Synthetic Approaches to Marine Labdane Diterpenes

THESIS

Submitted in fulfilment of the requirements
for the Degree of

MASTER OF SCIENCE

of Rhodes University

by

GREGORY ALBERT WISCH

January 2002

ACKNOWLEDGMENTS

Foremost I wish to thank my supervisor, Prof. M.T.Davies-Coleman, for the endless support, encouragement and guidance given throughout this project. I also wish to thank past and present members of my research group (Prof. D.E.A.Rivett, Dr. D.Beukes, Dr L.Collett, Dr K.McPhail, C.Gray, E.Antunes and T. Dzeha), whose expertise, support and friendship helped me through the best and worst of times.

A further thanks needs to be given to Mr J.Buys and Mr A.Sonneman of Rhodes University for technical assistance, Prof. M.Caira of the University of Cape Town for X-ray crystallographic studies and Dr L.Fourie of Potchefstroom University for high resolution mass spectrometry. Many thanks to the National Research Foundation for the financial support over the past two years.

I wish to convey my greatest thanks, love and eternal gratitude to my parents, Les and Denise, and brother, Michael, whose love and unconditional support paves my journey through life, opening up a future for me that has no boundaries. Finally, a heartfelt thanks to all those people in my life, my family and friends, who will always be an inspiration to me.

TABLE OF CONTENTS

LIST OF FIGURES, SCHEMES AND TABLES

LIST OF ABBREVIATIONS

br	broad (related to NMR and IR spectroscopy)
COSY	^{1}H-^{1}H homonuclear COrrelation SpectroscopY
DEPT	Distortionless Enhancement by Polarisation Transfer
d	doublet
EIMS	Electron Impact Mass Spectrometry
EtOAc	ethyl acetate
GLC	Gas-Liquid Chromatography
HMBC	Heteronuclear Multiple Bond Correlation
HMQC	Heteronuclear Multiple Quantum Coherence
HPLC	High Performance Liquid Chromatography
HRFABMS	High Resolution Fast Atom Bombardment Mass Spectrometry
IR	Infra Red
m	multiplet
mpt	melting point
NMR	Nuclear Magnetic Resonance
q	quartet
R	unspecified alkyl group
s	singlet
SCUBA	Self Contained Underwater Breathing Apparatus
t	triplet
TLC	Thin Layer Chromatography
UV	Ultra Violet

ABSTRACT

The work presented in this thesis describes the synthesis of labd-13-en-8β,15-diol (**46**) a stable reduced derivative of an unstable marine natural product aldehyde [8β-hydroxylabd-13E-en-15-al (**6**)] isolated by Cimino, *et al.*[8] from the skin of a Notaspidean mollusc *Pleurobranchaea meckelii*. The rationale for the synthesis was to provide sufficient **46** for eventual mild oxidation to **6** and investigation of the biological activity of this latter compound.

(-)-Sclareol (**32**), a common diterpene synthetic precursor, was the starting point for the ten step synthesis of **46** described in this thesis. A search of the literature revealed that only one non stereospecific synthesis of **46** had been previously reported. To provide the necessary background to the synthetic component of this thesis, both the occurrence of labdane and *ent*-labdane in the marine environment and the use of sclareol in the synthesis of marine natural products, was reviewed.

The initial step in the synthesis of **46** was the potassium permanganate oxidation of sclareol to give a bisnorlabdane ketone. Reduction of this ketone with lithium aluminium hydride produced a mixture of diols which, through *t*-butyldimethysilyl triflate protection, afforded a quantitative route to protecting the C-13 ketone in an effort to prevent unwanted intramolecular cyclization reactions. Dehydration of the tertiary alcohol moiety at C-8 with phosphorous oxychloride yielded the $\Delta^{8,15}$exocyclic alkene required for the next oxidation step. Ozonolysis succeeded in producing the desired C-8 ketone after much deliberation and research into alternative oxidation strategies. Methyl lithium methylation quantitatively afforded the desired α-methyl substitution at C-8, identified at the onset as the key step in the synthesis. Tetra-butylammonium fluoride deprotection yielded the 8β,13-dihydoxylated product, which was in turn subjected to a Swern oxidition to give the desired 8β-hydroxy-bisnorlabda-13-one. A modified Horner-Wadsworth-Emmons reaction allowed for elaboration at C-13 to yield a Δ^{13} olefin with a terminal C-15 ethyl ester. Diisobutylaluminium hydride reduction of the ester produced the desired labd-13-en-8β,15-diol (**46**) in a low overall yield of 3.4%. Although opportunities for optimization of at least three steps in the synthesis exist, time constraints prevented both optimization of these steps and an investigation of the oxidation of **46** to **6**.

CHAPTER 1

INTRODUCTION

1. INTRODUCTION

1.1 The Role of Synthesis in Marine Natural Product Chemistry

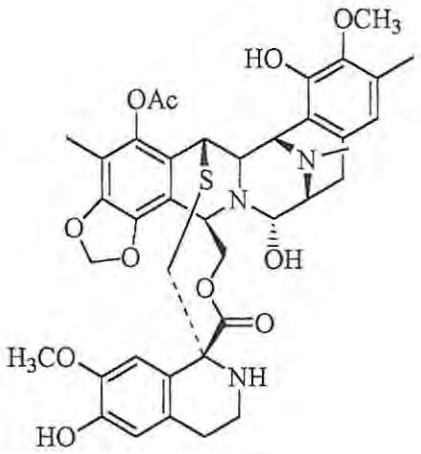
The marine environment is an exceptional reservoir of bioactive natural products, many of which exhibit structural features not found in terrestrial natural products.[1] The evolution of some phyla of marine organisms, *e.g.* sponges, over enormous time periods (500 million years) has meant that these organisms have been able to evolve unique biosynthetic pathways or adapt established pathways to produce complex natural products.[2] The secondary metabolites produced by certain organisms *e.g.* Coelenterata (soft corals, sea fans), Porifera (sponges), Bryozoa (bryozoans) and Echinodermata (sea stars, sea cucumbers),[2] are thought to act as antifeedants or toxins in preventing predation on the producing organism. Other roles for these metabolites include growth inhibition of competing organisms on a marine reef in an environment characterized by limited nutrients and space.

One of the major problems facing marine natural product chemists, interested in exploring the bioactivity of secondary metabolites, either in chemical ecology studies or as potential pharmaceuticals or agrochemicals, is the often extremely low abundance of marine natural products in marine organisms. Several reasons have been proposed for the paucity of these secondary metabolites in the organisms that produce them. These reasons include the enhanced toxicity or bioactivity of marine natural products, relative to the secondary metabolites produced by terrestrial plants, thus requiring less of the compounds to elicit a physiological response in another organism. Other reasons include the variations in the biosynthesis of marine natural products, a process which has been shown to be related to several factors including reproduction, predation, competition for habitable space and seasonal changes relating to physical changes (*e.g.* temperature). Frustratingly, as a result of our very limited knowledge of the factors affecting biosynthesis in marine organisms, underwater collections often do not coincide with periods of maximum biosynthetic activity.

There are three approaches to overcome the problem of the supply of sufficient amounts of marine natural products for biological screening. The first approach is to make larger collections of

invertebrate and/or algal marine material. Most marine natural product studies are carried out on one kilogram or less wet mass of a marine invertebrate or algae. Given the sensitivity of marine reefs to over-exploitation, the collection of more than this amount would have negative ecological ramifications, with permits for such collections being denied by government conservation agencies. Ideally, collections should encompass as many species as possible and should be collected from a large number of sites in order to be representative of the diversity found in the marine environment.

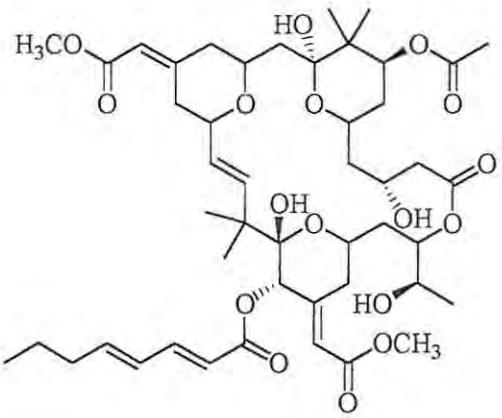
A good example of bioassay guided isolation of a bioactive marine natural product and its current status as an anti-tumour drug candidate is ecteinascidin 743 (**1**). Isolated from the ascidian *Ecteinascidia turbinata*, sufficient quantities of the tetrahydroisoquinolone alkaloid ecteinascidin 743 for clinical trials were obtained by harvesting natural populations of the ascidian.[3]



(1)

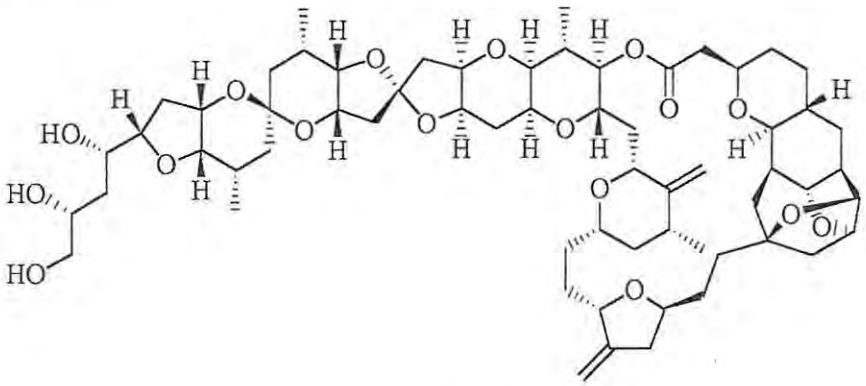
The second approach to providing sufficient material for bioactivity studies is the aquaculture of the marine organisms producing the desired bioactive metabolite. Due to the non-invasive means of acquiring more of the desired metabolite via laboratory fermentation or aquaculture of the marine organism, there is an increasing shift in this direction by research organizations. An example of this approach is the isolation of the anti-cancer metabolite bryostatin (**2**) from the bryozoan *Bugula neritina*. Although an adequate sufficient supply of bryostatin required for preclinical trials was

provided by harvesting wild populations, due to the low yield and large variability from collection to collection exhibited in harvesting, sufficient compound required for Phase I and Phase II clinical trials was provided via an aquaculture approach sponsored by the United States National Cancer Institute.[4]



(2)

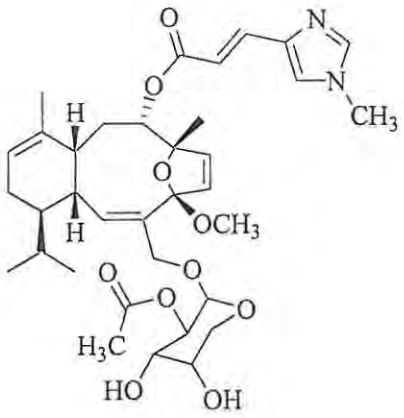
Another example of a successful marine metabolite produced *via* aquaculture is halichondrin B (**3**), a complex macrolide isolated from the sponge *Halochondria okadai*. This compound was identified as a potent inhibitor of tubulin polymerization, showing promising *in vivo* activity against a number of human cancer cell lines. The very limited supply of halichondrin B from naturally occurring sponges prompted the *in situ* production of the compound in the sponge *Lissodendoryx* via aquaculture techniques.[5]



(3)

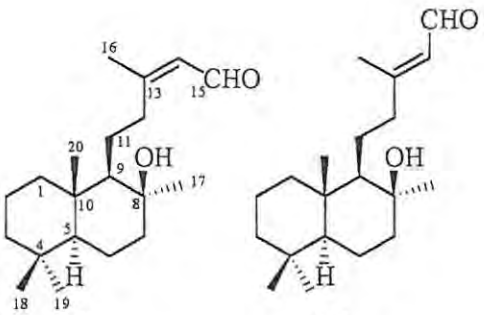
The third approach to solving the marine natural product supply problem is the laboratory synthesis of bioactive, or potentially bioactive marine secondary metabolites. Academic syntheses can also provide valuable structure-activity relationship data. Unfortunately, few synthetic endeavours are short enough or use reagents that are cost effective or safe enough to scale up to large-scale industrial syntheses. These efforts therefore often favour the use of the natural product as a 'lead' structure for synthetic medicinal chemistry, helping to identify synthetically accessible and simpler analogues that exhibit the same or similar bioactivity.[1]

Despite their complexity, several important bioactive marine natural products have been synthesized successfully, most notably the anti-tumour agent eleutherobin (**4**). Eleutherobin, isolated from the rare soft-coral *Eleutherobia albiflora*, was found to possess significant cytotoxicity against breast, renal, ovarian and lung cancer cell lines.[6] Chen, *et al.*[7] reported the first total laboratory synthesis of eleutherobin in 1999. This important synthesis has allowed further investigations into the biological activity of the natural product and the development of possible synthetic analogues.



(4)

We have adopted the latter synthetic approach to the bioactive marine natural product supply problem and in continuation of four decades of diterpene natural product research at Rhodes University and a developing collaboration with Professor Cimino of the Instituto per la Chimica di Molecole di Interesse Biologico (Napoli), we present in this thesis our synthetic approach to the diterpene aldehydes (**5** and **6**) isolated by Cimino, *et al.*[8] from the opisthobranch mollusc *Pleurobranchaea meckelli*.

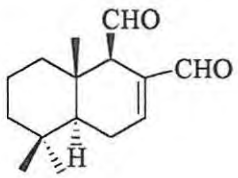


(5) (6)

1.2 Labdane Aldehydes from the Opisthobranch Mollusc *Pleurobranchaea meckelli*

1.2.1. Opisthobranch Molluscs

Many shell-less opisthobranchs, more commonly referred to as nudibranchs or sea slugs, are brightly coloured, using this aposematic colouration to warn potential predators that they contain toxic metabolites. Hence, there have been numerous studies of the chemical defence strategies responsible for the almost complete absence of predation on these seemingly unprotected molluscs. Most nudibranchs sequester toxic or deterrent bioactive metabolites from their prey species, efficiently moving these metabolites from their gut to glands in their outer mantle or skin tissue without appearing to suffer any ill effects. As nudibranchs prey primarily on sponges, bryozoans and coelenterates,[9] these organisms serve as sources of the nudibranch's sequestered metabolites, with the highest incidence and diversity of these metabolites being found in habitats such as coral reefs that are characterised by intense competition and feeding pressure.[10] In some instances opisthobranch molluscs are able to create new chemical defense metabolites through the biotransformation of dietary compounds in a process known as *de novo* biosynthesis.[11] Geographical variance in the metabolites of certain nudibranch species have served as a good predictor of the *de novo* biosynthetic origin of the metabolites.[12] With cold water species often exhibiting this form of chemical defence, the *de novo* biosynthesis of polygodial (7) by *Dendrodoris grandiflora* has been established.[13]



(7)

Opisthobranchs are divided into eight orders, namely: Cephalaspidea; Saccoglossa; Anaspidea; Acochlidiacea; Thecosomata; Gymnosomata; Nudibranchia and Notaspidea. Past research has focused on the chemistry of the sea slugs from the orders Cephalaspidea, Saccoglossa, Anaspidea and Nudibranchia with very little attention given to the study of Notaspidean chemistry.[8,14] *Pleurobranchaea meckelli*, which produced the metabolites we attempted to synthesize in this thesis, is a member of the order Notaspidea which displays very interesting evolutionary characteristics. The order Notaspidea contains species protected by a relic shell (*Umbruculum umbruculum*, *Tylodina perversa*), those that possess an internal shell (*Pleurobranchus memranaceus*, *Berthella aurantiaca*) and those that are completely shell-less (*Pleurobranchaea meckelli*).[8] Although four nudibranchs of the *Pleurobranchus* species occur off the South African coast, the natural product chemistry of the South African species has not been studied.

Figure 1: The Notaspidean nudibranch *Pleurobranchaea meckelli*.

Nudibranchs have yielded a variety of metabolites, such as monoterpenes, sesquiterpenes, diterpenes, sesterterpenes, steroids, diacylguanidine, and the degradation products thereof.[15] Overall, nudibranch skin metabolites tend to be primarily terpenoid, with sesquiterpenes being the most abundant, followed by diterpenes.[12] Studies of the sequestered or *de novo* biosynthesized natural product chemistry of nudibranchs is complicated by three factors. Firstly, the low abundance of nudibranchs in the marine environment. Secondly, the small size of the organism (<5cm in length) and thirdly, the conservation restrictions imposed to prevent the negative impact of the wholesale removal of nudibranchs from the marine ecosystem. Therefore, often the only viable route to follow to explore the bioactivity of nudibranch metabolites is the laboratory synthesis of these metabolites.

1.2.2. The Isolation of the Diterpenes from *Pleurobranchaea meckelli*

The first chemical investigation of the shell-less notaspidean mollusc *Pleurobranchaea meckelli* was reported by Cimino, *et al.*[8] and yielded the isomeric aldehydes 8β-hydroxylabd-13E-en-15-al (**5**) and 8β-hydroxylabd-13Z-en-15-al (**6**). *P.meckelli* was collected in the Gulf of Naples by SCUBA at a depth of 6 meters in June, 1993. *P.meckelli* was found to produce an acid secretion that is a characteristic of most Notaspideans and was initially considered as the main protection of these molluscs against predation. To isolate the natural products, the live animal was sonicated in acetone, the acetone extract concentrated and the concentrate extracted further with diethyl ether. TLC analysis indicated the presence of two UV absorbing compounds, with final separation of these two compounds achieved by preparative TLC to yield **5** (3mg) and **6** (4mg). The structures of compounds **5** and **6** were established from ^{1}H-NMR and ^{13}C-NMR data with supportive 2D NMR experiments (^{1}H-^{1}H-COSY, HMQC, HMBC, ^{1}H-^{1}H decoupling).[8]

Cimino noted that these metabolites were unstable and hence their isolation was performed as quickly as possible. The biological role of these two compounds, located in the skin of the mollusc, were correlated to their probable involvement in the protection of the mollusc against predation, as established for other bioactive aldehydes.[8] The biological role of these molecules has still to be investigated and it is hoped that our synthetic endeavours presented here will enable these investigations to be carried out. It must also be noted here that Cimino, *et al.*[8] postulated that the

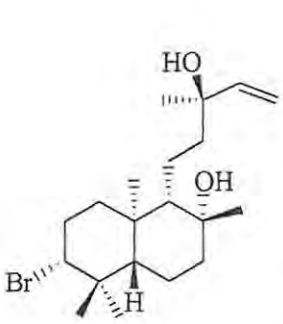
labdane aldehydes **5** and **6** were sequestered by *P.meckelli* from its invertebrate or algal prey and were not produced by *de novo* biosynthesis.

1.3 Labdane and *ent*-Labdane Diterpene Metabolites in the Marine Environment

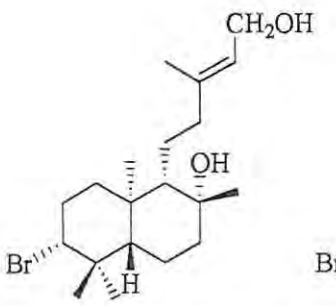
Over the last three decades, marine natural products investigations have yielded a variety of related terpenoid metabolites. Using the computer database MarinLit,[16] we reviewed only diterpenes isolated from marine organisms. Our choice for the search encompassed compounds possessing a basic 20 carbon diterpene skeleton, and the metabolic derivatives thereof with fewer carbon atoms. The connection between the different terpenoid metabolites and their structural similarities all stem from the fact that marine organisms share similar biosynthetic pathways. This review serves to illustrate the fairly limited occurrence of labdane and *ent*-labdane diterpenes in marine organisms, given that approximately 10000 marine natural products are known.

1.3.1. Diterpene Metabolites Isolated from Marine Algae

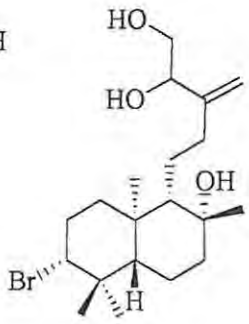
Algae comprise a range of aquatic plants from single-celled to filamentous species and include the taxonomic groups Rhodophyta (red algae), Chlorophyta (green algae) and Phaeophyta (brown algae). Halogenated sesquiterpenes and diterpenes predominate in algae. Due to their abundance in shallow waters and ease of collection, algae were one of the first groups of marine organisms whose natural products chemistry was extensively studied.[1]



(8)



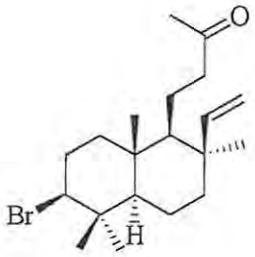
(9)



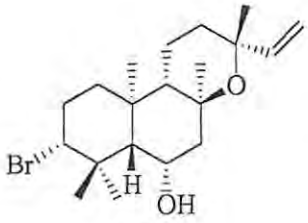
(10)

The chemistry of red algae has also been extensively investigated because of their propensity to

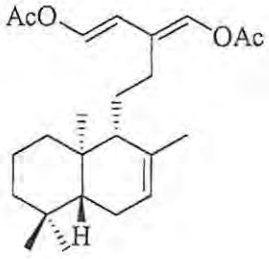
incorporate halogens (bromine, and occasionally chlorine) in their secondary biosynthetic pathways. The marine red alga *Laurencia snyderae*, collected off the coast of Mexico, yielded the brominated *ent*-labdane diterpene isoconcinndiol (**8**).[17] Isoconcinndiol is related to one of the early pioneer marine diterpene metabolites aplysin-20 (**9**), isolated from the Japanese sea hare *Aplysia kurodai*.[18] A brominated *ent*-labdane diterpene triol closely related to **9**, venustanol (**10**), was obtained from the Japanese red alga *L. venusta* by Suzuki, *et al.*.[19] *Aplysia* sea hares commonly sequester their defensive chemicals from *Laurencia* seaweeds.



(11)



(12)



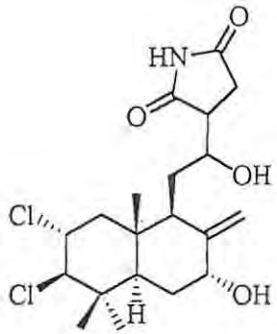
(13)

Another halogenated diterpene, 3β-bromobisnorlabda-8-eth-18-ene-13-one (**11**), obtained from the red alga *L. perforata* collected on the Canary and Madeira archipelagos, was isolated by González, *et al.*.[20] The red algae *L. paniculata* yielded an *ent*-labdane bromoditerpene (-)-paniculatol (**12**).[21] Interestingly, paniculatol was the first tetrahydropyran containing *ent*-bromolabdane to be found in a marine organism. Finally, a bicyclic diterpene with a labd-7-ene skeleton (**13**) was found in several species of green algae, *Caulerpa trifaria*, *C. brownii*, *C. flexilis*, *C. peltata* and *C. racemosa*.[22]

1.3.2. Diterpene Metabolites Isolated from Marine Ascidians

Ascidians, also known as tunicates or sea squirts, usually inhabit shallow waters, but have also been found up to depths of 2000m. The natural products chemistry investigations of these organisms have primarily unearthed new amino acid derived biologically active metabolites and labdane diterpenes are rare.[23] A single example of an ascidian labdane diterpene of mixed biosynthesis is the cytotoxic

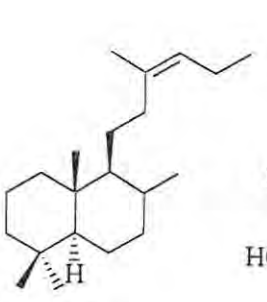
dichlorolissoclimide (**14**), isolated from *Lissoclinum voeltzkowi*, collected off New Caledonia.[24] Dichlorolissoclimide was the first chlorinated compound to be isolated from the phylum Urochordata and exhibited strong cytotoxic activity against human carcinoma KB cells and P388 leukemia cells.



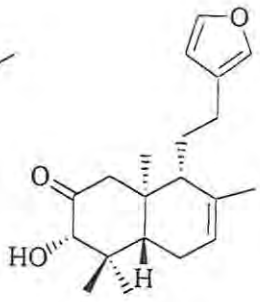
(14)

1.3.3. Diterpene Metabolites Isolated from Marine Sponges

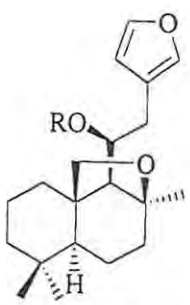
Sponges (phylum Porifera) are the simplest multicellular organisms and are mostly of marine origin. They differ from other groups of invertebrates in that they maintain an almost protozoan independence for their constituent cells, which as a consequence form no true tissue layers or organs.[1] Sponges have become one of the dominant sources of biologically active marine natural products because they are abundant, easy to collect and as discussed earlier, because of their long evolutionary history, able to biosynthesize a wide variety of natural product structural classes.



(15)



(16)



(17) R = Ac
(18) R = H

Agelosine-D (**15**) is one of four novel bicyclic diterpenoids isolated from the orange Okinawan sea sponge *Agelas sp.*.[25] Interestingly, agelosine-D has been shown to act as powerful inhibitors of Na,K-ATPase enzymes and also exhibits strong anti-microbial properties. Ciavatta, *et al.*[26] obtained blanesin (**16**), an *ent*-labdane furanoditerpenoid with an α-hydroxyketone moiety, from the red sponge *Raspaciona aculeata* collected off the coast of Spain. The furanoditerpenoids cacofuran A (17) and B (**18**) were the first bridged tetracyclic diterpenes isolated from the marine sponge *Cacospongia* sp.[27] Both **17** and **18** showed moderate cytotoxicity against P388 and K562 cancer cell lines.

1.3.4. Diterpene Metabolites Isolated from Marine Pulmonate Molluscs

Pulmonate molluscs are a subclass of intertidal gastropods possessing secondary gills, a muscular foot and a shell. The Pacific ocean pulmonate *Trimusculus reticulatus* was found to contain 6β-isovaleroxylabda-8,13-dien-7α,15-diol (**19**) and 2α,7α-diacetoxy-6β-isovaleroxylabda-8,13-dien-15-ol (**20**).[28] *T. reticulatus*, from the family Trimusculidae, was the first pulmonate molluscan source of labdane diterpenoids. Preliminary chemical ecology studies revealed that these metabolites function as a repellant to predatory starfish. The related mollusc *T. peruvianus*, collected on the coast of central Chile, yielded five similar labdane metabolites : 6β-acetoxylabda-8,13-dien-7α,15-diol (**21**); 7α,15-diacetoxylabda-8,13-dien-16β-ol (**22**); 6β,7α,15-triacetoxylabda-8,13-diene (**23**); 6β,7α-diacetoxylabda-8,13-dien-15-ol (**24**) and 6β-acetoxylabda-8,13-dien-15-ol.[29] Gray, *et al.*[30] isolated 6β,7α-diacetoxyladba-8,13-15-ol (**25**) and 2α,6β,7α-triacetoxylabda-8,13-dien-15-ol (**26**) from the only South African member of this species *T. costatus*. Both the latter labdanes exhibited marked antifeedant properties in bioassays carried out with the omnivorous South African predatory fish *Pomadasys commersonnii* (spotted grunter). The structures of the compounds discussed above are presented in Table 1.

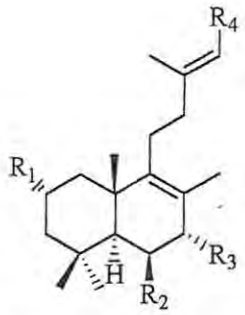
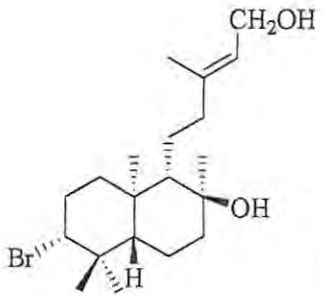


Table 1: A summary of labdane diterpenes isolated from *Trimusculus sp.*

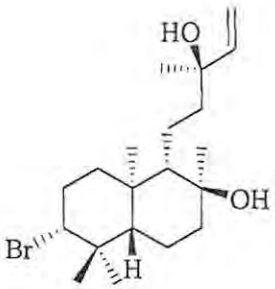
Compound	Substituents	
19	$R^1 = H$	$R^2 = OCOCH_2CH(CH_3)_2$
	$R^3 = OH$	$R^4 = CH_2OH$
20	$R^1 = OAc$	$R^2 = OCOCH_2CH(CH_3)_2$
	$R^3 = OAc$	$R^4 = CH_2OH$
21	$R^1 = H$	$R^2 = OAc$
	$R^3 = OH$	$R^4 = CH_2OH$
22	$R^1 = H$	$R^2 = OH$
	$R^3 = OAc$	$R^4 = CH_2OAc$
23	$R^1 = H$	$R^2 = OAc$
	$R^3 = OAc$	$R^4 = CH_2OAc$
24	$R^1 = H$	$R^2 = OAc$
	$R^3 = H$	$R^4 = CH_2OH$
25	$R^1 = H$	$R^2 = OAc$
	$R^3 = OAc$	$R^4 = CH_2OH$
26	$R^1 = OAc$	$R^2 = OAc$
	$R^3 = OAc$	$R^4 = CH_2OH$

1.3.5. Diterpene Metabolites Isolated from Marine Opisthobranch Molluscs

Opisthobranch molluscs (nudibranchs and sea hares) are an order of molluscs in which the shell and mantle cavity are absent. As discussed in section 1.2.1., the loss of the shell in opisthobranch molluscs requires those organisms to utilize chemical defense to protect themselves from predation.

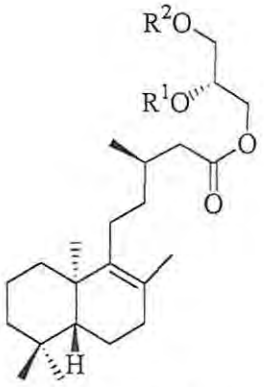


(27)

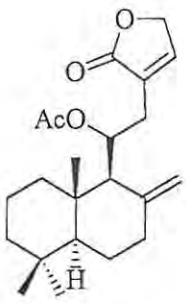


(28)

Aplysin 20 (**9**) was first isolated from the Japanese sea hare *Aplysia kurodai* by Yamamura, *et al.*.[31] Further investigation of *A. kurodai* yielded the new brominated ladbane-type diterpenes, *epi*-aplysin 20 (**27**) and *ent*-isoconcinndiol (**28**).[17]



(29) R^1=H ; R^2=Ac
(30) R^1=Ac ; R^2=H

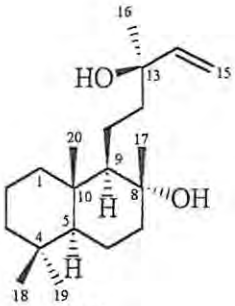


(31)

The antarctic nudibranch *Austrodaris kerguelenesis* is thought to produce the 1,3-glyceryl ester, 3'-acetoxyglyceryl(5R,10R,13R)-labda-8-en-15-oate (**29**), and its corresponding 1,2-derivative, 2'-acetoxyglyceryl(5R,10R,13R)-labda-8-en-15-oate (**30**) by *de novo* biosynthesis.[32,33] Both these metabolites show potent activity as activators of the protein kinase C enzyme. Finally, a study of the skin extracts and egg mass collections of the Northeastern Pacific dorid nudibranch *Cadlina luteomarginata* gave rise to the isolation and identification of several terpenoid metabolites, including the labdane diterpene lutenolide (**31**).[34]

1.4 Sclareol as a Synthetic Precursor for the Synthesis of Marine Metabolites

(-)-Sclareol (labd-14-ene-8α,13β-diol, **32**) is a commercially available natural product ideally suited for use as a chiral synthon in labdane diterpene syntheses and was first isolated from the terrestrial plant *Salvia sclarea* (Labiatae).[35] A complete synthesis of **32**, in nine steps from podocarp-8(14)-en-7-one, was reported by Bigley, *et al.*[36] in 1960.



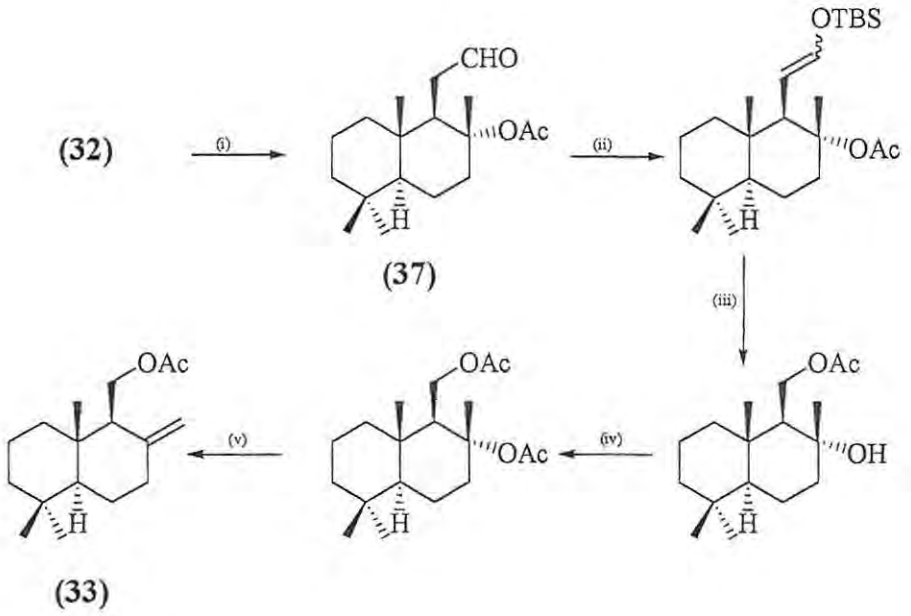
(32)

Sclareol possesses significant biological activity including fungal growth regulation, plant growth inhibition, cytotoxic activity and good antibacterial activity.[37,38,39] Commercially, sclareol has become widely used in a number of industries, where it is used as a fixative and synthon for the preparation of Ambra odourants in the perfume industry, as a flavouring agent in the tobacco industry, as a flavourant in food products and fragrance component in soaps, detergents, creams lotions and tabac-type fragrances.[35]

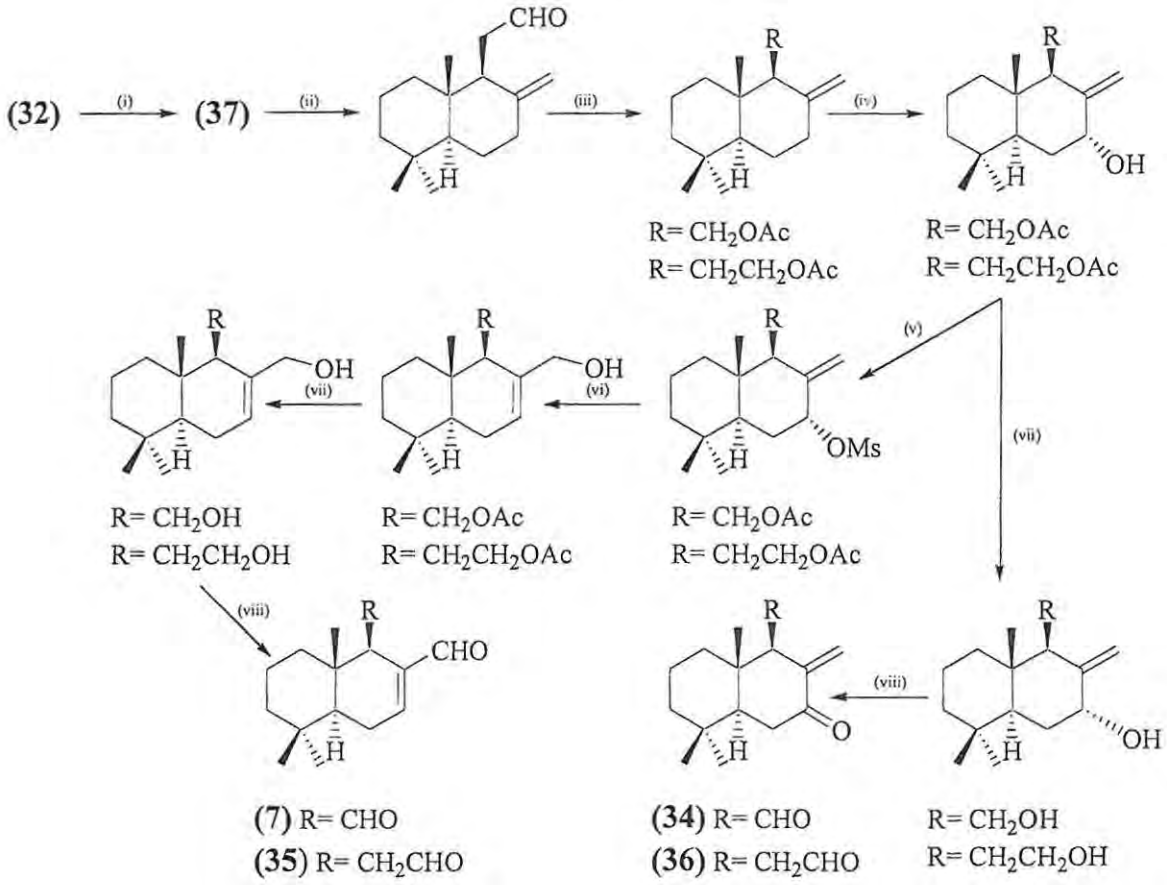
The stereochemistry of sclareol, makes it ideal for use as a synthetic precursor for the synthesis of the labdane diterpenes described in this thesis. A review of the use of sclareol as a synthetic precursor reveals the widespread use of **32** in the syntheses of both terrestrial and marine metabolites. Highlights of the successful use of sclareol in the synthesis of marine metabolites are presented below.

1.4.1. The Synthesis of Polygodial and Albicanyl Acetate from (-)-Sclareol

Polygodial (7) and albicanyl acetate (**33**) are both biologically active drimanes possessing potent fish antifeedant properties. The synthesis of these two marine natural products was undertaken to provide sufficient material to test for antifeedant activity using chemoreceptor protein studies. Some new structurally related active drimanes were concurrently synthesized from **32** in an effort to compare their antifeedant, antitumour and antimicrobial properties with those of polygodial. These compounds included 7-oxo-8,12-drimen-11-al (**34**), 13,14,15,16-tetranorlabd-7-ene (**35**) and 7-oxo-13,14,15,16-tetranorlabd-8(17)-en-12-al (**36**).[40] Interestingly the latter two proved to be significantly more bioactive than polygodial. The synthesis of compound **33** is represented in Scheme 1. The synthesis of compounds 7, **34**, **35** and **36** are presented in Scheme 2.



SCHEME 1 : The synthesis of albicanyl acetate from (-)-sclareol [40] (i) $OsO_4/NaIO_4$, Pr^iOH, 45°C, 6h, 73%. (ii) TBSCl, NaH, THF, -78°C, 4h, 99%. (iii) O_3, $MeOH/CH_2Cl_2$, -78°C, $NaBH_4$, MeOH, rt, 30min., 95%. (iv) Ac_2O, Et_3N, 4-DMAP, THF, reflux, 18h, 92%. (v) Collidine, reflux.

