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SYNTHESIS OF INHIBITORS OF 2,3-OXIDOSQUALENE-LANOSTEROL CYCLASE

By

Dharmpal Singh Dodd

B. Sc. University of British Columbia, 1987

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Abstract

2,3-Oxidosqualene-lanosterol cyclase (OSC) is suspected to bind (3S)-2,3-oxidosqualene in a chair-boat-chair conformation. It then mediates the sequential formation of four new ring forming bonds through a series of rigidly-held carbocationic intermediates producing a protosterol intermediate which is subsequently transformed by the enzyme *via* a series of hydride and methyl migrations to lanosterol, the precursor to cholesterol in mammals and cycloartenol in photosynthetic plants. This thesis describes the synthesis of two types of "mechanism-based" inhibitors of OSC, the structures of which should allow the existence of three of the presumptive cationic intermediates to be probed. The first group of inhibitors are aza-monocyclic analogues of the monocyclic cation postulated to be initially formed in the cyclization. The second group of OSC inactivators are 9-thia and 16-thia 2,3-oxidosqualenes, which were prepared to examine the existence of presumptive bicyclic and tricyclic cationic intermediates, respectively.

The synthesis of the aza-monocyclic mimics involved the application of two different methodologies. The first involved the conjugate addition of organocuprate reagents to 5,6-dihydro-4-pyridones to give C2-C3 substituted 4-piperidones and the second involved cyclocondensation of C2 substituted Nazarov's reagent with imines to give C2, C3 substituted 4-piperidones. The substituted 4-piperidones were then transformed to a structurally related group of azacyclic compounds that were evaluated as inhibitors on yeast and pig-liver OSC's. The best inhibitor exhibited an IC_{50} of 0.23 μM against *C. albicans* OSC. This inhibitor contained all the structural features of the intermediate it was designed to mimic except that the cationic center in the inhibitor was a tertiary nitrogen.

Synthesis of all *trans* 9-thia and 16-thia, 2,3-oxidosqualenes involved the preparation of novel sulfur substituted Wittig-Horner reagents (α-thioterpenoidyldiphenylphosphine oxides). These reagents were condensed with the appropriate aldehydes at low temperature to generate α-hydroxy diphenylphosphinoyl adducts as mixtures of *erythro/threo* diastereoisomers. The diastereoisomers were separated by chromatography and the *erythro* diastereoisomers transformed to the vinyl-thia (*E*) double bond.

DEDICATION

This thesis is dedicated my parents
Sardar Rattan Singh and Sardarni Swarn Kaur Dodd
and to my wife Baljit

ACKNOWLEDGEMENT

I would like to thank Professor A. C. Oehlschlager for allowing me the opportunity to work in his laboratory. I am grateful to him for giving me total control over the formulation of the synthetic strategies, the execution of the synthesises and the preparation of the resulting manuscripts.

The assistance of those post-doctoral fellows, research associates, graduate-students and technicians that have contributed to the success of my projects is very much appreciated. I am very grateful to Albert Zheng and Alice Perez for their assistance in the preparation of my defence seminar. I will fondly remember our numerous discussions and the exchange of research ideas. I am particularly indebted to Dr. S. Mohan Singh for both academic guidance and moral support throughout my Ph. D. Program.

I also wish to acknowledge Dr. N. Georgopapadakou at Hoffmann-La Roche Ltd in New Jersey and Drs. A.-M. Polak and P. G. Hartman at Hoffmann-La Roche Ltd. in Basel, Switzerland for providing us the results of the biological evaluation of the aza-analogues (Chapter 3) as inhibitors of 2,3-oxidosqualene-lanosterol cyclase of *Candida albicans*. I also wish to thank Alice Perez for carrying out the inhibition study on these analogues on pig-liver 2,3-oxidosqualene-lanosterol cyclase.

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List of Abbreviations

Ac_2O	acetic anhydride
b	broad (NMR, IR)
n-Bu_4NF	tetrabutylammonium fluoride
CI	chemical ionization (mass spectroscopy)
Cp	cyclopentadienyl
m-CPBA	*meta*-chloroperbenzoic acid
d	doublet (NMR)
δ	chemical shift in ppm downfield from tetramethyl silane (^{1}H NMR)
DHP	3,4-2*H*-dihydropyran
DIBAL-H	diisobutylaluminum hydride
DMAP	*N*,*N*-dimethylaminopyridine
DME	1,2-dimethoxyethane
DMF	*N*,*N*-dimethylformamide
DMS	dimethyl sulfide
EI	electron impact (mass spectroscopy)
eq.	equivalent
EtOH	ethanol
FAB	fast atom bombardment (mass spectroscopy)
g	grams
GC	gas chromatography
HMPA	hexamethylphosphoramide
H.O.	higher order (cuprates)
HRMS	high resolution mass spectroscopy
N-Imid	*N*-imidazole

IC_{50}	concentration of Inhibitor required to decrease enzymatic activity by 50% (Biological inhibition assays)
LDA	lithium diisopropylamide
m	multiplets (NMR, IR)
m/z	mass to charge ratio (mass spectroscopy)
MIC	minimum concentration of inhibitor required to completely inhibit cell growth (biological assays)
m. p.	melting point
MeOH	methanol
NBS	*N*-bromosuccinimide
NCS	*N*-chlorosuccinimide
NMR	nuclear magnetic resonance (spectroscopy)
nCe	nuclear Overhauser enhancement (NMR)
NOEDS	nuclear Overhauser enhancement difference spectroscopy
NOESY	nuclear Overhauser enhancement spectroscopy (2-D, NMR)
OSC	2,3-oxidosqualene cyclase
OTBDMS	*tert*-butyldimethylsilyloxy
Py	pyridine
q	quartet (NMR)
s	singlet (NMR)
SDS-PAGE	sodium dodecylsulfate-polyacrylamide gel electrophoresis
t	triplet

2-Th	2-thienyl
THF	tetrahydrofuran
TBDMSCl	*tert*-butyldimethylsilyl chloride
TMSCl	trimethylsilyl chloride
p-TsCl	*para*-toluenesulfonyl chloride
p-TsOH	*para*-toluenesulfonic acid

Chapter 1

I: Introduction

2,3-Oxidosqualene cyclases (OSC)[1] comprise a class of enzymes that catalyse the cyclization of (3S)-2,3-oxidosqualene (**1**) to tetracyclic sterols. Lanosterol (**2**) (Figure 1-1) is the product in fungi and mammals, whereas cycloartenol or β-amyrin are the products in photosynthetic plants[1a]. The cyclizations and rearrangements carried out by this enzyme constitute some of the most complex and fascinating biochemical transformations in nature. These unique enzymatic transformations have been the subject of intense interest for nearly four decades.[2] The goal of most work has been to understand the mechanism of these cyclizations and to harness OSC's for the preparation of novel sterols.[2-16] It is suspected that the cyclization of 2,3-oxidosqualene (**1**) to protosterol (**3**) (Figure 1-2) proceeds through a series of enzymatically stablized, conformationally rigid carbocationic intermediates.[6,9] The work presented in this thesis focuses on the synthesis of compounds designed to probe the existence of three of these cationic intermediate by inhibiting OSC in a mechanism-based manner.

