH_2Cl_2 (15 mL) at 0 °C was added Et_3N (720 μL, 5.16 mmol) and the reaction mixture was stirred for 10 minutes. TIPSOTf (1.1 mL, 3.97 mmol) was then added dropwise and the mixture was stirred for 45 minutes. A solution of NaOH (1%, 1 mL) was added to the reaction mixture and after another 10 minutes of stirring, the mixture was poured into Et_2O (50 mL) and water (50 mL) and extracted with Et_2O (2 x 50 mL). The combined extracts were dried ($MgSO_4$) and concentrated *in vacuo*. The resulting liquid was rapidly purified by flash chromatography (hexanes 100%) on a short column of silica gel (1-2 cm) to afford 4-(chloromethyl)-2-(triisopropylsilyloxy)-furan **149** (839 mg, 74%) as a colorless oil. TLC R_f = 0.42 (hexanes 100%); IR (film): υ_{max} 2930s, 2857s, 1618s, 1518s, 1457m, 1335m, 1292s, 1259m, 1205s, 1110m, 992m, 953s, 877s, 835s, 684s cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 6.85 (s, 1H), 5.22 (s, 1H), 4.38 (s, 2H), 1.23 (m, 3H) 1.09 (d, J = 7.1 Hz, 18H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 167.5, 129.6, 123.5, 84.2, 38.1, 17.4, 12.0 ppm.

The spectral data were in accord with those reported in the literature.[84]

2.2 Synthesis of butenolide 152 and γ-hydroxybutenolide 153

4-[4-methyl-3-pentenyl]-2-(triisopropylsilyloxy)-furan 151
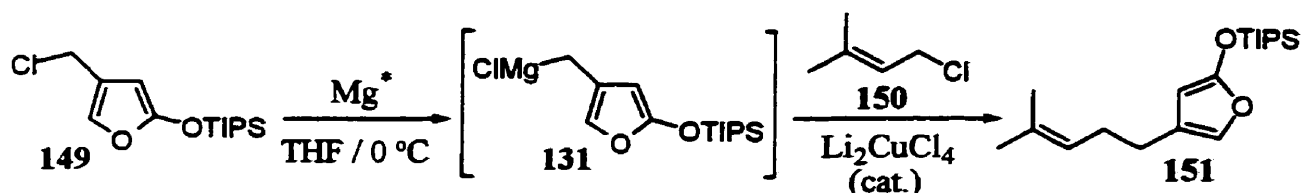
Magnesium turnings (212 mg, 8.70 mmol) were activated by washing successively with aq. 10% HCl, water, acetone and ether, and dried in a vaccum dessicator. The turnings were then flame heated, allowed to cool, and 1,2-dibromoethane (75 μL, 0.87 mmol) and dry THF (4 mL) were added under a nitrogen atmosphere. The mixture was heated to reflux, stirred for 15 minutes, and the THF was cannuled out and replaced with dry THF (3 mL). The resulting suspension was cooled to 0 °C and 4-(chloromethyl)-2-(triisopropylsilyloxy)-furan 149 (835 mg, 2.89 mmol) was added at once. Stirring was continued for one hour after which no more starting material could be detected by TLC. Dry THF (2 mL) was added, the reaction mixture was divided into two parts placed into two separate dry vials at 0 °C. In each of these vials was then added 1-chloro-3-methyl-2-butene (150; 80 μL, 0.70 mmol) at 0 °C, followed immediately by a solution of Li$_2$CuCl$_4$ (0.1 M, 350 μL, 0.035 mmol) in THF. Each reaction mixture was stirred for 20 minutes at that temperature and then poured (in parallel fashion) into water (50 mL) and Et$_2$O (50 mL), and a solution of aq. sat. NH$_4$Cl was added until the two layers separated. The product was extracted with Et$_2$O (3 x 50 mL), washed with brine (25 mL), dried (MgSO$_4$), and concentrated *in vacuo* to afford crude product 151 as a yellowish oil that was used in the next step without further purification; TLC R$_f$ = 0.81 (EtOAc/hexanes, 1:9); ^{1}H NMR (300 MHz, CDCl$_3$): δ 6.57 (s, 1H), 5.12 (t, J = 7.0 Hz, 1H), 5.02 (s, 1H), 2.31 (m, 2H), 2.18 (t, J = 7.3 Hz, 2H), 1.66 (s, 3H), 1.57 (s, 3H), 1.24 (m, 3H), 1.07 (d, J = 7.0 Hz, 18H); ^{13}C NMR (75 MHz, CDCl$_3$): δ 156.5, 131.6, 127.5, 126.5, 123.9, 85.1, 28.1, 25.8, 25.5, 17.4, 15.1, 12.0 ppm.