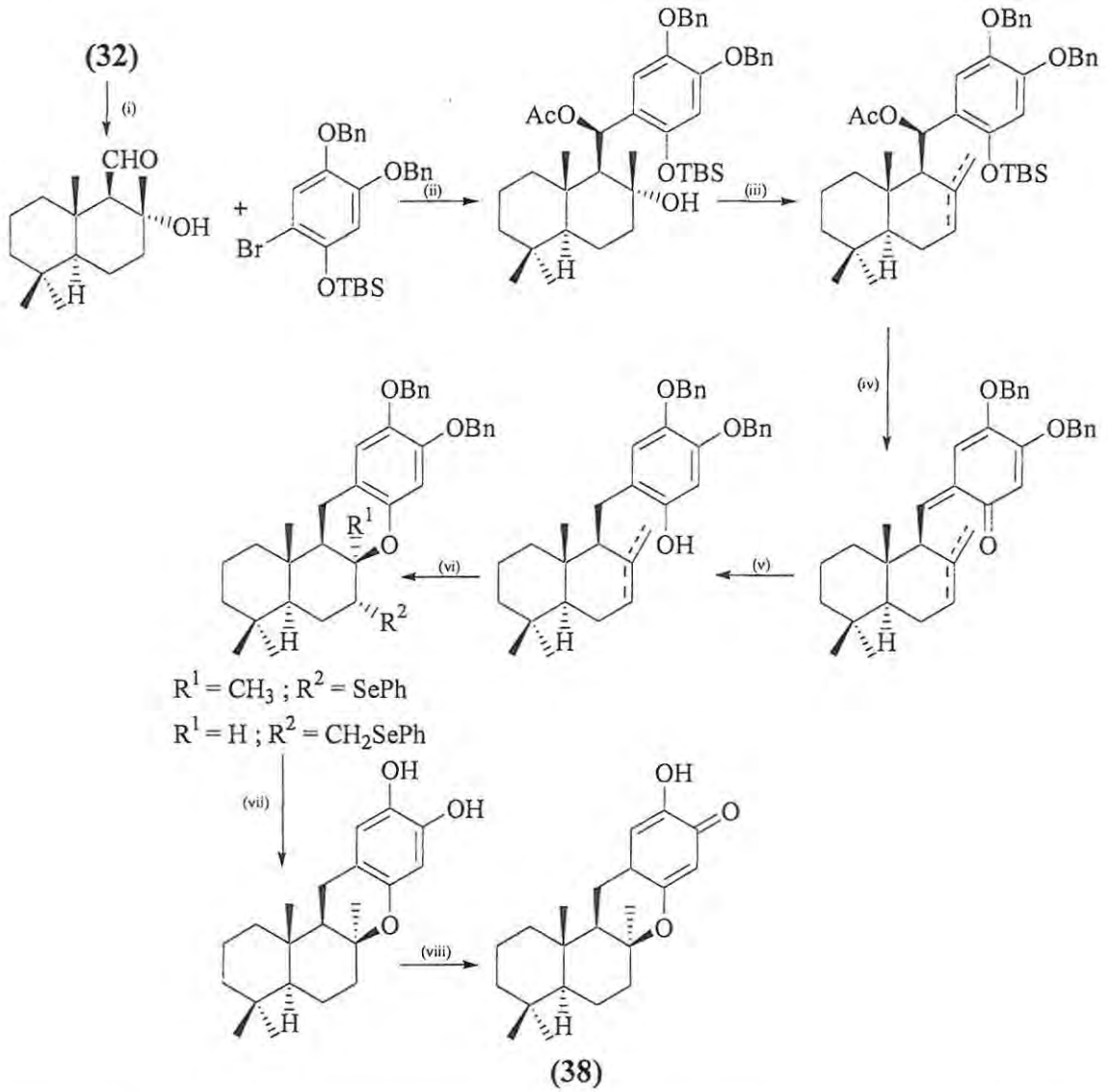


SCHEME 2 : The synthesis of polygodial and related compounds from (-)-sclareol.[40] (i) $OsO_4/NaIO_4$, Pr^iOH, 45°C, 6h, 73%. (ii) Collidine, 170°C, 8h, 60%. (iii) $NaBH_4$, MeOH, rt, 30min, 93%; Ac_2O, Py, rt, 2h, 94%. (iv) t-BuOOH, SeO_2, CH_2Cl_2, rt. (v) MsCl, Py, rt. (vi) NaOAc, acetone-H_2O, reflux. (vii) 2N KOH/MeOH, rt. (viii) $(ClCO)_2$/DMSO, CH_2Cl_2, Et_3N, -78°C, 15min.

1.4.2. The Synthesis of (+)-Puupehenone from (-)-Sclareol

(+)-Puupehenone (**38**) is a very bioactive marine natural products possessing a wide variety of activities including cytotoxic, antiviral, antifungal and immunomodulatory properties.[41,42] Some (+)-puupehenone derivatives are showing inhibition of HIV and cholesteryl ester transfer protein (CETP).[43] Barrero, *et al.*[43] reported the first enantiospecific synthesis of **38** in an effort to allow for further investigation into the bioactivity of this compound and analogues thereof. The synthesis,

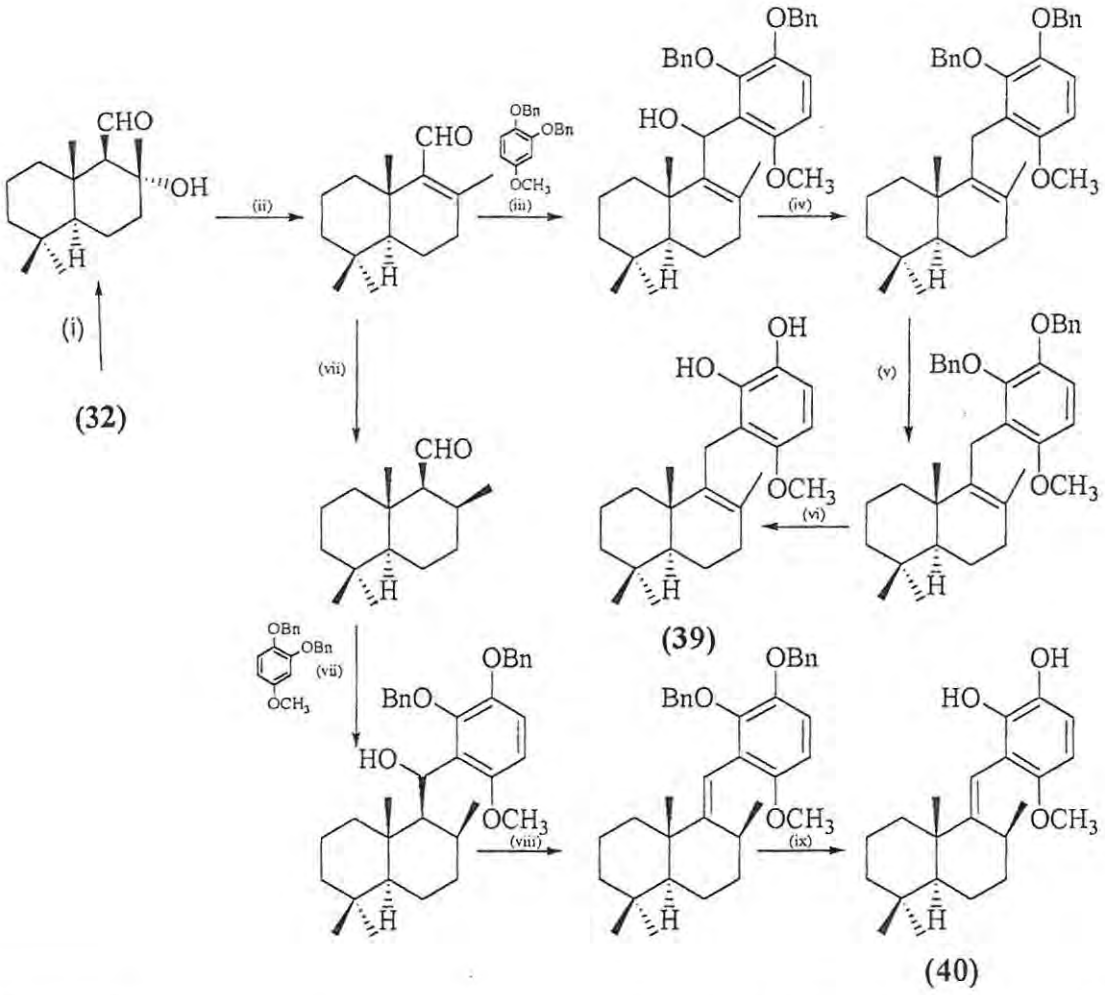
represented in Scheme 3, included the use of an aromatic synthon prepared from protocatechualdehyde.



SCHEME 3 : The synthesis of (+)-puupehenone from (-)-sclareol.[43] (i) $OsO_4/NaIO_4$, Pr^iOH, 45°C, 6h. (ii) t-BuLi, Et_2O, -78°C, 88%. (iii) $SOCl_2$, Py, rt, 1h, 94%. (iv) TBAF, THF, rt, 15 min, 81%. (v) $NaBH_4$, EtOH, rt, 20 min, 91%. (vi) NPSP, $SnCl_4$, CH_2Cl_2, -78°C, 2h, 91%. (vii) Raney Ni, THF, rt, 20h, 75%. (viii) PDC, CH_2Cl_2, rt, 3h, 70%.

1.4.3. The Synthesis of Wiedendiol-A and Wiedendiol-B from (-)-Sclareol

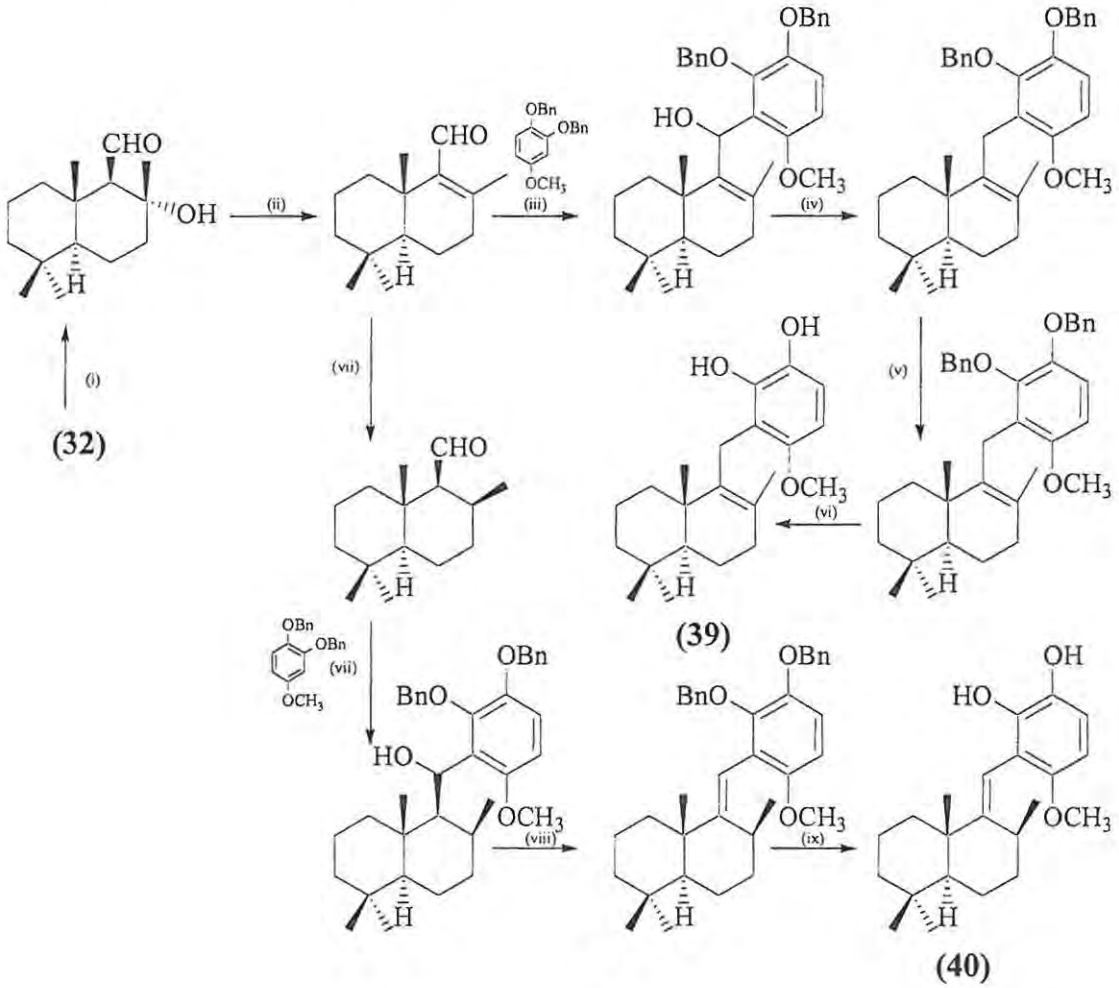
Wiedendiol A (**39**) and wiedendiol B (**40**) are marine natural products consisting of drimane and polyphenolic moieties resulting from mixed biosynthesis. Both compounds are of great interest because of their ability to inhibit CETP and hence can potentially be used to reduce the risk of coronary heart disease.[44,45] This synthesis was undertaken in order to provide a route for the synthesis of these bioactive compounds, aiding research relating to this class of compounds. This was the first enantiospecific synthesis of wiedendiol A and wiedendiol B from **32** and (+)-*cis*-abienol (Scheme 4).[46]



SCHEME 4 : The synthesis of wiedendiol-A and wiedendiol-B from (-)-sclareol.[46] (i) $OsO_4/NaIO_4$, PrOH, 45°C, 6h. (ii) Collidine, 200°C, 3h, 78%. (iii) *n*-BuLi, Et_2O -TMEDA, -78°C to 0°C, 1h. 65%. (iv) ZnI_2-$NaBH_3CN$, CH_2Cl_2, rt, 50 min, 71%. (v) Raney Ni, H_2O-THF, rt, 20h, 81%. (vi) H_2, Pd-C, MeOH-EtOAc, 0°C, 1h, 70%. (vii) *n*-BuLi, Et_2O-TMEDA, -40°C to 0°C, 1h 30 min, 55%. (viii) TsOH, Benzene, 35°C, 13h, 82%. (ix) H_2, Pd-C, MeOH-EtOAc, 0°C, 2h, 93%.

1.4.4. The Synthesis of *ent*-Chromazonarol and Related Compounds from (-)-Sclareol

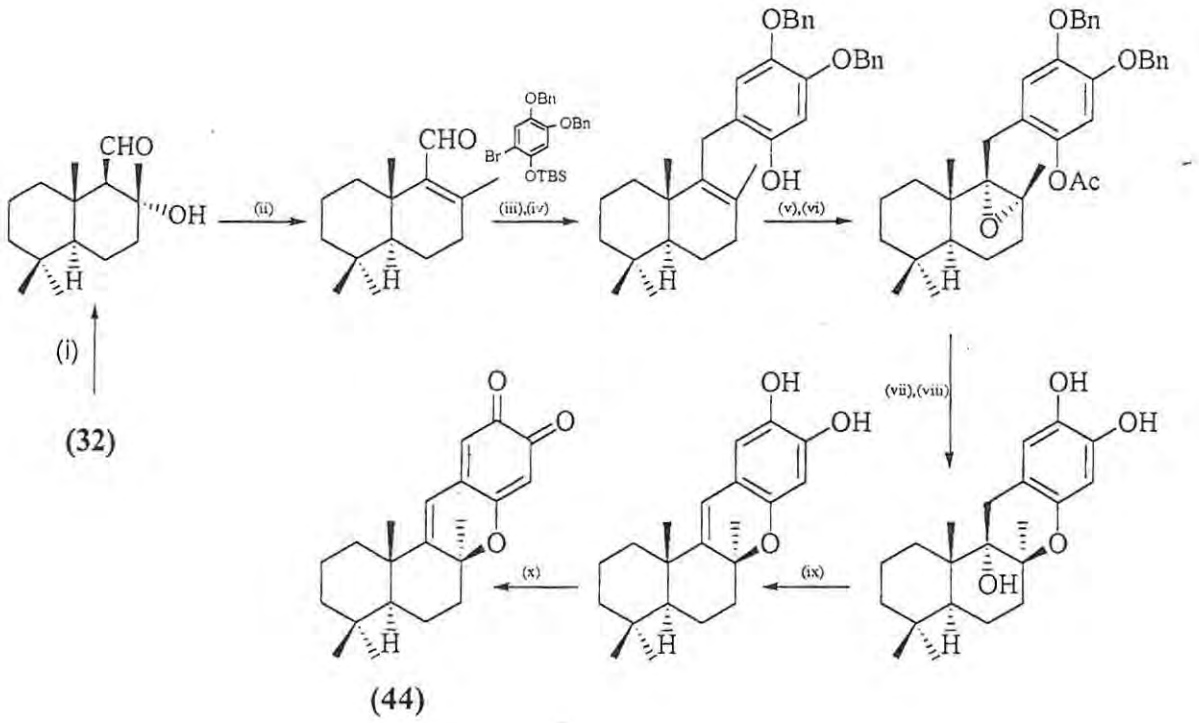
ent-Chromazonarol (**41**), isolated from the sponge *Disidea pallescens*, has been shown to possess antifeedant, antifungal and antitumour activity.[47] *ent*-Isozonarol (**42**) and *ent*-isozonarone (**43**) exhibited similar bioactivity. Compounds **41- 43** were prepared as part of a structure-activity relationship study of this class of compounds (Scheme 5).



SCHEME 5 : The synthesis of *ent*-chromazonarol and related compounds from (-)-sclareol.[47] (i) $OsO_4/NaIO_4$, Pr'OH, 45°C, 6h. (ii) *n*-BuLi, Et_2O, -78°C, 1h 30 min, 91%. (iii) Cl_2SO, Py, -14°C, 20 min, 97%. (iv) TBAF, EtOH, rt, 5 min. (v) $NaBH_4$, EtOH, rt, 25 min, 40%. (vi) 2N KOH-MeOH, rt, 10 min, 85%. (vii) $NaBH_4$, EtOH, reflux, 15h, 47%. (viii) TBAF, THF, rt, 15 min, 90%. (ix) Jones, acetone, 0°C, 5 min, 93%. (x) $BF_3.OEt_2$, CH_2Cl_2, -14°C, 5 min, 90%.

1.4.5. The Synthesis of Puupehedione from (-)-Sclareol

As we have seen previously, compounds of mixed biosynthesis consisting of drimane and polyphenolic moieties are among the most active of marine metabolites. Their biological activities range from cytotoxicity, antifungal and immunomodulatory properties to CETP inhibition.[48,49] There have been several approaches to their syntheses. The Verongid sponge metabolite puupehedione (**44**), related to (+)-puupehenone (**38**), was prepared *via* an enantiospecific synthesis in order to obtain sufficient material for comparative antitumour assays.[50] The synthesis of **44** is represented in scheme 6.



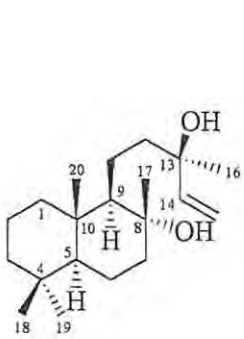
SCHEME 6 : The synthesis of puupehedione from (-)-sclareol.[50] (i) $OsO_4/NaIO_4$, Pr^iOH, 45°C. (ii) Collidine, 200°C. (iii) t-BuLi, Et_2O, -78°C, 50 min, Ar; Et_2O, -78°C, 40 min; Et_3SiH, TFA, CH_2Cl_2, -78°C, 45 min, 79%. (iv) TBAF, THF, rt, 15 min, 95%. (v) Ac_2O, NaAcO, reflux, 2h, 98%. (vi) *m*-CPBA, $NaHCO_3$, CH_2Cl_2, -20°C, 2h, 98%. (vii) 2N-KOH-MeOH, rt, 48h, 95%. (viii) H_2, 10% Pd/C, MeOH, rt, 1h, 91%. (ix) Silica gel, 45%. (x) $NaIO_4$, EtOH-H_2O, rt, 15 min, 80%.

CHAPTER 2
RESULTS AND DISCUSSION

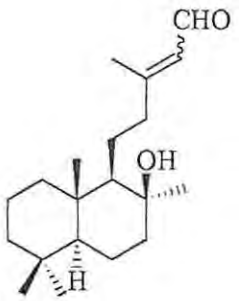
2. RESULTS AND DISCUSSION

2.1 An Overview of the Synthetic Approach Adopted for the Synthesis of the Labdane Alcohols 45 and 46.

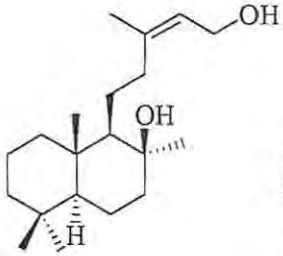
Initially the primary objective of our synthesis was to synthesize the two isomeric marine natural products 8β-hydroxylabd-13E-en-15-al (**5**) and 8β-hydroxylabd-13Z-en-15-al (**6**). However, we noted that Cimino, *et al.*,[8] during their structural studies of these metabolites, first reduced the unstable aldehydes **5** and **6** with lithium aluminium hydride ($LiAlH_4$) to give the more stable alcohols **45** and **46**. We anticipated similar stability problems of the aldehydes arising towards the end of our synthesis and therefore rather directed our synthesis towards the preparation of the alcohols **45** and **46**. We assumed that mild oxidation of the alcohols would give the corresponding aldehyde natural products if so required.



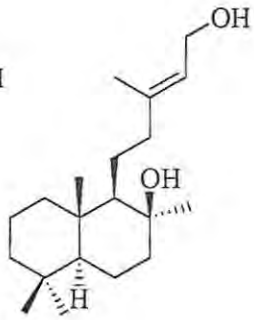
(32)



(5) Z
(6) E



(45)

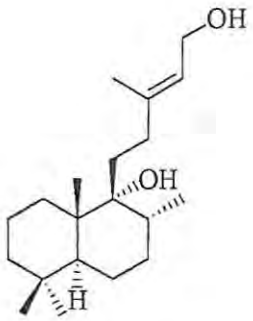


(46)

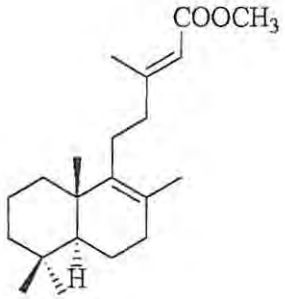
Having decided on sclareol (**32**) as the starting point of our synthesis, we envisioned that the main challenge in this synthesis would be an inversion of the substituents on C-8. Obviously the preservation of the chirality of C-5, C-9 and C-10 in **32** during synthetic transformations would also be critical.

Before we embarked on our synthesis of the aldehydes, an extensive search of available literature

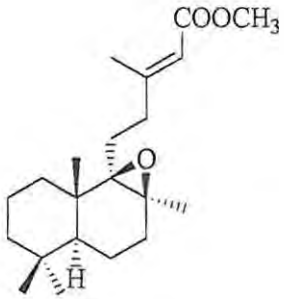
resources was undertaken to identify syntheses of identical or similar structures to either **5** and **6** or **45** and **46**. While there are no syntheses of **5** and **6** reported, this search yielded a single reference for a synthesis reported in 1970 by Popa, *et al.*[51] in which the synthesis of the natural product peregrinol (**47**) from sclareol was described.



(47)

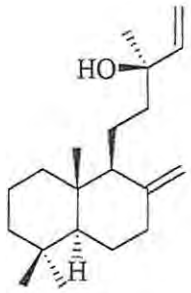


(48)

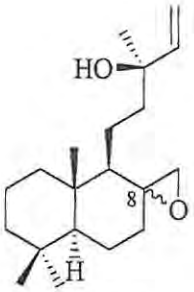


(49)

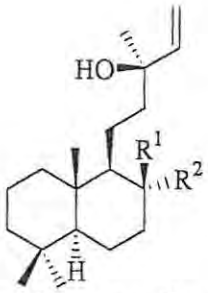
Popa's synthetic strategy initially involved the introduction of a Δ^8 double bond (**48**) into the starting material, labd-8(20),13-dien-15-ol, by regioselective epoxidation with monoperoxyphthalic acid (MPPA), followed by $LiAlH_4$ reduction of the proposed C-8 - C-9 α-epoxide to give peregrinol. A mixture of epoxides were separated prior to the reduction step. From the limited available data, the supposed β-epoxide (**49**) was suggested as the minor product. Although $LiAlH_4$ reduction of **49** gave **46,** the evidence given in support of the structure of **46** was tenuous.



(50)



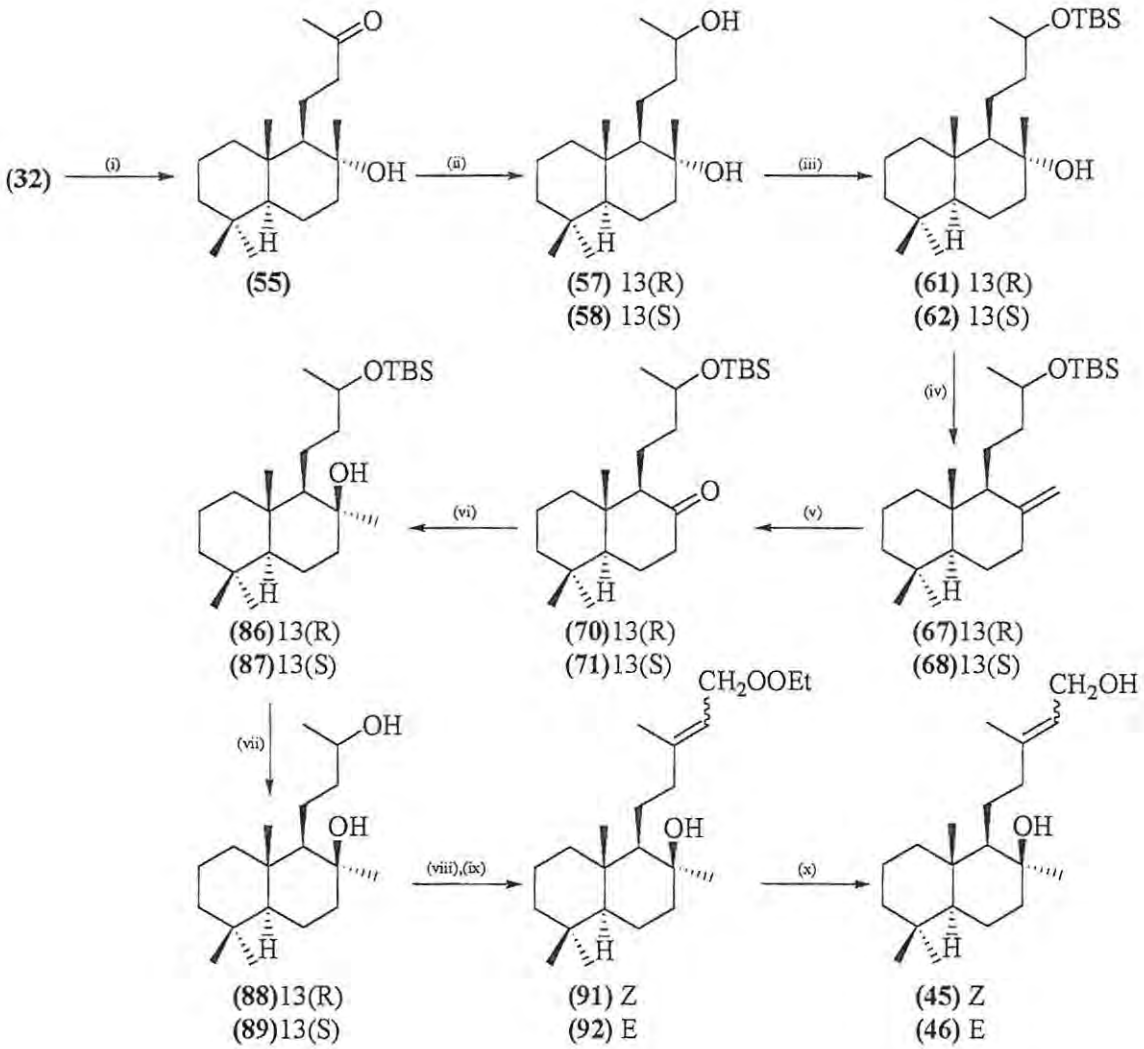
(51) 8(R)
(52) 8(S)



(53) R^1= OH ; R^2= CH_3
(54) R^1= CH_3 ; R^2 = CH_3

A further low yielding preparation, introducing a C-8 - C-17 epoxide at C-8, was reported by Leite, *et al.*.[52] The *m*-chloroperbenzoic acid (*m*-CPBA) epoxidation of manool (**50**) yielded a mixture of

8,17-epoxy-14-labden-13-ols (**51** and **52**) in a 3:1 ratio respectively. $LiAlH_4$ reduction of these epoxides resulted in the formation of the 8,17-epoxy-14-labden-13-ols (**53** and **54**). Even though this procedure offers the formation of the desired stereochemistry at C-8 in the former alcohol, the reactions were non-stereoselective and low yielding. The lack of stereoselectivity and low yields in the two published syntheses necessitated that we approach the synthesis of **45** and **46** from another direction and Scheme 7 represents a summary of our proposed synthetic approach to the synthesis of **45** and **46**.



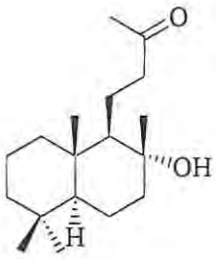
SCHEME 7 : Summary of our proposed synthetic approach to compounds **45** and **46**. (i) $KMNO_4$ Oxidation (ii) $LiAlH_4$ Reduction (iii) TBDMS Triflate Protection (iv) $POCl_3$ Dehydration (v) Ozonolysis (vi) MeLi Methylation (vii) TBAF Deprotection (viii) Swern Oxidation (ix) Horner-Wadsworth-Emmons Reaction (x) DIBALH Reduction.

It should be noted at the outset that all synthetic products were fully characterised, where possible, by Nuclear Magnetic Resonance (NMR) spectroscopy, in addition to Infra Red (IR) and Mass Spectrometric (MS) techniques. NMR experiments used for signal assignments included ^{1}H and ^{13}C spectra, Distortionless Enhancement by Polarisation Transfer (DEPT), ^{1}H-^{1}H Correlation Spectroscopy (COSY), Heteronuclear Multiple Quantum Coherence (HMQC) and ^{1}H-^{13}C Heteronuclear Multiple Bond Correlation (HMBC) spectra. Molecular formulae were provided from High Resolution Fast Atom Bombardment Mass Spectrometric (HRFABMS) data. Where applicable, if synthetic intermediates were known compounds, spectroscopic data was supported by comparison of melting point and optical rotation with published data.

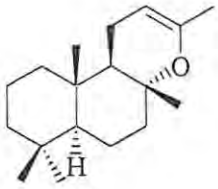
In all the compounds prepared in this thesis there is very little change in the NMR chemical shifts of the protons and carbon atoms in ring A which greatly facilitated the assignment of these resonances in compounds with previously unassigned NMR data. The structural modifications to ring B and the side chain at C-9 obviously affected the chemical shifts of proton and carbon atoms in these regions of the molecules. Accordingly, to avoid unnecessary repetition, only the assignment of resonances affected by the synthetic transformations are discussed where necessary in support of proposed structures.

2.2 Oxidation of Sclareol.

As discussed in Section 1.4.1., the stereochemistry at C-5, C-9 and C-10 in sclareol (**32**) favoured its use as the precursor in our proposed synthesis. Initial oxidative removal of the terminal alkene in **32** would yield a ketone at C-13 and allow for the stereoselective introduction of a carbon-carbon double bond at C-13 at a later stage in the synthesis. Accordingly, loss of the terminal double bond was achieved by potassium permanganate ($KMnO_4$)oxidation in acetone as described by Bigley, *et al.*,[53] a well established procedure in our laboratory having being used with success in syntheses of other related diterpenes. The reaction yielded an equilibrium mixture of the required ketone, (**55**), and the cyclized enol ether (**56**).



(55)



(56)

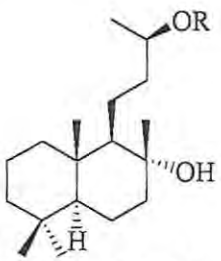
Obviously a method of shifting the equilibrium in favour of **55** was desirable. A procedure reported by Ruzicka, *et al.*,[54] involving hydration of the enol ether with either methanolic acetic acid or semicarbazide acetate, apparently yielded mostly the ketone. Bigley, *et al.*[53] later refuted this method, reporting that interconversion could only be obtained through treatment of **56** with an aqueous oxalic acid dihydrate solution, followed by extraction of the ketone with ether. In our hands, neither method provided satisfactory conversion of **55** to **56**. The separation of **55** and **56** by silica gel chromatography was further hampered by the facile conversion of **55** to **56** on contact with silica gel. Purification of the ketone was finally achieved by recrystallization from cold hexane, to give an overall modest isolated yield of 51% for this compound.

An alternative method of oxidizing sclareol has been described by Barrero, *et al.*[55] who used an osmium tetroxide-sodium periodate mixture to improve the yields of **55** to 85%. The improved yield of **55** was attributed to the more favourable reaction conditions, *ie.* 10°C, and shorter reaction time, as opposed to the relatively higher temperatures and longer reaction duration required for the $KMnO_4$ oxidation. Unfortunately, the cost and the hazardous properties of osmium tetroxide precluded its use in our laboratory.

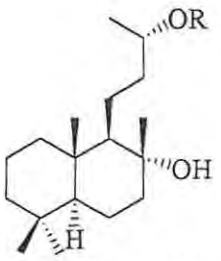
Spectroscopic data established the structure of the ketone **55**, with ^1H and ^{13}C spectra confirming the product purity. The ^{13}C spectrum of **55** clearly showed the presence of the C-13 ketone (δ_C 210.4) and the loss of the C-14 and C-15 vinylic carbons (δ_C 147.5 and 110.7). HRFABMS established the molecular formula of **55** as $C_{18}H_{32}O_2$ (280.2402, Δmmu -0.3). The IR spectrum exhibited a prominent carbonyl absorption band at ν_{max}1712 cm^{-1}, while the melting point (81-82°C) and the optical rotation ($[\alpha]_D$ = +5°) were consistent with literature values (78-80°C)[53,55] and (+6.7°).[56]

2.3 Reduction of the Aliphatic Ketone 55.

The ease with which the hydroxyl group at C-8 cyclizes with the C-13 ketone necessitated protection of the ketone early in our synthetic strategy. Reduction of the ketone to form the secondary alcohol was carried out as a prerequisite for the *t*-butyldimethylsilyl protection of this functionality.



(57) R = H
(59) R = (S)-MTPA
(61) R = (R)-MTPA



(58) R = H
(60) R = (S)-MTPA
(62) R = (R)-MTPA

The reduction of ketones to form secondary alcohols is a well documented procedure and using the same $LiAlH_4$ reduction reported by Hinder and co-workers,[57] for the reduction of **55**, we obtained diol (**57**) as a colourless oil and diol (**58**) as white crystals in an overall yield of 96% and in a ratio of 3:2 respectively. The diastereomeric mixture of these alcohols was easily separable by silica gel flash column chromatography (EtOAc:hexane/70:30).

HRFABMS data established the molecular formulae of **57** and **58** as $C_{18}H_{34}O_2$ (282.2551, Δmmu -0.8). The melting point (97-99°C) and the $[\alpha]_D$ (+8°) of **58** were consistent with literature melting point (102°C) and $[\alpha]_D$ (+11°) values for this compound.[57] The ^1H and ^{13}C NMR data for both compounds were assigned using a combination of 2D NMR techniques and are presented in the experimental section (Chapter 3). As an example of the effective use of HMBC and COSY data in the structure elucidation of the compounds prepared in our synthesis, the HMBC and COSY correlations for compound **57** are presented in Figures 2 and 3. HMQC data in conjunction with HMBC correlations were crucial in resolving the problem of overlapped signals in the proton methylene envelope.

The absolute configuration of the C-13 secondary alcohol moiety in **57** and **58** was established using

Figure 2: The HMBC NMR spectrum ($CDCl_3$, 400MHz) for compound **57** with some key HMBC correlations shown in the accompanying figure.

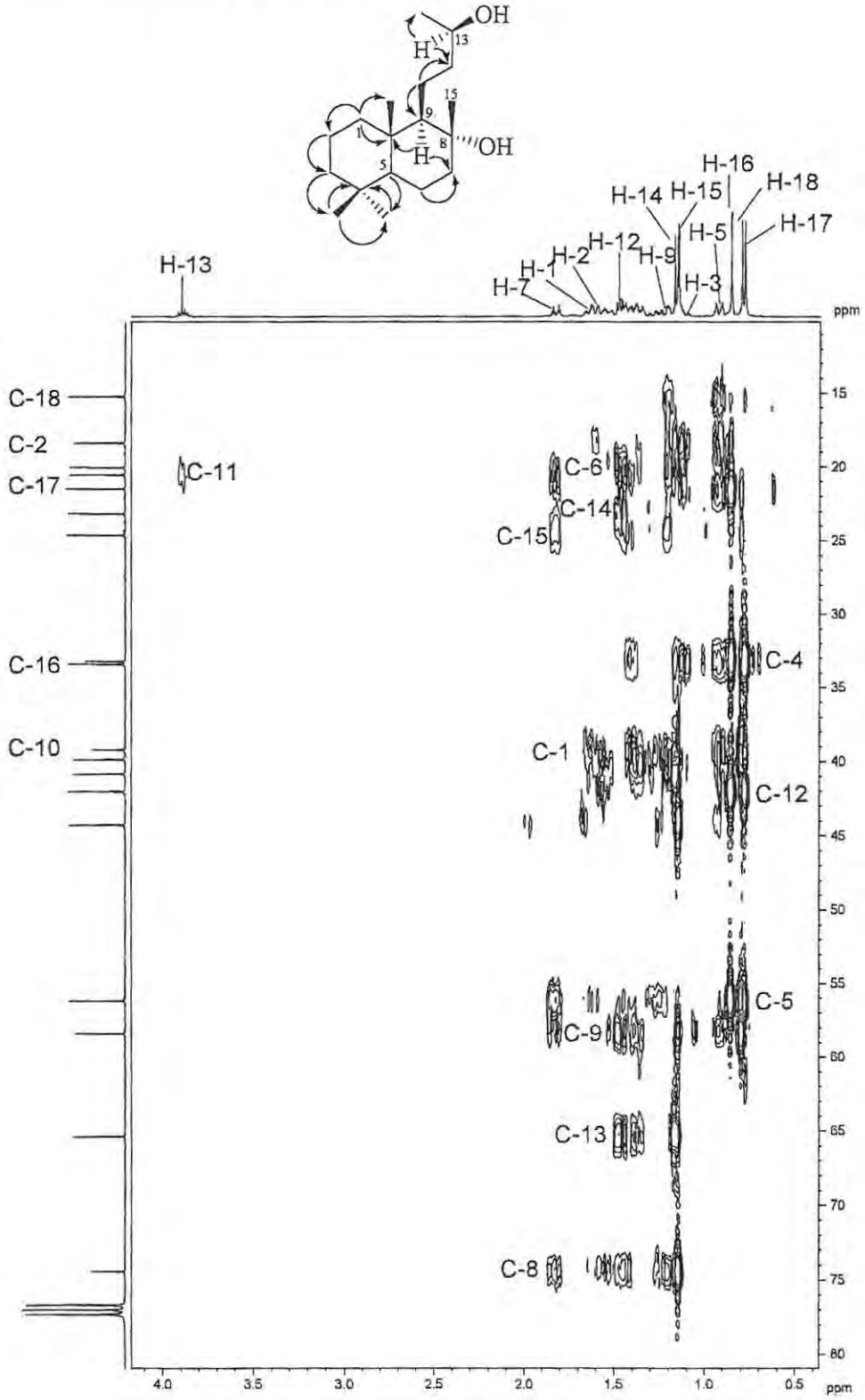
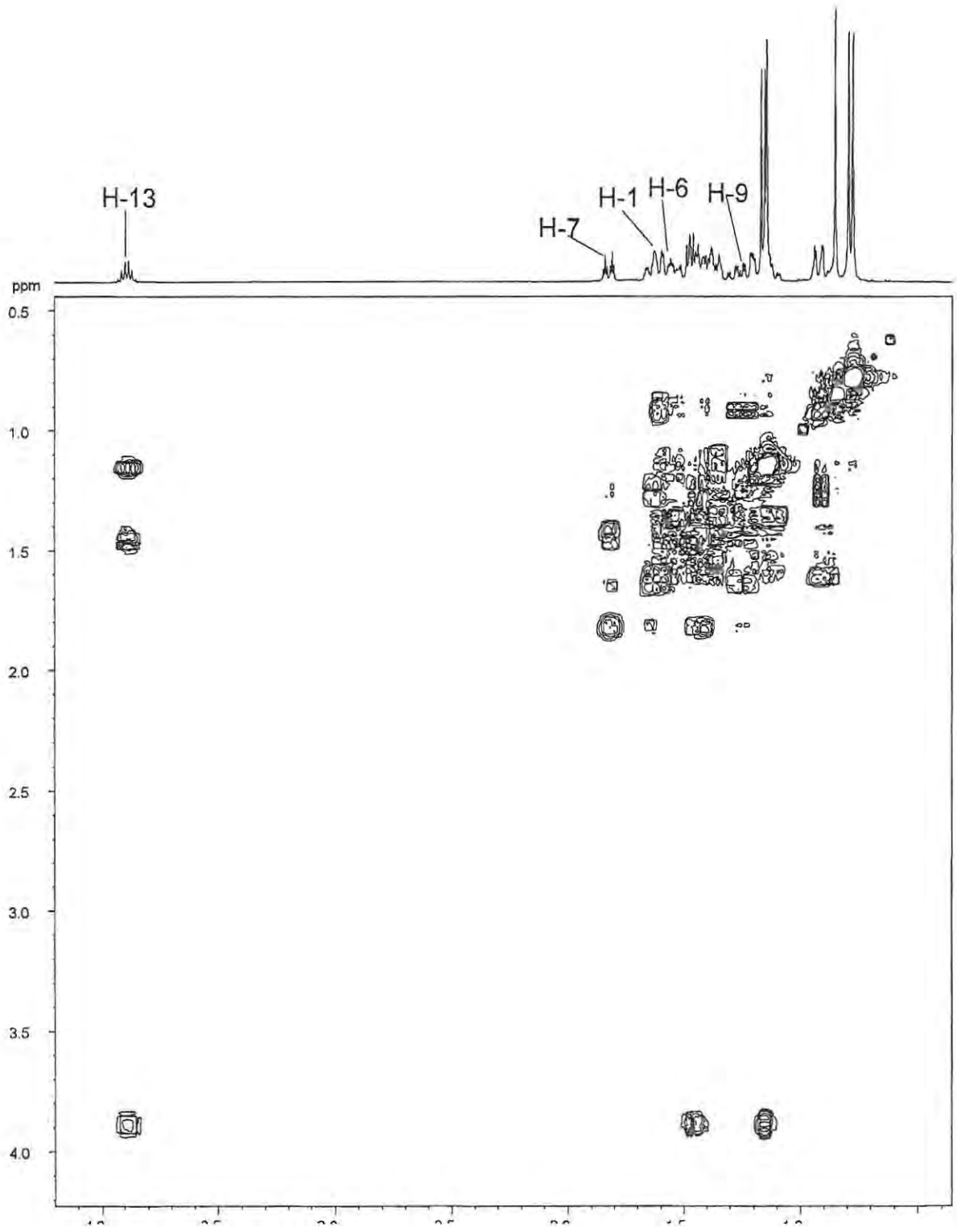
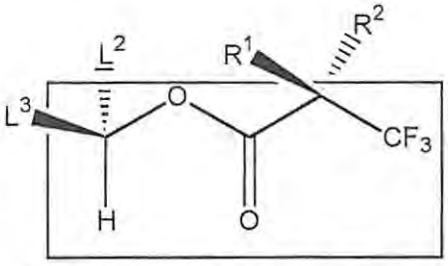


Figure 3: The COSY NMR spectrum ($CDCl_3$, 400MHz) of compound **57**.