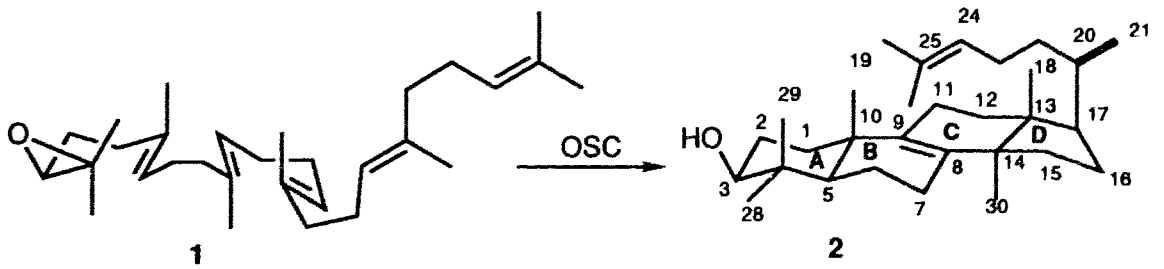


Figure 1-1: Enzymatic conversion of 2,3-oxidosqualene (**1**) to lanosterol (**2**).

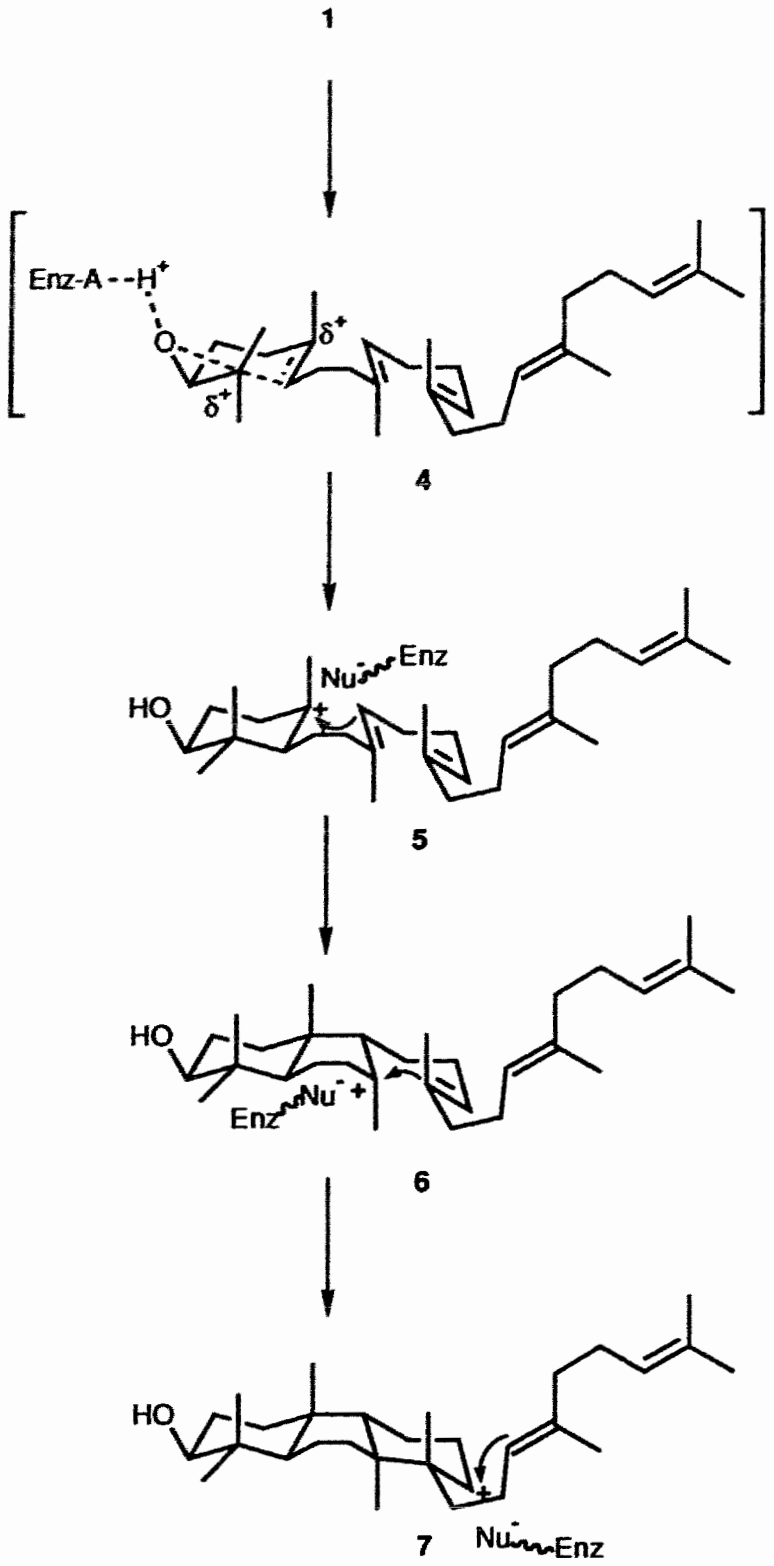


Figure 1-2: Proposed mechanism of lanosterol (**2**) biosynthesis from 2,3-oxidosqualene (**1**).