The spectral data were in accord with those reported in the literature.[84]

4-[4-methyl-3-pentenyl]-2-(5H)-furanone 152

$$\text{151} \xrightarrow[\text{Me}_2\text{CO / H}_2\text{O / rt}]{\text{Amberlyst-15}} \text{152}$$

84%
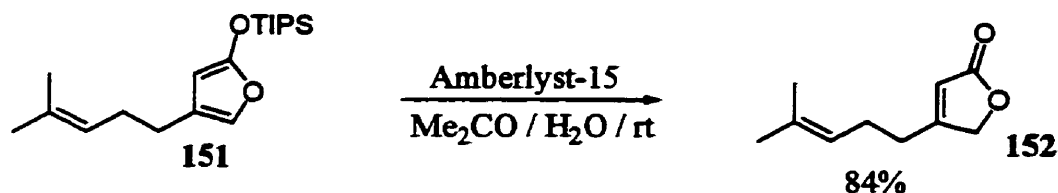
To a solution of crude silyloxyfuran **151** (approximately 112 mg) in Me$_2$CO (10 ml) and water (5 drops) was added Amberlyst-15 (25 mg) and the mixture was stirred at rt for 45 minutes. The resin was filtered off, washed with Me$_2$CO (15 mL), and the solvent was evaporated *in vacuo*. The resulting oil was purified by flash chromatography on silica gel (EtOAc/hexanes, 15:85, then 2:8) to afford pure 4-[(3E)-4-methyl-3-pentenyl]-2-(5H)-furanone **152** as a colorless oil (98 mg, 84%). TLC R$_f$ = 0.24 (EtOAc/hexanes, 2:8); IR (film): u$_{max}$ 2910s, 1778s, 1746s, 1634m, 1442m, 1167m, 1129m, 1039m, 880m cm^{-1}; ^{1}H NMR (300 MHz, CDCl$_3$): δ 5.75 (t, J = 1.3 Hz, 1H), 5.00 (dt, J = 1.1, 6.9 Hz, 1H), 4.66 (d, J = 0.9 Hz, 1H), 2.38 (t, J = 7.3 Hz, 2H), 2.21 (m, 2H), 1.62 (s, 3H), 1.54 (2, 3H); ^{13}C NMR (75 MHz, CDCl$_3$): δ 174.0, 170.3, 133.6, 121.9, 115.3, 73.0, 28.5, 25.5, 25.4, 17.6 ppm.
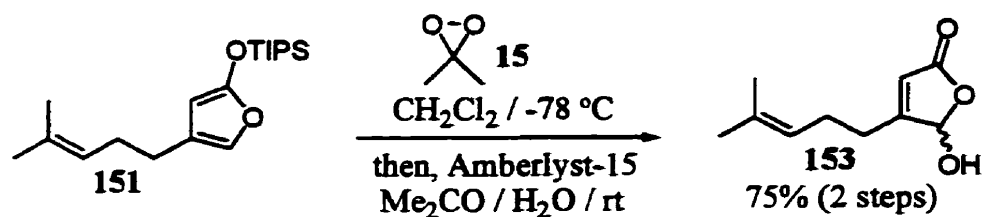
The spectral data were in accord with those reported in the literature.[84]