Mosher's method.[58] Unfortunately, the paper providing the physical data for diastereomer **58**[57] only came to our attention after we had established the absolute chemistry of **57** and **58**. 2-Methoxy-2-phenyl-2-(trifluoromethyl)acetic acid (MTPA), is the most frequently used Moshers derivatising agent and has been successfully used for such determinations in other marine terpenoids.[59,60] Mosher, *et al.*[58] hypothesized that the most stable conformation of the molecule in solution is one in which the carbinyl proton, the ester carbonyl and the trifluoromethyl groups of the MTPA moiety lie in the same plane (Figure 4). In this conformation the proton signals of the alkyl chain (L^2) on the same side of the plane to that of the phenyl substituent of the (R)-MTPA ester will be shielded (*ie.* they will appear upfield in the NMR spectra) as a result of the diamagnetic effect of the benzene ring. Conversely, the protons of the other alkyl chain (L^3) will be deshielded (*ie.* they will appear downfield in the NMR spectra) by the methoxy functionality (R^1). Hence, in summary, the signals of L^2 protons of the (R)-MTPA ester will be shielded relative to the L^2 protons in the corresponding (S)-MTPA ester.

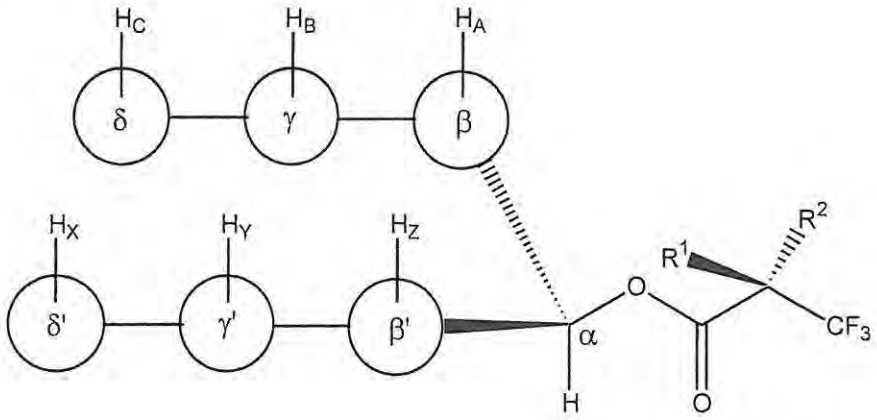


(R)-MTPA ; R^1=OMe, R^2=Ph
(S)-MTPA ; R^1=Ph, R^2=OMe

Figure 4: The most stable conformation of (R)-MTPA and (S)-MTPA esters as proposed by Mosher.[59]

Mosher put this hypothesis forward at a time when the complete assignment of protons in complex organic molecules was not viable as the available NMR instruments operated only in the 60-100MHz range. Mosher, *et al.* made use of ^{19}F NMR or lanthanide shift reagents to disperse the overlapping ^1H signals, with the knowledge that steric repulsion between the MTPA phenyl moiety and the β- and β'-substituents (Figure 5) resulted in the chemical shift difference of the CF_3 (^{19}F) or OMe (^1H) functionalities. The advent of Fourier Transform NMR overcame the limitations inherent in assigning the absolute configuration from only two data points.

Ohtani, *et al.*[59] proposed a modification to Moshers method by using high-field ^{1}H-NMR spectroscopy to determine the absolute configuration of secondary alcohols. High-field FT NMR, in addition to two-dimensional NMR experiments, enables one to calculate the chemical shift differences ($\Delta\delta = \delta_S - \delta_R$) between several of the assigned protons in the (R)- and (S)-MTPA esters and hence permits the use of a larger number of data points for a more reliable determination of absolute stereochemistry. Figure 5 illustrates the assumed conformation of the respective (R)- and (S)-MTPA esters, wherein the H_A, H_B and H_C protons of the (R)-MTPA ester will be upfield relative to those in the (S)-MTPA ester. Conversely, the opposite holds true for the H_X, H_Y and H_Z protons left of the MTPA plane.



(R)-MTPA ; R^1=OMe, R^2=Ph
(S)-MTPA ; R^1=Ph, R^2=OMe

Figure 5 : The most stable conformation of (R)-MTPA and (S)-MTPA esters as proposed by Ohtani.[59]

As a result of the difference in chemical shifts, we see a negative $\Delta\delta$ value for the protons on the left and a positive $\Delta\delta$ value for the protons on the right of the model structure illustrated in Figure 6. Once the $\Delta\delta$ values for the protons in the molecule have been determined, it is possible to elucidate the stereochemistry of the chiral secondary alcohol. Generally, it was also noted that validity of the hypothesis was enhanced if the $\Delta\delta$ values were proportional to the distance of the protons from the MTPA moiety.[59]

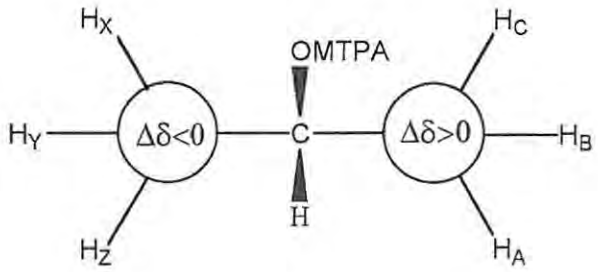


Figure 6: Model used in the modified Moshers method to determine the absolute configuration of secondary alcohols.

The Moshers (S)-MTPA esters (**59** and **60**) and (R)-MTPA esters (**61** and **62**) were prepared using 20mg of each alcohol (**57** and **58**) respectively. Assignment of the proton signals for each MTPA ester was confirmed using COSY experiments. The $\Delta\delta$ values for each pair of diastereomers was determined and is represented in Table 2. The tabulated data in Table 2 are reproduced in Figures 7 and 8 in order to clearly illustrate how the (13R) and (13S) configurations were assigned in compounds **57** and **58**.

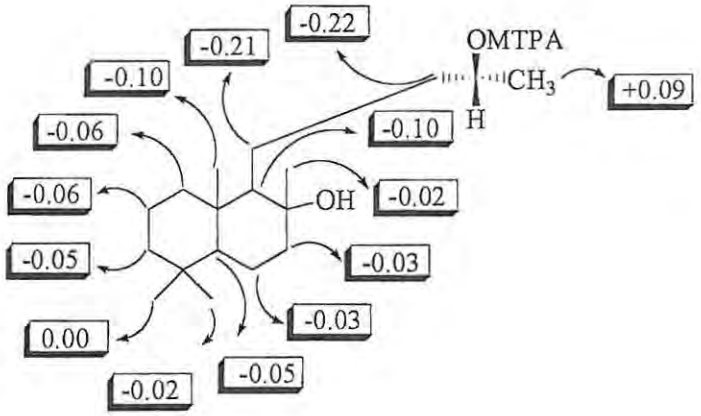


Figure 7: $\Delta\delta$ values calculated for the MTPA esters of compound **57**.

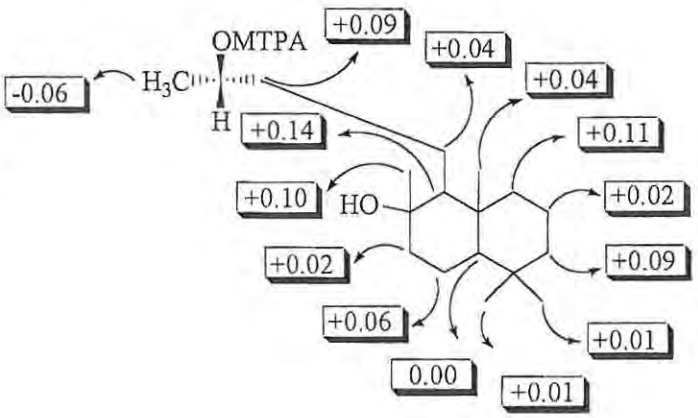


Figure 8: $\Delta\delta$ values calculated for the MTPA esters of compound **58**.

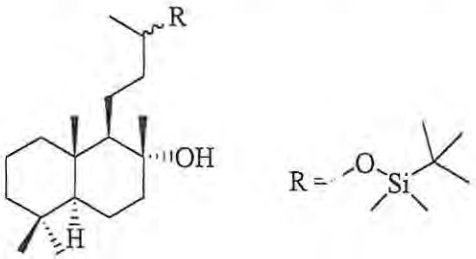
Table 2: Results from the Modified Mosher's Method (δ represented in ppm).

Proton	Mosher's Esters for Compound **57**			Moshers Esters for Compound **58**		
	δ (S)-MTPA	δ (R)-MTPA	$\delta_S - \delta_R$	δ (S)-MTPA	δ (R)-MTPA	$\delta_S - \delta_R$
H-1	1.67	1.73	-0.06	1.67	1.56	+0.11
H-2	1.46	1.52	-0.06	1.39	1.37	+0.02
H-3	1.34	1.39	-0.05	1.31	1.22	+0.09
H-5	0.83	0.88	-0.05	0.89	0.89	0.00
H-6	1.62	1.65	-0.03	1.57	1.53	+0.04
H-7	1.81	1.84	-0.03	1.81	1.79	+0.02
H-9	0.90	1.00	-0.10	1.02	0.88	+0.14
H-11	1.15	1.36	-0.21	1.22	1.18	+0.04
H-12	1.30	1.52	-0.22	1.63	1.54	+0.09
H-13	5.13	5.12	+0.01	5.12	5.10	+0.02
H-14	1.34	1.25	+0.09	1.27	1.33	-0.06
H-15	1.10	1.12	-0.02	1.09	0.99	+0.10
H-16	0.83	0.83	0.00	0.85	0.84	+0.01
H-17	0.74	0.76	-0.02	0.77	0.76	+0.01
H-18	0.63	0.73	-0.10	0.75	0.71	+0.04

2.4 *t*-Butyldimethylsilyl (TBDMS) Protection of the Aliphatic Alcohols 57 and 58.

In addition to the prevention of the cyclization mentioned in Section 2.3, the TBDMS protection of the C-13 alcohols also conferred stability of this hydroxyl functionality to the organometallic reagents, bases and acids we planned to use in the next few steps of our synthesis. Although acetate, tosyl and trimethylsilyl protecting groups were considered, TBDMS protection was chosen as TBDMS ethers are reported to be very stable.[61] It was also noted that *t*-butyldimethylsilyl ethers could be rapidly cleaved back to the alcohol by treatment with tetra-*n*-butylammonium fluoride (TBAF).

Protection was first achieved using *t*-butyldimethylchlorosilane, a standard method described in numerous syntheses.[62] Accordingly, a solution of **57** in CH_2Cl_2 was stirred at 0°C with imidazole and 4-dimethylaminopyridine (4-DMAP) under nitrogen for 5 hours to yield **63** (71%). We were disappointed that a theoretically quantitative reaction should only give a 71% yield and tried another approach to the TBDMS protection of the alcohol. Using 2,6-lutidine and *t*-butyldimethylsilyl trifluoromethane sulphonate under slightly altered reaction conditions (CH_2Cl_2, -78°C, 1hr) resulted in an improved yield of **63** (97%). The TBDMS ether of **58** was similarly prepared (**64**).



(63) = β-R
(64) = α-R

Identification of the TBDMS ethers was achieved using NMR spectroscopy where the dimethyl signals of the TBDMS moiety are characteristically seen as isolated peaks at δ_H 0.04 and δ_C -4.62. The signals of the *t*-butyl methyl groups are, likewise, also easily identifiable appearing as single large peaks at δ_H 0.87 and δ_C 25.92 as depicted in Figure 9 and 10. HRFABMS data determined the molecular formulae of **63** and **64** as $C_{24}H_{48}SiO_2$ ($[M+1-H_2O]^+$ 379.3395, Δmmu -0.1 and $[M+1-H_2O]^+$ 379.3397, Δmmu -0.09 respectively). ^1H and ^{13}C NMR data for **63** and **64** data have not previously been reported and these data, carefully assigned from 2D NMR experiments, are presented in Chapter 3.

2.5 Dehydration of the TBDMS Protected Alcohols 63 and 64.

In order to facilitate the introduction of a ketone at C-8, we first needed to prepare the $\Delta^{8,15}$ exocyclic alkene. The most readily identifiable means to achieve this was by regioselective dehydration of the tertiary alcohol at C-8. Reagents used for such transformations include phosphorous oxychloride ($POCl_3$) and thionyl chloride ($SOCl_2$).[63] However, at the outset we anticipated the formation of a

Figure 9: ^{1}H NMR spectrum ($CDCl_3$, 400MHz) of compound **63**.

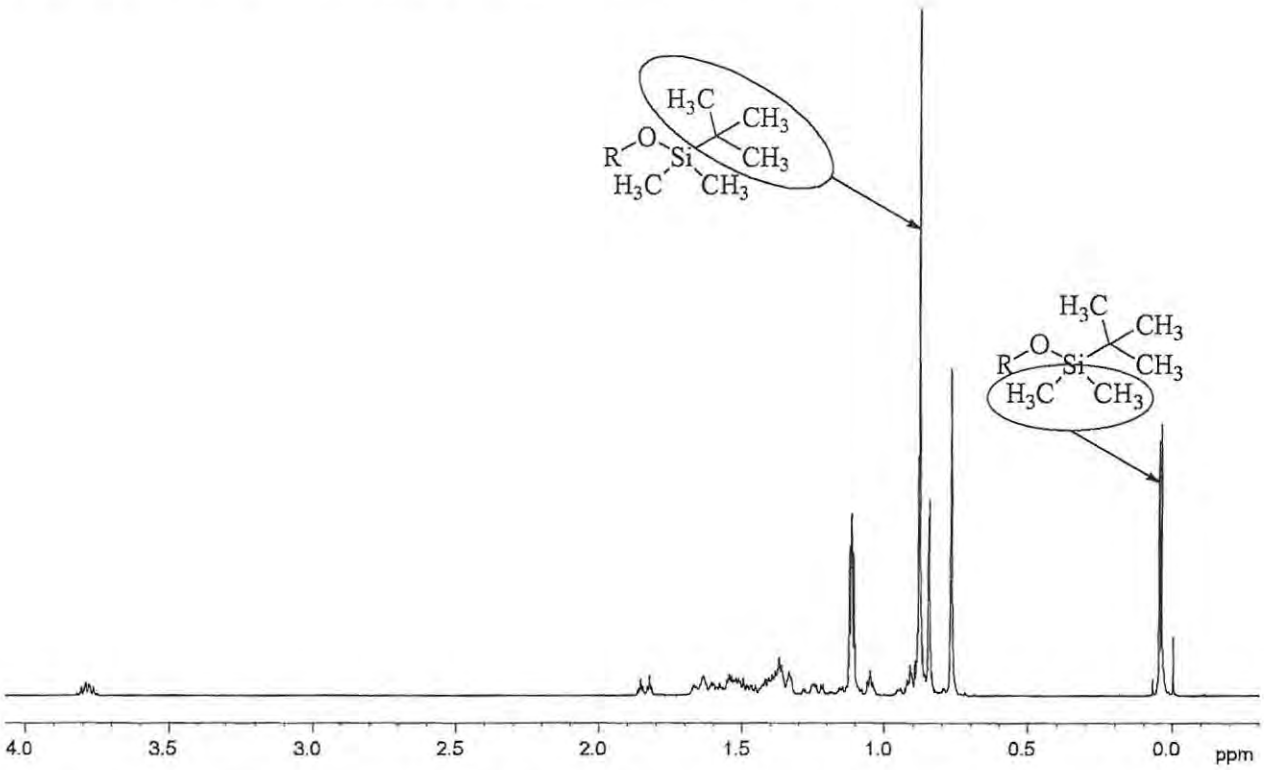
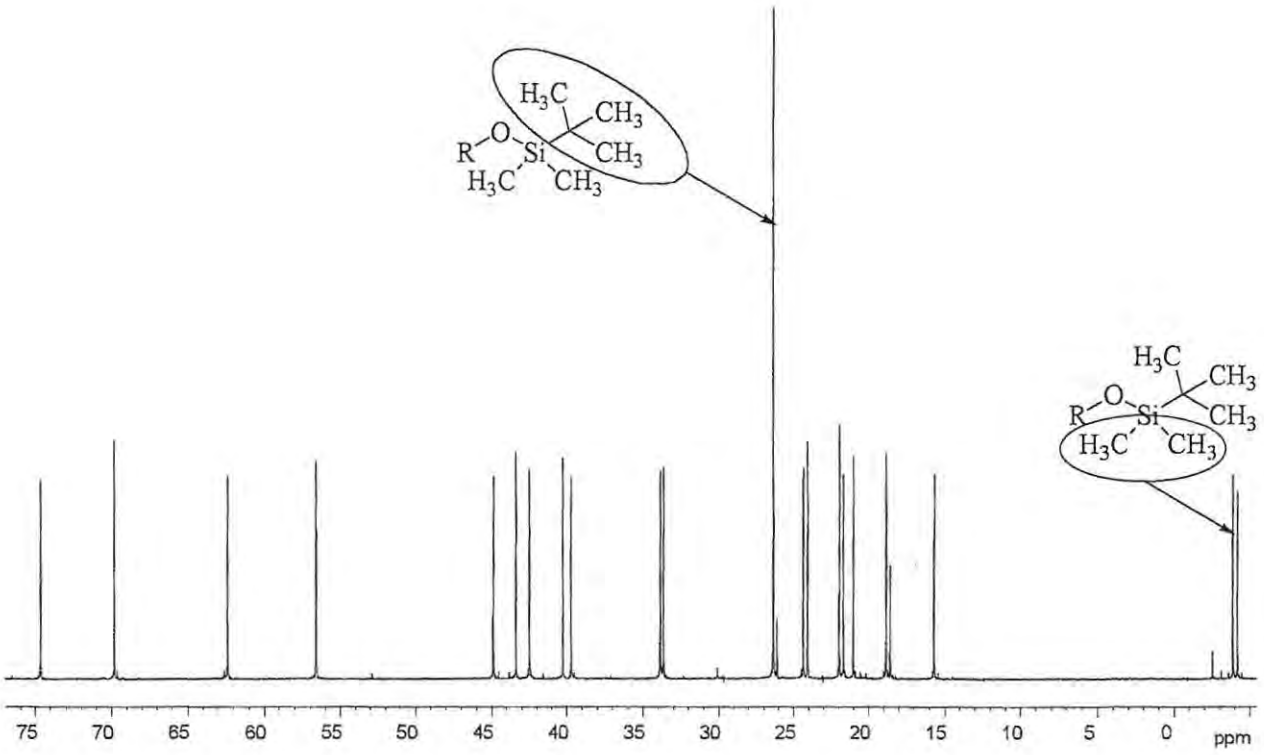
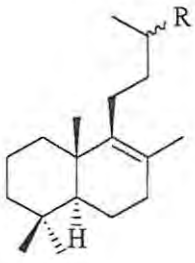


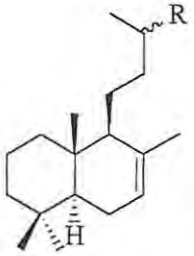
Figure 10: ^{13}C NMR spectrum ($CDCl_3$, 100MHz) of compound **63**.



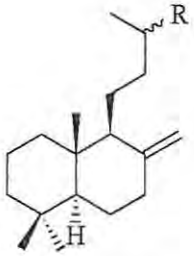
mixture of the endocyclic and exocyclic dehydration products (*e.g.* **65, 66** and **67**). The procedure followed was that of McCreadle and co-workers[64] for a series of labdadienol interconversions in which a similar exocyclic dehydration was achieved.



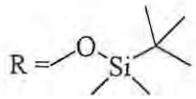
(65)



(66)



(67) = β-R
(68) = α-R



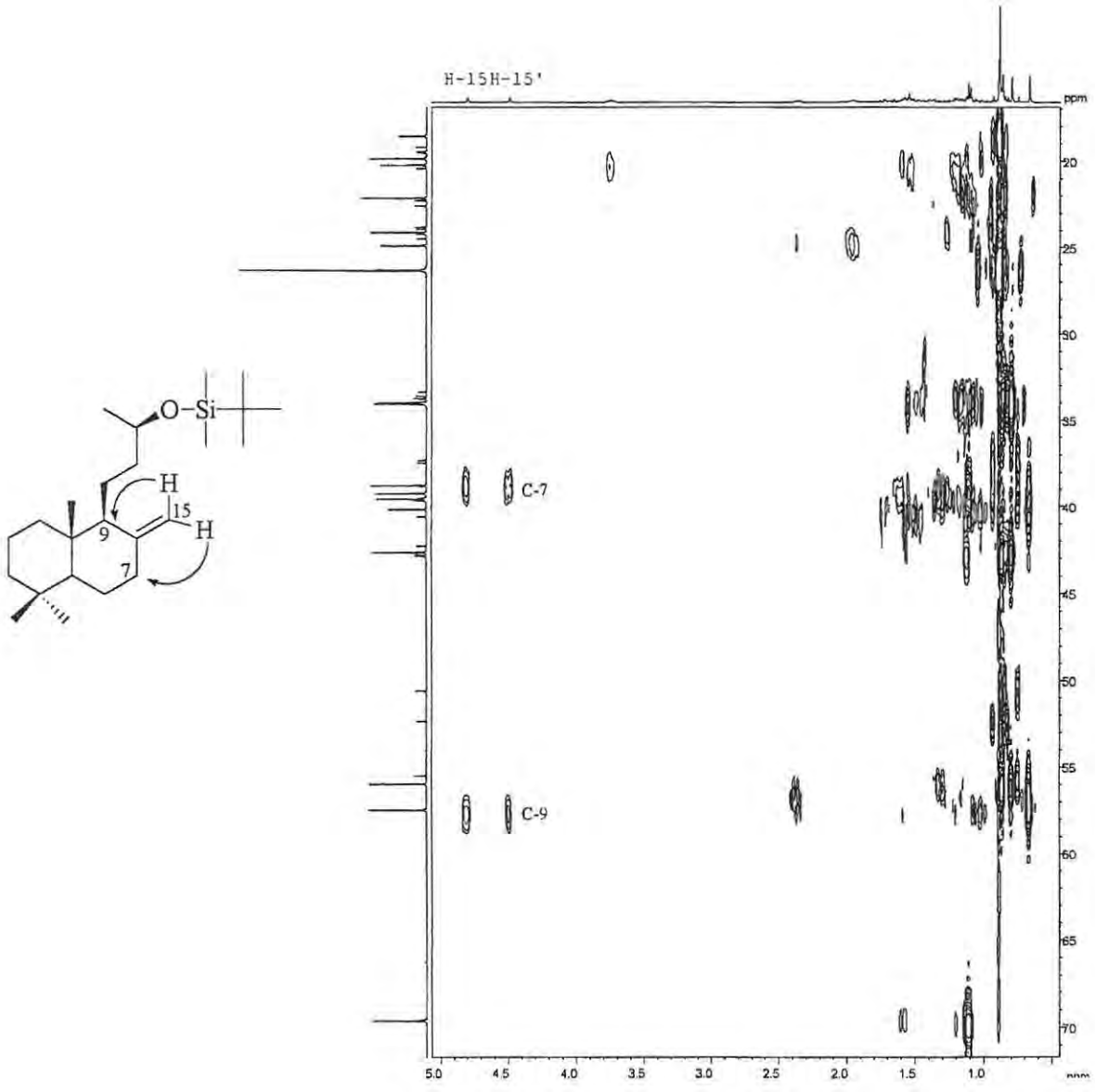
Initially using the thionyl chloride dehydration protocol of McCreadle *et al.* we obtained a mixture of products. After purification of the mixture using normal phase HPLC (hexane:EtOAc-95:5), the desired exocyclic alkene was obtained in a disappointing 41% yield. In order to improve the yield of the desired product, we considered the use of a milder dehydrating agent, *i.e.*$POCl_3$.[63]

A solution of $POCl_3$, **63** and 4-DMAP in pyridine was stirred at room temperature for three hours. Final purification of the reaction products was achieved *via* flash column chromatography to give the desired exocyclic alkene in 73% isolated yield. The structures of **67** and **68** were confirmed using ^1H and ^{13}C NMR spectroscopy in conjunction with 2D NMR experiments (HMQC, HMBC and COSY). The protons of the $\Delta^{8,15}$ vinylic functionality appeared in the ^1H NMR spectrum as a distinctive doublet at 4.64ppm. Significant changes in chemical shifts were also seen in the ^{13}C NMR spectrum, with regards to the C-15 carbon resonance which shifts from δ_C 23.63 in **63** to δ_C 106.36 in **67** and the C-8 carbon signal which shifts from δ_C 74.17 in **63** to δ_C 148.82 in **67**. Three bond HMBC correlations from H_2-15 to C-7 and C-9 unequivocally confirmed the presence of the $\Delta^{8,15}$ exocyclic alkene (Figure 11).

Following the precepts of *Zaitsev's Rule*,[63] which predicts that in elimination reactions the more highly substituted alkene is formed, it is somewhat surprising that although we did encounter a

mixture of products, including $\Delta^{8,9}$ and $\Delta^{8,7}$ alkenes in small amounts, the least substituted $\Delta^{8,15}$ alkene predominates in this reaction. HRFABMS data provided the molecular formulae of **67** and **68** as $C_{24}H_{46}SiO$ (378.3318, ∆mmu -0.05 and $[M+1]^+$ 379.3396, ∆mmu -0.05 respectively). The fully assigned ^1H and ^{13}C NMR data for these TDBMS derivatives have not previously been reported and are provided in Chapter 3.

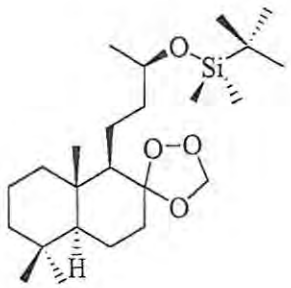
Figure 11 : The HMBC NMR spectrum ($CDCl_3$, 400MHz) of compound **67** showing two key HMBC correlations.



2.6 Ozonolysis of the TBDMS Protected Alkenes 67 and 68.

Ozonolysis has frequently been used as an efficient means of introducing a ketone into a compound *via* a carbon carbon double bond.[65,40] The ketone arises from a dipolar addition of ozone across the double bond resulting in the eventual formation of an ozonide. Common reagents used for the reduction of ozonides to ketones include zinc/acetic acid, H_2/Pd, dimethyl sulphide (Me_2S) and triphenyl phosphine (TPP).[66] Of these possible reduction methods we chose to use Me_2S because of the success encountered previously with this method in our laboratory and the ease with which the oxidized product, dimethyl sulphoxide (DMSO), could be removed from the reaction products.

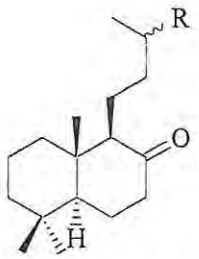
Initial attempts at ozonolysis of either **67** or **68** followed by work up with up to four equivalents of Me_2S proved unsuccessful. The identification of the desired ketone product in the reaction mixture was based primarily on the appearance of the ketone carbon signal in ^{13}C NMR spectra and TLC. Development of the TLC plate with 2,4-dinitrophenylhydrazine reagent (2,4-DNP) produced a yellow spot on the TLC if the ketone was present. NMR analysis of the reaction products revealed a complex mixture and not the single expected. After numerous unsuccessful attempts at ozonolysis, it was decided to start a parallel investigation into alternative methods of oxidizing the alkene to a ketone (see Section 2.7).

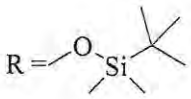


(69)

Frustrated by our lack of success with a straight forward ozonolysis reaction, we eventually isolated a product *via* normal phase HPLC (hexane:EtOAc/90:10) of the reaction mixture from a fairly large scale ozonolysis of **67** in CH_2Cl_2 with a Me_2S (4 equivalents) reductive work up.[67,68] Using ^1H and ^{13}C NMR spectroscopy, combined with 2D experiments, we identified the ozonolysis product as being

being the ozonide (**69**). The ^{1}H NMR spectrum of **69** showed the loss of the double doublet at 4.64ppm in **67** and the appearance of a doublet at 5.03ppm. Changes in chemical shifts were also seen in the ^{13}C NMR spectrum, where the C-15 carbon resonance shifted from δ_C 106.36 in **67** to δ_C 93.37 in **69** and the C-8 carbon signal which shifted from δ_C 148.82 in **67** to δ_C 111.50 in **69**. Unfortunately, the ozonide degraded into several products within a period of 72 hours before we were able to obtain mass data. So the conclusion drawn was that we did indeed form the ozonide after ozonolysis. However, our means of reductive cleavage appeared to be non-effective and we therefore required an alternative reductive work up procedure.

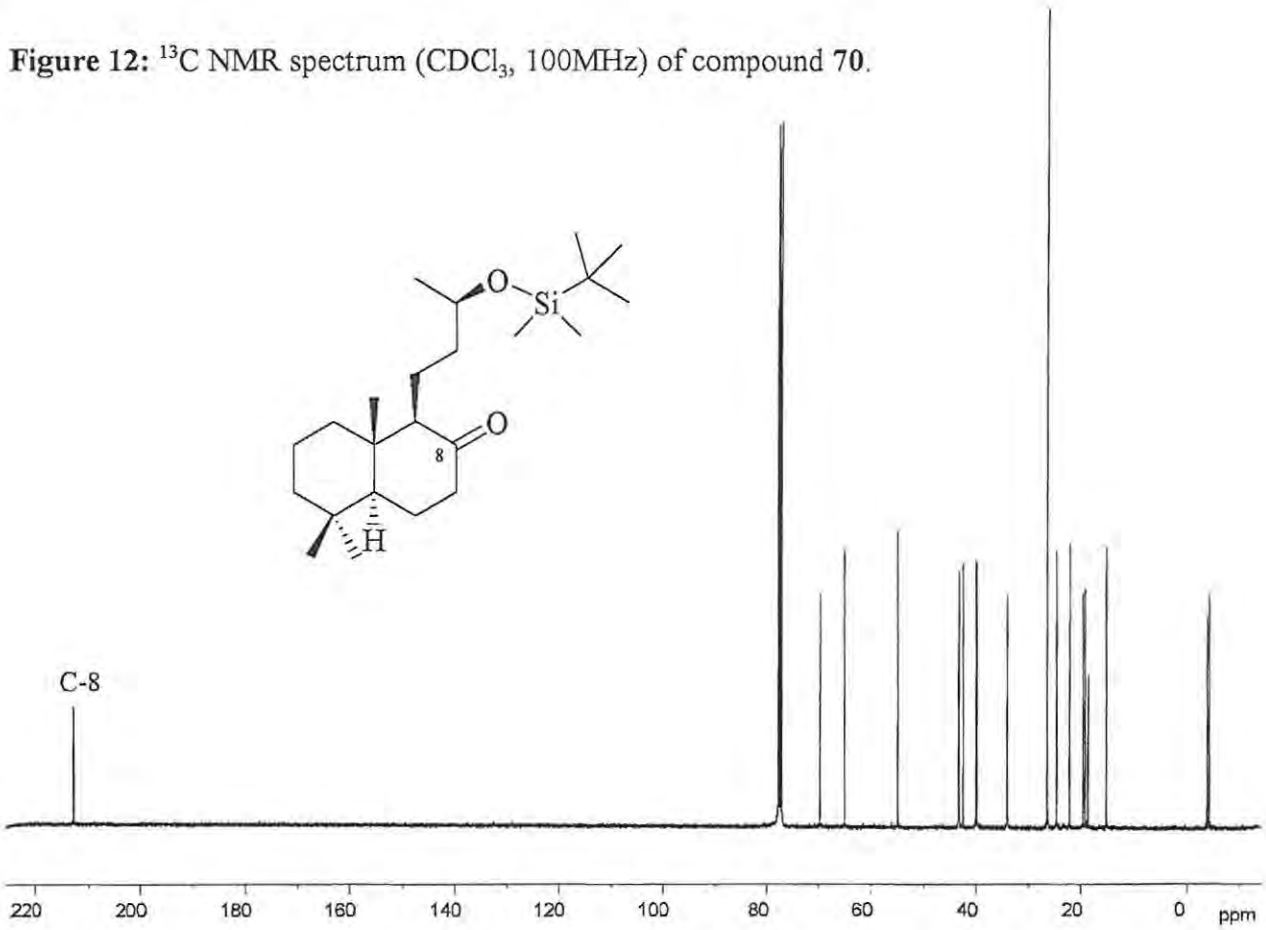




(70) = β-R
(71) = α-R

Accordingly, we then focused our attention on the ozonolysis of **67** in CH_2Cl_2 with a TPP reductive work up.[69] The presence of unreacted TPP reagent made identification of the ketone difficult and it proved vital to sufficiently remove all traces of unreacted TPP from the final product. We found a hydrogen peroxide wash of the reaction mixture to be very effective and excess TPP was removed as triphenyl phosphine oxide to yield **70** (97%). Purification of the ketone was achieved by normal phase HPLC (hexane:EtOAc/95:5). The ozonolysis of **68** was also undertaken to give (**71**) in similar yields. ^{1}H and ^{13}C NMR spectroscopy, in addition to 2D NMR experiments, confirmed the structure of the ketones **70** and **71**. The loss of the C-15 signal at δ_C 106.86 and the shifting of the C-8 carbon signal at δ_C 148.82 to 211.95 reflected the change at C-8 (see Figure 12). HRFABMS data indicated that the molecular formulae of the isomers **70** and **71** was $C_{23}H_{44}SiO_2$ (380.3119, Δmmu = -0.8 and $[M+1]^+$ 381.3188, Δmmu = -0.07 respectively). ^{1}H and ^{13}C NMR data for these TDBMS derivatives have not been reported before and are presented in Chapter 3.

Figure 12: ^{13}C NMR spectrum ($CDCl_3$, 100MHz) of compound **70**.



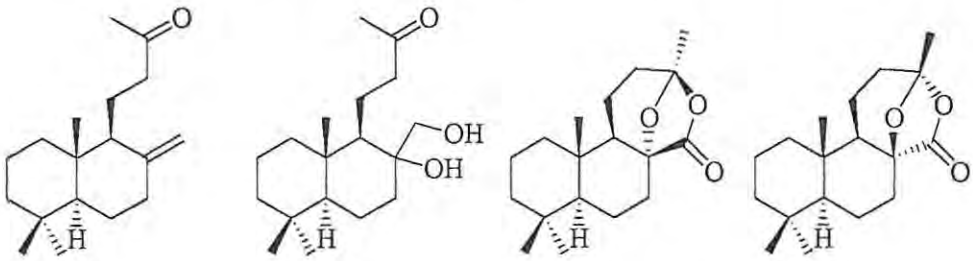
2.7 Alternative Oxidation Strategies.

As mentioned previously, the initial problems associated with the ozonolysis of **67** necessitated the investigation of an alternative approach for introducing a C-8 ketone in **67**. Preliminary studies were carried out on the TBDMS protected alkenes (**67** and **68**) and the ketone (**72**). No sufficiently high yielding oxidation alternative to ozonolysis was identified.

2.7.1 Ruthenium Trichloride Oxidation of Compounds 67 and 72.

Ruthenium compounds are recognized as powerful oxidizing agents which have been used for the cleavage of alkenes to yield either ketones, aldehydes or carboxylic acids. Ruthenium chloride-periodic acid oxidation was attempted using the procedure reported by Beukes, *et al.*.[70]

The TBDMS protected alkene (**67**) was reacted with periodic acid and a catalytic amount of $RuCl_3$ in a CH_2Cl_2/acetonitrile/H_2O solvent mixture over 4 hours to yield unchanged starting material. It was thought that the TBDMS moiety might, due to its steric bulk, have inhibited the formation of possible ruthenium oxidation complexes. Therefore the same oxidation was attempted using the unprotected keto-olefin (**72**), prepared by $POCl_3$ dehydration of compound **55**.[64] The oxidation procedure followed was the same as described above, however, the solution was stirred vigorously for 13 hours. The reaction yielded the dihydroxylated product (**73**, 63%) and the lactone ether isomers (**74** and **75**, 23%). Although **74** had been prepared previously by the by Francis, in his PhD research, in a series of oxidations of manool using $KMnO_4$ and Jones reagent (CrO_3), no mention of the isomeric lactone **75** was made in the paper describing the PhD thesis work.[71] Compounds **74** and **75** were separated using normal phase HPLC (hexane:EtOAc/95:5). When the reaction mixture was heated to 30°C over a period of 16 hours, we observed a change in the yields of each compound, with the yields of **73** reduced to 32% and **74** and **75** increased to 58%. It stands to reason that **73** is probably an intermediate product in which facile oxidation of the primary alcohol followed by cyclization of the tertiary alcohol moiety and the C-13 ketone occurs with heating.



(**72**) (**73**) (**74**) (**75**)

^{13}C NMR data for **73** indicated that conversion to the cyclized products had occurred on standing in $CDCl_3$, and hence, the NMR data for **73** could not be fully assigned. The ^1H and ^{13}C NMR for "pure" **73**, represented in Figure 13, reveals the signals used for the identification of the dihydroxylated product in the mixture induced by $CDCl_3$. The molecular formulae of the isomers **74** and **75** ($C_{18}H_{28}O_3$), ($[M+1]^+$ 293.2115, Δmmu = -0.19 and $[M+1]^+$ 293.2116, Δmmu = -0.07 respectively) established from HRFABMS data, suggested a double bond equivalence of five for both these compounds. A single lactone carbonyl ester resonance (δ_C 175.29 and 175.37) in the ^{13}C NMR spectra of **74** and **75** respectively, accounted for one of the double bond equivalents required by the

by the molecular formulae. The absence of any olefinic signals in the ^{1}H and ^{13}C NMR spectra of **74** and **75** would therefore require these compounds to be tetracyclic. The only tetracyclic structure that would feasibly accommodate the three oxygen atoms, suggested by the molecular formulae, is the unusual lactone ether structure shown here for **74** and **75**. As mentioned earlier, **74** has been prepared previously and a comparison of the ^{1}H and ^{13}C NMR data of **74** and **75** with those of compound **74** prepared by Francis is presented in Table 3 (Note : the majority of the ^{1}H NMR signals were not assigned by Francis).

Table 3: Comparison of relevant ^{1}H and ^{13}C NMR data of **74** and **75** with the limited reported data for **74** prepared by Francis.[72]

Carbon	Compound **74**		Compound **74**[72]		Compound **75**	
	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C
C-1	0.87 (m), 1.62 (m)	39.3	-	39.2	0.87 (m), 1.62 (m)	39.2
C-2	1.43 (m)	18.3	-	18.3	1.59 (m)	18.3
C-3	1.15 (m), 1.42 (m)	41.8	-	41.8	1.15 (m), 1.43 (m)	41.8
C-4	-	33.1	-	33.1	-	33.1
C-5	0.93 (m)	55.0	-	55.0	0.97 (m)	55.0
C-6	1.71 (m)	19.1	-	19.0	1.70 (m)	19.1
C-7	1.50 (m), 2.07 (dt)	32.5	-	32.5	1.77 (m), 2.09 (dt)	32.5
C-8	-	81.1	-	81.1	-	81.3
C-9	1.39 (m)	51.9	-	51.9	1.42 (m)	51.9
C-10	-	37.5	-	37.5	-	37.5
C-11	1.53 (m), 1.80 (m)	17.9	-	18.0	1.41 (m), 1.74 (m)	17.9
C-12	1.94 (m)	32.7	-	32.7	1.54 (m), 1.94 (m)	32.7
C-13	-	108.0	-	108.0	-	108.0
C-14	1.54 (s)	23.8	1.54 (s)	23.8	1.59 (s)	24.3
C-15	-	175.3	-	175.4	-	175.4
C-16	0.89 (s)	33.7	0.91 (s)	33.7	0.90 (s)	33.7
C-17	0.84 (s)	21.8	0.86 (s)	21.8	0.85 (s)	21.8
C-18	0.91 (s)	13.4	0.93 (s)	13.4	0.92 (s)	13.4

Figure 13: ^{1}H NMR spectrum ($CDCl_3$, 400MHz) of compound **73**.