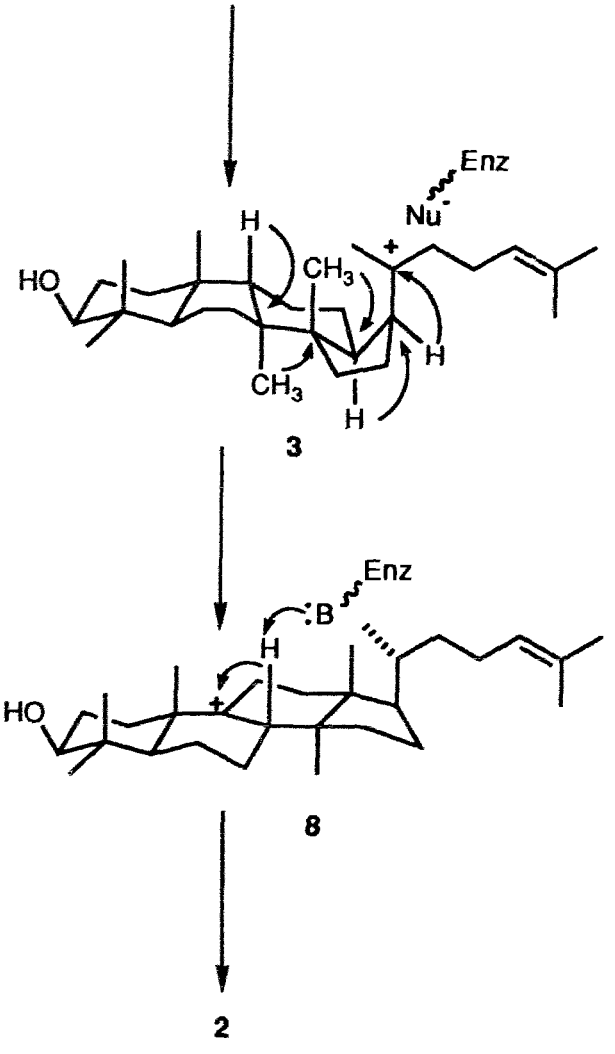


Figure 1-2: Proposed mechanism of lanosterol (**2**) biosynthesis from 2,3-oxidosqualene (**1**).

II: Lanosterol Biosynthesis

A: 2,3-Oxidosqualene-lanosterol Cyclase

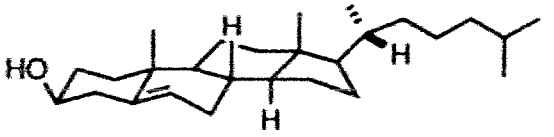
Oxidosqualene cyclases (OSC) are membrane-bound enzymes that, until recently, eluded all attempts to purify them to homogeneity. All early cyclization studies utilised crude microsomal homogenates from animal sources. Recently methods for purification of OSC from hog-liver[10,11] and other

vertebrates[11] have been reported. Optimisation of the partial purification[13] of the less stable yeast cyclase has led to the purification of 2,3-oxidosqualene-lanosterol cyclase from *Saccharomyces cerevisiae*.[14] The yeast cyclase appears to be a single polypeptide with a molecular weight of ~26 kDa[14] and has a pH optimum of 6.2[13] (*vs* 7.2 for hog-liver OSC)[10a,e] This molecular weight is substantially lower than the 75 kDa , 78 kDa and 55 kDa (from SDS-PAGE) reported for the OSC from hog-liver[11b], rat[11] and plants,[12] respectively. Yeast OSC was found to be highly negatively charged, even at low pH (<6) and required high concentrations of potassium phosphate buffer for optimum activity.[13] Apparently no cofactors are required. Hog-liver OSC requires a soluble protein factor and anionic phospholipids.[10d] Chemical modification using *N*-ethylmaleimide of purified hog-liver[10e] and yeast 2,3-oxidosqualene-lanosterol[13] cyclase suggests the presence of a thiol residue (Cysteine-SH) is required for activity of OSC. Inhibition of yeast OSC using *N*-(ethoxycarbonyl)-2-ethoxy-1,2-dihydroquinoline (EEDQ) also implicates a carboxylate residue.[13]

B: Proposed Mechanism of 2,3-Oxidosqualene Cyclization

1: Involvement of Cationic Intermediates

Mammalian OSC is suspected to bind (3S)-2,3-oxidosqualene (**1**) in a chair-boat-chair-boat (five membered ring) conformation and mediate the sequential formation of four new carbon-carbon bonds leading to the highly strained protosterol, **3**. This strain is then relieved by series hydride and methyl migrations[8] to give enzymatically stabilized carbocationic intermediate **8**. The elimination of the hydrogen from C-8, presumably by the assistance of a basic or negatively charged amino acid, then gives lanosterol (**2**)(Figure 1-2), the precursor of cholesterol (**9**) in mammals.



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Since the initial demonstration that lanosterol (**2**) was derived from 2,3-oxidosqualene[5] and not squalene[2,3] (**10**) (Figure 1-3) as previously thought, mechanism of this enzymatic cyclization has been a topic of debate. It can be viewed as a "concerted" single step process[4a-c] in which all four rings of **3** are generated in a synchronous process. This is similiar to the original proposal by Professor Eschenmoser[3a,c] for the cationic "concerted" cyclization of squalene (**10**) *via* a B-ring boat conformation giving **3**. This postulate requires that 2,3-oxidsqualene, **1**, must be initially constrained in a highly restrictive chair-boat-chair-five membered boat conformation in which the reacting centres are within the requisite distances for bond formation. This hypothesis was consistent with the observation that no products other than lanosterol (**2**) were detected from the enzymatic cyclization of 2,3-oxidosqualene, **1**.

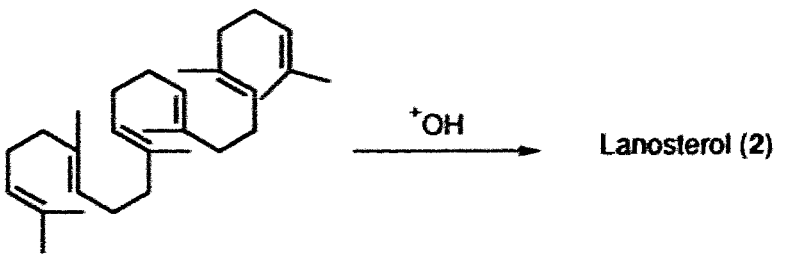

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Figure 1-3: Proposed electrophilic induced cyclization of squalene (**10**) to lanosterol (**2**)

The other hypothesis holds that the enzymatic cyclization of 2,3-oxidosqualene (**1**) to protosterol (**3**) involves the formation of enzymatically stabilized carbocationic intermediates produced during the formation of each

ring (as shown in Figure 1-2). This hypothesis, proposed by van Tamelen, has been referred to as the "stepwise" mechanism.[4c,9] This hypothesis was originally proposed by Stork[3b] to explain cationic "biomimetic" cyclizations of 1,5-polyenes.and as well as the cyclization of squalene (**10**) to lanosterol (**2**) (Figure 1-3). Work of van Tamelen's group supported this hypothesis, in part, from results obtained from the chemically induced cyclizations of **11**, **13a**, **13b**, and **13c**. These showed that (i) the ring A is formed with a high degree of neighbouring π-bond participation during S_N2-like epoxide ring opening, and (ii) the overall annulation process is not completely concerted but involves a series of conformationally rigid carbocyclic cationic intermediates.