5-Hydroxy-4-[4-methyl-3-pentenyl]-2-(5H)-furanone 153

To a solution of crude silyloxyfuran **151** (approximately 112 mg) in dry CH_2Cl_2 (10 mL) at -78 °C was added a solution of dimethyldioxirane (**15**; 0.1M, approximately 0.4 mL) until no more starting material could be detected by TLC. The reaction mixture was stirred at -78 °C for one hour and concentrated *in vacuo* at this temperature. The crude oil was dissolved in Me_2CO (10 ml) and water (5 drops) was added followed by Amberlyst-15 (25 mg). The mixture was then stirred at rt for 1.5 hour after which the Amberlyst-15 was filtered off, washed with Me_2CO (15 mL), and the solvent evaporated *in vacuo*. The crude oil was purified by flash chromatography on silica gel (EtOAc/hexanes, 2:8, then 25:75) to afford pure 5-Hydroxy-4-[(3E)-4-methyl-3-pentenyl]-2-(5H)-furanone **153** as a colorless oil (88 mg, 75%). TLC R_f = 0.32 (EtOAc/hexanes, 3:7); IR (film): u_{max} 3300s, 2910s, 1740s, 1648s, 1442m, 1377m, 1332m, 1272m, 1180m, 1125s, 948s, 885m, 730w cm^{-1}; ^{1}H NMR (300 MHz, CDCl$_3$): δ 6.01 (s, 1H), 5.81 (s, 1H), 5.76 (s, 1H), 5.06 (t, J = 6.5 Hz, 2.50 (m, 1H), 2.39 (m, 1H), 2.28 (m, 2H), 1.67 (s, 3H), 1.60 (s, 3H); ^{13}C NMR (75 MHz, CDCl$_3$): δ 172.2, 170.0, 133.5, 122.1, 117.2, 99.4, 27.6, 25.5, 25.0, 17.6 ppm.

The spectral data were in accord with those reported in the literature.[84]

2.3 Completion of the synthesis of luffariellolide (2)

(E,E)-1-Chloro-3,7-dimethyl-9-(2,6,6-trimethyl-1-cyclohexen-1-yl)-2,6-nonadiene 154
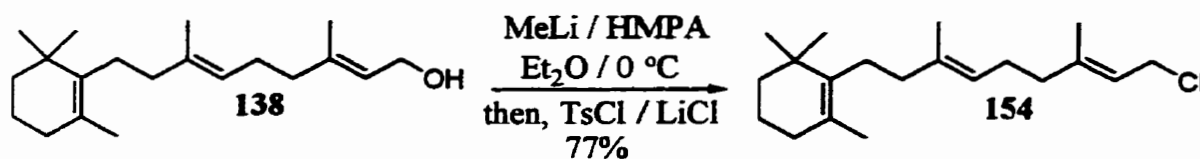
To a solution of allylic alcohol **138** (30 mg, 0.103 mmol) in dry Et$_2$O (1.5 mL) at 0 °C under a nitrogen atmosphere was successively added triphenylmethane (2 mg) as indicator, HMPA (40 mL, 0.35 mL/mmol), and MeLi (1.3 M, 80 mL, 0.105 mmol) over a period of 15 minutes. A solution of tosyl chloride (22 mg, 0.1081 mmol) in dry Et$_2$O (0.5 mL) was added dropwise to the reaction mixture over a period of 15 minutes. Solid LiCl (5 mg, 0.103 mg) was finally added at 0 °C and the reaction mixture was warmed to rt and stirred for another 5 hours. Subsequently, Et$_2$O (50 mL) was added and the organic phase was washed successively with water (2 x 20 mL) and brine (20 mL), dried (MgSO$_4$), and the solvent was evaporated *in vacuo*. The resulting oil was filtered on silica gel (hexanes 100%) to afford pure allylic chloride **154** (25 mg, 77%). TLC R$_f$ = 0.65 (hexanes 100%); IR (film): u$_{max}$ 2908s, 1660w, 1445m, 1377m, 1358m, 1252m, 1188w, 1177m, 1094w cm^{-1}; ^{1}H NMR (300 MHz, CDCl$_3$): δ 5.45 (t, J = 8.0 Hz, 1H), 5.10 (m, 1H), 4.10 (d, J = 8.0 Hz, 2H), 2.13 (m, 4H), 1.99 (m, 4H), 1.90 (s, 3H), 1.88-1.52 (m, 10H), 1.41 (m, 2H), 1.00 (s, 6H); ^{13}C NMR (75 MHz, CDCl$_3$): δ 142.6, 137.0, 136.5, 126.8, 122.7, 120.2, 41.0, 40.1, 39.7, 39.3, 34.9, 32.6, 29.4, 28.5, 27.8, 25.9, 19.7, 19.4, 16.0, 15.9 ppm; HRMS (EI, 70 eV) m/z 308.2271 [M$^+$; Calcd for C$_{20}$H$_{33}$Cl, 308.2265]