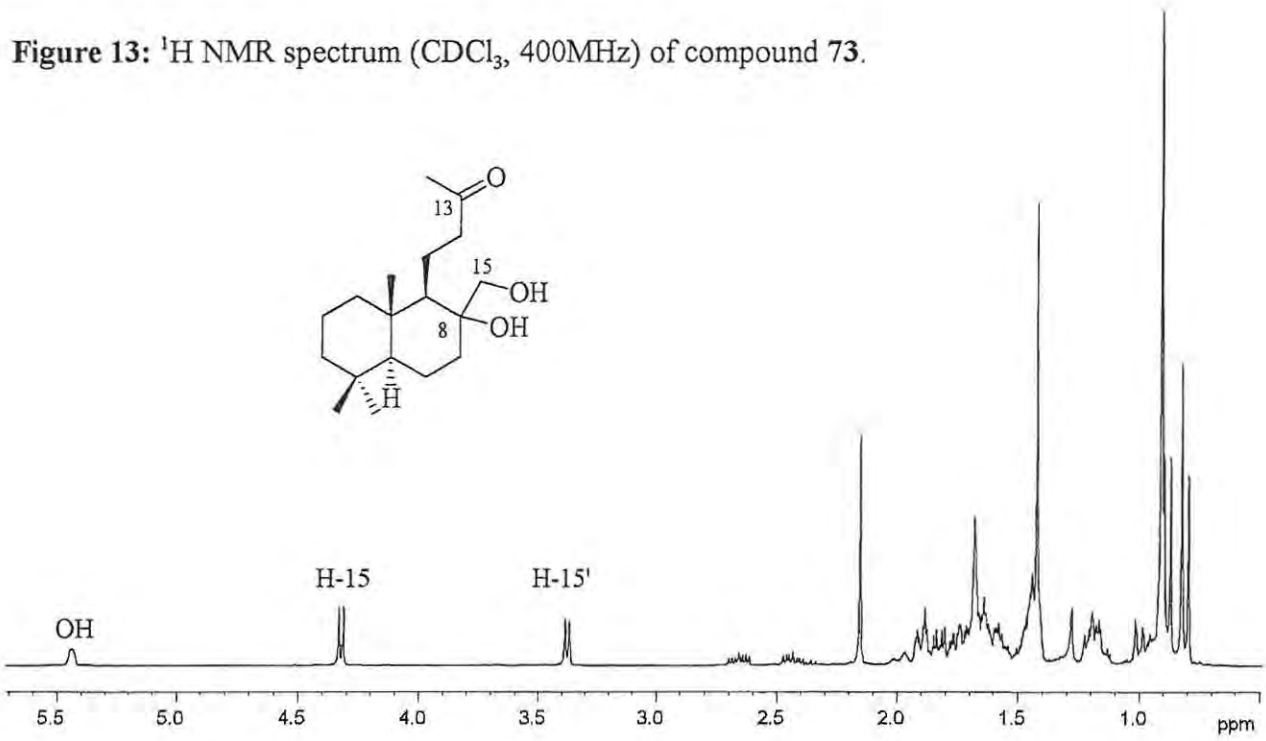
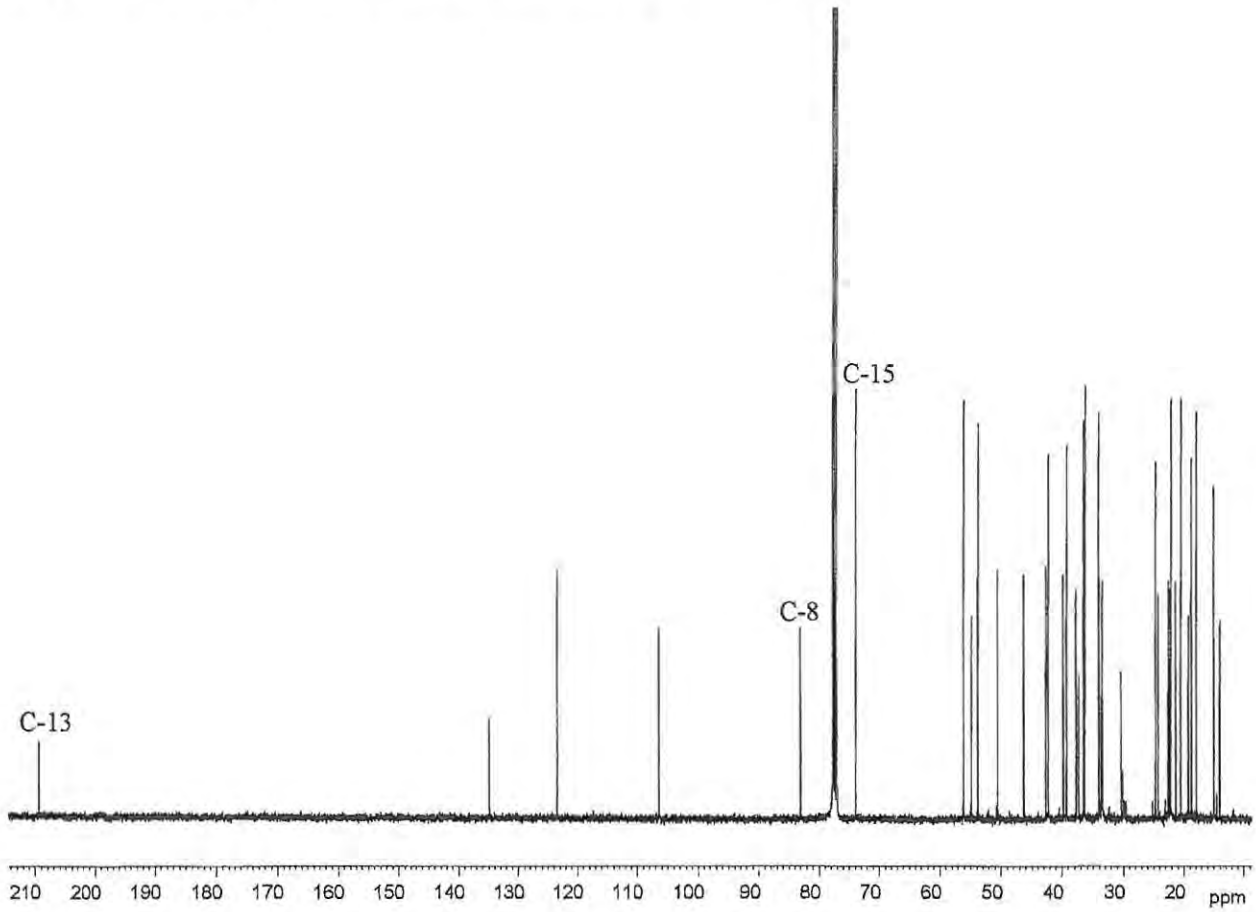


Figure 14: ^{13}C NMR spectrum ($CDCl_3$, 100MHz) of compound **73**.



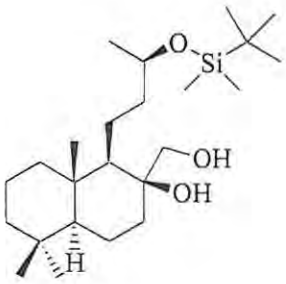
Unfortunately the physical data ([α_D] and melting point) for **74** was not reported by Grant, *et al.*[72] and because Francis' thesis was not accessible to us, our assignment of the stereochemistry of **74** and **75** was based on tenuous comparison of ^1H and ^{13}C NMR data of our compounds with the limited published data (Table 3). In particular, we noted a small, but significant shift in the C-14 methyl resonance of **74** (δ_C 23.80) versus **75** (δ_C 24.33) which aided our stereochemical assignment. The absolute stereochemistry of **74** was provided by the crystallographic data reported by Godfrey, *et al.* which identified **74** as 13(R)-8α,13:13,17-diepoxy-14,15-bisnorlabdan-17-one.[73] Hence, the data suggests that **75** is 13(S)-8β,13:13,17-diepoxy-14,15-bisnorlabdan-17-one.

Even though ruthenium oxidation proved unsuccessful with regards to the production of the desired ketone (**70** and **71**), it provided an interesting alternative to the preparation of the dihydroxylated product (**73**) which may undergo oxidative cleavage to give the resulting C-8 ketone as discussed in Section 2.7.2. However, the ease with which **73** cyclizes to **74** and **75** necessitated the handling of this compound with care.

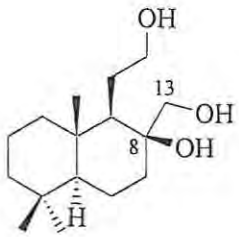
2.7.2 Attempted Cetyl Trimethyl Ammonium Permanganate *cis*-Hydroxylation and Oxidative Cleavage of Compound 67.

The oxidative cleavage of 1,2-diols using sodium metaperiodate or lead tetra-acetate was identified as an alternative means of introducing a ketone at C-8. Phase transfer catalysis has been used as a means to improve the permanganate oxidations of alkenes to 1,2-diols.[74] This alternative method of oxidation was considered as our substrates, unlike $KMNO_4$, are all insoluble in water which may mean that the $KMnO_4$ concentrations are often too low in the organic phase containing the substrate, in these mixed phase systems, to result in successful oxidation. Another way to overcome this problem is to find a solvent in which both reagents and substrate are soluble. We had used acetone previously (Section 2.2) as a solvent for permanganate oxidation, however, no oxidation was observed when these reaction conditions were applied to the TBDMS protected alkene **67**. To overcome solubility problems associated with permanganate oxidations, Bhushan, *et al.*[74] reported the preparation of the phase transfer catalyst cetyl trimethyl ammonium permanganate (CTAP ; $[n\text{-}C_{16}H_{33}\text{-}N(CH_3)_3]^{\oplus}MnO_4^{\ominus}$) and its use in the *cis*-hydroxylation of alkenes.

The general procedure for the preparation of CTAP is given in Chapter 3. Bhushan, *et al.*[74] reported two methods for the *cis*-hydroxylation of alkenes. The first method involved the dissolution of CTAP in CH_2Cl_2 and its dropwise addition to a solution of the substrate in CH_2Cl_2 at room temperature (RT) with stirring for 1 to 5 hours. When this method was applied to the hydroxylation of **67**, no change in **67** could be identified by NMR spectroscopy. An alternative method reported by Bhushan, *et al.* involved the dissolution of CTAP in a solvent mixture of *t*-butanol and H_2O and its dropwise addition to a solution of the substrate in *t*-butanol at RT with stirring for 1 to 5 hours. When the latter method was applied to compound **67**, after a tedious work-up, the dihydroxylated product **76** was identified in an undesirably low yield of 22%.



(76)



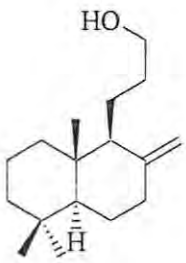
(77)

^1H and ^{13}C were used to confirm the formation of the dihydroxylated product **76** by identification of characteristic signals seen in a related compound (**77**) prepared by Grant, *et al.*[72] possessing a C-13 methylene (δ_H 3.60, 3.51, J = 11.0Hz and δ_C 63.8) and quartenary C-8 (δ_C 74.0). Compound **76** showed similar chemical shifts for the corresponding C-15 methylene (δ_H 3.59, 3.50, J = 12.2Hz and δ_C 67.7) and quarternary C-8 (δ_C 76.1). HRFABMS determined the molecular formula of **76** to be $C_{24}H_{44}SiO_3$ ($[M+1]^+$ 413.3451, Δmmu -0.17).

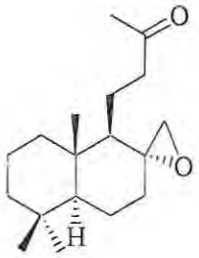
As was discussed previously the purpose of the synthesis of **76** was to produce the C-8 ketone by 1,2-diol oxidative cleavage. The oxidative cleavage of **76** was unsuccessfully attempted firstly using $Pb(OAc)_4$ and secondly using $NaIO_4$. Reagent insolubility in the organic solvent used was thought to be a possible limiting factor. However, even with the use of an aqueous *t*-butanol solvent system, only starting material was found.

2.7.3 Epoxidation followed by Oxidative Cleavage of 67 and 72.

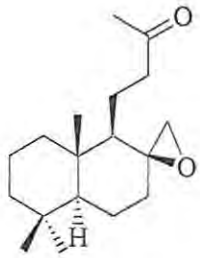
As we continued to be frustrated by the limited success of ozonolysis and other potential oxidation strategies for introducing a ketone at C-8, we decided to reassess the introduction of oxygen onto C-8 by epoxidation. As was mentioned in Section 2.1, epoxidation has previously been used with questionable success in introducing a β-epoxide at C-8.[52] This nevertheless provided us with an alternative means of deriving the desired 8β-hydroxyl through the opening of the β-epoxide ring with $LiAlH_4$. Chauvet, *et al.*[75] reported an epoxidation of the $\Delta^{8,17}$ olefin in compound **78** using *m*-chloroperbenzoic acid (*m*-CPBA) to produce the α-epoxide in a quantitative yield. Confusingly, Chauvet's quantitative preparation of the α-epoxide with *m*-CPBA appears to contradict the findings of Leite, *et al.*[52] who carried out an *m*-CPBA epoxidation of manool (**50**), mentioned previously in Section 2.1, to get a mixture of α- and β-epoxides. We therefore decided to investigate the *m*-CPBA epoxidation of our compounds **67** and **72**. The procedure for the $LiAlH_4$ reduction of epoxides used has been reported in numerous publications.[76,77] As the epoxidation results in a mixture of the α- and β-epoxides, we used reported epoxide ^1H shifts to identify the α- and β-epoxides in these mixtures. Grant, *et al.*[78] reported the ^1H chemical shift of the exocyclic methylene protons in the α-epoxide (**79**) to be 2.79ppm (J = 1.8Hz) and 2.47ppm (J = 4.0Hz) and in the β-epoxide (**80**) to be 2.31ppm (J = 4.0Hz) and 2.52ppm.



(78)



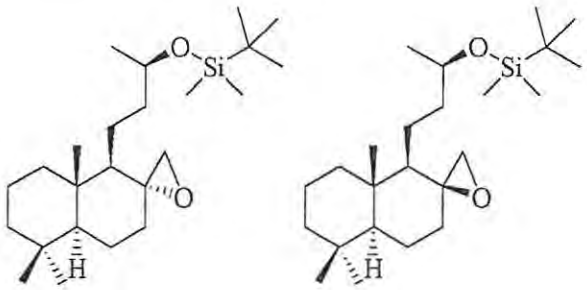
(79)



(80)

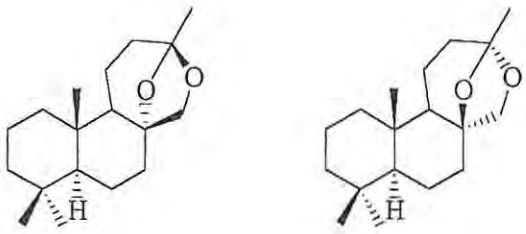
Compound **67** was reacted with *m*-CPBA in CH_2Cl_2 at -78°C to yield a mixture of epoxides, identified by ^1H and ^{13}C NMR (Figure 15). Separation of the epoxides was achieved by normal phase HPLC (hexane:EtOAc-97:3) which gave the α-epoxide (**81**, 31%) and the desired β-epoxide (**82**, 8%). Due to the instability of the compounds, complete 2-D NMR data could not be collected

degradation took place at RT. Given the poor yield of the β-epoxide, we decided to discontinue any further investigation of this alternative method for the preparation of the C-8 ketone from **67**.



(81) **(82)**

Realizing that the TBDMS functionality may be adversely affecting the stereoselectivity of the epoxidation reaction, we decided to attempt the epoxidation on the unprotected keto-olefin (**72**) as described by Grant, *et al.*[78] The *m*-CPBA epoxidation of **72** yielded a mixture of α- and β-epoxides identifiable from ^{1}H NMR spectrum (Figure 16). Rather than carrying out $LiAlH_4$ and thereby losing the C-13 ketone functionality required for later chain extension, we opted instead for an oxidative cleavage with periodate.[79] Oxidation using periodic acid (H_5IO_6) yielded a mixture of cyclized crystalline products although TLC indicated a single spot. The mixture was finally separated using normal phase HPLC (hexane:EtOAc/90:10) to give the acetal (**83**,13% yield).



(83) **(84)**

There have been several reported syntheses of **83** and **84**, commonly known as ambraketal and nor-ambraketal respectively.[79,80,81] These isomeric acetals (**83** and **84**) were first prepared by Jeger, *et al.*[81] from the permanganate oxidation of manool (**50**). Ambraketal has also been prepared by Martres, *et al.*[80]

Figure 15: ^{1}H NMR spectrum ($CDCl_3$, 400MHz) of the epoxide mixture of **79** and **80**.

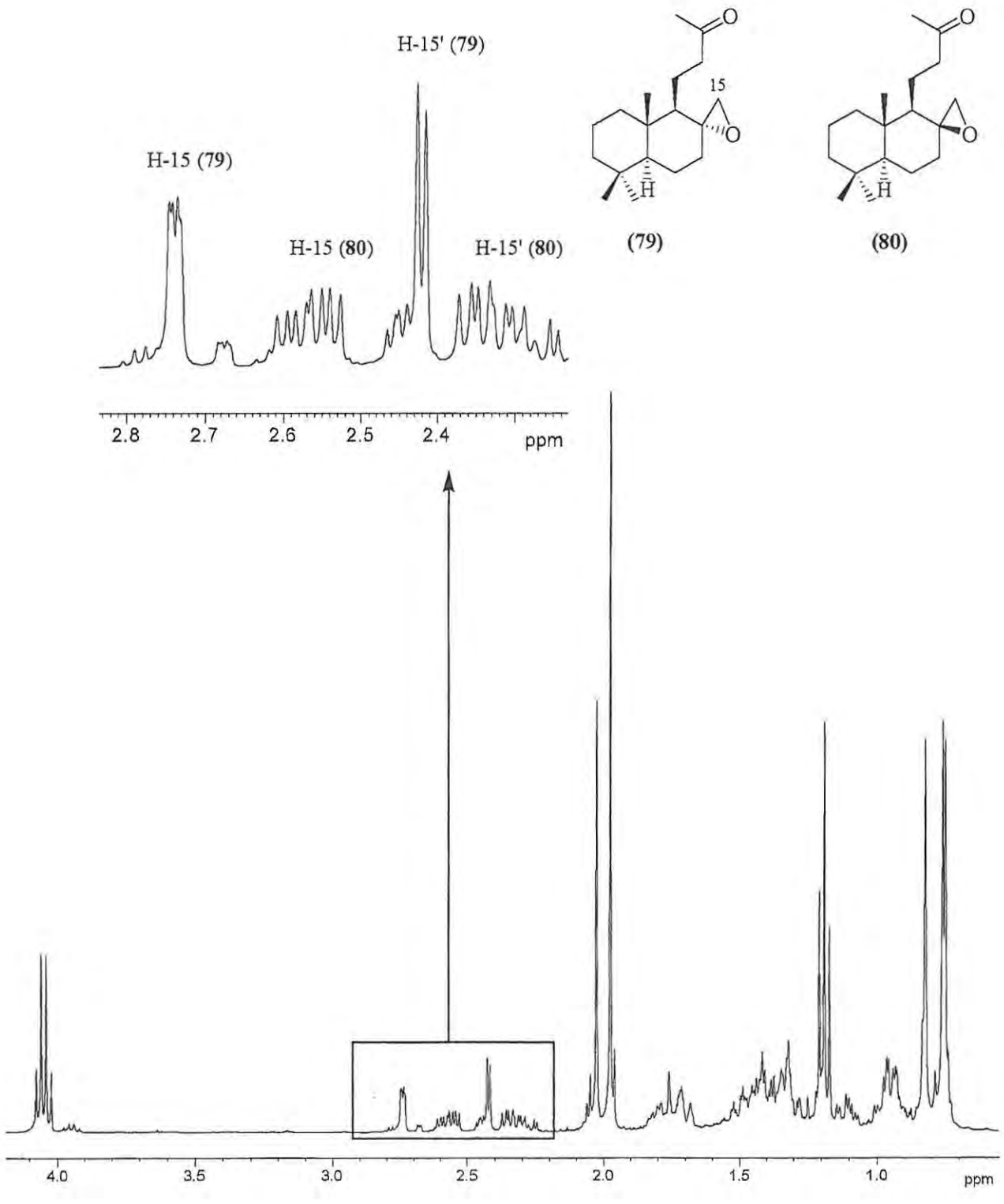
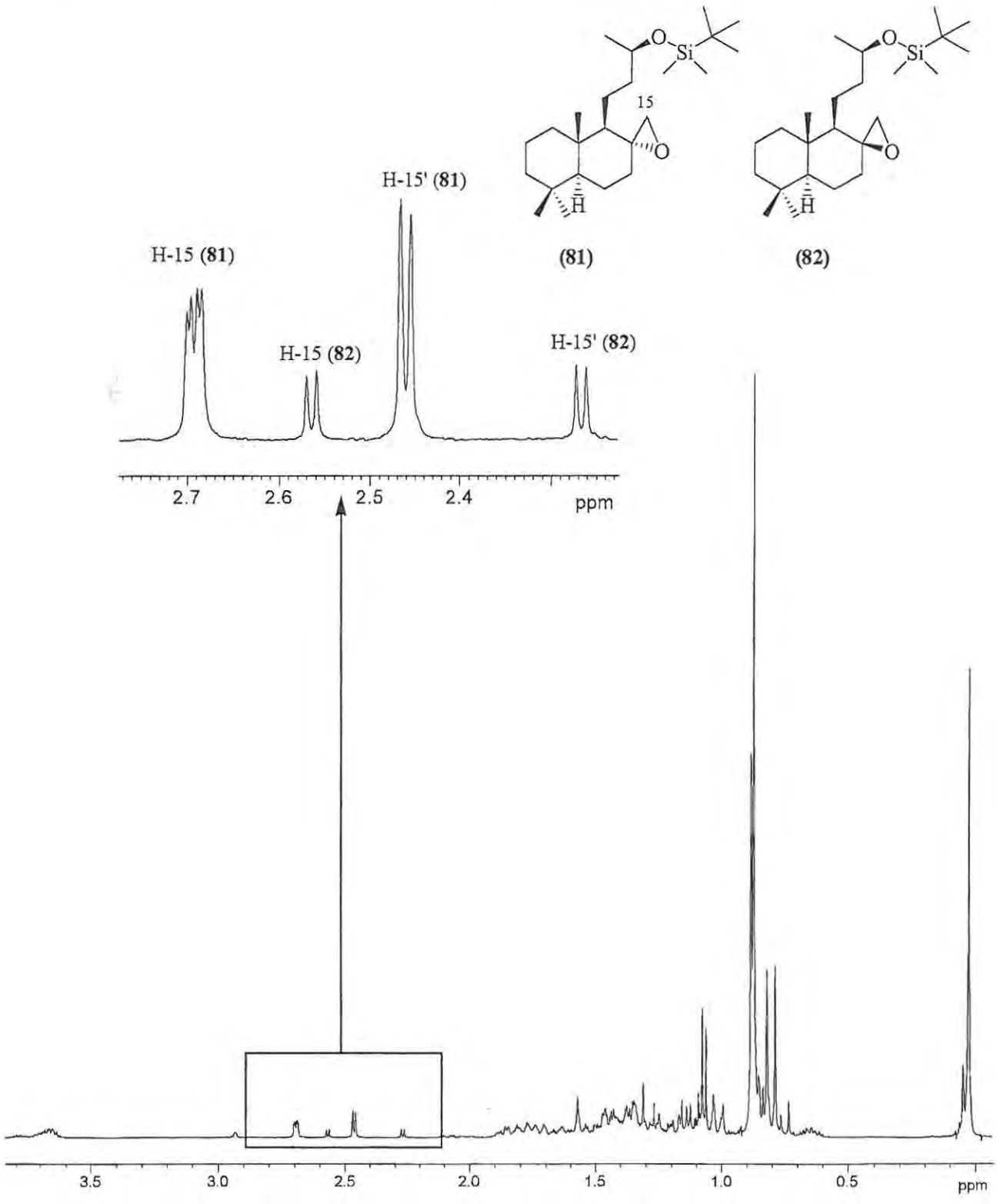


Figure 16: ^{1}H NMR spectrum ($CDCl_3$, 400MHz) of the epoxide mixture of **81** and **82**.



via the *m*-CPBA epoxidation of the keto-olefin (**72**) followed by copper sulphate work up. Ours is the first reported synthesis of ambraketal *via* periodic acid cleavage of an epoxide.

HRFABMS data identified the molecular formula of **83** to be $C_{18}H_{30}O_2$ (278.2246, Δmmu -0.39). Physical data obtained for **83** ($[\alpha]_D$ = -2°, Mpt = 117-119°C) corresponds well to literature values ($[\alpha]_D$ = -9°, Mpt = 120-121°C)[81] respectively. The structure of **83** was unequivocally confirmed by X-ray analysis. (Figure 17, Appendix 1).

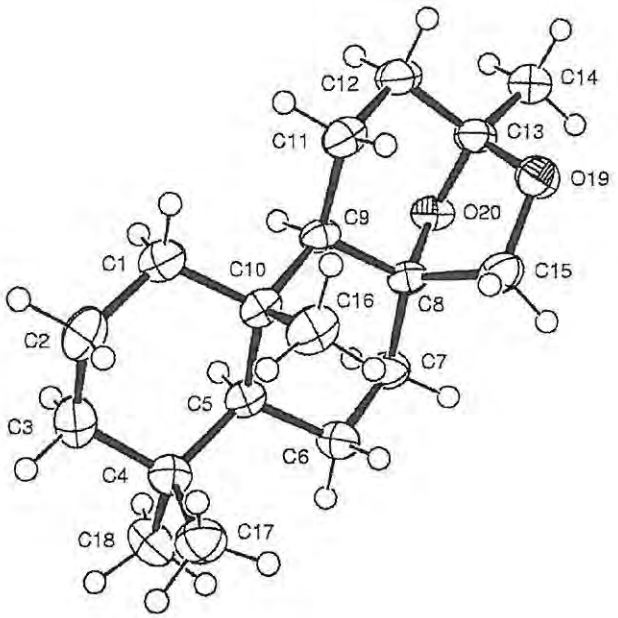


Figure 17: A view of compound **83** from the crystal structure showing the numbering scheme employed in the analysis. Anisotropic displacement of ellipsoids for the non-hydrogen atoms are shown at the 50% probability level. Hydrogen atoms are displayed with an arbitrarily small radius.

The ^{1}H NMR data of **83** was compared to the selected ^{1}H NMR data reported by Jeger, *et al.*[81] (Table 4). We believe Jeger, *et al.* incorrectly reported compound **83** as being (13S)-8α,13:13,20-diepoxy-

14,15-bisnorlabdane. The ^{1}H chemical shift of the C-18 methyl and the C-15 methylene protons of Jeger, *et al.*'s nor-ambraketal are consistent with ambraketal.

Table 4: Comparison of selected ^{1}H NMR data of our compound **83** with those of compound **83** and **84** as reported by Jeger, *et al.*.[81]

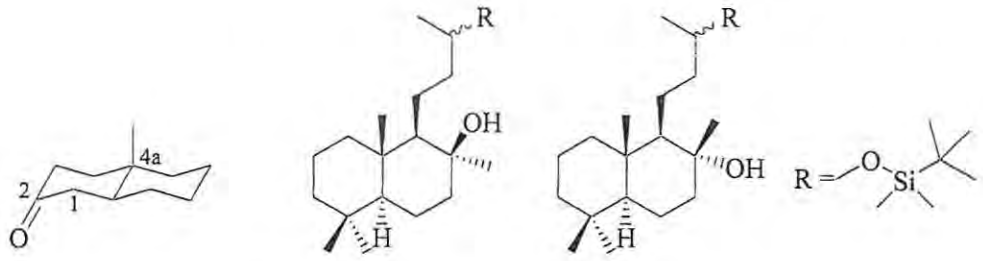
Carbon	Compound **83**[81](δ_H)	Compound **84**[81](δ_H)	Compound **83**(δ_H)
C-14	1.41 (s)	1.43 (s)	1.41 (s)
C-15	3.36 (d), 4.31 (d)	3.31 (d), 3.77 (d)	3.35 (d), 3.74 (d)
C-16	0.91 (s)	0.89 (s)	0.87 (s)
C-17	0.91 (s)	0.89 (s)	0.86 (s)
C-18	0.81 (s)	1.11 (s)	1.08 (s)

2.8 Preparation of the TDBMS Protected Alcohols 86 and 87.

The eventual successful preparation of the ketones **70** and **71** *via* ozonolysis facilitated the next step in our synthesis, namely the stereoselective alkylation of the ketone at C-8. The α-face (equatorial) delivery of a methyl nucleophile at the C-8 ketone is crucial in order to produce the required axial 8β-hydroxy group. In doing so, we would achieve the inversion of C-8 stereochemistry required in our synthesis of the marine natural product derivatives **45** and **46**.

With numerous methods of alkylation available *e.g.* Grignard reagents and organometallic reagents,[63] we needed to identify which procedure provided exclusive α-face delivery. MacDonald. *et al.*[83] reported results from a study involving the addition of nucleophiles to ketones. They found that methyllithium (MeLi) reacted with 4a-methyl-*trans*-2-decalone (**85**), a similar system to our bicyclic diterpenes, to produce an axial alcohol with high stereoselectivity and in a high yield. Variable temperature studies also indicated that α-face methylation was enhanced by lower reaction temperatures *i.e.*-78°C. A similar but more in depth study of the carbonyl alkylation process in cyclohexanone systems was reported by Maruoka, *et al.*.[84] They discussed the use of MeLi and

several Grignard reagents in producing axial and equatorial alcohols in differing ratios. It was found that Grignard reagents preferentially result in the formation of equatorial alcohols, but always in a mixture with the axial alcohol. They also found that the formation of the axial alcohol, by alkylation with MeLi, is enhanced when the targeted ketone possesses β-alkyl substitution, resulting in a quantitative yield of axial hydroxylated product. Given the substantial β-alkyl substitution in **70** and **71** we concluded that MeLi was the reagent of choice for the methylation step in our synthesis.



(85) **(86)** = β-R **(87)** = α-R **(63)** = β-R **(64)** = α-R

The procedure for organolithium alkylation was taken from the PhD thesis of P.T. Kaye.[85] MeLi was add dropwise to a solution of TBDMS protected ketone (**70**) in aprotic anhydrous THF cooled (-78°C) under N_2. TLC of the reaction mixture indicated the formation of a polar product. With purification using normal phase flash column chromatography, we obtained **86** in an isolated yield of 73%. The isomer **87** was similarly prepared from **71**.

Ashby, *et al.*[86] in a study of the stereoselective alkylation of cyclic ketones used GLC as a means of determining relative yields of axial and equatorial mixtures. When GLC chromatograms of our diastereomeric methylation products (**86** and **87**) were compared with GLC chromatograms of their epimers (**63** and **64** respectively), it was apparent that we had quantitatively prepared the 8β-hydroxy product. The combined GLC chromatogram for compounds **63**, **64**, **86** and **87** is shown in Figure 18. HRFABMS data provided the molecular formulae of **86** and **87** ($C_{24}H_{48}SiO_2$, 396.3424, Δmmu +0.07 and 396.3425, Δmmu +0.13 respectively). IR spectra indicated the presence of a hydroxyl functionality (ν_{max} = 3427 cm^{-1}) in both **86** and **87** and the loss of the carbonyl stretching frequency (ν_{max} = 1710 cm^{-1}) in both **70** and **71**. The differences in the ^{13}C chemical shift of **63** and **86** are highlighted in Table 5.

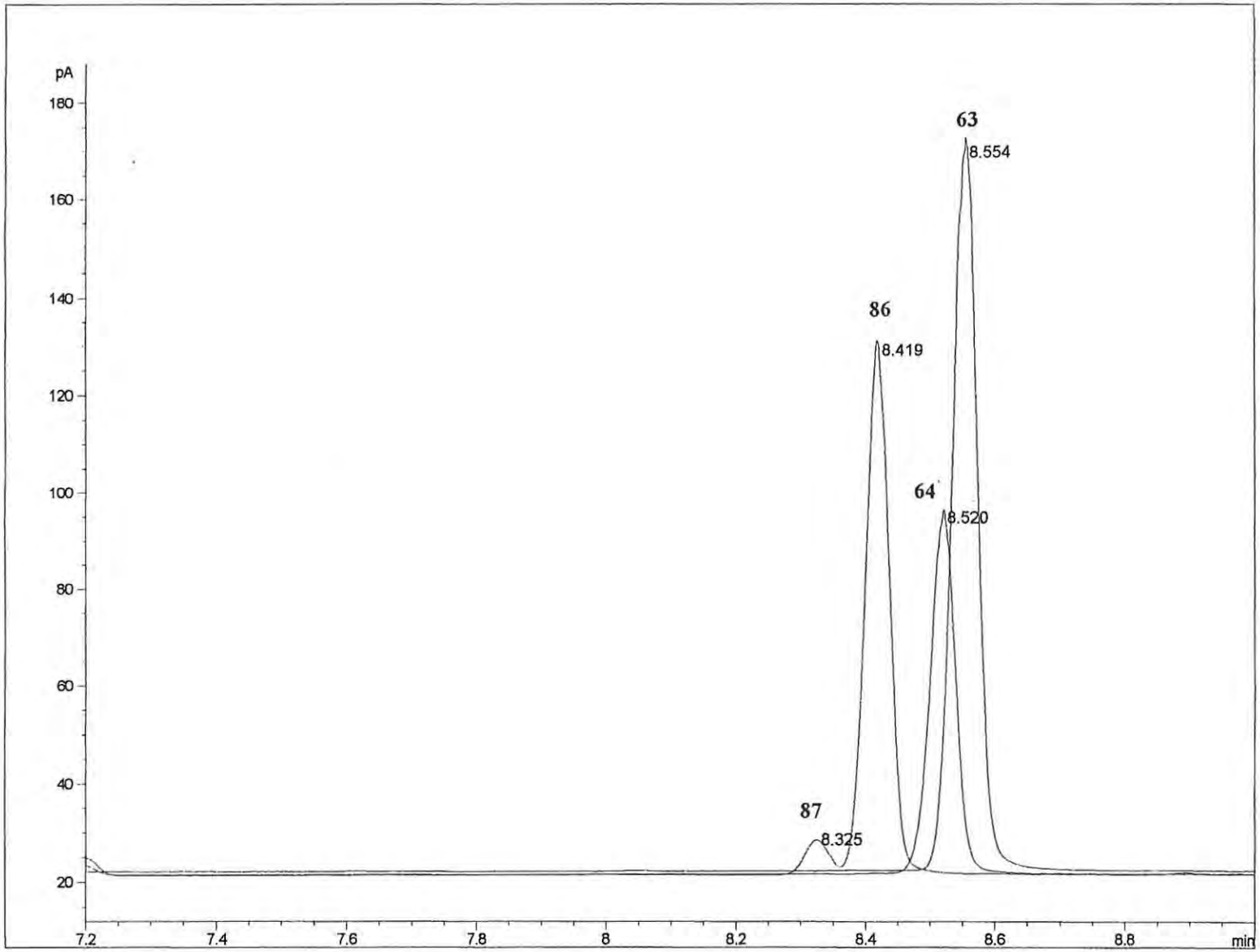


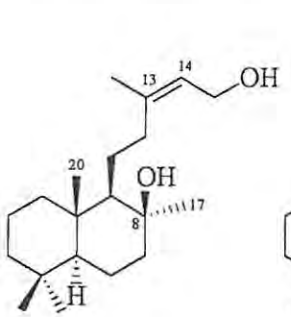
Figure 18 : The overlaid GLC chromatograms of the methylated products **63**, **64**, **86** and **87**.

[Chromatogram recorded on an HP6890C gas chromatograph, HP-5 capillary column (5% phenylmethyl siloxane) gradient temperature profile : initial temp. 75°C, ramped to 250°C @ 5°C min^{-1}, He flow rate 2mL.min^{-1}, split ratio 75:1, split flow 151mL.min^{-1}, FID detection]

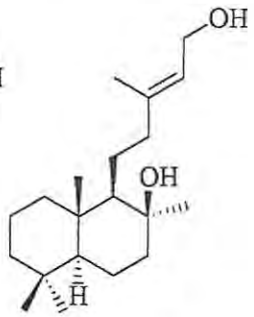
A change in stereochemistry around C-8 results in chemical shift changes primarily in the α and β-carbons closest to the stereocenter. As can be seen in Table 5, this holds true for the inversion of stereochemistry at C-8 in compound **86**. The optical rotations of **86** ($[\alpha]_D = +15°$) and **87** ($[\alpha]_D = +11°$) differ significantly from the epimers **63** ($[\alpha]_D = -14°$) and **64** ($[\alpha]_D = -9°$) respectively.

Table 5: ^{13}C Chemical shift (100MHz, $CDCl_3$)comparison between compound **86** and compound **63**, with significant changes in chemical shifts indicated by shading.

Carbon	Compound **86** (δ_C)	Compound **63** (δ_C)
C-1	39.1	39.8
C-2	18.3	18.4
C-3	43.8	43.0
C-4	33.2	33.2
C-5	55.9	56.2
C-6	18.1	20.6
C-7	42.1	44.5
C-8	73.2	74.2
C-9	59.1	61.9
C-10	39.0	39.83
C-11	21.3	21.2
C-12	42.1	42.1
C-13	69.1	69.4
C-14	23.6	23.6
C-15	30.5	23.9
C-16	33.4	33.4
C-17	21.7	21.5
C-18	15.0	15.3
C-19	-4.4	-4.3
C-20	-4.7	-4.6
C-21	18.2	18.1
C-22, 23, 24	25.9	25.9



(45)

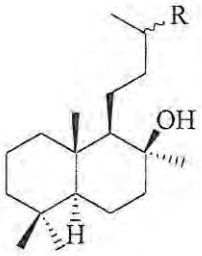


(46)

Finally, Cimino, *et al.*[8] used two pieces of NMR evidence to support their assignment of an axial alcohol functionality at C-8 in the alcohols **45** and **46** prepared from the natural products **5** and **6**. Firstly, α or β orientation of the alcohol moiety at C-8 apparently influences the ^{1}H chemical shift of the methyl group (H_3-20). An α-equatorial alcohol at C-8 induces an upfield shift of the H_3-20 methyl protons (δ_H 0.80) while a β-axial alcohol C-8 alcohol causes a downfield shift of H_3-20 methyl protons (δ_H 0.96).[87,88] In **86** and **87** the equivalent methyl protons are H_3-18 which are shifted upfield (δ_H 0.93 for both), thus implying a C-8 axial hydroxyl functionality in these two compounds. The second piece of evidence provided by Ciminio, *et al.*[8] in support of the C-8 stereochemistry in **44** and **45** was the ^{13}C chemical shift of the methyl carbon C-17. An α-equatorial methyl substituent at C-8 has a downfield ^{13}C chemical shift (δ_C 30.0) while a β-orientated methyl group resonance appears upfield (δ_C 25.0).[87,88] The equivalent methyl carbon in **63**, **64**, **86** and **87** is C-15. From the chemical shifts of C-15 in these four compounds (Table 5), it is clear that the methyl substituent at C-8 in **86** and **87** is α-equatorial and in compounds **63** and **64** it is β-axial.

2.9 Synthesis of the α,β-Unsaturated Esters 91 and 92 from the Protected Alcohols 86 and 87

In order to facilitate chain extension at C-13, we required the reintroduction of the ketone functionality at this carbon. TDBMS deprotection was therefore necessary to expose the side-chain hydroxyl group for further oxidation to the ketone. TBAF has been identified as a means to readily cleave the TDBMS moiety to generate the free alcohol[61] and has been used as an effective method of deprotection on numerous occasions in marine natural product syntheses.[89,90] Therefore individual solutions of the 8β-hydroxy TBDMS protected products (**86** and **87**) and TBAF in anhydrous THF were refluxed for four hours to produce the 8β,13-diols (**88** and **89**) in isolated yields of 85% and 74% respectively.