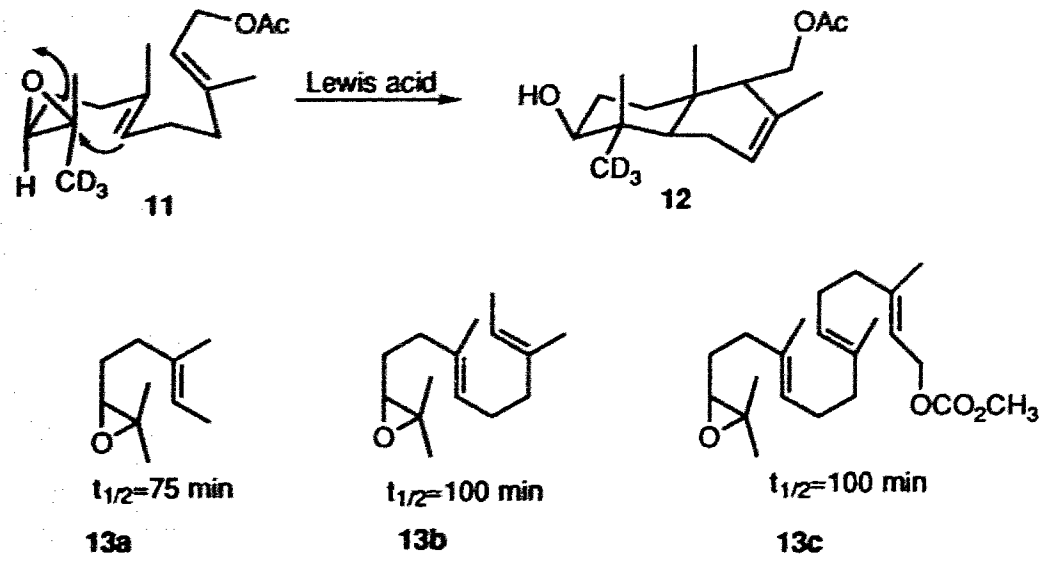

Figure 1-4: Lewis acid initiated cyclization of oxido-1,5-polyenes

To detect anchimeric assistance in the epoxide opening by the first π-bond, isotopically labelled **11** was subjected to Lewis acid catalysed cyclization (Figure 1-4).[9b] Racemic 12,12,12-trideutero-10,11-oxido-(2(*E*),6(*E*)-farnesyl acetate (**11**), in which the 10-*H* and 11-CD_3 are *cis*, cyclized to give stereospecifically trideuteromethyl hydroxydecalin (**12**). Since the isotopically

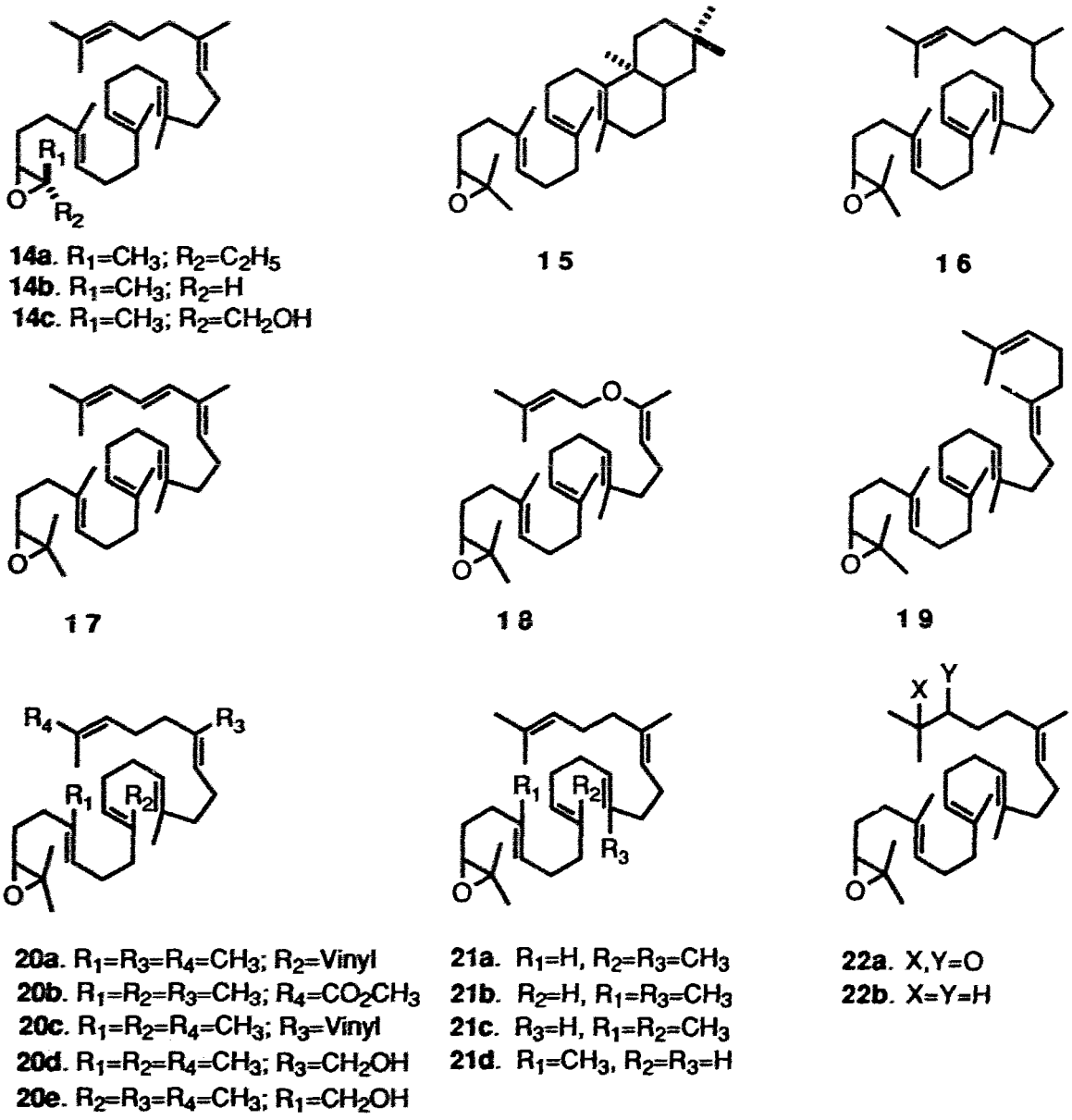

Figure 1-5: Substrates accepted and cyclized by 2,3-oxidosqualene-lanosterol cyclase (OSC).

labelled methyl group maintained its stereochemical relationship with respect to the internal double bond, this result was interpreted to confirm the concerted formation of the first ring with a S_N2-like participation of the immediate neighbouring δ,γ–π-bond. To determine if additional π-bonds significantly affected the overall rate of polycyclization, van Tamelen subjected mono-ene

epoxide **13a**, diene epoxide **13b** and triene epoxide **13c** to the Lewis acid catalysed cyclization conditions and compared the half-lives of these cyclizations. Half-lives for **13a**, **13b**, and **13c** were found to be ~75, 100 and 100 min, respectively, suggesting that the initial epoxide opening is the slowest step in the cyclization and additional π-bonds do not accelerate the rate of cyclization.