4-[(E,E)-4,8-dimethyl-10-(2,6,6-trimethyl-1-cyclohexen-1-yl)-3,7-decadienyl]-2-(triisopropylsilyloxy)-furan 142
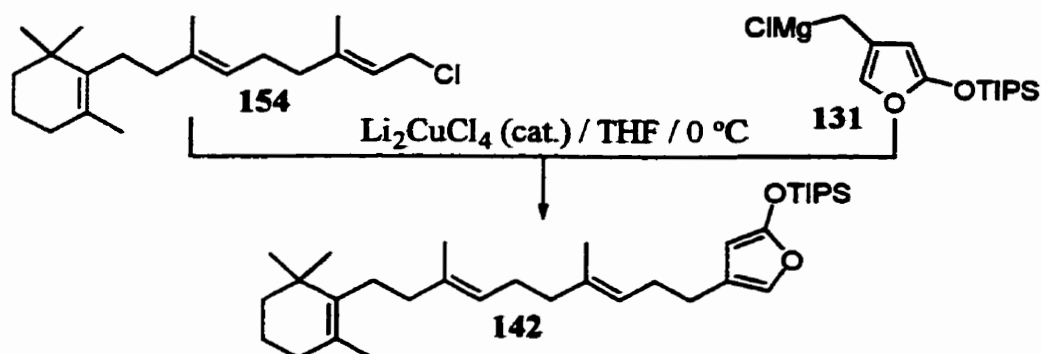
Magnesium turnings (76 mg, 3.12 mmol) were activated by washing successively with aq. 10% HCl, water, acetone and ether, and dried in a vaccum dessicator. The turnings were then flame heated, allowed to cool, and 1,2-dibromoethane (30 μL, 0.312 mmol) and dry THF (2 mL) was added under a nitrogen atmosphere. The mixture was heated to reflux, stirred for 15 minutes, and the THF was cannuled out and replaced with dry THF (1 mL). The resulting suspension was cooled to 0 °C and the 4-(chloromethyl)-2-(triisopropylsilyloxy)-furan **149** (300 mg, 1.04 mmol) was added. Stirring was continued for one hour after which no starting material could be detected by TLC. Dry THF (2 mL) was added and 10% of the volume was transfered to a dry vial at 0 °C. The allylic chloride **154** (15 mg, 0.048 mmol) in dry THF (1 mL) was added to organometallic reagent **131** at 0 °C followed immediately by a solution of Li_2CuCl_4 (0.1 M, 50 μL, 0.005 mmol) in THF. The reaction mixture was stirred for 20 minutes at this temperature and then poured into water (40 mL) and Et_2O (40 mL), and portions of aq. sat. NH_4Cl were added until the layers separated. This mixture was extracted with Et_2O (3 x 40 mL), dried ($MgSO_4$), and concentrated *in vacuo*. The so obtained oil (**142**) was used in the next step without further purification. TLC R_f = 0.77 (hexanes 100%); IR (film): u_{max} 2908s, 1660w, 1445m, 1377m, 1358m, 1252m, 1188w, 1177m, 1094w cm^{-1}; ^{1}H NMR (300 MHz, CDCl$_3$): δ 6.58 (s, 1H), 5.21-4.94 (m, 3H), 2.37-1.89 (m, 14H), 1.65-1.53 (m, 11H), 1.42 (m, 2H), 1.31-1.17 (m, 3H), 1.09 (d, J = 7.1 Hz, 18H), 1.06 (s, 6H); ^{13}C NMR (75 MHz, CDCl$_3$): δ 156.5, 137.1, 135.9, 135.3, 127.6, 126.8, 126.5, 123.8, 123.5, 85.1, 40.2, 39.7, 39.6, 34.9, 32.6, 29.6, 28.5, 28.0, 27.8, 26.5, 25.9, 19.7, 19.4, 17.4, 15.9, 12.1, 10.5 ppm.