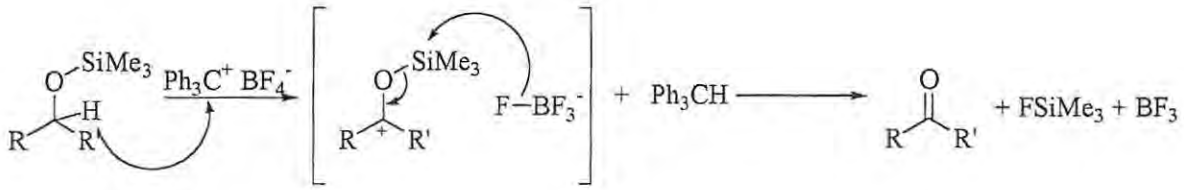


(88) R = β-OH
(89) R = α-OH

Compounds **88** and **89** were both fully characterized using ^{1}H, ^{13}C and 2D NMR experiments. The ^{1}H NMR spectra indicated the loss of the protective group methyl proton peaks (δ_H 0.03ppm and 0.87ppm) and the ^{13}C NMR spectra showed the loss of the protective group methyl carbon signals (δ_C -4.72, -4.45 and 25.90). HRFABMS data gave the molecular formulae of **88** and **89** as $C_{18}H_{34}O_2$ (282.2559, Δmmu = +0.19 and 282.2558, Δmmu= -0.66 respectively). The optical rotations of **88** ($[\alpha]_D$ = +8°) and **89** ($[\alpha]_D$ = +18°) also differed from the isomeric forms of **57** ($[\alpha]_D$ = -30°) and **58** ($[\alpha]_D$ = +8°) respectively. The presence of the secondary alcohol functionality at C-13 was further confirmed by the H-13 multiplet (δ_H 3.75) in the ^{1}H NMR spectrum and the deshielded oxymethine carbon resonance (δ_C 68.46) in the ^{13}C NMR spectrum.

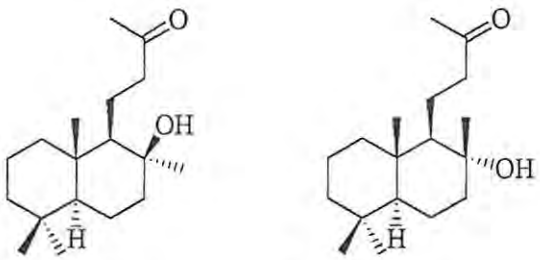
To prevent dehydration of the tertiary alcohol at C-8 and reduce potential cyclic hemi-acetal formation during oxidation, we needed to identify a method of mild oxidation for our next synthetic step. Interestingly, during our literature search of mild oxidation processes, we identified a neat method for alcohol oxidation to a ketone *via* hydride abstraction of trimethylsilyl ethers.[91] Jung reported that the oxidation was facilitated using triphenylcarbenium (trityl) tetrafluoroborate as the hydride extractor. The oxidation takes place through the formation of a carbocation intermediate followed by fluoride ion attack from BF_4^- to give the desired ketone (Scheme 8). Jung also mentioned that the bulky trimethylsilyl group reduces complexation between the trityl salt and the ether oxygen. We concluded that this oxidation method could possibly be effectively applied to our protected alcohols which possessed the bulkier *t*-butyldimethylsilyl group. Using commercially available trityl fluoroborate and following Jung's procedure, we attempted the oxidation of **86** in CH_2Cl_2 at room temperature. NMR indicated no change in the starting material and this method of oxidation was

pursued any further.



Scheme 8: Mechanism of trityl tetrafluoroborate oxidation of TMS protected alcohols.[91]

Other alternatives for mild oxidation included pyridinium chlorochromate,[92] tetrapropylammonium perruthenate,[93] raney nickel,[94] and chromium trioxide (Collin's oxidation)[95] oxidations. We, however, decided to opt for Swern oxidation, which was a well established procedure in our laboratory.[96,97] Accordingly, the 8β,13-diols (**88** and **89**) were independently added to cooled (-78°C) solutions of oxalyl chloride and dimethylsulphoxide in CH_2Cl_2 under N_2. Triethylamine was added to the mixtures and the reaction stirred for one hour and extracted to yield the crude ketone (**90**).

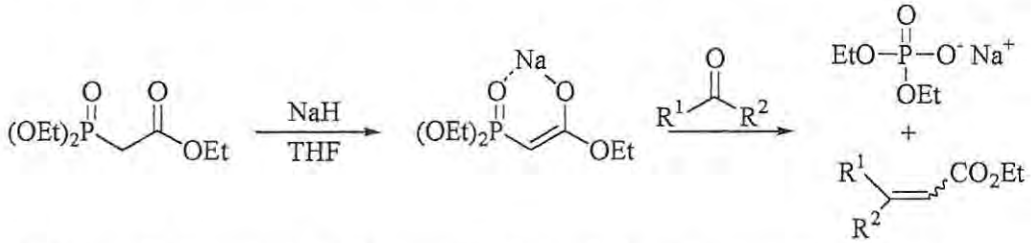


(90) **(55)**

^{13}C NMR showed the undeniable presence of a ketone functionality ($\delta_C = 209.9$). The IR spectrum also confirmed the presence of a ketone functionality ($\nu_{max} = 1720\ cm^{-1}$). Due to the problem of cyclic hemi-acetal formation as described in Section 2.2, we were reluctant to purify **90** and opted to proceed with the crude ketone instead.

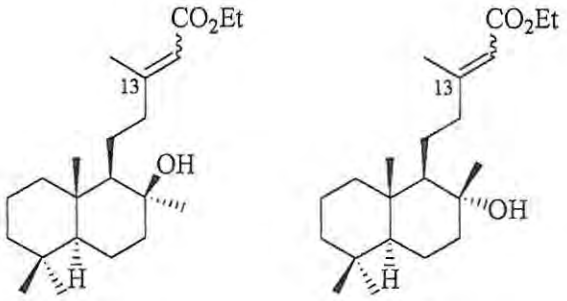
With the successful formation of the 8β-hydroxy ketone (**90**), we were now in a position to reintroduce a Δ^{13} olefin as required to synthesize our marine natural product derivatives (**45** and **46**). The Horner-Wadsworth-Emmons (HWE) modification of the Wittig reaction is a well known

synthetic strategy used to introduce carbonyl derived olefinic functionalities.[98,99] In our synthesis, we anticipated that the HWE procedure would utilise the reaction of metal enolates [derived from triethyl phosphonoacetate (TEPA) and sodium hydride (NaH)] and the ketone (**90**) to yield the desired olefinic ester (**91** and **92**) and a water soluble phosphate ester (Scheme 9).



Scheme 9: A generalized Horner-Wadsworth-Emmons reaction mechanism.

Variations of the HWE procedure have been used for the stereoselective preparation of both E- and Z-isomers, however, this stereoselectivity is somewhat diminished when the HWE reaction is applied to ketones.[100,101,102] Both E- or Z-isomers, would lead to either one of our target molecules (**45** or **46**), so stereoselectivity was not initially of concern to us. We, however, decided to attempt the HWE reaction on **55**, easily prepared from sclareol (See Section 2.2), to validate this synthetic approach and to prevent the unnecessary use of an already dwindling supply of the 8β-hydroxy substrate, **90**.



(**91**) Δ^{13}Z
(**92**) Δ^{13}E

(**93**) Δ^{13}Z
(**94**) Δ^{13}E

Compound **55** was added to a stirred solution of NaH and TEPA in anhydrous THF under N_2. The reaction gave a mixture of the α,β-unsaturated esters in a 94% overall yield. Separation *via* normal phase HPLC yielded Z-isomer (**93**) and the E-isomer (**94**) in a ratio of 2:1 respectively.

Table 6: ^{1}H and ^{13}C Chemical shift (100MHz and 400MHz respectively, $CDCl_3$) for compounds **91**, **92**, **93** and **94** (multiplicities in parentheses, coupling constants given in Chapter 3).

Carbon	Compound **91**		Compound **92**		Compound **93**		Compound **94**	
	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C
C-1	0.87(m) 1.65(m)	39.1	0.96(m) 1.79(m)	39.1	0.94(m) 1.64(m)	39.8	0.96(m) 1.74(m)	37.4
C-2	1.40(m) 1.57(m)	18.2	1.39(m) 1.56(m)	18.3	1.41(m) 1.56(m)	18.4	1.41(m) 1.56(dt)	19.5
C-3	1.14(m) 1.38(m)	41.7	1.11(m) 1.38(m)	44.6	1.16(m) 1.36(m)	42.0	1.19 (m) 1.34 (m)	39.3
C-4	-	33.2	-	33.3	-	33.3	-	33.3
C-5	0.82(m)	55.9	0.90(m)	55.9	0.91(m)	56.1	0.91(m)	56.1
C-6	1.48(m)	18.1	1.49(m)	18.2	1.24(m) 1.63(m)	20.6	1.22(m) 1.62(m)	20.3
C-7	1.44(m) 1.72(m)	42.2	1.49(m) 1.74(m)	42.0	1.37(m) 1.85(dt)	44.8	1.44(m) 1.84(dt)	43.2
C-8	-	73.1	-	73.3	-	74.2	-	73.9
C-9	0.77(m)	58.8	0.83(m)	59.3	1.04(t)	61.5	1.11(m)	61.5
C-10	-	39.0	-	39.1	-	39.2	-	38.8
C-11	1.38(m) 1.55(m)	23.6	1.16(m) 1.43(m)	23.8	1.37(m) 1.56(m)	23.6	1.46(m)	24.3
C-12	2.12(m)	44.6	2.56(ddd) 2.74(ddd)	42.4	2.16(m) 2.28(m)	44.4	2,21(m) 2.93(m)	42.0
C-13	-	160.2	-	159.9	-	160.9	-	161.7
C-14	5.65(s)	115.3	5.60(s)	115.9	5.66(s)	115.2	5.64(s)	115.6
C-15	-	166.9	-	166.2	-	167.0	-	166.6
C-16	1.24(m)	14.3	1.23(m)	14.3	1.28(t)	14.3	1.23(t)	14.3
C-17	1.12(s)	30.6	1.23(s)	30.6	1.14(s)	24.0	1.16(s)	24.2
C-18	0.80(s)	21.6	0.82(s)	21.7	0.78(s)	21.5	0.77(s)	21.5
C-19	0.85(s)	33.4	0.86(s)	33.4	0.86(s)	33.4	0.85(s)	33.4
C-20	0.93(s)	15.1	0.94(s)	15.1	0.78(s)	15.4	0.75(s)	15.5
C-21	4.11(m)	59.4	4.13(m)	59.4	4.14(q)	59.4	4.12(q)	56.8
C-22	2.15(s)	19.0	1.90(s)	19.0	2.15(s)	19.1	1.89(d)	25.7

Each isomer (**93** and **94**) was identified by ^{1}H, ^{13}C and 2D NMR techniques, and the fully assigned NMR data are presented in Table 6. The ^{1}H NMR spectra for compounds **93** and **94** showed the distinct chemical shift of the C-14 olefinic proton (δ_H 5.66 and δ_H 5.64 respectively) and the C-21 ethyl ester methylene (δ_H 4.14 and δ_H 4.12), which confirmed the introduction of the vinylic functionality and the anticipated C-15 ester. The ^{13}C NMR chemical shifts of C-13 (δ_C 160.9 and δ_C 161.7 respectively) and C-14 (δ_C 115.2 and δ_C 115.6 respectively), in conjunction with the C-15 carbonyl shifts (δ_C.167.0 and δ_C.166.6, respectively) in the ^{13}C NMR spectra of **93** and **94** further confirmed the formation of the $\Delta^{13,14}$ moiety and the anticipated ethyl ester. The difference in vinyl methyl chemical shifts between E- and Z-isomers has been reported by Bates, *et al.*[103] who stated that the methyl proton signals (C-17 in this case) in E-isomers appear further upfield in ^{1}H NMR spectra when compared to vinyl methyl protons in Z-isomers. We applied this principle to the identification of **93** and **94**, and as can be seen in Table 6, the chemical shift of H_3-17 in **93** varies significantly from that in **94**. IR spectra of both compounds showed characteristic carbonyl (ν_{max} = 1715 cm^{-1}) and hydroxyl (ν_{max} = 3500 cm^{-1}) absorbances.

HRFABMS data provided the molecular formulae of **93** and **94** ($C_{22}H_{38}O_3$), ($[M+1-CH_3]^+$ 335.2429, Δmmu = - 0.12 and $[M+1-CH_3]^+$ 335.2429, Δmmu = - 0.05 respectively). The spectroscopic data thus confirmed the structures of **93** and **94** as 13(Z)-8α-hydroxy-ladb-13-en-15-ethanoate and 13(E)-8α-hydroxy-ladb-13-en-15-ethanoate respectively.

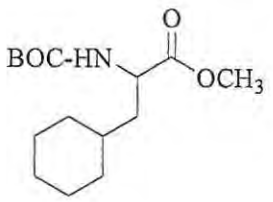
Having established that the HWE olefination was viable, we carried out the same reaction on our limited amount of compound **90**. It should be noted that again the Swern oxidation of the 8β,13-diols (**88** and **89**) was directly followed, after work-up, with the HWE reaction in order to reduce hemi-acetal cyclization and hence, in theory improve yields. The HWE of compound **90** gave the anticipated mixture of E and Z isomers (**91** and **92**) in a low overall yield of 35%. The low yield could be possibly explained by our inability to establish the rigorous anhydrous environment necessary when performing the HWE reaction using crude **90** directly from the Swern oxidation. Normal phase HPLC was used to achieve separation of the E-isomer (**92**) and Z-isomer (**91**) in a ratio of 10:1, respectively.

Compound **91** and **92** were identified as per **93** and **94** from NMR and mass data. The assigned NMR data for compounds **91** and **92** are also presented in Table 6. The ^{1}H NMR spectra for compounds **91** and **92** showed the C-14 olefinic proton (δ_H 5.65 and δ_H 5.60 respectively) and the C-21 ethyl ester methylene (δ_H 4.11 and δ_H 4.13). The ^{13}C NMR spectra of compounds **91** and **92** showed the desired C-13 (δ_C 160.2 and δ_C 159.9 respectively), C-14 (δ_C 115.3 and δ_C 115.9 respectively) and C-15 (δ_C.166.9 and δ_C.166.2 respectively) chemical shifts. IR spectra also confirmed the presence of the carbonyl moiety (ν_{max} = 1717 cm^{-1}) and the hydroxyl functionality (ν_{max} = 3450 cm^{-1}). From the available data, we identified compounds **91** and **92** as(13Z)-8β-hydroxy-labd-13-en-15-ethanoate and (13E)-8β-hydroxy-labd-13-en-15-ethanoate respectively. We were unable to explain the different E:Z ratios encountered when the HWE reaction was carried out on **55** and **90**. Interestingly, the HRFABMS spectra of **91** and **92** provided the molecular ions directly and did not show the loss of CH_3 encountered with **93** and **94**. No published spectroscopic data could be found for compounds **91** - **94** and we assume that these are new compounds.

2.10 DIBALH Reduction of the Esters 91, 93 and 94.

Initially we had hoped that the reduction of the terminal C-15 ethyl ester functionality would give rise directly to the aldehyde of the target natural products **6** and **7**. A precedent for the reduction of an ester directly to an aldehyde was provided by Luly, *et al.*[43] who successfully prepared Boc-(cyclohexyl)-alaninal (**95**) by diisobutylaluminium hydride (DIBALH) reduction of the corresponding ester (**96**). Even though **96** is not related to our α,β-unsaturated esters (**91** and **92**), it does demonstrate the possible conversion of a side chain ester to an aldehyde with DIBALH.

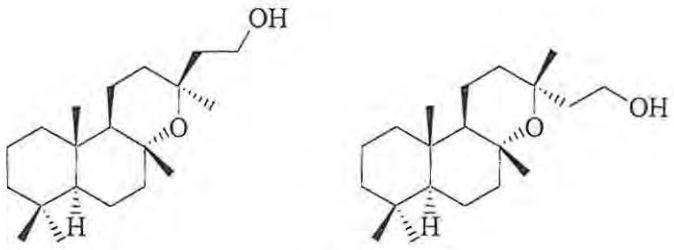
(95)



(96)

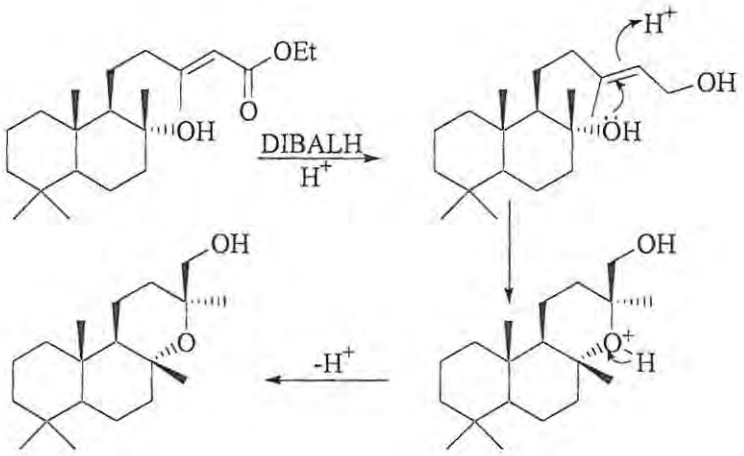
Although the DIBALH (1.2 equivalents) reduction was first attempted on **93** and **94** to ascertain the viability of this reaction, unexpected products were obtained. After purification by normal phase

silica column chromatography, both **97** and **98** were identified using ^{1}H, ^{13}C and 2D NMR experiments. HRFABMS data gave the molecular formulae of **97** and **98** ($C_{20}H_{36}O_2$), ($[M+1]^+$ 309.2795, Δmmu = -0.13 and $[M+1]^+$ 309.2793, Δmmu = -0.07).



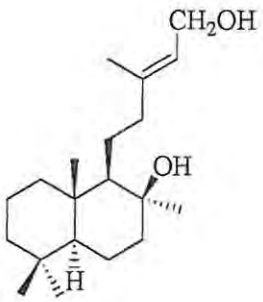
(97) **(98)**

The molecular formula of **97** and **98** suggested three degrees of unsaturation and as there were no olefinic or carbonyl resonances evident in the ^{13}C NMR spectra of these two isomers implied that both compounds possessed a tricyclic skeleton. The COSY NMR spectrum of **97** clearly showed coupling between an oxymethylene (H_2-15, δ_H 3.75) and two methylene protons (H_2-14, δ_H 1.52 and 1.66). No further coupling between this isolated spin system and the other protons was observed. The two oxygenated quaternary carbon resonances in the ^{13}C NMR spectrum of **97** (δ_C 75.8 and 75.9) were assigned to a cyclic ether moiety with two bridgehead methyl substituents and proved crucial in establishing the structure of **97** and **98**. As is shown in Scheme 10, we propose that cyclization may have occurred after the reduction of **97** and **98** during the purification step on silica gel. The stereochemistry at C-13 could not be established by a NOESY NMR spectrum and is arbitrarily assigned.



Scheme 10 : A possible mechanism for the formation of compound **97** with a trace of acid.

We decided to attempt the reduction of compound **91** using one equivalent of DIBALH despite the results obtained on the test compounds **93** and **94** (the small amount of compound **92** was retained for future studies). No change in the starting material was observed after the reaction of **91** with one equivalent of DIBALH. Compound **91** was further reacted with three equivalents of DIBALH to give the corresponding C-15 alcohol **46** in an isolated yield of 46% after purification *via* normal phase HPLC (hexane:EtOAc/1:1).



(46)

HRFABMS data gave the molecular formula of **46** as $C_{20}H_{36}O_2$ (308.2715, Δmmu = -0.04). The structure of compound **46** was resolved using ^1H, ^{13}C and 2D NMR experiments. The ^1H NMR spectrum of compound **46** confirmed the presence of the C-14 olefinic proton (δ_H 5.41), the loss of the C-21 ethyl ester methylene (δ_H 4.11) and the formation of a C-15 methylene (δ_H 4.14). The ^{13}C NMR spectrum of compound **46** further substantiated the still intact olefinic moiety (C-13, δ_C 140.30; C-14, δ_C 123.10) and the loss of the C-15 carbonyl moiety (δ_C 166.85). The previously unassigned ^1H and ^{13}C NMR data for **46** are shown in Table 7. Finally, the optical rotation of **46** ($[\alpha]_D = +19°$) was consistent with that reported by Cimino, *et al.*[8] ($[\alpha]_D = +32°$) confirming that we had successfully synthesized our target molecule. The ^1H and ^{13}C spectra of **46** are presented in Figures 19 and 20 respectively.

Table 7 : The ^{1}H and ^{13}C chemical shifts (400MHz and 100MHz respectively, $CDCl_3$) for compound **46** (multiplicities and coupling constants in parentheses).

Carbon	Compound **46**	
	δ_H	δ_C
C-1	0.84 (m), 1.66 (m)	39.2
C-2	1.42 (m), 1.59 (dt)	18.2
C-3	1.12 (m),1.38 (m)	42.1
C-4	-	33.3
C-5	0.90 (m)	55.9
C-6	-	18.3
C-7	2.03 (m)	43.3
C-8	-	73.2
C-9	0.79 (m)	58.8
C-10	-	39.0
C-11	1.38 (m), 1.52 (m)	24.0
C-12	1.46 (m),1.73 (m)	42.2
C-13	-	140.4
C-14	5.41 (t, J = 6.8Hz)	123.1
C-15	4.14 (d, J = 6.9Hz)	59.4
C-16	1.69 (s)	16.4
C-17	1.14 (s)	30.6
C-18	0.82 (s)	21.7
C-19	0.86 (s)	33.4
C-20	0.95 (s)	15.1

Figure 19: ^{1}H NMR spectrum ($CDCl_3$, 400MHz) of compound **46**.

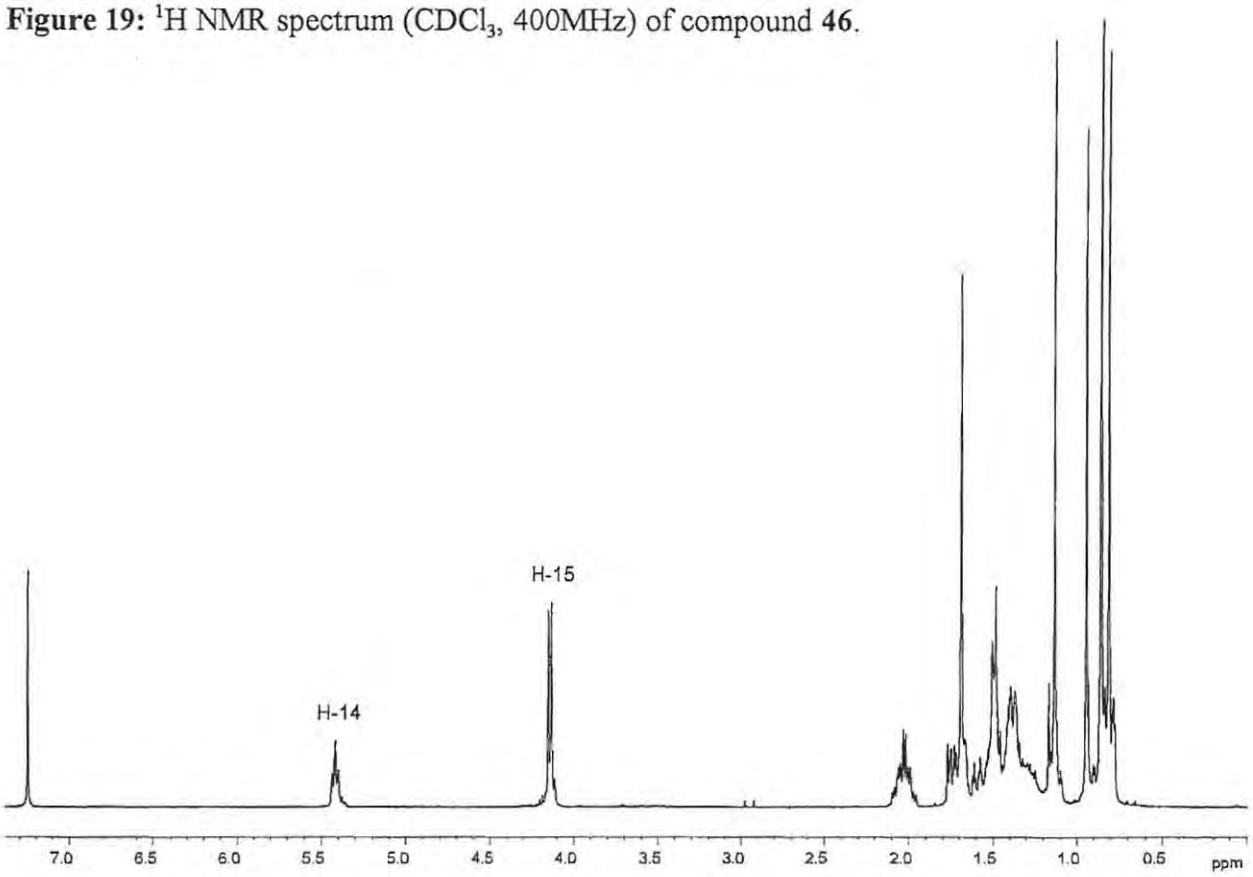
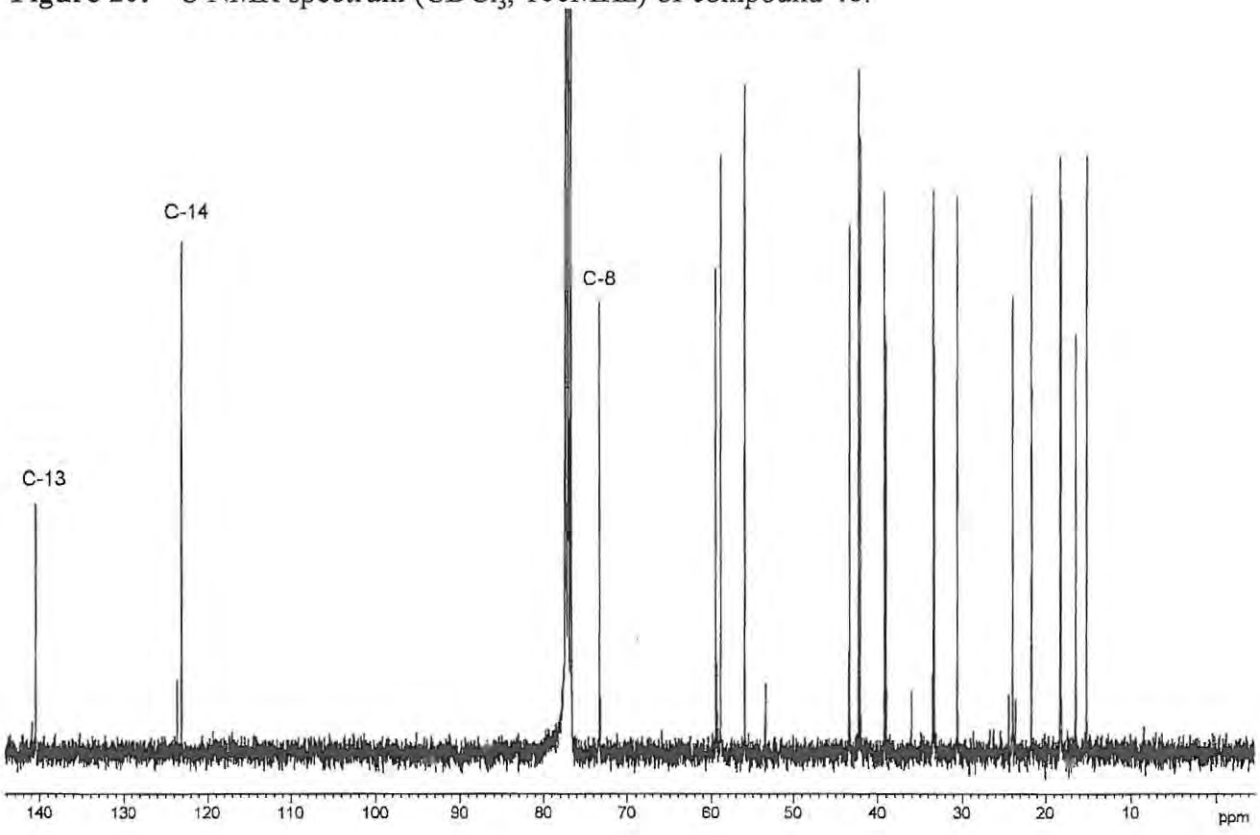


Figure 20: ^{13}C NMR spectrum ($CDCl_3$, 100MHz) of compound **46**.

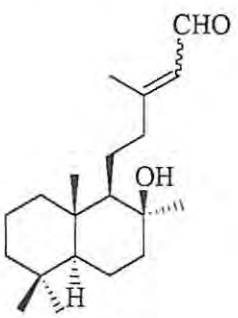


CHAPTER 3

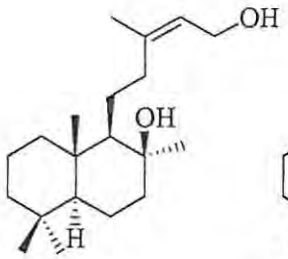
CONCLUSION

3. CONCLUSION

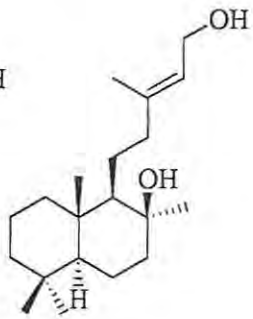
The initial focus of our synthesis was to identify an optimized method of preparing the two isomeric marine natural products 8β-hydroxylabd-13E-en-15-al (**5**) and 8β-hydroxylabd-13Z-en-15-al (**6**) isolated by Cimino, *et al.* Having identified that the aldehydes (**5** and **6**) were unstable, our synthesis was redirected towards the synthesis of their alcohol derivatives (**45** and **46**). Accordingly labd-13E-en-8β,15-diol (**46**) was prepared in ten steps from sclareol (**32**) in an overall yield of 3.4%. The preparation of the labd-13Z-en-8β,15-diol (**45**) would also have been undertaken had it not been for time constraints and limited supplies of the required precursor (**91**).



(5) Δ^{13}E
(6) Δ^{13}Z



(45)



(46)

Problems associated with yields arose in the first oxidation step which gave **55** (51%), the HWE step which yielded **91** and **92** (35%) and the final DIBALH reduction step which produced **46** (46%). Given adequate time, optimization of these reactions could have been investigated. With the preparation of sufficient quantities of **46**, the mild oxidation of the C-15 hydroxyl group could be attempted to synthesize the aldehyde natural product. Suitably activated manganese dioxide is a possible mild oxidant for synthesizing the aldehydes **5** and **6**, from compounds **45** and **46** respectively and providing adequate amounts of these compounds for bioassay studies.

It is hoped that the work presented in this thesis will facilitate future marine labdane diterpene synthetic studies at Rhodes University.

CHAPTER 4

EXPERIMENTAL

4. EXPERIMENTAL

4.1 General Experimental

Dried solvents were prepared as per procedures described by Perrin, *et al.*[105] THF was dried over benzophenone and sodium wire under nitrogen. All solvents used were redistilled prior to use. Anhydrous reactions were performed in flame dried glassware under dry nitrogen. Distilled solvents used for anhydrous reactions were stored over 4Å molecular sieves. HPLC solvents used were of analytical grade.

Normal phase TLC was carried out on Merck DC-Plastikfolien Kieselgel 60 F_{254} and viewed under a 254nm UV light. TLC plates were developed using a 10% H_2SO_4 in MeOH spray followed by heating on a hotplate. Iodine and 4-DNP staining were used as alternate methods of development when required. Column chromatography was performed using Merck 7734 Kieselgel 60 silica (70-230 mesh). Flash column chromatography was performed using Merck 9385 Kieselgel 60 silica (230-400 mesh). Normal phase HPLC separations were achieved using a system comprising of a Spectra-Physics IsoChrom LC or Spectra-Physics SpectraSERIES P100 pump, a Waters R401 differential refractometer, a Whatman Magnum 9-Partisil 10 column and a Rikadenki chart recorder.

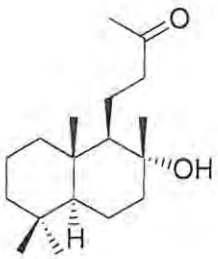
^1H (400MHz), ^{13}C (100MHz), DEPT and 2D NMR spectra were acquired using a Bruker AMX400 NMR spectrometer. Spectra were run in $CDCl_3$, with ^1H and ^{13}C shifts being referenced to 7.25ppm and 77ppm respectively. Chemical shifts are reported in δ units (ppm) and coupling constants in hertz (Hz). IR spectra were recorded on a Perkin Elmer Spectrum 2000 FT-IR spectrophotometer. Optical rotations were measured on a Perkin-Elmer 141 polarimeter, with samples being run in $CHCl_3$. Low resolution mass spectra (EIMS) were obtained on a Finnigan Matt GCQ spectrometer. High resolution fast atom bombardment mass spectra (HRFABMS) were obtained by Dr Louis Fourie of the mass spectrometry unit at the University of Potchefstroom. The X-ray structure of ambraketal was determined by Professor Mino Caira of the University of Cape Town. Melting points were determined using a Kofler hot-stage apparatus.

4.2 Synthetic Procedures

4.2.1 Preparation of Ketone 55.

Compound **32** (40.00g) was dissolved in 4.0L of acetone. Powdered $KMnO_4$ (73.50g) was added over 2 hours at a temperature between 15°C-20°C. H_2O (40mL) was added and the mixture was stirred for a further 5 hours at 15°C-20°C. The mixture was then stirred overnight at RT (22°C), and then allowed to stand for 24 hours. The supernatant was removed with a pipette. NaOH (10g) in H_2O (50mL) was added to the MnO_2 residue, which was further extracted with acetone (3x1.5L). The combined acetone fractions were filtered through a cotton wool plug and concentrated under vacuum to give a crude crystalline product. The product was dried under vacuum and recrystallized from hexane to yield pure **55** (18.00g; 51%).

Compound **55**:



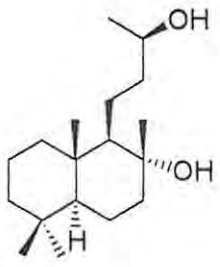
Light pink crystals. Mpt = 81-82°C. $[\alpha]_D^{27} = +5.0°$ ($CHCl_3$; *c* 1.08). IR ν_{max} cm^{-1}(NaCl): 3422(br), 2925, 2859, 1712, 1447, 1387, 1155, 1117, 1074. EIMS (70 eV) *m/z* (rel.int.) : 280 $[M]^+$ (2.5), 262(H_2O-79), 244(41), 229(95), 217(12), 201(22), 191(57), 177(48), 159(30), 149(70), 135(60), 121(65), 109(100), 95(91), 81(82), 67(52), 55(20), 41(26). HRFABMS *m/z* : 280.240463. $C_{18}H_{32}O_2$ requires 280.240230. ^1H NMR (400 MHz, $CDCl_3$) : δ 0.77 (3H, s, Me-18), 0.79 (3H, s, Me-16), 0.85 (3H, s, Me-17), 0.88 (1H, m, H-5), 0.90 (1H, d, $J_{1,2}$ = 2.4Hz, H-1), 1.06 (1H, s, H-9), 1.14 (3H, s, Me-14), 1.21 (1H, d, *J* = 17.4, H-3), 1.25 (1H, m, H-6), 1.38 (1H, dd, *J* = 4.2Hz, 16.6Hz, H-3), 1.50 (1H, m, H-2), 1.56 (1H, t, *J* = 2.2Hz, H-11),1.59 (1H, d, *J* = 2.5Hz, H-11'), 1.61 (1H, m, H-1'),

1.68 (1H, m, H-6'), 1.72 (1H, m, H-2'), 1.84 (2H, dt, J = 3.1Hz, 12.2Hz, H-7), 2.11 (3H, s, Me-15), 2.53-2.68 (2H, m, H-12). ^{13}C NMR (100 MHz, $CDCl_3$) : δ 15.2 (C-18), 18.4 (C-6), 18.8 (C-2), 20.5 (C-11), 21.5 (C-17), 24.1 (C-14), 30.0 (C-15), 33.2 (C-4), 33.4 (C-16), 39.3 (C-10), 40.1 (C-1), 41.9 (C-3), 44.5 (C-7), 46.3(C-12), 56.1 (C-5), 60.7 (C-9), 73.7 (C-8), 210.4 (C-13).

4.2.2 Preparation of Diols 57 and 58.

A solution of **55** (2.000g) in anhydrous THF (10mL) was cooled to 0°C and stirred. A suspension of $LiAlH_4$ (0.542g) in anhydrous THF was added gradually to the stirred solution. The mixture was allowed to stir for a further 2 hours. The reaction was quenched with the addition of cooled saturated NH_4Cl solution (4mL) and the resulting precipitate dissolved with the addition of 1M HCl (10mL). The mixture was then extracted with EtOAc (3x30mL). Organic and aqueous fractions were separated and the aqueous fraction washed with EtOAc (30mL). The EtOAc fractions were combined and washed with saturated $NaHCO_3$ (50mL), H_2O (50mL) and saturated brine (50mL), dried over $MgSO_4$ and reduced under vacuum. Purification was achieved *via* flash column chromatography(hexane:EtOAc/70:30) to give the diol **57** (1.101g) as a colourless oil and the diol **58** (0.822g) as white needles in a 96% combined isolated yield.

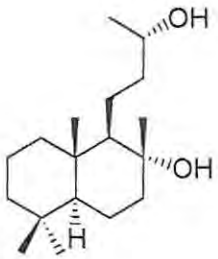
Compound **57**:



Colourless oil. $[\alpha]_D^{25}$ = -30° ($CHCl_3$; *c* 0.23). IR ν_{max} cm^{-1}(NaCl) : 3347(br), 2930, 2871, 1460, 1382, 1131, 1080, 1025, 987, 936. EIMS (70 eV) *m/z* (rel.int.) : 279(1.5), 264 (2.5), 249 (25), 231 (30), 221 (18), 203 (10), 191 (22), 177 (100), 161 (19), 149 (69), 135 (29), 123 (45), 109 (59), 95 (98), 81 (48), 67 (55), 55 (19). HRFABMS : 282.2551. $C_{18}H_{34}O_2$ requires 282.2559. ^{1}H NMR (400

MHz, $CDCl_3$) : δ 0.78 (3H, s, Me-17), 0.80 (3H, s, Me-18), 0.86 (3H, s, Me-16), 0.86 (1H, m, H-1), 0.92 (1H, dd, J = 2.3Hz, 12.2Hz, H-5), 1.10 (1H, m, H-3), 1.15 (3H, d, J = 6.2Hz, Me-14), 1.61 (3H, s, Me-15), 1.21 (1H, m, H-9), 1.24-1.35 (1H, m, H-6), 1.35 (1H, m, H-11), 1.37-1.41 (1H, m, H-2), 1.40 (1H, m, H-3'), 1.44-1.49 (2H, m, H-12), 1.45 (1H, m, H-7), 1.51 (1H, m, H-11'), 1.53-1.59 (1H, m, H-2'), 1.60-1.63 (1H, m, H-1'), 1.66 (1H, m, H-6'), 1.83 (1H, dt, J = 3.2Hz, 12.2Hz, H-7'), 3.90 (1H, m, H-13). ^{13}C NMR (100 MHz, $CDCl_3$) : δ 15.2 (C-18), 18.4 (C-2), 20.0 (C-11), 21.5 (C-17), 23.2 (C-14), 24.6 (C-15), 33.2 (C-4), 33.4 (C-16), 39.2 (C-10), 39.9 (C-1), 40.1 (C-12), 42.0 (C-3), 44.3 (C-7), 56.2 (C-5), 58.4 (C-9), 65.4 (C-13), 74.4 (C-8).

Compound **58**:

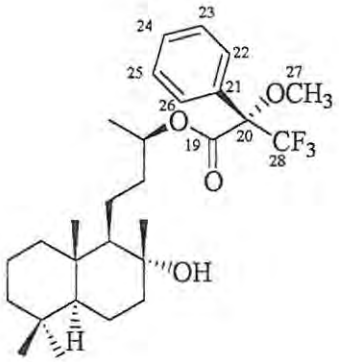


White needles. Mpt = 97°C-99°C. $[\alpha]_D^{25}$ = +8° ($CHCl_3$; *c* 0.27). IR ν_{max} cm^{-1}(NaCl): 3346, 2931, 2859,1460, 1381, 1129, 1082, 936, 756 . EIMS (70 eV) *m/z* (rel.int.): 279(2.5), 264 (8), 249 (29), 231 (30), 221 (12), 206 (8), 191 (20), 177 (100), 161 (13), 149 (28), 135 (20), 123 (41), 109 (41), 95 (48), 81 (39), 67 (40), 56 (18). HRFABMS : 282.2551. $C_{18}H_{34}O_2$ requires 282.2558. ^1H NMR (400 MHz, $CDCl_3$): δ 0.77 (3H, s, Me-17), 0.79 (3H, s, Me-18), 0.84 (3H, s, Me-16), 0.93 (1H, dd, J = 2.0Hz, 12.1Hz, H-5), 1.00 (1H, m, H-1), 1.13 (3H, s, Me-14), 1.15 (3H, s, Me-15), 1.22 (1H, s, H-9), 1.24-1.29 (1H, m, H-11), 1.28 (1H, m, H-12), 1.33 (1H, m, H-6), 1.49-1.55 (1H, m, H-6'), 1.39 (1H, m, H-3), 1.44 (1H, m, H-7), 1.52-1.59 (1H, m, H-2), 1.60 (1H, m, H-1'), 1.61 (1H, m, H-11'), 1.63 (1H, m, H-12'), 1.64 (1H, m, H-2'), 1.81 (1H, dt, J = 3.1Hz, 12.3Hz, H-7'), 1.91 (1H, m, H-3'), 2.64 (1H, br s, OH), 3.76 (1H, m, H-13). ^{13}C NMR (100 MHz, $CDCl_3$): δ 15.2 (C-18), 18.4 (C-2), 20.5 (C-11), 21.5 (C-17), 22.1 (C-6), 23.8 (C-15), 24.3 (C-14), 33.2 (C-4), 33.4 (C-16), 39.2 (C-10), 39.7 (C-1), 42.0 (C-3), 42.3 (C-12), 44.2 (C-7), 56.0 (C-5), 61.5 (C-9), 69.8 (C-13), 74.6 (C-8).