van Tamelen also subjected unnatural 2,3-oxidosqualene analogues (**14a**, **14b**, **16** figure 1-5) and partially cyclized substrate **15** to mammalian-liver OSC's mediated cyclizations.[6] These experiments revealed that a full pocket fit or the "lock and key" is not essential for either substrate binding or cyclization. This suggested that entropic control by this enzyme is minimal and the oxide-tetra-π-bond sequence of squalene constitutes the essential substrate requirement for tetracyclization. A chiral trisubstituted oxide and the adjacent two double bonds seem to represent the minimum requirement for significant cyclase action. Several cleverly designed unnatural substrates (Figure 1-5) have been shown to be accepted by OSC from yeast and animals.[6,7]

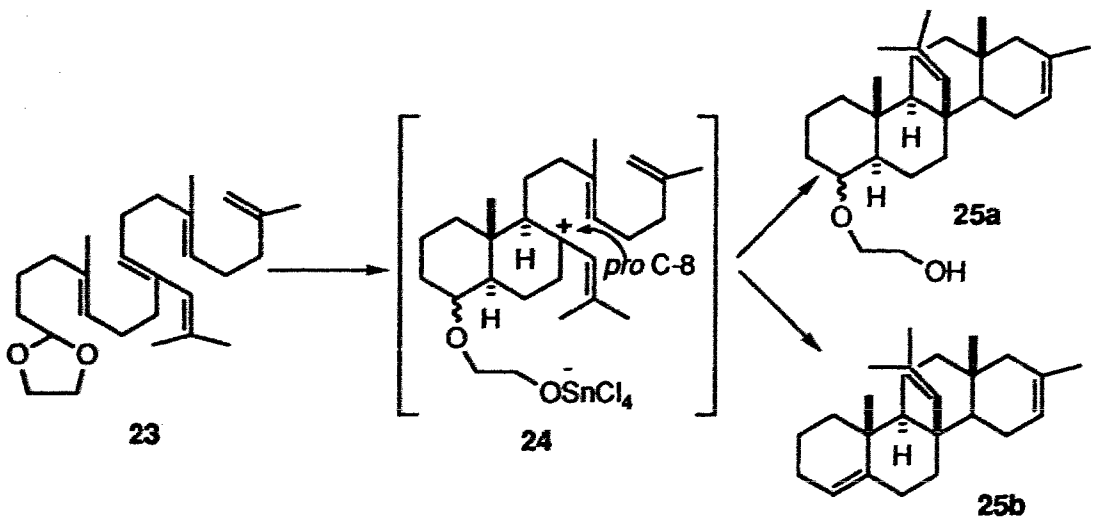

Figure 1-6: The use of cation-stabilizing auxiliary to increase yields of biomimetic cyclizations

Chemically induced cyclizations of 1,5-polyenes are now generally considered to proceed *via* the "stepwise" mechanism.[15,16] The Johnson group[16] has presented some of the strongest evidence in favour of the "stepwise" mechanism for "biomimetic" cyclizations. They have found that the yields in polyene cyclizations increased dramatically when cation-stabilizing auxiliaries were added to the polyenes at positions that allow them to stabilize the cationic charges generated. For example, Lewis acid induced cyclization of **23**, substituted with a cation-stabilizing auxiliary (methylidene group) at *pro* C-8 (*pro*-steroid numbering, Figure 1-6), gave tetracyclic products (**25a** and **25b**) in combined yield of 77%, as compared to 30% yield from the analogous polyene lacking the auxiliary at *pro* C-8 position.

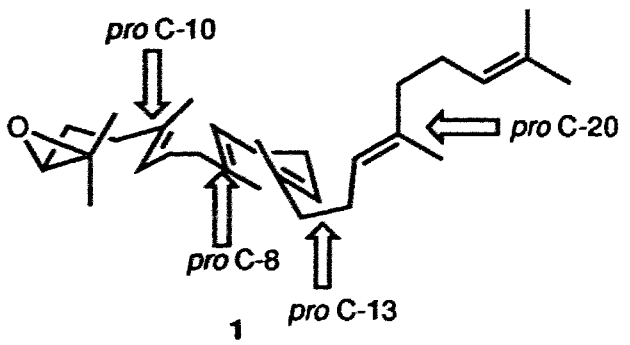

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Figure 1-7: Sites of carbocation generation that could be stablized by nucleophilic residues in the cyclase catalytic pocket

Johnson postulated that the OSC's may act to stabilize cationic intermediates by ion pairing. They might deliver these point charges as shown in Figure 1-7. For example, rate of closure of ring B in a boat conformation might be enhanced by providing a point charge to the *pro*-C-8 centre from the α-face, thereby lowering the activation energy of closure to a boat relative a chair conformation. Similarly the anti-Markovnikov closure of ring-C may be favoured by delivery of a point charge at *pro*-C-13 also from the α-face. The

point charge delivery to *pro*-C-10 from the β-face, although not specified by Johnson, may stabilize the cationic species at this centre. Attack by the Δ^{10} π-bond of 2,3-oxidosqualene from the α-face would result in the β-configuration of the C-10 methyl.