The spectral data were in accord with those reported in the literature.[85]

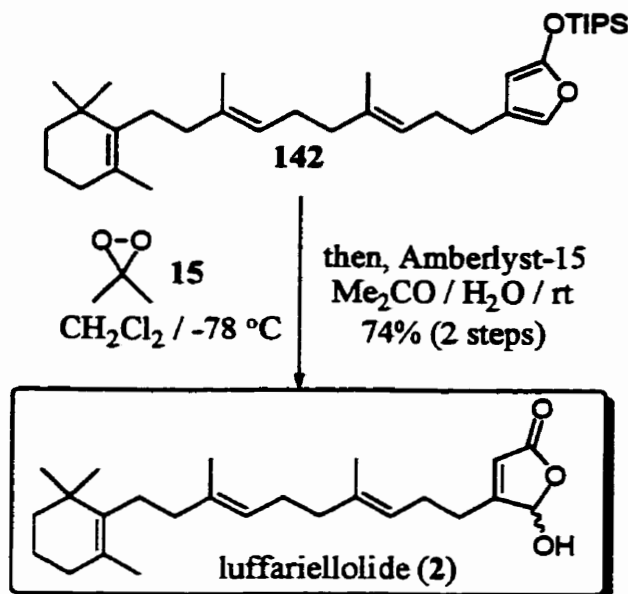## Luffariellolide 2

To a solution of silyloxyfuran **142** in dry CH_2Cl_2 (10 mL) at -78 °C was added sequentially a solution of dimethyldioxirane (**15**; 0.1M; approximately 100 μL) until no starting material could be detected by TLC. After stirring the reaction mixture at -78 °C for one hour, the solvent was removed *in vacuo* at this temperature. The oil obtained was dissolved in Me_2CO (10 ml), water (5 drops) was added followed by Amberlyst-15 (25 mg), and the mixture was stirred at rt for 1.5 hours. The resin was then filtered off, washed with Me_2CO (20 mL), and the solvent evaporated *in vacuo*. The resulting oil was purified by flash chromatography over silica gel (EtOAc/hexanes, 2:8, then 25:75) to afford pure luffariellolide **2** as a colorless oil (88 mg, 74%). TLC R_f = 0.33 (EtOAc/hexanes, 3:7); IR (film): u_{max} 3300br, 2900s, 1760s, 1632w, 1432m, 1369w, 1347w, 1322w, 1259w, 1117m, 936m, 872w, 841w, 723w cm^{-1}; ^{1}H NMR (300 MHz, CDCl$_3$): δ 6.00 (d, J = 7.6 Hz, 1H), 5.86 (s, 1H), 5.11 (t, J = 6.5 Hz, 2H), 4.21 (br, 1H), 2.59-2.28 (m, 4H), 2.09-1.98 (m, 8H), 1.91 (t, J = 6.1 Hz, 2H), 1.64 (s, 6H), 1.60-1.53 (m, 5H), 1.43-1.39 (m, 2H), 0.99 (s, 6H); ^{13}C NMR (75 MHz, CDCl$_3$): δ 171.1, 169.1, 137.3, 137.0, 136.2, 126.8, 123.1, 121.8, 117.8, 98.7, 40.2, 39.7, 39.5, 34.9, 32.6, 28.5, 27.8, 27.6, 26.4, 25.1, 19.7, 19.4, 16.1, 15.9 ppm.

The spectral data were in accord with those reported in the literature.[3]

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