4.2.3 Preparation of the (S)-MPTA Esters of Diols 57 and 58.

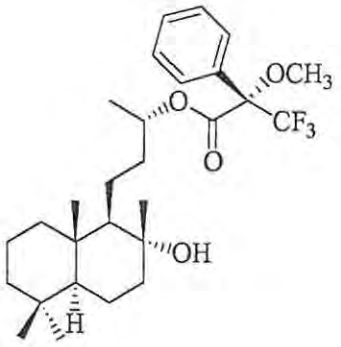
This procedure is representative of both diol esterifications. A solution of **57** (20mg) and 4-DMAP (30mg) in anhydrous CH_2Cl_2 (2mL) was added to a solution of (S)-MPTA (100mg) and dicyclohexylcarbodiimide (147mg) in anhydrous CH_2Cl_2 (2ml) under N_2. The mixture was stirred overnight at RT (20°C), diluted with EtOAc (15mL) and H_2O (2mL) and filtered. The filtered EtOAc solution was then washed with 0.2M HCl (10mL), H_2O (10mL), saturated $NaHCO_3$ (10mL) and saturated brine (10mL), dried over $MgSO_4$, filtered and reduced under vacuum. Purification was achieved *via* gradient elution flash column chromatography (hexane:EtOAc/80:20) to yield **59** (21mg).

Compound **59**:



Colourless oil. ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.63 (3H, s, Me-18), 0.72 (1H, m, H-1), 0.74 (3H, s, Me-17), 0.79 (1H, m, H-12), 0.83 (3H, s, Me-16), 0.83 (1H, m, H-5), 0.90 (1H, t, J= 4.0Hz, H-9), 1.04 (1H, m, H-3), 1.10 (3H, s, Me-15), 1.51 (1H, m, H-11), 1.21 (1H, m, H-6), 1.30 (1H, m, H-12'), 1.33 (3H, d, J= 6.3Hz, Me-14), 1.34 (1H, m, H-3'), 1.40 (1H, m, H-7), 1.46 (1H, m, H-2), 1.61 (1H, m, H-11'), 1.62 (1H, m, H-6'), 1.67 (1H, m, H-1'), 1.81 (1H, dt, J= 3.1Hz, 12.2Hz, H-7'), 3.57 (3H, s, Me-27), 5.13 (1H, m, H-13), 7.38 (2H, m, H-23, H-25), 7.39 (1H, m, H-24), 7.54 (2H, m, H-22, H-26). ^{13}C NMR (100 MHz, $CDCl_3$): δ 15.3 (C-18), 18.4 (C-2), 20.1 (C-14), 20.5 (C-6), 21.1 (C-11), 21.5 (C-17), 24.0 (C-15), 33.2 (C-4), 33.4 (C-16), 39.0 (C-10), 39.2 (C-1), 39.4 (C-12), 41.9 (C-3), 44.6 (C-7), 55.4 (C-27), 56.0 (C-5), 61.5 (C-9), 74.1 (C-8), 74.8 (C-13), 122.1 (C-20), 125.1 (C-28), 127.2 (C-22, C-26), 128.3 (C-24), 129.4 (C-23, C-25), 132.7 (C-21), 166.2 (C-19).

Compound **60**:

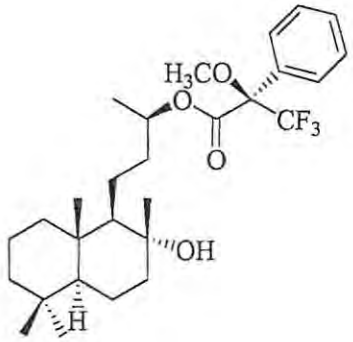


Colourless oil. ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.75 (3H, s, Me-18), 0.77 (3H, s, Me-17), 0.85 (3H, s, Me-16), 0.87 (1H, m, H-12), 0.89 (1H, m, H-5), 1.02 (1H, t, *J* = 4.0Hz, H-9), 1.09 (3H, s, Me-15), 1.11 (1H, m, H-3), 1.20 (1H, d, *J* = 3.2Hz, H-6), 1.22 (1H, m, H-11), 1.27 (3H, d, J = 6.25Hz, Me-14), 1.31 (1H, m, H-3'), 1.38 (1H, m, H-7), 1.39 (1H, m, H-2), 1.57 (1H, m, H-6'), 1.63 (1H, m, H-12'), 1.67 (1H, m, H-1), 1.81 (1H, dt, *J* = 3.3Hz, 12.1Hz, H-7'), 1.82 (1H, m, H-1'), 3.54 (3H, s, Me-27), 5.12 (1H, m, H-13), 7.38 (2H, m, H-23, H-25), 7.40 (1H, m, H-24), 7.53 (2H, m, H-22, H-26). ^{13}C NMR (100 MHz, $CDCl_3$): δ 15.3 (C-18), 18.4 (C-2), 19.4 (C-14), 20.5 (C-6), 21.0 (C-11), 21.5 (C-17), 24.0 (C-15), 33.2 (C-4), 33.4 (C-16), 39.0 (C-1, C-10), 39.8 (C-12), 41.9 (C-3), 44.5 (C-7), 55.3 (C-27), 56.1 (C-5), 61.5 (C-9), 74.1 (C-8), 74.8 (C-13), 121.9 (C-20), 124.8 (C-28), 127.5 (C-22, C-26), 128.4 (C-24), 129.5 (C-23, C-25), 132.4 (C-21), 166.2 (C-19).

4.2.4 Preparation of the (R)-MPTA Esters of Diols 57 and 58.

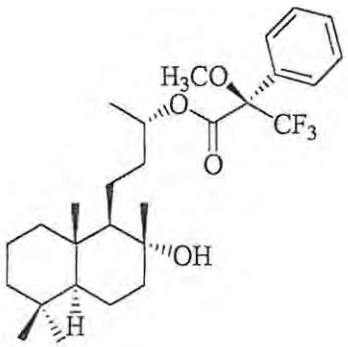
This procedure is representative of both diol esterifications. A solution of **57** (20mg) and 4-DMAP (30mg) in anhydrous CH_2Cl_2 (2ml) was added to a solution of (R)-MPTA (100mg) and DCC (147mg) in anhydrous CH_2Cl_2 (2mL) under N_2. The mixture was stirred overnight at RT (20°C). The mixture was diluted with EtOAc (15mL) and H_2O (2mL) and filtered. The filtered EtOAc solution was washed with 0.2M HCl (10mL), H_2O (10mL), saturated $NaHCO_3$ (10mL) and saturated brine (10mL), dried over $MgSO_4$, filtered and reduced under vacuum. Purification was achieved *via* flash column chromatography (hexane:EtOAc/80:20) to yield **61** (22mg).

Compound **61**:



Colourless oil. δ 0.73 (3H, s, Me-18), 0.76 (3H, s, Me-17), 0.83 (3H, s, Me-16), 0.84 (1H, m, H-11), 0.86 (1H, m, H-12), 0.88 (1H, m, H-5), 1.00 (1H, t, *J* = 4.0Hz, H-9), 1.12 (3H, s, Me-15), 1.18 (1H, m, H-3), 1.25 (3H, d, J = 6.2Hz, Me-14), 1.33 (1H, m, H-7), 1.36 (1H, m, H-11'), 1.39 (1H, m, H-3'), 1.52 (1H, m, H-12'), 1.52 (1H, m, H-2), 1.61 (1H, m, H-2'), 1.65 (1H, m, H-6), 1.73 (1H, m, H-1),1.84 (1H, dt, *J* = 3.2Hz,12.2Hz, H-7'), 5.12 (1H, m, H-13), 7.38 (2H, m, H-23, H-25), 7.40 (1H, m, H-24), 7.53 (2H, m, H-22, H-26). ^{13}C NMR (100 MHz, $CDCl_3$): δ 15.3 (C-18), 18.4 (C-2), 19.7 (C-14), 20.5 (C-6), 21.3 (C-11), 21.5 (C-17), 24.0 (C-15), 33.2 (C-4), 33.4 (C-16), 39.0 (C-10), 39.1 (C-1), 39.6 (C-12), 41.9 (C-3), 44.7 (C-7), 55.3 (C-27), 56.1 (C-5), 61.5 (C-9), 74.1 (C-8), 74.9 (C-13), 122.1 (C-20), 125.0 (C-28), 127.5 (C-22, C-26), 128.4 (C-24), 129.5 (C-23, C-25), 132.4 (C-21), 166.3 (C-19).

Compound **62**:



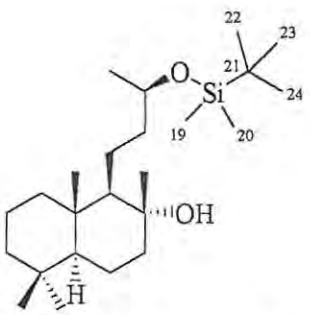
Colourless oil. ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.71 (3H, s, Me-18), 0.76 (3H, s, Me-17), 0.84 (3H, s, Me-16), 0.88 (1H, m, H-9, H-12), 0.89 (1H, m, H-5), 0.99 (3H, s, Me-15), 1.18 (1H, m, H-11), 1.22 (1H, m, H-3), 1.25 (1H, m, H-6), 1.29 (1H, m, H-7), 1.33 (3H, s, Me-14), 1.37 (1H, m, H-2),

1.53 (1H, m, H-6'), 1.54 (1H, m, H-3', H-12'), 1.56 (1H, m, H-1), 1.61 (1H, m, H-11'), 1.78 (1H, m, H-2'), 1.79 (1H, dt, J = 3.0Hz, 12.7Hz, H-7'), 1.81 (1H, m, H-1'), 3.56 (3H, s, Me-27), 5.10 (1H, m, H-13), 7.37 (1H, m, H-23, H-25), 7.38 (1H, m, H-24), 7.54 (1H, m, H-22, H-26). ^{13}C NMR (100 MHz, $CDCl_3$): δ 15.3 (C-18), 18.4 (C-2), 19.8 (C-14), 20.4 (C-6), 20.9 (C-11), 21.5 (C-17), 24.0 (C-15), 33.2 (C-4), 33.4 (C-16), 38.9 (C-10), 39.0 (C-1), 39.7 (C-12), 41.9 (C-3), 44.2 (C-7), 55.4 (C-27), 56.0 (C-5), 61.5 (C-9), 74.1 (C-8), 74.7 (C-13), 122.1 (C-20), 125.1 (C-28), 127.2 (C-23, C-25), 128.3 (C-24), 129.5 (C-22, C-26), 132.6 (C-21), 166.1 (C-19).

4.2.5 Preparation of the TBDMS Protected Ethers 63 and 64.

The procedure for the t-butyldimethylsilyl protection presented here is representative. A solution of **57** (0.800g) was dissolved in anhydrous CH_2Cl_2 (10mL) and cooled (-78°C) under N_2. 2,6-Lutidine (661μL) was added to the solution, followed by the dropwise addition of TBDMS triflate (776μL). After stirring for 2 hours, TLC of the solution indicated that the reaction was complete. The reaction was accordingly quenched with 50% aqueous acetic acid (30mL) and stirred for a further 30 minutes. The mixture was extracted with EtOAc (3x30mL) and the organic and aqueous fractions separated. The aqueous fraction was washed with EtOAc (3x15ml). All the EtOAc fractions were combined and washed with H_2O (30mL), saturated $NaHCO_3$ (2x30mL), H_2O (30mL) and saturated brine (30mL), dried over $MgSO_4$ and reduced under vacuum to yield **63** (1.087g; 97%) as an oil. No further purification was required.

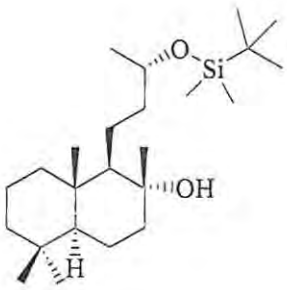
Compound **63**:



Colourless oil. $[\alpha]_D^{25}$ = -14° ($CHCl_3$; *c* 0.31). IR ν_{max} cm^{-1}(NaCl): 3427, 2931, 2859, 1464, 1381, 1251, 1132, 1089, 1042, 1001, 834, 774. EIMS (70 eV) *m/z* (rel.int.): 396 (2), 355 (2), 339 (12),

321 (22), 299 (3), 279 (3), 264 (4), 247 (37), 231 (20), 221 (5), 204 (14), 191 (50), 177 (44), 163 (44), 149 (100), 135 (32), 121 (40), 107 (44), 95 (65), 81 (55), 67 (50), 55 (18). HRFABMS: [M+1-H_2O] 379.3395. $C_{24}H_{43}O_1Si$ requires 379.3396. ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.04 (6H, s, Me_2-Si), 0.77 (6H, s, Me-17, Me-18), 0.79 (1H, m, H-11), 0.84 (3H, s, Me-16), 0.87 (9H, s, *t*-butyl-Si), 0.88 (1H, t, *J* = 4.2Hz, H-5), 0.91 (1H, m, H-1), 1.05 (1H, t, *J* = 3.9Hz, H-9), 1.11 (3H, s, Me-15), 1.11 (3H, d, *J* = 6.1Hz, Me-14), 1.14 (1H, m, H-12), 1.21-1.25 (1H, d, *J* = 1.8, H-6), 1.34 (1H, m, H-11'), 1.36 (1H, m, H-7), 1.38 (1H, m, H-12'), 1.41 (1H, m, H-2), 1.51 (1H, m, H-3), 1.57 (1H, m, H-2'), 1.66 (1H, m, H-1'), 1.69 (1H, m, H-6'), 1.84 (1H, dt, *J* = 2.9Hz, 12.2Hz, H-7'), 3.79 (1H, m, H-13). ^{13}C NMR (100 MHz, $CDCl_3$): δ -4.3 (C-19), -4.6 (C-20), 15.3 (C-18), 18.1 (C-21), 18.4 (C-2), 20.6 (C-6), 21.2 (C-11), 21.5 (C-17), 23.6 (C-14), 23.9 (C-15), 25.9 (C-22, C-23, C-24), 33.2 (C-4), 33.4 (C-16), 39.3 (C-10), 39.8 (C-1), 42.1 (C-12), 43.0 (C-3), 44.5 (C-7), 56.2 (C-5), 61.9 (C-9), 69.4 (C-13), 74.2 (C-8).

Compound **64**:



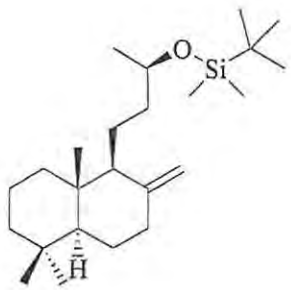
Colourless oil. $[\alpha]_D^{25}$ = -9.0° ($CHCl_3$; *c* 0.26). IR ν_{max} cm^{-1}(NaCl): 3435, 2931, 2858, 1630, 1455, 1382, 1253, 1131, 1081, 1036, 998, 834, 769. EIMS (70 eV) *m/z* (rel.int.): 380 (2), 363 (3), 339 (17), 321 (16), 303 (2), 279 (5), 264 (7), 247 (48), 231 (22), 221 (6), 205 (11), 191 (74), 177 (50), 163 (43), 149 (100), 135 (35), 121 (42), 109 (43), 96 (97), 81 (50), 55 (14). HRFABMS: [M+1-H_2O]$^+$ 379.3397. $C_{24}H_{43}O_1Si$ requires 379.3396. ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.04 (6H, s, Me_2-Si), 0.77 (6H, s, Me-17, Me-18), 0.85 (3H, s, Me-16), 0.87 (9H, s, *t*-butyl-Si), 0.91 (1H, m, H-5), 0.95 (1H, m, H-1), 1.02 (1H, t, *J* = 3.6Hz, H-9), 1.11 (3H, s, Me-15), 1.13 (3H, d, *J* = 6.1Hz, Me-14), 1.14 (1H, m, H-12), 1.22-1.26 (1H, m, H-6), 1.38 (1H, m, H-7), 1.35 (1H, m, H-12'), 1.40 (1H, m, H-2), 1.47-1.53 (1H, m, H-11), 1.55 (1H, m, H-2'), 1.58 (1H, m, H-3), 1.64 (1H, m, H-1'), 1.65 (1H, m, H-6'), 1.84 (1H, dt, *J* = 3.2Hz, 12.2Hz, H-7'), 3.75 (1H, m, H-13). ^{13}C NMR (100 MHz, $CDCl_3$):

δ -4.4 (C-19), -4.6 (C-20), 15.4 (C-18), 18.0 (C-21), 18.4 (C-2), 20.5 (C-11), 21.2 (C-6), 21.5 (C-17), 23.6 (C-15), 24.0 (C-14), 25.9 (C-22, C-23, C-24), 33.2 (C-4), 33.4 (C-16), 39.1 (C-10), 39.8 (C-1), 42.0 (C-12), 43.4 (C-3), 44.1 (C-7), 56.1 (C-5), 62.1 (C-9), 69.2 (C-13), 74.4 (C-8).

4.2.6 Preparation of the Unsaturated Ethers 67 and 68.

The dehydration described here is representative. Compound **63** (1.268g) was dissolved in dry, redistilled pyridine (8mL). 4-DMAP (0.392g) was added to the stirred solution at RT (25°C) and $POCl_3$ (585μL) was added dropwise to the cooled solution (0°C). After stirring for 3 hours, TLC of the reaction mixture indicated a single spot with reduced polarity, *c.f.* **63**. The reaction was accordingly quenched with ice. Glacial acetic acid (6.4mL) was added to remove excess pyridine and the mixture stirred for 30 minutes after which it was extracted with EtOAc (3x50mL). The EtOAc fractions were combined, washed with H_2O (30mL), saturated $NaHCO_3$(50mL), H_2O (50mL) and saturated brine (50mL). The combined EtOAc fraction was finally dried over $MgSO_4$ and reduced under vacuum. The product was passed through a plug of silica to remove any remaining pyridine salts. The reaction yielded **67** (0.925g, 73%) as an oil. Final purification was achieved *via* normal phase HPLC (hexane:EtOAc/95:5).

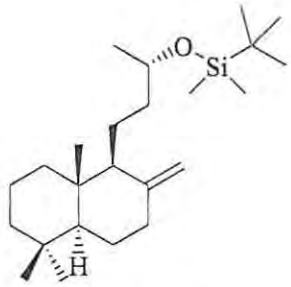
Compound **67**:



Colourless oil. $[\alpha]_D^{25} = +20°$ ($CHCl_3$; *c* 0.23). IR ν_{max} cm^{-1}(NaCl): 2928, 2856,1642, 1460, 1386, 1253, 1134, 1095,1048, 1003, 888, 835, 772. EIMS (70 eV) *m/z* (rel.int.): 379 $[M+1]^+$ (2), 321 (90), 303 (5), 279 (12), 265 (3), 245 (62), 231 (6), 204 (22), 189 (20), 171 (30), 157 (35), 135 (12.5), 121 (22), 110 (23), 95 (32), 75 (100), 55 (5). HRFABMS: $[M]^+$ 378.3317. $C_{24}H_{46}SiO$ requires 378.3318. ^1H NMR (400 MHz, $CDCl_3$): δ 0.05 (6H, s, Me_2-Si), 0.65 (3H, s, Me-18), 0.80 (3H, s, Me-17), 0.86 (3H, s, Me-16), 0.89 (9H, s, *t*-butyl-Si), 1.01 (1H, m, H-6), 1.02 (1H, m, H-3), 1.09 (1H, m, H-5),

1.11 (3H, d, J = 6.0Hz, Me-14), 1.17 (1H, m, H-12), 1.18 (1H, m, H-6'), 1.31 (1H, dd, J = 4.2Hz, 8.6Hz, H-11), 1.36 (1H, m, H-12'), 1.45 (1H, m, H-2), 1.58 (1H, m, H-9), 1.73 (1H, m, H-11'), 1.76 (1H, m, H-3'), 1.84 (1H, m, H-1), 1.97 (1H, m, H-7), 2.37 (1H, dt, J = 3.2Hz, 12.7Hz, H-7'), 3.75 (1H, m, H-13), 4.49 (1H, s, H-15), 4.80 (1H, s, H-15'). ^{13}C NMR (100 MHz, $CDCl_3$): δ -4.4 (C-19), -4.6 (C-20), 14.5 (C-18), 18.2 (C-21), 19.5 (C-2), 19.8 (C-6), 21.8 (C-17), 23.7 (C-14), 24.5 (C-11), 25.9 (C-22, C-23, C-24), 33.2 (C-4), 33.7 (C-16), 38.4 (C-1), 38.8 (C-7), 39.2 (C-3), 39.3 (C-10), 42.2 (C-12), 55.6 (C-5), 57.1 (C-9), 69.2 (C-13), 106.4 (C-15), 148.8 (C-8).

Compound **68**:



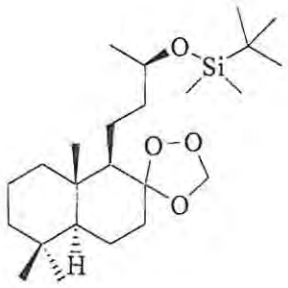
Colourless oil. $[\alpha]_D^{25}$ = -5° ($CHCl_3$; *c* 0.33). IR ν_{max} cm^{-1}(NaCl): 2928, 2856, 1643, 1461, 1442, 1372, 1255, 1137, 1093, 1043, 1003, 888, 836, 774. EIMS (70 eV) *m/z* (rel.int.): 379 $[M+1]^+$ (3), 321 (81), 303 (9), 279 (8), 245 (61), 231 (15), 204 (42), 189 (26), 171 (30), 157 (36), 137 (17), 121 (22), 107 (19), 95 (30), 76 (100), 56 (5). HRFABMS: $[M+1]^+$ 379.3396. $C_{24}H_{47}SiO$ requires 379.3396. ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.04 (6H, s, Me_2-Si), 0.67 (3H, s, Me-18), 0.80 (3H, s, Me-17), 0.86 (3H, s, Me-16), 0.89 (9H, s, *t*-butyl-Si), 0.93 (1H, m, H-6), 0.97 (1H, m, H-3), 1.06 (1H, m, H-5), 1.10 (3H, d, J = 6.2Hz, Me-14), 1.18 (1H, m, H-12), 1.31 (1H, dd, J = 4.3Hz, 12.9Hz, H-11), 1.38 (1H, m, H-12'), 1.42 (1H, m, H-2), 1.49 (1H, m, H-9), 1.60 (1H, m, H-2'), 1.73 (1H, m, H-11'), 1.74 (1H, m, H-1), 1.84 (1H, m, H-3'), 1.95 (1H, m, H-7), 2.37 (1H, dt, J = 3.2Hz, 12.7Hz, H-7'), 3.74 (1H, m, H-13), 4.54 (1H, s, H-15), 4.80 (1H, s, H-15'). ^{13}C NMR (100 MHz, $CDCl_3$): δ -4.4 (C-19), -4.6 (C-20), 14.4 (C-18), 18.1 (C-21), 19.5 (C-2), 19.8 (C-6), 21.8 (C-17), 23.9 (C-14), 24.5 (C-11), 25.9 (C-22, C-23, C-24), 33.6 (C-4), 33.7 (C-16), 38.4 (C-1), 38.8 (C-7), 39.2 (C-3), 39.7 (C-10), 42.3 (C-12), 55.6 (C-5), 57.1 (C-9), 69.2 (C-13), 106.6 (C-15), 148.6 (C-8).

4.2.7 Ozonolysis of the Unsaturated Ethers 67 and 68.

4.2.7.1 Dimethyl Sulphide (DMS) Workup of the Ozonolysis Reaction.

Compound **67** (250mg) was dissolved in dry CH_2Cl_2 (5mL) and cooled (-78°C). O_3 was bubbled through the solution for 30 minutes. N_2 was bubbled through the solution for 30 minutes to remove excess O_3. DMS (1.0mL) was added and allowed to stir for 2 hours. The solution was extracted with CH_2Cl_2 (3x30mL). The combined CH_2Cl_2 fractions were washed with saturated $NaHCO_3$ (30mL), H_2O (30mL) and saturated brine (30mL), dried over $MgSO_4$ and reduced under vacuum. Final purification was achieved *via* normal phase HPLC (hexane:EtOAc/90:10) to yield **69** (mg, 83%) as a colourless oil.

Compound **69**:

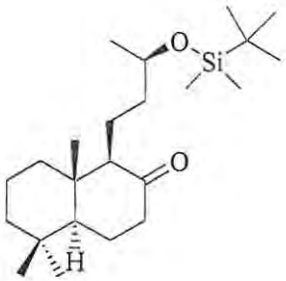


Colourless oil. ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.03 (6H, s, Me_2-Si), 0.80 (6H, s, Me-16, Me-17), 0.86 (9H, s, *t*-butyl-Si), 0.87 (3H, s, Me-15), 0.95 (1H, m, H-1), 1.09 (3H, d, J = 6.1Hz, Me-14), 1.10 (1H, m, H-12), 1.20-1.26 (1H, m, H-3), 1.39-1.42 (2H, m, H-2, H-2'), 1.72 (1H, m, H-1'), 0.92 (1H, m, H-5), 1.61 (1H, m, H-11), 1.54 (1H, m, H-6), 1.68 (1H, m, H-12'), 1.57 (1H, m, H-9), 1.83 (1H, m, H-7), 2.07 (1H, m, H-7'), 3.69 (1H, m, H-13), 5.03 (2H, d, J = 149.2, H-18, H-18'). ^{13}C NMR (100 MHz, $CDCl_3$): δ -4.5 (C-19, C-20), 14.4 (C-17), 18.1 (C-21), 18.6 (C-2), 19.7 (C-11), 21.6 (C-16), 19.7 (C-6), 23.7 (C-14), 25.9 (C-21, C-22, C-23), 33.5 (C-15), 33.2 (C-4), 42.7 (C-12), 38.9 (C-1), 42.0 (C-3), 36.7 (C-7), 39.5 (C-10), 54.9 (C-5), 57.0 (C-9), 69.1 (C-13), 93.4 (C-18), 111.5 (C-8).

4.2.7.2 Triphenylphosphine (TPP) Workup of the Ozonolysis Reaction.

The ozonolysis procedure is representative for our ozonolysis of both protected alkenes **67** and **68**. Compound **67** (1.000g) was dissolved in dry CH_2Cl_2 (5mL) and cooled (-78°C). O_3 was bubbled through the solution for 30 minutes. N_2 was bubbled through the solution for 30 minutes to remove excess O_3. TPP (2.771g) was added and the solution allowed to stir for 2 hours. Excess TPP was removed as triphenylphosphine oxide by the addition of H_2O_2 (30%, 5mL). After the triphenylphosphine oxide was removed by filtration, the solution was extracted with CH_2Cl_2 (3x30mL). The combined CH_2Cl_2 fractions were washed with saturated $NaHCO_3$ (30mL), H_2O (30mL) and saturated brine (30mL), dried over $MgSO_4$ and reduced under vacuum. An EtOAc solution of the product was passed through a plug of silica to remove any remaining TPP. Final purification was achieved *via* normal phase HPLC (hexane:EtOAc/95:5) to yield **70** (0.975g, 97%) as a colourless oil.

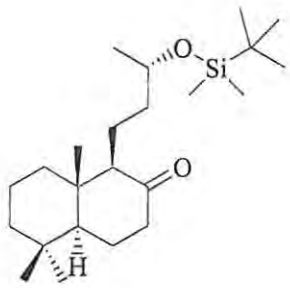
Compound **70**:



Colourless oil. $[\alpha]_D^{25} = -37°$ ($CHCl_3$; *c* 0.34). IR ν_{max} cm^{-1}(NaCl): 2947, 2924, 2847, 1710, 1455, 1368, 1250, 1143, 1093, 1047, 998, 830, 770. EIMS (70 eV) *m/z* (rel.int.): 381 $[M+1]^+$ (1), 365 (2), 323 (65), 281 (22), 305 (3), 281 (22), 267 (3), 249 (5), 231 (100), 215 (4), 199 (28), 189 (26), 175 (35), 161 (30), 149 (39), 119 (21), 107 (18), 95 (36), 76 (38), 67 (9), 55 (4). HRFABMS: 380.3119. $C_{23}H_{44}SiO_2$ requires 380.3111. ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.04 (6H, s, Me_2-Si), 0.70 (3H, s, Me-17), 0.84 (3H, s, Me-16), 0.86 (9H, s, *t*-butyl-Si), 0.95 (3H, s, Me-15), 1.01 (1H, m, H-1), 1.11 (3H, d, *J*=6.1Hz, Me-14), 1.12 (1H, m, H-12), 1.22-1.25 (1H, m, H-3), 1.36 (1H, m, H-2), 1.40 (1H, m, H-1'), 1.41 (1H, m, H-5), 1.48 (1H, m, H-3'), 1.57 (1H, m, H-11), 1.65 (1H, m, H-6), 1.74 (1H, m, H-12'), 1.98 (1H, d, $J_{9,11}$ = 9.9Hz, H-9), 2.03 (1H, m, H-6'), 2.26 (1H, m, H-7), 2.39 (1H, ddd, *J* = 2.1Hz, 7.0Hz, 13.1Hz, H-7'), 3.74 (1H, m, H-13). ^{13}C NMR (100 MHz, $CDCl_3$): δ -4.3 (C-18, C-

19), 14.7 (C-17), 18.1 (C-20), 18.7 (C-2), 19.1 (C-11), 21.7 (C-16), 24.1 (C-6), 24.2 (C-14), 25.9 (C-21, C-22, C-23), 33.5 (C-15), 33.7 (C-4), 39.3 (C-12), 39.5 (C-1), 42.0 (C-3), 42.7 (C-7), 42.8 (C-10), 54.3 (C-5), 64.5 (C-9), 69.2 (C-13), 212.4 (C-8).

Compound **71**:



Colourless oil. $[\alpha]_D^{25} = -32°$ ($CHCl_3$; *c* 0.33). IR ν_{max} cm^{-1}(NaCl): 2952, 2929, 2856, 1714, 1461, 1386, 1367, 1254, 1184, 1140, 1097, 1055, 1006, 834, 773. EIMS (70 eV) *m/z* (rel.int.): 381 $[M+1]^+$ (1), 365 (1), 323 (29), 305 (2), 281 (15), 249 (3), 231 (100), 215 (3), 199 (13), 189 (18), 175 (30), 161 (25), 149 (50), 133 (12), 119 (17), 107 (17), 95 (25), 76 (32), 55 (4). HRFABMS: $[M+1]^+$ 381.3188. $C_{23}H_{45}SiO_2$ requires 381.3189. ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.04 (6H, s, Me_2-Si), 0.69 (3H, s, Me-17), 0.82 (3H, s, Me-16), 0.86 (9H, s, *t*-butyl-Si), 0.94 (3H, s, Me-15), 1.09 (3H, d, *J* = 6.0Hz, Me-14), 1.15 (1H, m, H-12), 1.21 (1H, m, H-3), 1.47 (1H, m, H-2), 1.55 (1H, m, H-1'), 1.49 (1H, m, H-5), 1.41 (1H, m, H-3'), 1.07 (1H, m, H-11), 1.69 (1H, m, H-11'), 1.62 (1H, dd, *J* = 4.9Hz, 13.1Hz, H-6), 1.75 (1H, m, H-12'), 2.04 (1H, m, H-9), 2.03 (1H, m, H-6'), 2.28 (1H, m, H-7), 2.38 (1H, ddd, *J* = 2.0Hz, 6.9Hz, 13.1Hz, H-7'), 3.77 (1H, m, H-13). ^{13}C NMR (100 MHz, $CDCl_3$): δ -4.6 (C-18, C-19), 14.6 (C-17), 18.1 (C-20), 17.0 (C-2), 19.0 (C-11), 21.7 (C-16), 24.1 (C-6), 24.2 (C-14), 25.9 (C-21, C-22, C-23), 33.5 (C-15), 33.7 (C-4), 39.1 (C-12), 39.3 (C-1), 42.0 (C-3), 42.7 (C-7), 42.8 (C-10), 54.4 (C-5), 64.5 (C-9), 68.7 (C-13), 212.0 (C-8).

4.2.8 Ruthenium Trichloride Oxidation of 67.

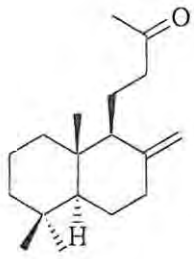
To a solution of **67** (25mg) dissolved in CCl_4 (0.50mL), H_5IO_6 (18mg) and a catalytic amount of $RuCl_3.3H_2O$, dissolved in acetonitrile (0.50mL) and H_2O (0.75mL), was added and allowed to stir for 4 hours. TLC indicated the formation of a less polar product. The mixture was extracted with

CH_2Cl_2 (3x5mL), the CH_2Cl_2 fractions combined and washed with H_2O (10mL) and saturated brine (10mL), dried over $MgSO_4$, filtered and reduced under vacuum. An EtOAc solution of the crude product was passed through a silica gel plug to remove any remaining ruthenium residues. The ruthenium trichloride oxidation product (21mg) was an inseparable mixture of compounds.

4.2.9 Preparation of the Unsaturated Ketone (72).

4-DMAP (230mg) was added to the stirred solution of **55** (500mg) in dry, redistilled pyridine (4mL) at RT (25°C). $POCl_3$ (342µL) was added dropwise to the cooled pyridine solution (0°C) and the solution stirred for 3 hours. TLC indicated a single spot less polar than **55**. The reaction was quenched with the addition of ice. Glacial acetic acid (3mL) was added to the mixture and stirred for 30 minutes to remove excess pyridine. The mixture was extracted with EtOAc (50mL), and the EtOAc fraction washed with H_2O (30mL), saturated $NaHCO_3$ (2x50mL), H_2O (50mL) and saturated brine (50mL), dried over $MgSO_4$ and reduced under vacuum. Excess pyridine was removed under high vacuum. Further purification was achieved by gradient elution column chromatography, with elution of **72** occurring in hexane:EtOAc (80:20). The reaction yielded **72** (344mg) as a yellow oil.

Compound **72**:



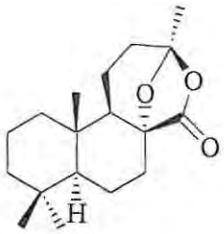
Yellow oil. $[\alpha]_D^{25} = +24°$ ($CHCl_3$; *c* 0.32). IR ν_{max} cm^{-1}(NaCl): 2939, 2845,1717, 1644, 1460, 1442, 1410, 1387, 1363, 1160, 889. EIMS (70 eV) *m/z* (rel.int.): 262 $[M]^+$ (10), 244 (38), 215 (2), 204 (31), 189 (33), 173 (49), 159 (79), 147 (22), 133 (24), 121 (26), 107 (28), 96 (52), 82 (39), 67 (19), 56 (5). HRFABMS: $[M]^+$ 262.2296. $C_{18}H_{30}O$ requires 262.2297. ^1H NMR (400 MHz, $CDCl_3$): δ 0.66 (3H, s, Me-18), 0.77 (3H, s, Me-17), 0.84 (3H, s, Me-16), 2.07 (3H, s, Me-14), 4.41 (1H, s, H-15), 4.83 (1H, s, H-15'). ^{13}C NMR (100 MHz, $CDCl_3$): δ 14.3 (C-18), 17.5 (C-11), 19.3 (C-2), 21.7

(C-17), 24.4 (C-6), 29.9 (C-14), 33.6 (C-4), 33.6 (C-16), 38.2 (C-7), 39.7 (C-10), 42.1 (C-3), 42.8 (C-12), 55.5 (C-9), 56.3 (C-5), 106.3 (C-15), 148.3 (C-8), 209.2 (C-13).

4.2.10 Ruthenium Trichloride Oxidation of 72.

To a solution of **72** (90mg) dissolved in CCl_4 (5mL), H_5IO_6 (387mg) and a catalytic amount of $RuCl_3.3H_2O$ dissolved in acetonitrile (5mL) and H_2O (10mL) was added and allowed to stir for 16 hours. TLC indicated the formation of two products. H_2O (10mL) was added and the mixture was extracted with CH_2Cl_2 (3x20mL). The CH_2Cl_2 solution was dried over $MgSO_4$, filtered and reduced under vacuum. The black crude product was purified by gradient elution flash column chromatography, with elution in hexane:EtOAc (80:20) yielding a mixture of the lactone ether isomers **74**, **75** (59mg) and the dihydroxylated product **73** (32mg). **74** and **75** were separated *via* normal phase HPLC (hexane:EtOAc/95:5) as white crystalline products.

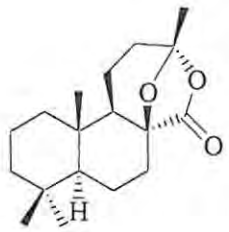
Compound **74**:



White needles. Mpt = 122-124°C. $[\alpha]_D^{25} = +43°$ ($CHCl_3$; *c* 0.30). IR ν_{max} cm^{-1}(NaCl): 2948, 2924, 2867, 2846, 1791, 1462, 1448, 1391, 1286, 1176, 1100, 1083, 1060, 972, 892. EIMS (70 eV) *m/z* (rel.int.): 277 (2), 259 (12), 248 (51), 233 (60), 215 (100), 199 (10), 190 (16), 175 (36), 159 (34), 149 (32), 125 (26), 105 (25), 95 (27), 79 (22), 67 (18), 55 (8). HRFABMS: $[M+1]^+$ 293.2115. $C_{18}H_{29}O_3$ requires 293.2117. ^1H NMR (400 MHz, $CDCl_3$): δ 0.84 (3H, s, Me-17), 0.87 (1H, m, H-1), 0.89 (3H, s, Me-16), 0.91 (3H, s, Me-18), 0.93 (1H, m, H-5), 1.15 (1H, m, H-3), 1.39 (1H, m, H-9), 1.42 (1H, m, H-3'), 1.43 (1H, m, H-2), 1.50 (1H, m, H-7), 1.51 (3H, s, Me-14), 1.53 (1H, m, H-11), 1.62 (1H, m, H-1'), 1.71 (1H, m, H-6), 1.80 (1H, m, H-11'), 1.94 (1H, m, H-12), 2.07 (1H, dt, *J* = 3.1Hz, 13.1Hz, H-7'). ^{13}C NMR (100 MHz, $CDCl_3$): δ 13.4 (C-18), 17.9 (C-11), 18.3 (C-2), 19.1 (C-6), 21.8

(C-17), 23.8 (C-14), 32.5 (C-7), 32.7 (C-12), 33.1 (C-4), 33.7 (C-16), 37.5 (C-10), 39.3 (C-1), 41.8 (C-3), 51.9 (C-9), 55.0 (C-5), 81.1 (C-8), 108.0 (C-13), 175.3 (C-15).

Compound **75**.



White needles. Mpt = 120-122°C. $[\alpha]_D^{25} = +41°$ ($CHCl_3$; *c* 0.38). IR ν_{max} cm^{-1}(NaCl): 2950, 2926, 2864, 2845, 1791, 1460, 1390, 1286, 1174, 1097, 1085, 1058, 1048, 973, 894, 884. EIMS (70 eV) *m/z* (rel.int.): 278 (5), 259 (8), 248 (32), 233 (39), 215 (54), 190 (12), 175 (22), 159 (24), 149 (100), 133 (15), 121 (20), 105 (22), 91 (23), 81 (25), 67 (19), 55 (10). HRFABMS: $[M+1]^+$ 293.2116. $C_{18}H_{29}O_3$ requires 293.2117. ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.85 (3H, s, Me-17), 0.87 (1H, m, H-1), 0.90 (3H, s, Me-16), 0.92 (3H, s, Me-18), 0.97 (1H, m, H-5), 1.15 (1H, m, H-3), 1.41 (1H, m, H-11), 1.42 (1H, m, H-9), 1.43 (1H, m, H-3'), 1.54 (1H, m, H-12), 1.59 (3H, s, Me-14), 1.59 (1H, m, H-2), 1.62 (1H, m, H-1'), 1.70 (1H, m, H-6), 1.74 (1H, m, H-11'), 1.77 (1H, m, H-7), 1.59 (1H, m, H-2'), 1.94 (1H, m, H-12'), 2.09 (1H, dt, *J* = 3.1Hz, 13.1Hz, H-7'). ^{13}C NMR (100 MHz, $CDCl_3$): δ 13.4 (C-18), 17.9 (C-11),18.3 (C-2), 19.1 (C-6), 21.8 (C-17), 24.3 (C-14), 32.5 (C-7), 32.7 (C-12), 33.1 (C-4), 33.7 (C-16), 37.5 (C-10), 39.2 (C-1), 41.8 (C-3), 51.9 (C-9), 55.0 (C-5), 81.3 (C-8), 108.0 (C-13), 175.4 (C-15).