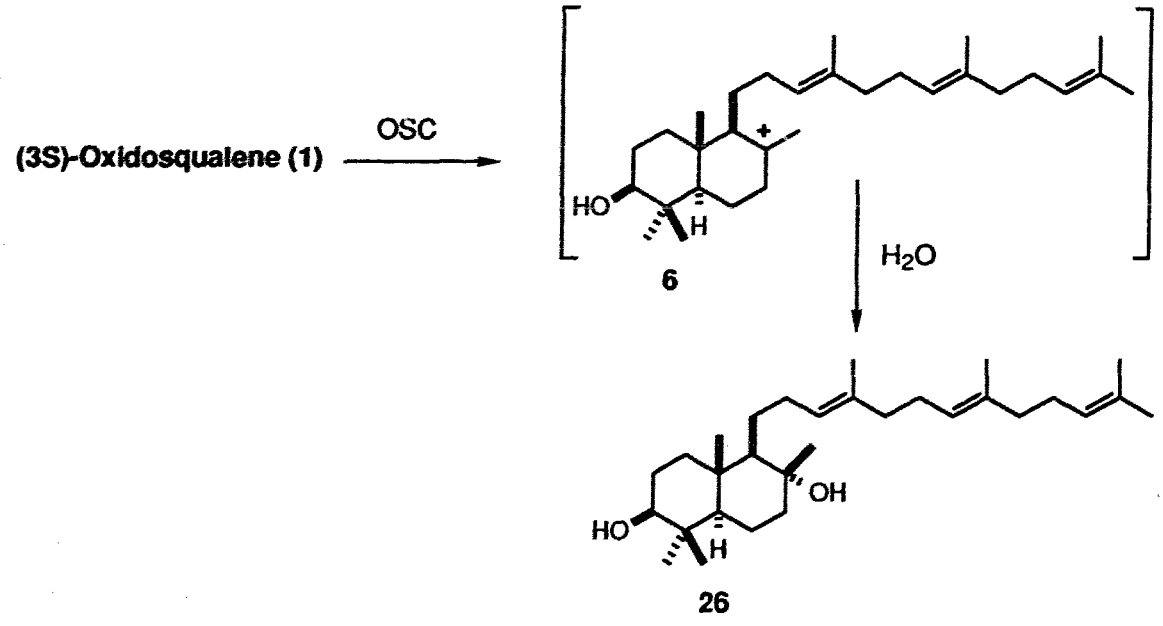


Figure 1-8: Isolation of bicyclic triterpenoid, **26**, from *Pistacia lentiscus* L, resulting presumibly from the trapping of cationic intermediate **6** with H_2O.

Finally the isolation of **26** (Figure 1-8) from the resin from the Mediterranean shrub *Pistacia lentiscus* L by Boar *et al.*[17] provides possibly the strongest evidence in support of the existence of cationic intermediate **6**. The structure and the absolute stereochemistry of this bicyclic triterpenoid is fully consistent with its formation by the interception of this carbocationic intermediate by H_2O. The equatorial (β) geometry of the C-8 hydroxyl group (steroid numbering) is that expected if **6** undergoes a chair-boat to chair-chair conformational change prior to reaction with water from the α-face. This compound is the only one, that is obviously derived from partially cyclized 2,3-oxidosqualene, to be isolated from natural sources.

Theoretical calculations[18] have also suggested the existence of olefin-carbenium ion π-complexes as distinct intermediates in reaction of carbocations with carbocations with olefins. These calculations support van Tamelen's postulation of "rigid" cationic intermediates fixed by π-complexation with the next olefinic bond.

OSC's must therefore exert most of the control by providing an electronic environment to sustain the presumed, conformationally rigid carbocyclic carbocationic intermediates (**5**→**6**→**7**→**3**, Figure 1-2) and guide the course of the reaction in the formation of the next carbocation ion intermediate.

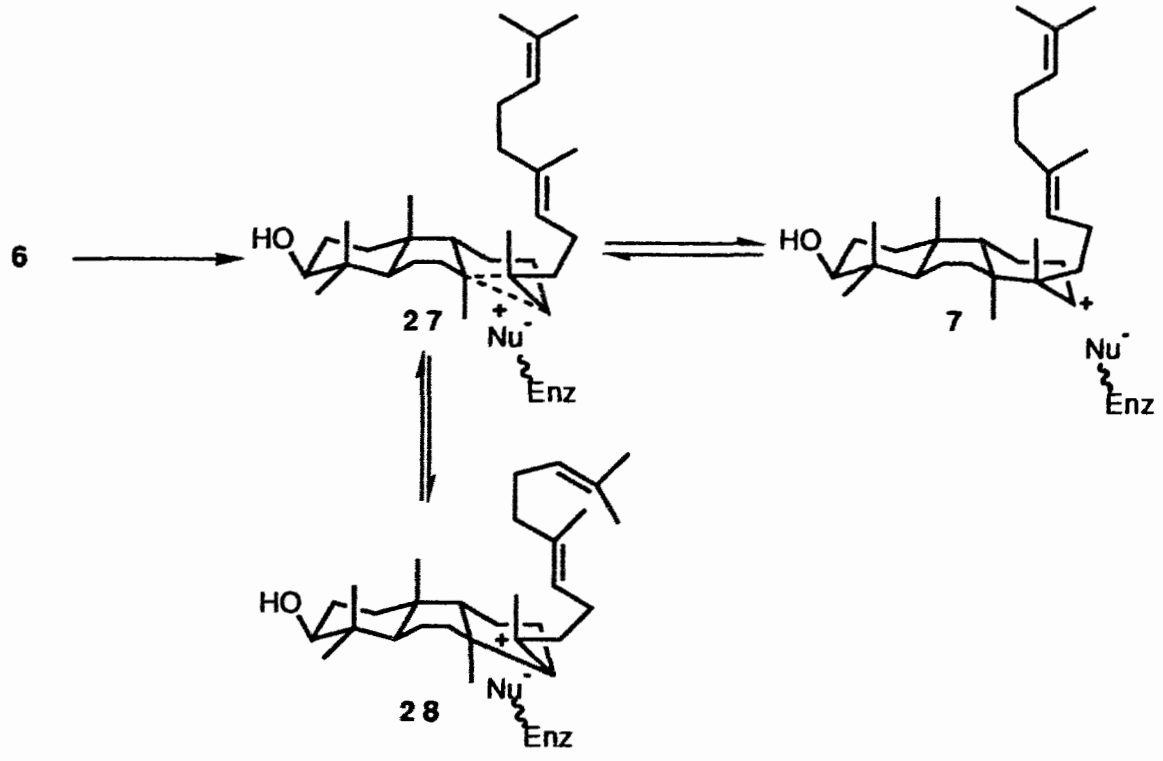


Figure 1-9: Proposed cyclization of ring C; involvement of an enzymatically stabilized five-membered Markovnikov intermediate (**28**).

2: Mechanism of Ring-C Formation

Another mechanistic question that has fascinated investigators is the process leading to formation of ring C. This ring must be formed either through