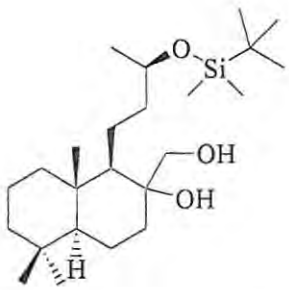
4.2.11 Preparation of Cetyl Trimethyl Ammonium Permanganate (CTAP).

A solution of cetyl trimethyl ammonium bromide (8.04g) in H_2O (100mL) was added dropwise to a stirred solution of $KMnO_4$ (3.17g) in H_2O (100mL) over a period of 20 minutes at RT (20°C). The mixture was allowed to stir for 1 hour. A light purple precipitate formed, which was collected via vacuum filtration. The product was repeatedly washed with H_2O until the washes were uncoloured. The product was dried over phosphorous pentoxide to yield CTAP (8.20g) as a purple solid.

4.2.12 CTAP Dihydroxylation of 67.

To a stirred solution of **67** (50mg) in *t*-butanol (1mL), a solution of CTAP (54mg) in *t*-butanol (5mL) and H_2O (1.5mL) was added dropwise at RT (20°C) and the mixture stirred for 19 hours. $CHCl_3$ (25mL) and 5% aq. NaOH (5mL) was added to the mixture which was stirred for a further 30 minutes. The mixture was extracted with $CHCl_3$ (3x25mL). The combined $CHCl_3$ fractions were washed with H_2O (50mL) and saturated brine (50mL), dried over $MgSO_4$, filtered and reduced under vacuum. Separation was achieved by gradient elution column chromatography, with elution in hexane:EtOAc (80:20) to yield **76** (19mg, 22%) as a colourless oil.

Compound **76**:



$[\alpha]_D^{25} = -7°$ ($CHCl_3$; *c* 0.30). IR ν_{max} cm^{-1}(NaCl): 3509 (br), 2926, 2855, 1715, 1461, 1385, 1370, 1254, 1136, 1046, 836, 773. EIMS (70 eV) *m/z* (rel.int.): 381 (2), 337 (6), 293 (2), 279 (6), 249 (22), 231 (11), 203 (6), 189 (18), 177 (5), 167 (29), 149 (100), 135 (12), 121 (15), 107 (16), 95 (22), 81 (18), 55 (7). HRFABMS: $[M+1]^+$ 413.3451. $C_{24}H_{49}SiO_3$ requires 413.3451. ^1H NMR (400 MHz, $CDCl_3$): δ 0.04 (6H, s, Me_2-Si), 0.76 (3H, s, Me-17), 0.77 (3H, s, Me-18), 0.84 (3H, s, Me-16), 0.85 (9H, s, *t*-butyl-Si), 1.20 (3H, d, *J* = 6.0Hz, Me-14), 2.28 (1H, dt, *J* = 3.2Hz, 12.2Hz, H-7), 3.50 (H, d, J = 7.0Hz, H-15), 3.59 (H, d, J = 7.0Hz, H-15'), 3.81 (1H, m, H-13). ^{13}C NMR (100 MHz, $CDCl_3$): δ -4.3 (C-19), -4.6 (C-20), 16.4 (C-18), 18.4 (C-21), 18.4 (C-2), 21.0 (C-11), 21.5 (C-6), 21.7 (C-17), 23.9 (C-14), 25.7 (C-22, C-23, C-24), 32.2 (C-4), 33.6 (C-16), 39.0 (C-10), 39.9 (C-1), 41.0 (C-12), 43.4 (C-3), 43.7 (C-7), 56.5 (C-5), 62.3 (C-9), 67.7 (C-15), 69.2 (C-13), 76.1 (C-8).

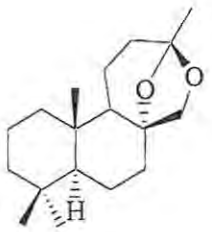
4.2.13 Epoxidation of Compound 72.

Compound **72** (83mg) was dissolved in anhydrous CH_2Cl_2 (5mL) and cooled (-10°C) under N_2. *m*CPBA (273mg) dissolved in anhydrous CH_2Cl_2 (2mL), was initially dried over $MgSO_4$. The dry *m*CPBA solution was transferred *via* a cannula to the reaction vessel. The solution was stirred vigorously for 1 hour then allowed to warm to 0°C. TLC indicated the formation of a mixture of products. Saturated $NaHCO_3$ (15mL) was added and the solution was stirred for a further 30 minutes. The solution was extracted with $CHCl_3$ (2x40mL). The combined $CHCl_3$ fractions were washed with aqueous $Na_2S_2O_3$ (20%, 30mL), H_2O (30mL) and saturated brine (30mL), dried over $MgSO_4$, filtered and reduced under vacuum to give an inseparable mixture of epoxide products **79** and **80** (81mg) as a colourless oil.

4.2.14 Periodic Acid Oxidation of the Epoxide Mixture 68.

Periodic acid (H_5IO_6, 250mg) was dissolved in diethyl ether (5mL) and cooled (0°C). Compound **68** (81mg), dissolved in diethyl ether (1mL), was added to the H_5IO_6 solution and stirred for 6.5 hours. The mixture was allowed to warm to RT (21°C) and stirred overnight. The reaction was quenched with saturated $NaHCO_3$ (10mL) and extracted with diethyl ether (3x30mL). The combined diethyl ether fractions were washed with saturated $NaHCO_3$ (30mL), H_2O (30mL) and saturated brine (30mL), dried over $MgSO_4$, filtered and reduced under vacuum to yield a mixture of products (79mg). Separation was achieved *via* normal phase HPLC (hexane:EtOAc/90:10) to give the acetal **83** (10.3mg, 13%) as a crystalline product.

Compound **83**.



White needles. Mpt = 117-119°C. $[\alpha]_D^{25} = -2°$ ($CHCl_3$; *c* 0.35). IR ν_{max} cm^{-1}(NaCl): 3430, 2925,

2868, 2844, 1634, 1451, 1386, 1261, 1207, 1111, 1037, 1027, 1005, 952, 916, 862. EIMS (70 eV) *m/z* (rel.int.): 279 $[M+1]^+$ (10), 261 (1), 229 (1), 206 (4), 177 (1), 167 (30), 149 (100), 121 (3), 91 (2), 71 (2), 55(2). HRFABMS: $[M]^+$ 278.2246. $C_{18}H_{30}O_2$ requires 278.2246. ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.82 (1H, m, H-1), 0.86 (3H, s, Me-17), 0.87 (3H, s, Me-16), 0.90 (1H, m, H-9), 1.02 (1H, d, J = 6.2Hz, H-5), 1.08 (3H, s, Me-18), 1.15 (1H, dd, J = 4.0Hz, 13.3Hz, H-3), 1.39 (1H, m, H-3'), 1.41 (3H, s, Me-14), 1.54 (1H, m, H-6), 1.55 (1H, m, H-2), 1.68-1.73 (1H, m, H-11), 1.82 (1H, m, H-11'), 1.83 (1H, m, H-1'), 1.85 (1H, m, H-7), 3.41 (1H, d, *J* = 6.8Hz, H-15), 3.75 (1H, d, *J* = 6.8Hz, H-15'). ^{13}C NMR (100 MHz, $CDCl_3$): δ 16.5 (C-18),16.9 (C-11), 18.5 (C-2), 19.3 (C-6), 21.9 (C-17), 25.0 (C-14), 33.2 (C-4), 33.7 (C-12), 33.9 (C-16), 35.3 (C-7), 38.6 (C-10), 40.1 (C-1), 42.0 (C-3), 50.4 (C-5), 54.9 (C-9), 76.2 (C-15), 81.8 (C-8), 108.5 (C-13)

4.2.15 Epoxidation of Compound 68.

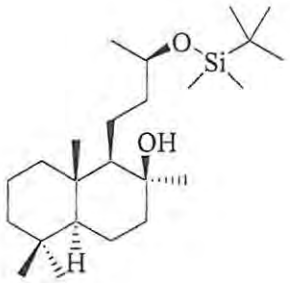
Compound **68** (85mg) was dissolved in anhydrous CH_2Cl_2 (1mL) and cooled (-10°C) under N_2. *m*CPBA (175mg) dissolved in anhydrous CH_2Cl_2 (1mL), was dried over $MgSO_4$ and the dry *m*CPBA solution was transferred *via* a cannula to the reaction vessel containing the solution of **68**. The mixture was stirred vigorously for 2 hours before warming to 0°C. TLC indicated the formation of a mixture of products. Saturated $NaHCO_3$ (15mL) was added and the mixture allowed to stir for a further 30 minutes. The mixture was extracted with CH_2Cl_2 (3x30mL), and the combined CH_2Cl_2 fractions was washed with aqueous $Na_2S_2O_3$(20%, 30mL), H_2O (30mL) and saturated brine (30mL), dried over $MgSO_4$, filtered and reduced under vacuum to give an inseparable mixture of epoxide products **81** and **82** (78mg) as a colourless oil.

4.2.16 Preparation of Compounds 86 and 87.

This procedure is representative for the methyl lithium methylation of the TDBMS protected ketones **70** and **71**. A solution of **70** (460mg) in anhydrous THF (5mL) was cooled (-78°C) under N_2. A standard 1.6M diethyl ether solution of MeLi (4.96mmol, 3.1mL) was added dropwise to the stirred solution. The mixture was warmed to RT (25°C) and allowed to stir overnight. TLC indicated conversion of **70** into a more polar product. The reaction was quenched by addition of an ice cold

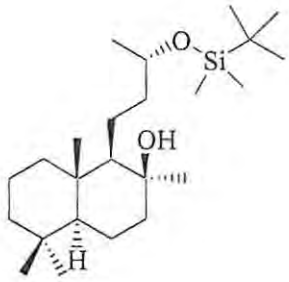
$NaHCO_3$ slurry. The mixture was extracted with diethyl ether (3x30mL) and the combined ether fractions washed with H_2O (2x30mL) and saturated brine (50mL), dried over $MgSO_4$ and reduced under vacuum. Purification was achieved by gradient elution column chromatography, with elution in hexane:EtOAc (90:10) to yield **86** (352mg, 73%).

Compound **86**:



Colourless oil. $[\alpha]_D^{25} = +15°$ ($CHCl_3$; *c* 0.30). IR ν_{max} cm^{-1}(NaCl): 3420, 2954, 2927, 2858, 1636, 1471, 1463, 1388, 1371, 1255, 1182, 1137, 1091, 1047, 1003, 902, 835, 773. EIMS (70 eV) *m/z* (rel.int.): 397 $[M+1]^+$ (1), 379 (2), 339 (9), 321 (4), 293 (1), 264 (4), 247 (50), 231 (17), 221 (3), 205 (9), 191 (83), 177 (38), 163 (48), 149 (71), 135 (35), 121 (41), 109 (45), 96 (100), 82 (81), 67 (41), 55 (17). HRFABMS: $[M]^+$ 396.3424. $C_{24}H_{48}SiO_2$ requires 396.3423. ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.04 (6H, s, Me_2-Si), 0.74 (1H, m, H-9), 0.81 (3H, s, Me-17), 0.82 (1H, m, H-5), 0.85 (1H, m, H-1), 0.85 (3H, s, Me-16), 0.87 (9H, s, *t*-butyl-Si), 0.93 (3H, s, Me-18), 1.11 (3H, s, Me-15), 1.12 (3H, s, Me-14), 1.13 (1H, m, H-12), 1.28 (1H, m, H-11), 1.36 (1H, m, H-12'), 1.40 (1H, m, H-2), 1.41 (1H, m, H-7), 1.43 (1H, m, H-11'), 1.44-1.45 (2H, m, H-3, H-3'), 1.48 (1H, s, H-6), 1.57 (1H, dt, *J* = 3.3Hz, 13.6Hz, H-6'), 1.69 (1H, m, H-1'), 1.73 (1H, m, H-7'), 3.73 (1H, m, H-13). ^{13}C NMR (100 MHz, $CDCl_3$): δ -4.5 (C-19), -4.7 (C-20), 15.0 (C-18), 18.1 (C-6), 18.2 (C-21), 18.3 (C-2), 21.3 (C-11), 21.7 (C-17), 23.6 (C-14), 25.9 (C-22, C-23, C-24), 30.5 (C-15), 33.2 (C-4), 33.4 (C-16), 39.0 (C-10), 39.1 (C-1), 42.1 (C-12), 42.1 (C-7), 43.8 (C-3), 55.9 (C-5), 59.1 (C-9), 69.1 (C-13), 73.2 (C-8).

Compound **87**:



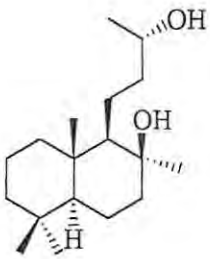
Colourless oil. $[\alpha]_D^{25} = +11°$ ($CHCl_3$; *c* 0.24). IR ν_{max} cm^{-1}(NaCl): 3427, 2955, 2928, 2858, 1640, 1458, 1388, 1373, 1253, 1136, 1098, 1043, 1004, 838, 772. EIMS (70 eV) *m/z* (rel.int.): 381 $[M+1-CH_3]^+$ (2), 363 (1), 339 (18), 321 (5), 303 (1), 279 (3), 264 (8), 247 (54), 231 (20), 221 (6), 204 (12), 191 (100), 177 (52), 163 (60), 149 (90), 135 (42), 121 (48), 109 (53), 95 (80), 82 (96), 67 (47), 55 (19). HRFABMS:$[M]^+$ 396.3425. $C_{24}H_{48}SiO_2$ requires 396.3424. ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.04 (6H, s, Me_2-Si), 0.74 (1H, m, H-9), 0.81 (3H, s, Me-17), 0.82 (1H, m, H-5), 0.84 (1H, m, H-1), 0.85 (3H, s, Me-16), 0.87 (9H, s, *t*-butyl-Si), 0.93 (3H, s, Me-18), 1.08 (1H, m, H-12), 1.11 (3H, s, Me-15), 1.11 (3H, d, *J*= 6.0Hz, Me-14), 1.22 (1H, m, H-11), 1.35 (1H, m, H-12'), 1.35 (1H, s, H-6), 1.42 (1H, m, H-3), 1.43 (1H, m, H-11'), 1.48 (1H, m, H-7), 1.49 (1H, m, H-2), 1.52 (1H, m, H-3'), 1.57 (1H, m, 13.6Hz, H-6'), 1.67 (1H, m, H-1'), 1.73 (1H, m, H-7'), 3.74 (1H, m, H-13). ^{13}C NMR (100 MHz, $CDCl_3$): δ -4.5 (C-19), -4.7 (C-20), 15.1 (C-18), 18.1 (C-6), 18.1 (C-21), 18.3 (C-2), 21.1 (C-11), 21.7 (C-17), 23.5 (C-14), 25.9 (C-22, C-23, C-24), 30.5 (C-15), 33.2 (C-4), 33.4 (C-16), 38.9 (C-10), 39.2 (C-1), 42.1 (C-12), 42.1 (C-7), 43.7 (C-3), 55.9 (C-5), 59.1 (C-9), 69.0 (C-13), 73.2 (C-8).

4.2.17 Deprotection of the Protected Diols 73 and 74.

This reaction is representative for the deprotection of both **73** and **74**. To a solution of **73** (230mg) in THF (5mL), 1M TBAF (3.5mL) was added and the solution refluxed for 4 hours. The solution was allowed to cool to RT (20°C) and stirred overnight. TLC indicated that a more polar product had formed. Saturated NH_4Cl (20mL) was added to the solution and the solution stirred for a further 30 minutes. The solution was extracted with EtOAc (3x30mL), the combined EtOAc fractions were

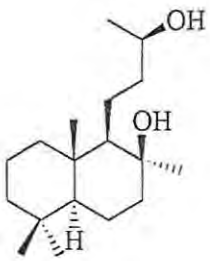
washed with H_2O (30mL) and saturated brine (50mL), dried over $MgSO_4$ and reduced under vacuum to yield the white crystalline product **88** (140mg, 85%). Final purification was achieved by gradient elution column chromatography, with elution in EtOAc:hexane (60:40). Compound **88** was recrystallized from hexane.

Compound **88**:



White powder. Mpt = 95°C-97°C. $[\alpha]_D^{25} = +8°$ ($CHCl_3$; *c* 0.30). IR ν_{max} cm^{-1}(NaCl): 3430, 2925, 1642, 1463, 1389, 1187, 1130, 1080, 1033, 983, 909. EIMS (70 eV) *m/z* (rel.int.): 264 $[M-H_2O]^+$ (8), 249 (20), 231 (30), 221 (20), 206 (10), 195 (12), 177 (100), 167 (18), 149 (80), 135 (21), 123 (45), 109 (53), 96 (96), 81 (49), 67 (53), 55 (19). HRFABMS: $[M]^+$ 282.2559. $C_{18}H_{34}O_2$ requires 282.2559. ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.78 (1H, m, H-9), 0.81 (3H, s, Me-17), 0.83 (1H, m, H-5), 0.85 (3H, s, Me-16), 0.86 (1H, m, H-1), 0.95 (3H, s, Me-18), 1.12 (3H, s, Me-15), 1.15 (1H, m, H-12), 1.19 (3H, d, *J* = 6.5Hz, Me-14), 1.38 (1H, m, H-12'), 1.38 (1H, m, H-11), 1.43 (1H, m, H-3), 1.44 (1H, m, H-6), 1.46 (1H, m, H-7), 1.49 (1H, m, H-3'), 1.50 (1H, m, H-11'), 1.52 (1H, m, H-2), 1.59 (1H, m, H-6'), 1.69 (1H, m, H-1'), 1.73 (1H, m, H-7'), 3.75 (1H, m, H-13). ^{13}C NMR (100 MHz, $CDCl_3$) : δ 15.1 (C-18), 18.3 (C-2), 21.3 (C-11), 21.7 (C-17), 23.4 (C-14), 30.6 (C-15), 33.2 (C-4), 33.4 (C-16), 39.0 (C-10), 39.2 (C-1), 42.0 (C-12), 42.2 (C-7), 43.3 (C-3), 55.9 (C-5), 59.1 (C-9), 68.6 (C-13), 73.2 (C-8).

Compound **89**:

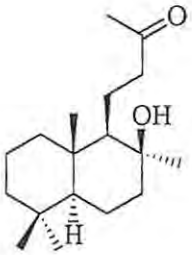


White powder. Mpt = 98°C-101°C. $[\alpha]_D^{25} = +18°$ ($CHCl_3$; *c* 0.26). IR ν_{max} cm^{-1}(NaCl): 3420, 2915,1638, 1460, 1387, 1371, 1217, 1187, 1131, 1075, 1030, 1022, 988. EIMS (70 eV) *m/z* (rel.int.): 264 $[M+H_2O]^+$ (8), 249 (22), 231 (25), 221 (15), 206 (10), 191 (12), 177 (100), 167 (15), 149 (60), 135 (18), 123 (40), 109 (50), 95 (59), 82 (73), 67 (51), 55 (17). HRFABMS: $[M]^+$ 282.2558. $C_{18}H_{34}O_2$ requires 282.2559. ^1H NMR (400 MHz, $CDCl_3$): δ 0.77 (1H, m, H-9), 0.81 (3H, s, Me-17), 0.82 (1H, m, H-5), 0.85 (3H, s, Me-16), 0.87 (1H, m, H-1), 0.94 (3H, s, Me-18), 1.12 (1H, m, H-12), 1.18 (3H, d, *J* = 6.2Hz, Me-14), 1.21 (3H, s, Me-15), 1.25 (1H, m, H-11), 1.37 (1H, m, H-12'), 1.39 (1H, m, H-6), 1.44 (1H, m, H-3), 1.45 (1H, m, H-7), 1.50 (1H, m, H-2), 1.51 (1H, m, H-3'), 1.54 (1H, m, H-11'), 1.59 (1H, m, H-6'), 1.67 (1H, m, H-1'), 1.74 (1H, m, H-7'), 3.76 (1H, m, H-13). ^{13}C NMR (100 MHz, $CDCl_3$) : δ 15.1 (C-18), 18.3 (C-2), 21.2 (C-11), 21.6 (C-17), 23.4 (C-14), 30.6 (C-15), 33.3 (C-4), 33.4 (C-16), 38.9 (C-10), 39.2 (C-1), 42.0 (C-12), 42.0 (C-7), 43.1 (C-3), 55.9 (C-5), 59.0 (C-9), 68.5 (C-13), 73.2 (C-8).

4.2.18 Oxidation of the Diols 88 and 89.

This procedure is representative for the oxidation of both diols **88** and **89**. Oxalyl chloride (70μL) was added to anhydrous CH_2Cl_2 (3mL) and cooled (-78°C) under N_2. DMSO (106μL) was added and the solution allowed to stir for 10 minutes. Compound **75** (140mg), dissolved in anhydrous CH_2Cl_2 (3mL), was transferred *via* a cannula to the reaction vessel containing the oxalyl chloride solution and stirred for 1 hour. Triethylamine (415μL) was added dropwise to the mixture and was stirred for 1 hour. The mixture was allowed to warm to 0°C and stirred for a further 2 hours. TLC with 2,4-DNP development indicated that the reaction was completed. Saturated NH_4Cl (5mL) was added to the oxidation reaction and the solution stirred for 30 minutes. The solution was extracted with EtOAc (3x30mL), and the combined EtOAc fractions washed with H_2O (30mL) and saturated brine (30mL), dried over $MgSO_4$, filtered and reduced under vacuum to yield the crude product **90** (139mg). No separation was attempted because of the compounds sensitivity to acidic media.

Compound **90**:

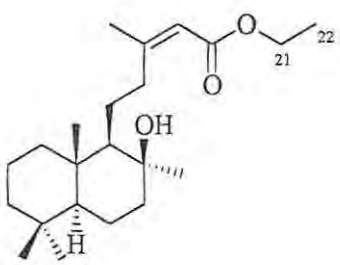


IR ν_{max} cm^{-1}(NaCl): 3417, 2918, 1712, 1463, 1388, 1371, 1190, 1130, 1078, 1025, 987, 915, 862, 734.

4.2.19 Preparation of the α,β-Unsaturated Esters 91 and 92.

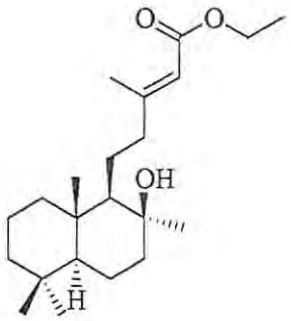
60% NaH (36mg) was washed with anhydrous THF (2x1mL) and resuspended in 1mL of the same solvent. Triethylphosphonoacetate (492μL) was added dropwise to the reaction vessel and the solution was stirred for 30 minutes. Compound **90** (138mg), dissolved in anhydrous THF (2mL), was transferred *via* a cannula to the reaction vessel under N_2. The mixture was allowed to stir overnight at RT (22°C) after which 5% aqueous NH_4Cl (10mL) was added and the mixture stirred for a further 30 minutes. The mixture was extracted with EtOAc (3x30mL), the EtOAc fractions washed with H_2O (30mL) and saturated brine (50mL), dried over $MgSO_4$, filtered and reduced under vacuum. Purification was achieved by gradient elution column chromatography, with elution in hexane:EtOAc (85:15) to give a mixture of E and Z isomers (60mg). Compounds **91** (12mg) and **92**(47mg) were finally separated using normal phase HPLC (hexane:EtOAc/90:10).

Compound **91**:



Colourless oil. $[\alpha]_D^{25} = +34°$ ($CHCl_3$; *c* 0.30). IR ν_{max} cm^{-1}(NaCl): 3450, 2920, 2934, 1712, 1638, 1467, 1387, 1219, 1142, 1070, 1032, 910. EIMS (70 eV) *m/z* (rel.int.): 350 $[M]^+$ (7), 333 (8), 317 (10), 304 (55), 289 (10), 280 (89), 258 (19), 243 (40), 229 (18), 218 (12), 205 (22), 192 (30), 177 (80), 163 (30), 149 (100), 135 (32), 123 (49), 110 (86), 95 (57), 81 (46), 67 (54), 55 (18). HRFABMS: $[M]^+$ 350.2820. $C_{22}H_{38}O_3$ requires 350.2821. ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.77 (1H, m, H-9), 0.80 (3H, s, Me-18), 0.82 (1H, m, H-5), 0.87 (1H, m, H-1), 0.85 (3H, s, Me-19), 0.93 (3H, s, Me-20), 1.12 (3H, s, Me-17), 1.14 (1H, m, H-12), 1.24 (3H, m, Me-16), 1.38 (1H, m, H-12'), 1.38 (1H, m, H-11), 1.40 (1H, m, H-2), 1.44 (1H, m, H-7), 1.48 (1H, m, H-6), 1.55 (1H, m, H-11'), 1.57 (1H, m, H-2'), 1.65 (1H, m, H-1'), 1.72 (1H, m, H-7'), 2.12 (1H, m, H-3), 2.15 (3H, s, Me-22), 4.11 (2H, m, H_2-21), 5.65 (1H, s, H-14). ^{13}C NMR (100 MHz, $CDCl_3$): δ 14.3 (C-16), 15.1 (C-20), 18.1 (C-6), 18.2 (C-2), 19.0 (C-22), 21.6 (C-18), 23.6 (C-11), 30.6 (C-17), 33.2 (C-4), 33.4 (C-19), 39.0 (C-10), 39.2 (C-1), 41.7 (C-12), 42.2 (C-7), 44.6 (C-3), 55.9 (C-5), 58.8 (C-9), 59.4 (C-21), 73.1 (C-8), 115.3 (C-14), 160.2 (C-13), 166.9 (C-15).

Compound **92**:



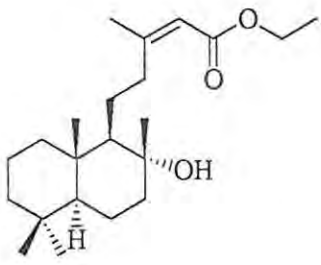
Colourless oil. $[\alpha]_D^{25} = +42°$ ($CHCl_3$; *c* 0.24). IR ν_{max} cm^{-1}(NaCl):3445, 2923, 2939, 2866, 2843, 1717, 1700, 1647, 1461, 1444, 1388, 1369, 1291, 1223, 1147, 1075, 1037, 914, 866. EIMS (70 eV) *m/z* (rel.int.): 350 $[M]^+$ (5), 333 (7), 317 (8), 304 (21), 289 (10), 280 (100), 259 (12), 243 (20), 229 (11), 218 (10), 205 (20), 192 (25), 177 (53), 163 (20), 149 (70), 135 (30), 121 (45), 110 (58), 95 (42), 81 (28), 68 (40), 55 (13). HRFABMS: $[M]^+$ 350.2821. $C_{22}H_{38}O_3$ requires 350.2821. ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.83 (1H, m, H-9), 0.82 (3H, s, Me-18), 0.90 (1H, m, H-5), 0.96 (1H, m, H-1), 0.86 (3H, s, Me-19), 0.94 (3H, s, Me-20), 1.11 (1H, m, H-3), 1.23 (3H, s, Me-17), 2.56 (1H, ddd, *J* = 5.4Hz, 12.2Hz, H-12), 1.23 (3H, m, Me-16), 2.74 (1H, ddd, *J* = 5.2Hz, 12.1Hz, H-12'),

1.16 (1H, m, H-11), 1.39 (1H, m, H-2), 1.49 (1H, m, H-7), 1.49 (1H, m, H-6), 1.43 (1H, m, H-11'), 1.56 (1H, m, H-2'), 1.79 (1H, m, H-1'), 1.74 (1H, m, H-7'), 1.38 (1H, m, H-3'), 1.90 (3H, s, Me-22), 4.13 (2H, m, H_2-21), 5.60 (1H, s, H-14). ^{13}C NMR (100 MHz, $CDCl_3$): δ 14.3 (C-16), 15.1 (C-20), 18.2 (C-6), 18.3 (C-2), 19.0 (C-22), 21.7 (C-18), 23.8 (C-11), 30.6 (C-17), 33.3 (C-4), 33.4 (C-19), 39.1 (C-10), 39.1 (C-1), 42.4 (C-12), 42.0 (C-7), 44.6 (C-3), 55.9 (C-5), 59.3 (C-9), 59.4 (C-21), 73.3 (C-8), 115.9 (C-14), 159.9 (C-13), 166.2 (C-15).

4.2.20 Preparation of the α,β-Unsaturated Esters 93 and 94.

60% NaH (79mg) was washed with anhydrous THF (3x1mL) and resuspended in 1mL of the same solvent. TEPA (396μL) was added dropwise to the reaction vessel and the solution was stirred for 30 minutes. Compound **47** (100mg), dissolved in anhydrous THF (1mL), was transferred *via* a cannula to the reaction vessel under N_2. The mixture was allowed to stir overnight at RT (26°C), after which 5% aqueous NH_4Cl (5mL) was added and the mixture stirred for a further 30 minutes. The mixture was extracted with diethyl ether (3x30mL), the EtOAc fractions washed with H_2O (30mL) and saturated brine (50mL), dried over $MgSO_4$, filtered and reduced under vacuum. Purification was achieved by gradient elution flash column chromatography, with elution in hexane:EtOAc (80:20) to give a mixture of E and Z isomers (118mg, 94%). Compounds **93** (34mg) and **94**(74mg) were finally separated using normal phase HPLC (hexane:EtOAc/80:20).

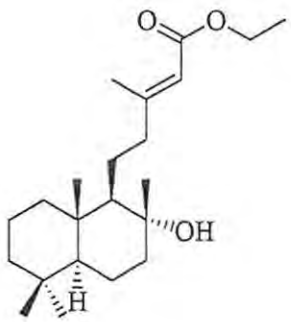
Compound **93**:



Colourless oil. $[\alpha]_D^{25} = +42°$ ($CHCl_3$; *c* 0.27). IR ν_{max} cm^{-1}(NaCl): 3519, 2935, 2869, 1698, 1645, 1460, 1444, 1387, 1377, 1238, 1176, 1143, 1037, 939, 858. EIMS (70 eV) *m/z* (rel.int.): 350 $[M]^+$ (22), 333 (21), 317 (40), 304 (100), 286 (20), 276 (83), 258 (26), 243 (70), 229 (22), 205 (38), 192

(40), 177 (70), 163 (32), 149 (55), 135 (25), 121 (49), 110 (98), 95 (70), 82 (40), 67 (38), 55 (18). HRFABMS: $[M\text{-}CH_3]^+$ 335.2428. $C_{21}H_{35}O_3$ requires 335.2430. ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.78 (3H, s, Me-20), 0.78 (3H, s, Me-18), 0.86 (3H, s, Me-19), 0.91 (1H, m, H-5), 0.94 (1H, m, H-1), 1.04 (1H, t, J = 3.2Hz, H-9), 1.14 (3H, s, Me-17), 1.16 (1H, m, H-3), 1.24 (1H, m, H-6), 1.28 (3H, d, J = 7.1Hz, Me-16), 1.37 (1H, m, H-11), 1.37 (1H, m, H-7), 1.41 (1H, m, H-2), 1.56 (1H, m, H-11'), 1.56 (1H, m, H-2'), 1.63 (1H, m, H-6'), 1.64 (1H, m, H-1'), 1.85 (1H, dt, J = 3.3Hz, 12.2Hz, H-7'), 2.15 (3H, s, Me-22), 2.16 (1H, m, H-12), 2.28 (1H, m, H-12'), 4.14 (2H, q, H_2-21), 5.66 (1H, s, H-14). ^{13}C NMR (100 MHz, $CDCl_3$): δ 14.3 (C-16), 15.4 (C-20), 18.4 (C-2), 19.1 (C-22), 20.6 (C-6), 21.5 (C-18), 23.6 (C-11), 24.0 (C-17), 33.3 (C-4), 33.4 (C-19), 39.2 (C-10), 39.8 (C-1), 42.0 (C-3), 44.4 (C-12), 44.8 (C-7), 56.1 (C-5), 59.4 (C-21), 61.5 (C-9), 74.2 (C-8), 115.2 (C-14), 160.9 (C-13), 167.0 (C-15).

Compound **94**:



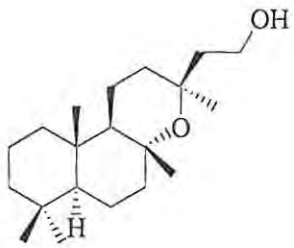
Colourless oil. $[\alpha]_D^{25}$ = +11° ($CHCl_3$; *c* 0.31). IR ν_{max} cm^{-1}(NaCl): 3500, 2931, 2868, 1715, 1698, 1644, 1463, 1444, 1387, 1367, 1350, 1224, 1147, 1076, 1040, 972, 940, 911, 861. EIMS (70 eV) *m/z* (rel.int.): 350 $[M]^+$(2), 332 (11), 317 (40), 304 (10), 289 (10), 280 (35), 259 (20), 244 (32), 229 (31), 205 (40), 191 (60), 177 (100), 161 (40), 149 (57), 135 (54), 122 (60), 107 (47), 95 (61), 81 (40), 67 (38), 55 (13). HRFABMS: $[M\text{-}CH_3]^+$ 335.2429. $C_{21}H_{35}O_3$ requires 335.2430. ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.75 (3H, s, Me-20), 0.77 (3H, s, Me-18), 0.85 (3H, s, Me-19), 0.91 (1H, m, H-5), 0.96 (1H, m, H-1), 1.11 (1H, m, H-9), 1.16 (3H, s, Me-17), 1.19 (1H, m, H-3), 1.34 (1H, m, H-3), 1.22 (1H, m, H-6), 1.23 (3H, d, J = 7.1Hz, Me-16), 1.46 (1H, m, H-11), 1.44 (1H, m, H-7), 1.41 (1H, m, H-2), 1.56 (1H, dt, J = 3.4Hz, 13.6Hz, H-2'), 1.62 (1H, m, H-6'), 1.74 (1H, m, H-1'), 1.84 (1H, dt, J = 3.2Hz, 12.5Hz, H-7'), 1.89 (3H, d, J = 1.3Hz, Me-22), 2.21 (1H, m, H-12), 2.93

(1H, m, H-12'), 4.12 (2H, q, H_2-21), 5.64 (1H, s, H-14). ^{13}C NMR (100 MHz, $CDCl_3$): δ 14.3 (C-16), 15.5 (C-20), 19.5 (C-2), 25.7 (C-22), 20.3 (C-6), 21.5 (C-18), 24.3 (C-11), 24.2 (C-17), 33.3 (C-4), 33.4 (C-19), 38.8 (C-10), 37.4 (C-1), 39.3 (C-3), 42.0 (C-12), 43.2 (C-7), 56.1 (C-5), 56.8 (C-21), 61.5 (C-9), 73.9 (C-8), 115.6 (C-14), 161.7 (C-13), 166.6 (C-15).

4.2.21 DIBALH Reduction of the Esters 93 and 94.

This reaction is representative for the reduction of both esters **93** and **94**. Compound **93** (220mg) was dissolved in anhydrous CH_2Cl_2 (2mL) and cooled (0°C) under N_2. DIBALH (1.88mL) was added dropwise and the solution stirred for 4 hours, after which saturated NH_4Cl (5mL) was added and stirred for a further 30 minutes. The mixture was extracted with EtOAc (3x30mL), the combined EtOAc fractions washed with H_2O (30mL) and saturated brine (50mL), dried over $MgSO_4$, filtered and reduced under vacuum. Purification was achieved *via* normal phase column chromatography, with elution in hexane:EtOAc (50:50) to yield **97** (184.7mg; 95%).

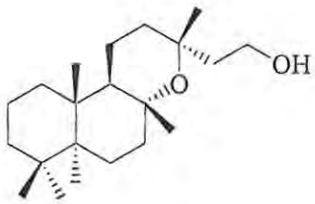
Compound **97**:



Colourless oil. $[\alpha]_D^{25} = +4°$ ($CHCl_3$; *c* 0.28). IR ν_{max} cm^{-1}(NaCl): 3435, 2930, 1630, 1455, 1371, 1150, 1097, 1040, 983. EIMS (70 eV) *m/z* (rel.int.): 309 $[M+1]^+$(2), 293 (6), 275 (11), 263 (9), 245 (100), 231 (6), 205 (3), 189 (7), 175 (7), 161 (6), 149 (12), 135 (6), 121 (9), 107 (8), 95 (9), 82 (12), 67 (6). HRFABMS: $[M+1]^+$ 309.2795. $C_{20}H_{37}O_2$ requires 309.2794. ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.73 (3H, s, Me-20), 0.75 (3H, s, Me-19), 0.81 (3H, s, Me-18), 0.83 (1H, m, H-1), 0.92 (1H, m, H-5), 1.10 (1H, m, H-3), 1.14 (1H, m, H-9), 1.21 (1H, m, H-6), 1.24 (3H, s, Me-17), 1.25 (1H, m, H-7), 1.26 (3H, s, Me-16), 1.34 (1H, m, H-3'), 1.40 (1H, m, H-2), 1.45-1.60 (2H, m, H_2-11), 1.52 (1H, m, H-14), 1.53 (1H, m, H-12), 1.58 (1H, m, H-1'), 1.60 (1H, m, H-6'), 1.61 (1H, m, H-2'), 1.62

(1H, m, H-12'), 1.66 (1H, m, H-14'), 1.72 (1H, m, H-7'), 3.75 (2H, ddd, J = 3.0Hz, 4.3Hz, 14.7Hz, H_2-15). ^{13}C NMR (100 MHz, $CDCl_3$): δ 15.1 (C-11), 15.7 (C-20), 18.5 (C-2), 19.7 (C-6), 21.2 (C-19), 24.5 (C-17), 27.5 (C-16), 33.2 (C-18), 33.2 (C-4), 36.8 (C-10), 36.9 (C-12), 39.0 (C-1), 42.0 (C-3), 43.1 (C-7), 45.7 (C-14), 56.2 (C-5), 58.1 (C-9), 59.6 (C-15), 75.8 (C-13), 75.9 (C-8).

Compound **98**:



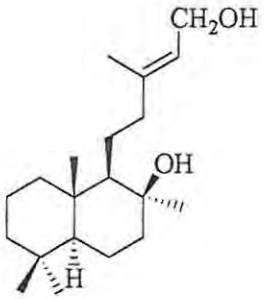
Colourless oil. $[\alpha]_D^{25}$ = +7° ($CHCl_3$; *c* 0.19). IR ν_{max} cm^{-1}(NaCl): 3437, 2932, 1648, 1633, 1383, 1121, 1028, 980. EIMS (70 eV) *m/z* (rel.int.): 293 (6), 275 (12), 263 (9), 245 (100), 231 (5), 205 (3), 189 (8), 175 (9), 161 (7), 149 (15), 135 (6), 121 (6), 107 (7), 96 (13), 81 (6), 67 (6). HRFABMS: $[M+1]^+$ 309.2793. $C_{20}H_{37}O_2$ requires 309.2794. ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.77 (3H, s, Me-20), 0.77 (3H, s, Me-19), 0.83 (3H, s, Me-18), 0.83 (1H, m, H-1), 1.40 (1H, m, H-5), 1.15 (1H, m, H-3), 1.38 (1H, dd, J = 2.5Hz, 9.7Hz, H-9), 1.14 (1H, m, H-6), 1.27 (3H, s, Me-17), 1.30 (1H, m, H-7), 1.20 (3H, s, Me-16), 1.33 (1H, m, H-3'), 1.36 (1H, m, H-2), 1.43 (1H, m, H-11), 1.58 (1H, m, H-11'), 1.42 (1H, m, H-14), 1.48 (1H, m, H-12), 1.57 (1H, m, H-1'), 1.61 (1H, m, H-6'), 1.56 (1H, m, H-2'), 1.86 (1H, m, H-12'), 1.81-1.90 (1H, m, H-14'), 1.70 (1H, dt, J = 3.3Hz, 5.8Hz, H-7'), 3.69 (1H, m, H-15), 3.88 (1H, m, H-15'). ^{13}C NMR (100 MHz, $CDCl_3$): δ 14.8 (C-11), 15.1 (C-20), 18.4 (C-2), 19.9 (C-6), 21.3 (C-19), 25.2 (C-17), 28.7 (C-16), 33.3 (C-18), 33.1 (C-4), 37.1 (C-10), 35.0 (C-12), 38.9 (C-1), 42.0 (C-3), 43.7 (C-7), 43.9 (C-14), 54.2 (C-5), 56.4 (C-9), 59.9 (C-15), 74.3 (C-13), 75.4 (C-8).