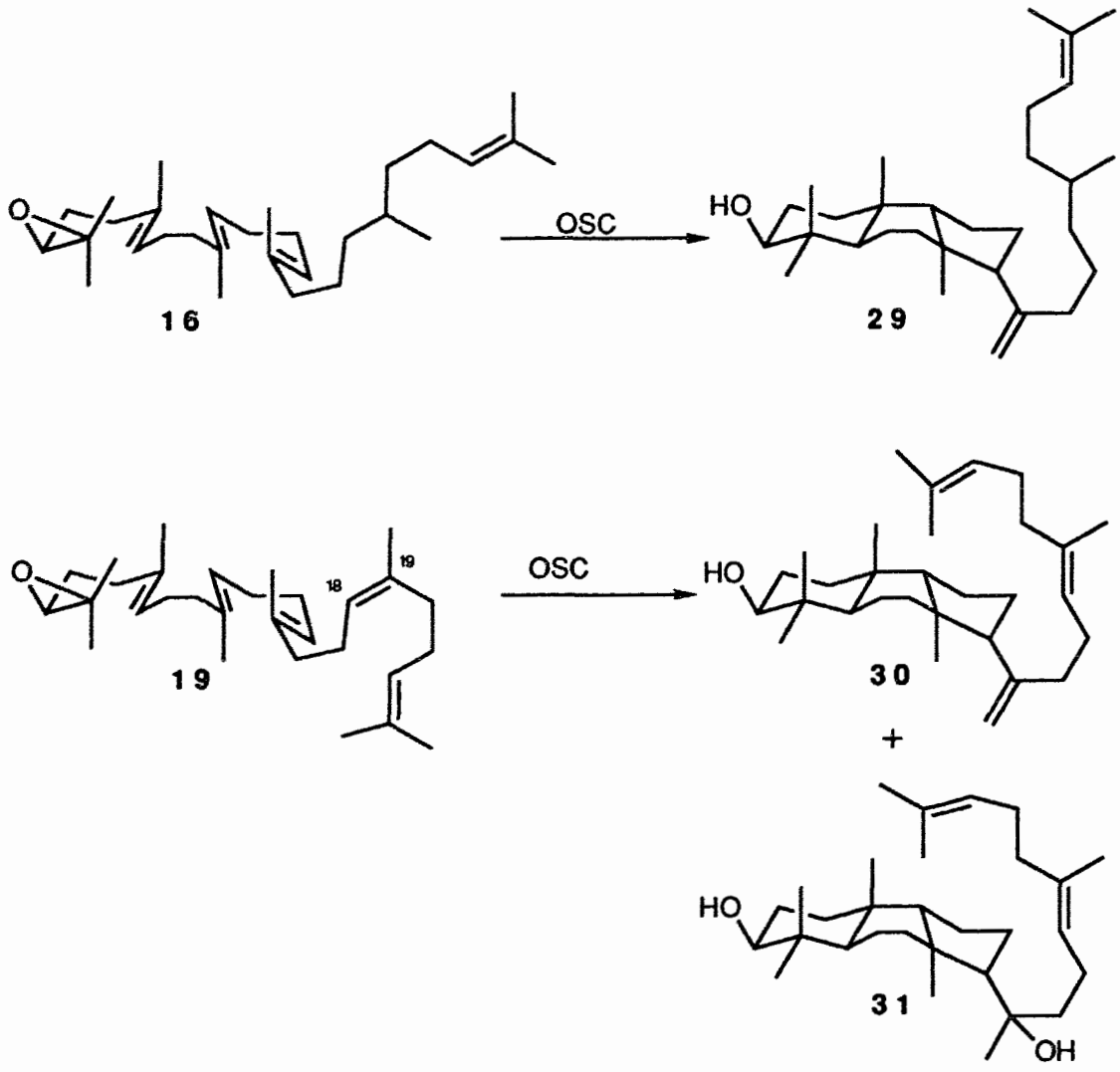


Figure 1-10: OSC mediated cyclization of C-18,C-19 dihydro-2,3-oxidosqualene (**16**) and 18(*Z*)-2,3-oxidosqualene (**19**): evidence for the existence of five-membered Markovnikov intermediate **28**.

an anti-Markovnikov cyclization of **6** to **7** in one step or *via* a Markovnikov cyclization to **28** (Figure 1-9). In the latter situation, ring expansion must occur *via* **27**, prior to subsequent reaction. The two cationic intermediates **28** and **7** could either equilibrate through π-complexed intermediate **27** or react as such depending on the nature of the oxidosqualene substrate. In the case of (3S),-2,3-oxidosqualene (**1**), **7** is the exclusive reactive intermediate. The reactive nature of **7** is most likely due to comformational and steric constraints set by

OSC's. This apparently is not the case for several other modified oxidosqualene substrates.

Consider the enzymatic treatment of 2,3-oxidosqualene analogue **16** lacking the Δ^{18} double bond[6b] and analogue **19** containing an unsaturation at C-18 with the *Z* geometry[7k] (Figure 1-10). van Tamelen[6b] found that the enzymatic cyclization of **16** resulted in the formation of tricyclic system **29** containing a six-six-five membered ring. This presumably involved a transition-state similar to **28** with loss of a hydrogen from the methyl at C-14 (steroid numbering). Similar results were obtained by Krief and colleagues[7k] from the enzymatic cyclization of **19**. They isolated the tricyclic sterols **30** and **31** with the six-six-five membered ring structure. The latter apparently arose from the capture of a H_2O molecule by a Markovnikov intermediate similar to **28** (steroid numbering).

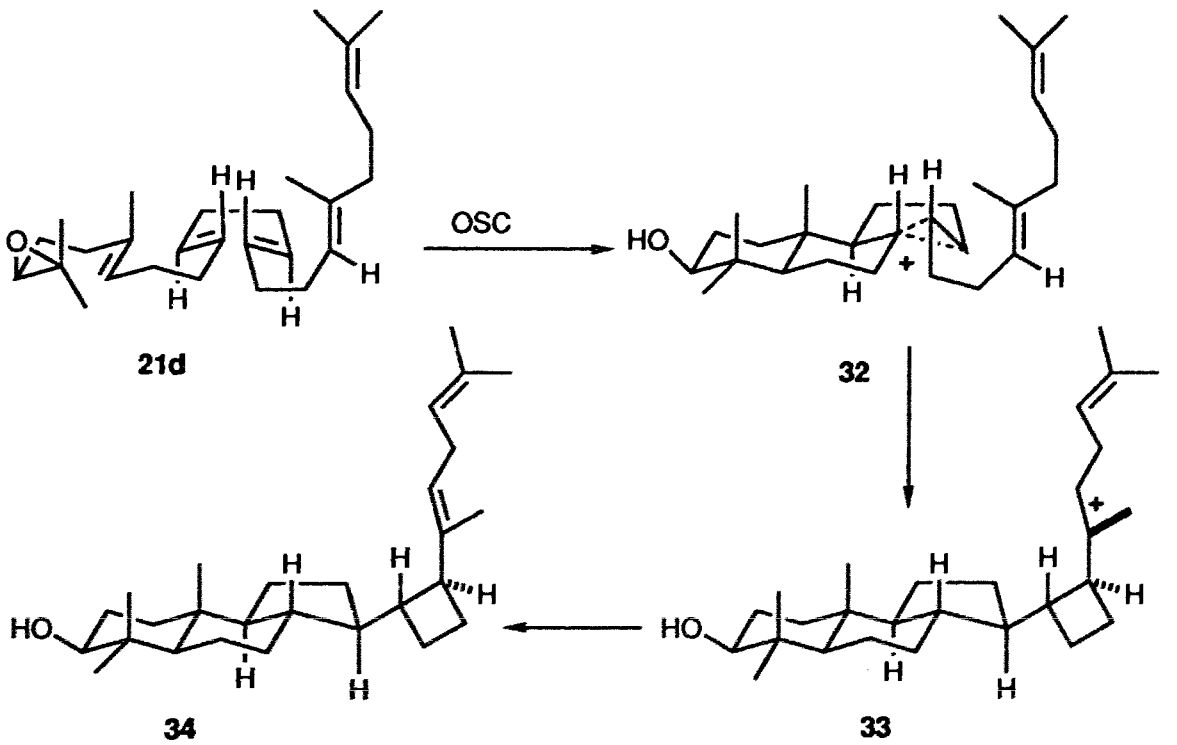


Figure 1-11: OSC mediated cyclization of **21d**, lacking methyl substituents at C-10 and C-15 of natural 2,3-oxidosqualene.