4.2.22 Preparation of the Target Compound 46 [Labd-13(14)-en-8α,15-diol].

Compound **92** (18mg) was dissolved in anhydrous CH_2Cl_2 (1mL) and cooled (0°C) under N_2. DIBALH (154μL) was added dropwise and the solution stirred for 4 hours. Saturated NH_4Cl (2mL) was added and the mixture stirred for a further 30 minutes. The mixture was extracted with EtOAc

(3x30mL), the combined EtOAc fractions washed with H_2O (10mL) and saturated brine (20mL), dried over $MgSO_4$, filtered and reduced under vacuum. Purification was achieved *via* normal phase HPLC (hexane:EtOAc/50:50) to yield **46** (8.2mg, 46%) as a white amorphous powder.

Compound **46**:



Amorphous powder. $[\alpha]_D^{25} = +19°$ ($CHCl_3$; *c* 0.35). IR ν_{max} cm^{-1}(NaCl): 3366, 2924, 2400, 1459, 1436, 1383, 1368, 1185, 1120, 1070, 1017, 907. EIMS (70 eV) *m/z* (rel.int.): 293 (3), 275 (4), 257 (8), 245 (10), 217 (4), 203 (5), 191 (20), 177 (100), 163 (18), 149 (52), 135 (20), 122 (69), 107 (42), 95 (38), 80 (39), 67 (29), 55 (9). HRFABMS: $[M]^+$ 308.271492. $C_{20}H_{36}O_2$ requires 308.271531. ^1H NMR (400 MHz, $CDCl_3$): δ 0.79 (1H, m, H-9), 0.82 (3H, s, Me-18), 0.84 (1H, m, H-1), 0.86 (3H, s, Me-19), 0.90 (1H, m, H-5), 0.95 (3H, Me-20), 1.12 (1H, m, H-3), 1.14 (3H, s, Me-17), 1.38 (1H, m, H-11), 1.38 (1H, m, H-3'), 1.42 (1H, m, H-2), 1.46 (1H, m, H-12), 1.52 (1H, m, H-11'), 1.59 (1H, dt, *J* = 3.3Hz, 13.6Hz, H-2), 1.66 (1H, m, H-1'), 1.69 (3H, s, Me-16), 1.73 (1H, m, H-12'), 2.03 (1H, m, H-7), 4.14 (2H, d, *J* = 6.9Hz, H_2-15), 5.41 (1H, t, *J* = 6.8Hz, H-14). ^{13}C NMR (100 MHz, $CDCl_3$): δ 15.1 (C-20), 16.4 (C-16), 18.2 (C-2), 18.3 (C-6), 21.7 (C-18), 24.0 (C-11), 30.6 (C-17), 33.3 (C-4), 33.4 (C-19), 39.0 (C-10), 39.2 (C-1), 42.1 (C-3), 42.2 (C-12), 43.3 (C-7), 55.9 (C-5), 58.8 (C-9), 59.4 (C-15), 73.2 (C-8), 123.1 (C-14), 140.4 (C-13).

REFERENCES

4. REFERENCES

1. Ireland, C.M.; Roll, D.M.; Molinski, T.F.; McKee, T.C.; Zabriske, T.M. and Swersey, J.C., *Uniqueness of the Marine Environment*, **1988**, 41-57.
2. Andersen, R.J. and Williams, D.E., "Pharmaceuticals from the Sea" in *Issues in Environmental Science and Technology*, No.13, Chemistry in the Marine Environment, Hester, R.E. and Harrison, R.M., Ed., **2000**, 55-79.
3. Rinehart, K.L.; Holt, T.G.; Freheau, N.L.; Stroh, J.G.; Keifer, P.A.; Sun, F.; Li, L.H. and Martin, D.G., *J. Org. Chem.*, **1991**, *56*, 1676-1678.
4. Pettit, G.R., *J. Nat. Prod.*, **1996**, *59*, 812-821.
5. Fodstad, O.; Breistol, K.; Pettit, G.R.; Shoemaker, R.H. and Boyd, M.R., *J. Exp. Ther. Oncol.*, **1996**, *1*, 119-125.
6. Lindel, T.; Jensen, P.R.; Fenical, W.; Long, B.H.; Casazza, J.; Carboni, J. and Fairchild, C.R., *J. Am. Chem. Soc.*, **1997**, *119*, 8744-8745.
7. Chen, X.; Bhattacharya, S.K.; Zhou, B.; Gutteridge, C.E.; Pettus, T.R.R. and Danishefsky, S.J., *J. Am. Chem. Soc.*, **1999**, *121*, 6563-6579.
8. Ciavatta, M.L.; Villani, G.; Trivellone, E. and Cimino, G., *Tetrahedron Lett.*, **1995**, *36*, 8673-8676.
9. Burreson, B.J.; Scheuer, P.J.; Finer, J.S. and Clardy, J., *J. Am. Chem. Soc.*, **1975**, *97*, 4763-4764.
10. Proksch, P., *Toxicon*, **1994**, *32*, 639-655.
11. Cimino, G.; Fontana, A. and Gavagnin, M., *Current Organic Chemistry*, **1999**, *3*, 327-372.
12. Gustafson, K. and Andersen, R.J., *Tetrahedron*, **1985**, *41*, 1101-1108.
13. Paul, V.J.; Seo, Y.; Woong Cho, K.; Rho, J.; Shin, J. and Bergquist, P.R., *J. Nat. Prod.*, **1997**, *60*, 1115-1120.
14. Faulkner, D.J., *Nat. Prod. Rep.*, **1995**, *12*, 223 (and references cited therein).
15. Gustafsen, K.; Andersen, R.J.; Chen, M.H.M.; Clardy, J. and Hochlowski, J.E., *Tetrahedron Lett.*, **1984**, *25*, 11-14.

16. Professor J.Blunt, University of Canterbury, New Zealand, j.blunt@chem.canterbury.ac.nz.
17. Howard, B.M. and Fenical, W., *Phytochemistry*, **1980**, *19*, 2774-2776.
18. Yamamura, S. and Hirata, Y., *Bull. Chem. Soc. Jpn.*, **1971**, *44*, 2560.
19. Suzuki, M.; Kurosawa, E. and Kurata, K., *Phytochemistry*, **1988**, *27*, 1209-1210.
20. González, A.G.; Ciccio, J.F.; Rivera, P.R. and Martin, J.D., *J. Org. Chem.*, **1985**, *50*, 1261-1265.
21. Briand, A. and Kornprobst, J., *Tetrahedron Lett.*, **1997**, *38*, 3399-3400.
22. Capon, R.J.; Ghisalberti, E.L. and Jefferies, P.R., *Phytochemistry*, **1983**, *22*, 1465-1467.
23. Davidson, B.S., *Chem. Rev.*, **1993**, *93*, 1771-1791.
24. Malochet-Grivois, C.; Cotelle, P.; Biard, J.F.; Hénichart, C.; Debitus, C.; Roussakis, C. and Verbist, J.F., *Tetrahedron Lett.*, **1991**, *32*, 6701-6702.
25. Nakamura, H.; Wu, H; Ohizumi, Y. and Hirata, Y., *Tetrahedron Lett.*, **1984**, *25*, 2989-2992.
26. Ciavatta, M.L.; Trivellone, E. and Cimino, G., *Tetrahedron Lett.*, **1994**, *35*, 7871-7874.
27. Tanaka, J.; Marriott, G.; Higa, T. and Higa, T., *J. Nat. Prod.*, **2001**, *64*, 1468-1470.
28. Manker, D.C. and Faulkner, D.J., *Tetrahedron*, **1987**, *43*, 3677-3680.
29. San-Martin, A.; Quezada, E.; Soto, P.; Palacious, Y. and Rovirosa, J., *Can. J. Chem.*, **1996**, *74*, 2741-2475.
30. Gray, C.; Davies-Coleman, M.T. and McQuaid, C., *Nat. Prod. Lett.*, **1998**, *12*, 47-53.
31. Yamamura, S. and Hirata, Y., *Tetrahedron*, **1963**, *19*, 1485.
32. Gavagnin, M.; De Napoli, A.; Cimino, G.; Iken, K.; Avila, C. and Garcia, F.J., *Tetrahedron: Asymmetry*, **1999**, *10*, 2647-2650.
33. Davies-Coleman, M.T. and Faulkner, C.J., *Tetrahedron*, **1991**, *47*, 9743-9750.
34. Dumdei, E.J.; Kubanek, J., Coleman, J.E.; Pika, J.; Andersen, R.J.; Steiner, J.R. and Clardy, J., *Can. J. Chem.*, **1997**, *75*, 773-789.
35. Kouzi, S.A. and McChesney, J.D., *J. Nat. Prod.*, **1991**, *54*, 483-490.
36. Bigley, D.B.; Rogers, N.A.J. and Barltrop, J.A., *J. Chem. Soc.*, **1960**, *4*, 4613-4627.
37. Bailey, J.A., Vincent, G.G. and Burden, R.S., *J. Gen. Microbiol.*, **1974**, *85*, 57.
38. Ulubelen, A.; Miski,M.; Johansson, C.; Lee, E.; Mabry, T.J. and Matlin, S.A., *Phytochemistry*,

1985, *24*, 1386.

39. Dimas, K.; Kokkinopoulos, D.; Demetzos, C.; Vaos, B.; Marselos, M.; Malamas, M. and Tzavaras, T., *Leukemia Research*, **1999**, *23*, 217-234.
40. Barrero, A.F.; Manzaneda, E.A.; Altarejos, J.; Salido, S.; Ramos, J.M.; Simmonds, M.S.J. and Blaney, W.M., *Tetrahedron*, **1995**, *51*, 7435-7450.
41. Hamann, M.T.; Scheuer, P.J. and Kelly-Borges, M.J., *J. Org. Chem.*, **1993**, *58*, 6565-6569.
42. Nasu, S.S.; Yeung, B.K.S.; Hamann, M.T.; Scheuer, P.J.; Kelly-Borges, M. and Goins, K., *J. Org.Chem.*, **1995**, *60*, 7290-7292.
43. Barrero, A.F.; Alvarez-Manzaneda, E.J. and Chaboun, R., *Tetrahedron Lett.*, **1997**, *38*, 2325-2328.
44. Chackalamannil,S.; Xia, Y.; Wang, Y.; Tsai, M.; Czarniecki, M.; Nang, S.; Clemmons, A.; Ahn, H.S. and Boykow, G., *Bioorg. Med. Chem. Lett.*, **1995**, *5*, 2005.
45. Marotti, K.R.; Castle, C.K.; Boyle, T.P.; Lin, A.H.; Murray, R.W. and Melchior, G.W., *Nature*, **1993**, *73*, 364.
46. Barrerro, A.F.; Alvarez-Manzaneda, E.J. and Chahboun, R., *Tetrahedron*, **1998**, *54*, 5635-5650.
47. Barrerro, A.F.; Alvarez-Manzaneda, E.J.; Mar Herrador, M. and Chahboun, R., Bioorg. *Med. Chem. Lett.*, **1999**, *9*, 2325-2328.
48. Barrero, A.F.; Alvarez-Manzaneda, E.A. and Chahboun, R., *Tetrahedron Lett.*, **1997**, *38*, 8101-8104.
49. Razmilic, I.; Sierra, I.; López, J. and Cortès, M., *Chem. Lett.*, **1985**, 1113-1114.
50. Barrero, A.F.; Alvarez-Manzaneda, E.J.; Chahboun, R.; Cortés, M. and Armstrong, V., *Tetrahedron*, **1999**, 15181-15208.
51. Popa, D.P.; Salei, L.A.; Titov, V.V. and Lazur'evskii, G.V., *Zhurnal Obshchei Khimii*, **1970**, *40*, 1413-1417.
52. Leite, M.A.F.; Sarragiotto, M.H.; Imamura, P.M. and Marsaioli, A.J., *J. Org. Chem.*, **1996**, *51*, 5409-5410.
53. Bigley, D.B.; Rogers, N.A.J. and Baltrop, J.A., *J. Chem. Soc.*, **1960**, *4*, 4613-4627.
54. Ruzicka, L.; Seidel C.F. and Engel, L.L., *Helv. Chim. Acta.*, **1942**, *25*, 621-630.
55. Barrero, A.F.; Alvarez-Manzaneda, E.J.; Altajeros, J.; Salido, S. and Ramos, J.M., *Tetrahedron*,

1993, *49*, 10405-10412.

56. Baltrop, J.A.; Littlehailes, J.D.; Rushton, J.D. and Rogers, N.A.J., *Tetrahedron Lett.*, **1962**, 429-432.
57. Hinder, M. and Stoll, M., *Helv. Chim.Acta.*, **1953**, *36*, 1984-1995.
58. Dale, J.A. and Mosher, H.S., *J. Am. Chem. Soc.*, **1973**, *95*, 512-519.
59. Ohtani, I.; Kusumi, T.; Kashman, Y. and Kakisawa, H., *J. Am. Chem. Soc.*, **1991**, *113*, 4092-4096.
60. Gray, C.A.; Davies-Coleman, M.T. and Schleyer, M.H., *J. Nat. Prod.*, **2000**, *63*, 1551-1553.
61. Corey, E.J. and Venkateswarlu, A., *J. Am. Chem. Soc.*, **1972**, *94*, 6190-6191.
62. Barrero, A.F.; Alvarez-Manzaneda, E.J. and Chahboun, R., *Tetrahedron Lett.*, **1997**, *38*, 2325-2328.
63. March, J., *Advanced Organic Chemistry*, **1992**, *4th Edition*, John Wiley & Sons, New York, pp 998.
64. McCreadle, T. and Overton, K.H., *J. Chem. Soc.*, **1971**, *C*, 312-316.
65. Barton, D.H.R.; Taylor, D.K. and Tse, C., *Tetrahedron Lett.*, **1994**, *51*, 9505-9508.
66. Smith, M.B., *Organic Synthesis*, **1994**, McGraw-Hill, New York, pp 306-310.
67. Barrero, A.F.; Alvarez-Manzaneda, M.J. and Chahboun, R., *Tetrahedron Lett.*, **1997**, *46*, 8101-8104.
68. Barrero, A.F.; Manzaneda, M.J.; Altarejos, J.; Salido, S.; Ramos, J.M.; Simmonds, M.S.J. and Planey, W.M., *Tetrahedron Lett.*, **1995**, *51*, 7435-7450.
69. Barrero, A.F.; Alvarez-Manzaneda, E.J.; Chahboun, R. and Paiz, M.C., *Tetrahedron Lett.*, **1998**, *39*, 9543-9544.
70. Beukes, D.R and Davies-Coleman, M.T., *Tetrahedron*, **1999**, *55*, 4051-4056.
71. Francis, M.J., Ph.D. Thesis, University of Otago, 1975.
72. Grant, P.K.; Hanton, L.R.; Lynch, G.P.; Robinson, W.T. and Wong, G., *Aust. J. Chem.*, **1994**, *47*, 71-90.
73. Godfrey, I.M.; Knox, J.R.; Raston, C.L. and White, A.H., *Aust. J. Chem.*, **1979**, *32*, 205-209.
74. Bhushan, V.; Rathore, R. and Chandrasekaran, S., *Synthesis*, **1984**, 431-433.
75. Chauvet, F.; Coste-Maniere, I.; Martres, P.; Perfetti, P.; Waegell, B. and Zahra, J., *Tetrahedron*

Lett., **1996**, *37*, 3695-3696.

76. Krishnamurthy, S.; Schubert, R.M. and Brown, H.C., *J. Am. Chem. Soc.*, **1973**, *95*, 8486-8487.
77. Benkeser, R.A.; Rappa, A. and Wolsieffer, L.A., *J. Org. Chem.*, **1986**, *51*, 3391-3393.
78. Grant, P.K. and Weavers, R.T., *Tetrahedron*, **1974**, *30*, 23885-2395.
79. Nagarkatti J. P and Ashley, K. R.. *Tetrahedron Lett.*, **1973**, 4599.
80. Martres, P.; Perfetti, P.; Zahra, J. and Waegell, B., *Tetrahedron Lett.*, **1994**, *35*, 97-98.
81. Scheidegger, U.; Schaffner, K, and Jeger, O., *Helv. Chim. Acta*, **1962**, *48*, 401-435.
82. Schenk, H.R.; Gutmann, H; Jeger, O. and Ruzicka, L., *Helv. Chim. Acta*, **1954**, *65*, 543-546.
83. MacDonald, T.L. and Still, W.C., *J. Am. Chem. Soc.*, **1975**, *97*, 5280-5281.
84. Maruoka, K.; Itoh, T. and Yamamoto, H., *J. Am. Chem. Soc.*, **1985**, *107*, 4573-4576.
85. Kaye, P.T., Ph.D. Thesis, University of Oxford, **1979**.
86. Ashby, E.C. and Willard, G.F., *J. Org. Chem.*, **1978**, *43*, 4094-4098.
87. San Feliciano, A.; Medarde, M.; Lopez, J.L.; Miguel de Corral, J.M.; Puebla, P. and Barrero, A.F., *Phytochemistry*, **1988**, *27*, 2241-2248.
88. Hugel, G.; Oehlsschlager, A.C. and Ourisson, G., *Tetrahedron*, **1966**, Suppl. 8, 203-216.
89. Barrero, A.F.; Alvarez-Manzaneda, E.J.; Chahboun, R.; Cortés, M. and Armstrong, V., *Tetrahedron*, **1999**, 15181-15208.
90. Barrero, A.F.; Alvarez-Manzaneda, E.J.; Mar Herrador, M.; Chahboun, R. and Galera, P., *Bio. Med. Chem. Lett.*, **1999**, *9*, 2325-2328.
91. Jung, E., *J. Org. Chem.*, **1976**, *41*, 1478-1482.
92. Corey, E.J. and Schmidt, G., *Tetrahedron*, **1979**, *5*, 399-402.
93. Ley, S.V.; Norman, J.; Griffith, W.P. and Marsden, S.P., *Synthesis*, **1994**, 639-665.
94. Krafft, M.E.; Crooks, W.J.; Zorc, B. and Milczanowski, S.E., *J. Org. Chem.*, **1988**, *53*, 3158-3163.
95. Collins, J.C.; Hess, H.W. and Frank, F.J., *Tetrahedron Lett.*, **1968**, 3363-3366.
96. Mancuso, A.J. and Swern,D., *Synthesis*, **1981**, 165-185.
97. McPhail, K.A., Ph.D. Thesis, Rhodes University, **2000**.
98. Horner, L.; Hoffman, H.; Wippel, H.G. and Klahre, G., *Chem. Ber.*, **1959**, *92*, 2499-2505.
99. Wadsworth, W.S. and Emmons, W.D., *J. Am. Chem. Soc.*, **1961**, *83*, 1733-1738.

100.Maryanoff, B.E. and Reitz, A.B., *Chem. Rev.*, **1989**, *89*, 863-927.

101.Boutagy, J. and Thomas, R., *Chem. Rev.*, **1974**, *74*, 87-99.

102.Kelly, S.E., *Comp. Org. Synth.*, Volume 1, **1991**, Pergamon Press, Oxford, 761-773.

103.Bates, R.B. and Gale, D.M., *J. Amer. Chem. Soc.*, **1960**, *82*, 5749-5751.

104.Luly, J.R.; Hsiao, C.; BaMaung, N. and Plattner, J.J., *J. Org. Chem.*, **1988**, *53*, 6109-6112.

105.Perrin, D.D. and Armarego, W.L.F., *Purification of Laboratory Chemicals*, 3rd Edition, **1988**, Wheaton and Co. Ltd, Exeter.

APPENDIX

Table 8 : Sample and crystal data for ambraketal (**83**).

Crystallization solvents	Ethyl acetate
Crystallization method	Slow evaporation
Empirical Formula	$C_{18}H_{30}O_2$
Temperature (K)	173
Wavelength (Å)	0.71073
Crystal Size (mm)	0.06 x 0.32 x 0.41
Crystal System	Monoclinic
Space group	C2 (No. 5)
Unit cell dimensions	a = 13.916(2)
	b = 6.0133(11)
	c = 19.703(4)
Volume	1566.1(5)
Z	4
Density (calculated) [g/cm^3]	1.181
Absorption coefficient (cm^{-1})	0.7
F(000)	616
Diffractometer	Nonius Kappa CCD
Data collection method (Type and range)	0.5° scans around j and w
Theta range for data collection	2.9° to 25.4°
Index ranges	-16: 13 ; -7: 7 ; -19: 23
Nref, Npar	2093, 185
Observed data [I > 2.0σ(I)]	1532
Minimum and Maximum residual density [e/Å^3]	-0.22, 0.17
Function minimized	w = 1/[σ2(Fo2)+(0.0416P)2+0.7404P]
	where P = (Fo^2+2Fc^2)/3
Data/Restraints/Parameters	2723, 2093, 0.025
R, wR, S	0.0514, 0.1152, 1.11

Table 9 : Atomic co-ordinates and equivalent isotropic thermal parameters for the non-hydrogen atoms for ambraketal (**83**).

Atom	x	y	z	U(eq) [Å^2]
O19	0.1374(19)	0.3760(3)	0.5526(14)	0.0419(9)
O20	0.0994(16)	0.6688(3)	0.6114(12)	0.0328(8)
C1	0.2874(3)	0.3734(6)	0.8444(2)	0.0387(14)
C2	0.2859(3)	0.2739(7)	0.9153(2)	0.0466(14)
C3	0.1918(3)	0.3434(6)	0.9330(2)	0.0422(14)
C4	0.0926(3)	0.2871(6)	0.8753(19)	0.0337(12)
C5	0.0987(2)	0.3805(5)	0.8027(19)	0.0291(11)
C6	0.0028(3)	0.3503(6)	0.7401(19)	0.0345(12)
C7	0.0025(3)	0.5088(6)	0.6794(19)	0.0342(12)
C8	0.0935(3)	0.4770(5)	0.6552(19)	0.0273(11)
C9	0.1931(2)	0.4719(5)	0.7172(18)	0.0275(11)
C10	0.1934(2)	0.3191(5)	0.7815(2)	0.0291(12)
C11	0.2817(3)	0.4411(5)	0.6882(2)	0.0380(14)
C12	0.2739(3)	0.6020(6)	0.6266(2)	0.0393(14)
C13	0.1670(3)	0.6008(6)	0.5743(2)	0.0345(12)
C14	0.1528(3)	0.7479(6)	0.5104(2)	0.0435(14)
C15	0.0835(3)	0.2942(6)	0.5991(2)	0.0396(12)
C16	0.1984(3)	0.0709(5)	0.7632(2)	0.0419(14)
C17	0.0715(3)	0.0369(6)	0.8747(2)	0.0449(14)
C18	0.0067(3)	0.4056(6)	0.8943(2)	0.0474(17)

U(eq) = 1/3 of the trace of the orthogonalized U Tensor

Table 10 : Hydrogen atom positions and isotropic thermal parameters for ambraketal (**83**).

Atom	x	y	z	U(iso) [Å²]
H5	0.10420	0.54510	0.81010	0.0350
H9	0.20120	0.62630	0.73710	0.0330
H11	0.34790	0.31770	0.83350	0.0460
H12	0.29380	0.53700	0.84960	0.0460
H21	0.28820	0.10970	0.91260	0.0560
H22	0.34670	0.32360	0.95390	0.0560
H31	0.19230	0.27010	0.97810	0.0510
H32	0.19450	0.50600	0.94130	0.0510
H61	-0.00150	0.19490	0.72290	0.0420
H62	-0.05690	0.38010	0.75580	0.0420
H71	-0.05940	0.48350	0.63850	0.0410
H72	0.00100	0.66410	0.69570	0.0410
H111	0.34600	0.46740	0.72690	0.0450
H112	0.28220	0.28620	0.67120	0.0450
H121	0.29150	0.75400	0.64560	0.0470
H122	0.32240	0.55760	0.60150	0.0470
H141	0.08280	0.73650	0.47890	0.0650
H142	0.19890	0.70080	0.48430	0.0650
H143	0.16750	0.90230	0.52610	0.0650
H151	0.01140	0.26770	0.57200	0.0480
H152	0.11360	0.15330	0.62210	0.0480
H161	0.20970	-0.01850	0.80660	0.0630
H162	0.25430	0.04700	0.74360	0.0630
H163	0.13460	0.02640	0.72780	0.0630
H171	0.05150	-0.00220	0.91660	0.0680

H172	0.01680	-0.00150	0.83120	0.0680
H173	0.13280	-0.04550	0.87590	0.0680
H181	-0.05860	0.35330	0.86260	0.0710
H182	0.01170	0.37280	0.94400	0.0710
H183	0.01230	0.56650	0.88840	0.0710

Table 11 : Anisotropic thermal parameters for ambraketal (**83**).

Atom	U(1,1) or	U(2,2)	U(3,3)	U(2,3)	U(1,3)	U(1,2)
O19	0.0531(17)	0.0328(15)	0.0461(17)	-0.0080(12)	0.0245(15)	-0.0103(12)
O20	0.0311(14)	0.0296(13)	0.0420(15)	0.0011(12)	0.0175(13)	-0.0006(11)
C1	0.032(2)	0.037(2)	0.045(3)	0.0039(19)	0.009(2)	-0.0018(18)
C2	0.036(2)	0.050(2)	0.045(3)	0.000(2)	0.000(2)	0.0012(19)
C3	0.056(3)	0.031(2)	0.036(2)	0.0013(18)	0.009(2)	-0.0012(18)
C4	0.034(2)	0.031(2)	0.038(2)	0.0001(18)	0.014(2)	0.0017(16)
C5	0.029(2)	0.0210(18)	0.037(2)	0.0020(16)	0.010(2)	0.0006(14)
C6	0.032(2)	0.036(2)	0.037(2)	0.0053(17)	0.013(2)	0.0012(16)
C7	0.026(2)	0.038(2)	0.038(2)	0.0031(19)	0.0093(19)	0.0023(16)
C8	0.026(2)	0.0241(18)	0.033(2)	0.0027(16)	0.0109(19)	-0.0018(15)
C9	0.025(2)	0.0219(18)	0.038(2)	-0.0028(17)	0.0134(19)	-0.0018(14)
C10	0.025(2)	0.023(2)	0.038(2)	-0.0001(16)	0.0078(19)	0.0005(14)
C11	0.029(2)	0.040(2)	0.044(3)	-0.0022(18)	0.010(2)	-0.0037(16)
C12	0.030(2)	0.044(2)	0.048(3)	-0.0016(19)	0.018(2)	-0.0048(17)
C13	0.033(2)	0.035(2)	0.040(2)	0.0008(17)	0.018(2)	-0.0053(16)
C14	0.040(2)	0.051(3)	0.043(2)	-0.002(2)	0.018(2)	-0.0070(18)
C15	0.040(2)	0.034(2)	0.046(2)	-0.005(2)	0.015(2)	-0.0104(17)
C16	0.045(2)	0.023(2)	0.057(3)	-0.0019(17)	0.015(2)	-0.0013(17)
C17	0.047(2)	0.038(2)	0.051(3)	0.005(2)	0.017(2)	-0.0067(19)
C18	0.054(3)	0.055(3)	0.039(3)	0.004(2)	0.023(2)	0.005(2)

Table 12 : Bond Distances (Å) for ambraketal (**83**).

O19	-C13	1.439(4)	C3	-H31	0.9900
O19	-C15	1.440(5)	C3	-H32	0.9901
O20	-C8	1.457(4)	C5	-H5	1.0000
O20	-C13	1.420(5)	C6	-H61	0.9894
C1	-C2	1.526(5)	C6	-H62	0.9893
C1	-C10	1.530(5)	C7	-H71	0.9898
C2	-C3	1.515(6)	C7	-H72	0.9901
C3	-C4	1.527(6)	C9	-H9	1.0005
C4	-C5	1.565(5)	C11	-H111	0.9901
C4	-C17	1.532(5)	C11	-H112	0.9906
C4	-C18	1.535(6)	C12	-H121	0.9891
C5	-C6	1.518(5)	C12	-H122	0.9900
C5	-C10	1.546(4)	C14	-H141	0.9801
C6	-C7	1.529(5)	C14	-H142	0.9811
C7	-C8	1.499(6)	C14	-H143	0.9798
C8	-C9	1.535(5)	C15	-H151	0.9910
C8	-C15	1.533(5)	C15	-H152	0.9899
C9	-C10	1.564(5)	C16	-H161	0.9802
C9	-C11	1.524(5)	C16	-H162	0.9812
C10	-C16	1.542(4)	C16	-H163	0.9793
C11	-C12	1.529(5)	C17	-H171	0.9788
C12	-C13	1.522(6)	C17	-H172	0.9794
C13	-C14	1.500(5)	C17	-H173	0.9804
C1	-H11	0.9901	C18	-H181	0.9797
C1	-H12	0.9902	C18	-H182	0.9798

C2	-H21	0.9899	C18	-H183	0.9805
C2	-H22	0.9905			

Table 13 : Bond Angles (°) for ambraketal (**83**).

C13	-O19	-C15	106.8(3)	C5	-C10	-C9	106.9(2)
C8	-O20	-C13	104.2(2)	C5	-C10	-C16	113.5(3)
C2	-C1	-C10	113.9(3)	C9	-C10	-C16	111.6(3)
C1	-C2	-C3	111.3(3)	C9	-C11	-C12	110.8(3)
C2	-C3	-C4	114.4(3)	C11	-C12	-C13	110.3(3)
C3	-C4	-C5	107.8(3)	O19	-C13	-O20	104.4(3)
C3	-C4	-C17	110.5(3)	O19	-C13	-C12	109.5(3)
C3	-C4	-C18	107.9(3)	O19	-C13	-C14	110.7(3)
C5	-C4	-C17	114.2(3)	O20	-C13	-C12	108.4(3)
C5	-C4	-C18	109.0(3)	O20	-C13	-C14	109.1(3)
C17	-C4	-C18	107.2(3)	C12	-C13	-C14	114.2(3)
C4	-C5	-C6	114.5(3)	O19	-C15	-C8	105.4(3)
C4	-C5	-C10	117.1(3)	C2	-C1	-H11	108.78
C6	-C5	-C10	111.1(3)	C2	-C1	-H12	108.75
C5	-C6	-C7	110.3(3)	C10	-C1	-H11	108.77
C6	-C7	-C8	112.0(3)	C10	-C1	-H12	108.81
O20	-C8	-C7	107.3(3)	H11	-C1	-H12	107.67
O20	-C8	-C9	106.5(3)	C1	-C2	-H21	109.39
O20	-C8	-C15	98.7(3)	C1	-C2	-H22	109.37
C7	-C8	-C9	113.0(3)	C3	-C2	-H21	109.40
C7	-C8	-C15	115.2(3)	C3	-C2	-H22	109.40
C9	-C8	-C15	114.5(3)	H21	-C2	-H22	107.94
C8	-C9	-C10	115.5(3)	C2	-C3	-H31	108.65
C8	-C9	-C11	109.8(3)	C2	-C3	-H32	108.63
C10	-C9	-C11	115.8(3)	C4	-C3	-H31	108.68

C1	-C10	-C5	108.4(3)	C4	-C3	-H32	108.71
C1	-C10	-C9	108.4(3)	H31	-C3	-H32	107.59
C1	-C10	-C16	107.8(3)	C4	-C5	-H5	104.15
C6	-C5	-H5	104.19	C13	-C14	-H143	109.51
C10	-C5	-H5	104.09	H141	-C14	-H142	109.34
C5	-C6	-H61	109.56	H141	-C14	-H143	109.53
C5	-C6	-H62	109.60	H142	-C14	-H143	109.44
C7	-C6	-H61	109.61	O19	-C15	-H151	110.66
C7	-C6	-H62	109.56	O19	-C15	-H152	110.71
H61	-C6	-H62	108.14	C8	-C15	-H151	110.60
C6	-C7	-H71	109.25	C8	-C15	-H152	110.72
C6	-C7	-H72	109.20	H151	-C15	-H152	108.73
C8	-C7	-H71	109.17	C10	-C16	-H161	109.48
C8	-C7	-H72	109.25	C10	-C16	-H162	109.45
H71	-C7	-H72	107.93	C10	-C16	-H163	109.50
C8	-C9	-H9	104.87	H161	-C16	-H162	109.41
C10	-C9	-H9	104.84	H161	-C16	-H163	109.50
C11	-C9	-H9	104.75	H162	-C16	-H163	109.48
C9	-C11	-H111	109.53	C4	-C17	-H171	109.47
C9	-C11	-H112	109.46	C4	-C17	-H172	109.46
C12	-C11	-H111	109.45	C4	-C17	-H173	109.45
C12	-C11	-H112	109.44	H171	-C17	-H172	109.47
H111	-C11	-H112	108.08	H171	-C17	-H173	109.45
C11	-C12	-H121	109.67	H172	-C17	-H173	109.53
C11	-C12	-H122	109.61	C4	-C18	-H181	109.48
C13	-C12	-H121	109.55	C4	-C18	-H182	109.43
C13	-C12	-H122	109.59	C4	-C18	-H183	109.42
H121	-C12	-H122	108.15	H181	-C18	-H182	109.53

C13	-C14	-H141	109.52	H181	-C18	-H183	109.48
C13	-C14	-H142	109.48	H182	-C18	-H183	109.49

Table 14 : Torsion Angles (°) for ambraketal (**83**).

C15	-O19	-C13	-O20	-21.6(4)
C15	-O19	-C13	-C12	94.3(4)
C15	-O19	-C13	-C14	-138.9(3)
C13	-O19	-C15	-C8	-6.1(4)
C13	-O20	-C8	-C7	-164.0(3)
C13	-O20	-C8	-C9	74.8(3)
C13	-O20	-C8	-C15	-44.1(3)
C8	-O20	-C13	-O19	42.5(3)
C8	-O20	-C13	-C12	-74.2(3)
C8	-O20	-C13	-C14	160.9(3)
C10	-C1	-C2	-C3	-55.5(4)
C2	-C1	-C10	-C16	-71.6(4)
C2	-C1	-C10	-C9	167.4(3)
C2	-C1	-C10	-C5	51.7(4)
C1	-C2	-C3	-C4	55.9(4)
C2	-C3	-C4	-C5	-51.8(4)
C2	-C3	-C4	-C17	73.7(4)
C2	-C3	-C4	-C18	-169.4(3)
C3	-C4	-C5	-C6	-176.5(3)
C17	-C4	-C5	-C10	-72.3(4)
C18	-C4	-C5	-C6	-59.6(4)
C18	-C4	-C5	-C10	167.8(3)
C17	-C4	-C5	-C6	60.3(4)
C3	-C4	-C5	-C10	50.9(4)
C10	-C5	-C6	-C7	-63.3(4)
C4	-C5	-C6	-C7	161.3(3)

C4	-C5	-C10	-C16	68.8(4)
C6	-C5	-C10	-C1	174.8(3)
C6	-C5	-C10	-C9	58.1(3)
C6	-C5	-C10	-C16	-65.4(4)
C4	-C5	-C10	-C1	-51.0(4)
C4	-C5	-C10	-C9	-167.7(3)
C5	-C6	-C7	-C8	57.1(4)
C6	-C7	-C8	-C9	-48.7(4)
C6	-C7	-C8	-O20	-165.8(3)
C6	-C7	-C8	-C15	85.6(4)
O20	-C8	-C9	-C11	-62.0(3)
C7	-C8	-C9	-C10	47.4(4)
C15	-C8	-C9	-C10	-87.2(4)
C15	-C8	-C9	-C11	45.9(4)
C7	-C8	-C9	-C11	-179.5(3)
O20	-C8	-C9	-C10	164.9(3)
C9	-C8	-C15	-O19	-82.4(3)
C7	-C8	-C15	-O19	144.0(3)
O20	-C8	-C15	-O19	30.3(4)
C8	-C9	-C10	-C5	-50.5(3)
C8	-C9	-C10	-C1	-167.2(3)
C11	-C9	-C10	-C5	179.2(3)
C8	-C9	-C10	-C16	74.1(4)
C11	-C9	-C10	-C1	62.5(4)
C10	-C9	-C11	-C12	-179.0(3)
C11	-C9	-C10	-C16	-56.2(4)
C8	-C9	-C11	-C12	48.1(3)

C9	-C11	-C12	-C13	-46.6(4)
C11	-C12	-C13	-C14	-177.9(3)
C11	-C12	-C13	-O19	-53.1(4)

Table 15 : Contact Distances (Å) for ambraketal (**83**).

O19	.H112	2.6210	C16	.H21	2.8250
O19	.H151_a	2.7491	C16	.H61	2.7465
O19	.H142_b	2.8043	C16	.H9_f	2.7251
O20	.H141_a	2.6336	C16	.H152	2.7047
C6	. C12_c	3.595(6)	C16	.H173	2.7426
C12	.C6_d	3.595(6)	C16	.H112	2.7648
C15	.C16	3.403(5)	C17	.H21	2.9043
C16	.C15	3.403(5)	C17	.H61	2.9967
C16	.C17	3.227(6)	C17	.H161	2.6877
C17	.C16	3.227(6)	C17	.H183_f	2.9819
C1	.H111	2.7432	C18	.H62	2.5967
C2	.H173	2.7922	H5	.C8	3.0375
C2	.H161	2.7171	H5	.H9	2.3116
C4	.H161	3.0408	H5	.H12	2.5071
C6	.H163	2.7376	H5	.H32	2.5070
C6	.H172	2.7416	H5	.H72	2.3729
C6	.H181	2.8010	H5	.H183	2.2948
C6	.H121_c	3.0040	H9	.C13	3.0958
C8	.H163	3.0341	H9	.C16_g	2.7251
C8	.H5	3.0375	H9	.H5	2.3116
C11	.H162	2.6858	H9	.H12	2.2529
C11	.H11	2.8188	H9	.H161_g	2.5199
C11	.H152	2.8737	H9	.H163_g	2.5641
C13	.H9	3.0958	H11	.C11	2.8188
C14	.H122_e	2.9873	H11	.H111	2.2777
C15	.H61	3.0838	H11	.H162	2.4578

C15	.H112	2.6850	H12	.H5	2.5071
C15	.H163	2.8985	H12	.H9	2.2529
H21	.C16	2.8250	H112	.H162	2.1450
H21	.C17	2.9043	H121	.C6_d	3.0040
H21	.H161	2.1732	H121	.H71_d	2.5319
H21	.H173	2.2566	H122	.H142	2.5584
H31	.H171	2.5502	H122	.C14_b	2.9873
H31	.H182	2.4684	H141	.O20_a	2.6336
H31	.H32_h	2.4415	H142	.H122	2.5584
H32	.H5	2.5070	H142	.O19_e	2.8043
H32	.H183	2.4484	H151	.H71	2.2757
H32	.H31_i	2.4415	H151	.O19_a	2.7491
H61	.C15	3.0838	H152	.C11	2.8737
H61	.C16	2.7465	H152	.C16	2.7047
H61	.C17	2.9967	H152	.H112	2.3790
H61	.H163	2.1234	H152	.H163	2.1504
H61	.H172	2.3818	H161	.C2	2.7171
H61	.H111_c	2.5468	H161	.C4	3.0408
H62	.C18	2.5967	H161	.C17	2.6877
H62	.H181	2.1179	H161	.H9_f	2.5199
H71	.H151	2.2757	H161	.H21	2.1732
H71	.H121_c	2.5319	H161	.H173	1.9874
H72	.H5	2.3729	H162	.C11	2.6858
H111	.C1	2.7432	H162	.H11	2.4578
H111	.H11	2.2777	H162	.H112	2.1450
H111	.H61_d	2.5468	H163	.C6	2.7376
H112	.O19	2.6210	H163	.C8	3.0341

H112	.C15	2.6850	H163	.C15	2.8985
H112	.C16	2.7648	H163	.H9_f	2.5641
H112	.H152	2.3790	H163	.H61	2.1234
H163	.H152	2.1504	H181	.C6	2.8010
H171	.H31	2.5502	H181	.H62	2.1179
H171	.H182	2.4228	H181	.H172	2.5380
H172	.C6	2.7416	H182	.H31	2.4684
H172	.H61	2.3818	H182	.H171	2.4228
H172	.H181	2.5380	H182	.H182_j	2.3292
H173	.C2	2.7922	H183	.C17_g	2.9819
H173	.C16	2.7426	H183	.H5	2.2948
H173	.H21	2.2566	H183	.H32	2.4484
H173	.H161	1.9874			

Translation of Symmetry Code to Equivalent Position

a =[2556.00] = -x,y,1-z

b =[4546.00] = 1/2-x,-1/2+y,1-z

c =[3445.00] = -1/2+x,-1/2+y,z

d =[3555.00] = 1/2+x,1/2+y,z

e =[4556.00] = 1/2-x,1/2+y,1-z

f =[1545.00] = x,-1+y,z

g =[1565.00] = x,1+y,z

h =[4547.00] = 1/2-x,-1/2+y,2-z

i =[4557.00] = 1/2-x,1/2+y,2-z

j =[2557.00] = -x,y,2-z