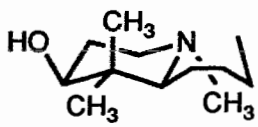
Corey[7h] has recently reported an investigation of the cyclization of **21d**, lacking methyl substituents at C-10 and C-15 (Figure 1-11). The isolation of **34** implicates a π-complexed cationic intermediate **32** which is analogous to **27**. It is noteworthy that in **34** ring B is in the chair form (rather than boat for normal 2,3-oxidosqualene) and that a cyclobutane ring has been formed rather than the normal five membered ring D. These results suggest that C-10 methyl of 2,3-oxidosqualene (**1**) is essential for folding of ring B in a boat conformation. It has been postulated that a bulky hydrophobic residue (possibly methyl group of valine, leucine or isoleucine) may be present in the active site of the OSC and which is large enough to prevent **1** from assuming the chair conformation of ring B because of a steric hindrance with the β oriented C-10 methyl.

III: Thesis overview

A: Synthetic Objectives

We have used two strategies to test for the involvement of carbocationic intermediates in the enzymatic conversion of 2,3-oxidosqualene to lanosterol. One approach was to study the inhibition of 2,3-oxidosqualene cyclase by rationally designed molecules that would mimic the presumptive cationic intermediates. The other approach also involves inhibiting 2,3-oxidosqualene-lanosterol cyclases. In this case, the enzyme would be irreversibly inactivated by 2,3 oxidosqualenoid substrate analogues in a mechanism-based manner.

We have probed the existence of presumptive cationic intermediate **5**, **6** and Markovnikov intermediate**28.** Azacyclic analogues **35** to **42** (Figure 1-12) were prepared as mimics of presumptive intermediate **5**. Thia-2,3-oxidosqualenes **43** and **44** were synthesized, as possible mechanism-based inactivators, to study the existence of intermediates **6** and **28**, respectively.



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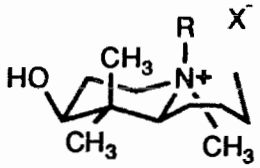
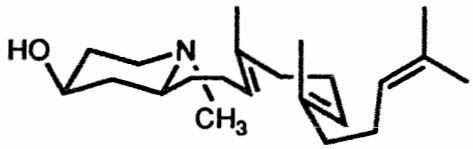


36a. R= H, X= Cl^-

36b. R= CH_3, X= I^-



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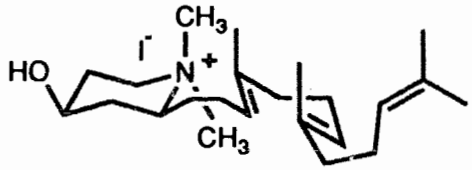

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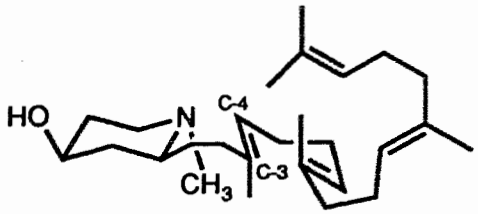

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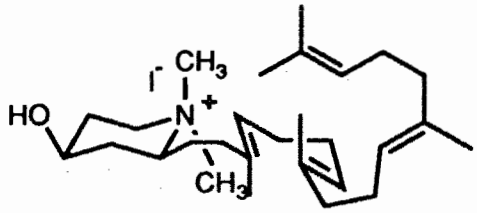

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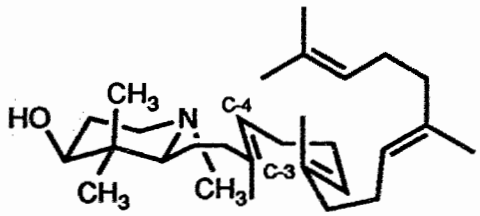

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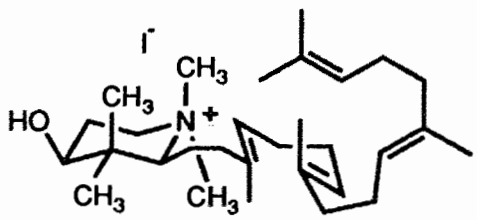

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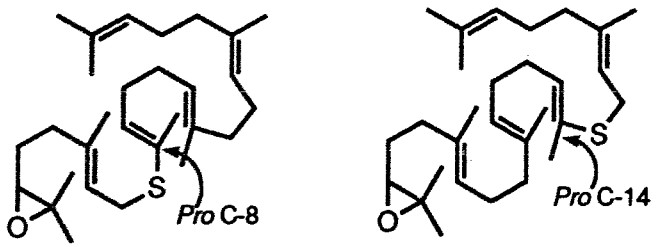
Figure 1-12: Aza-analogues of presumptive carbocation intermediate **5**.

The use of ammonium and sulfonium ions to mimic suspected cationic intermediates in enzymatic processes have been validated a number of times in our[1c,19,20] and other laboratories.[21,22,23,24,25] Some of the most potent inhibitors of sterol enzymes have been rationally the designed ammonium and sulfonium ion analogues of postulated cationic intermediates.[24h] A more thorough discussion on the use of heteroatom centred cations to mimic carbocation intermediates presumed to be generated in enzymatic processes is presented in Chapter 2.



43 **44**

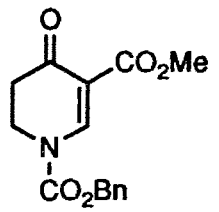
Figure 1-13: Vinyl-sulfur substituted oxidosqualenes **43** and **44**, possible mechanism-based inhibitors of 2,3-oxidosqualene cyclase.

The approach taken to evaluate the existence of intermediates **6** and **28** required the synthesis of thia-oxidosqualene analogues, **43** and **44**, respectively, (Figure 1-15) as mechanism based inactivators for the OSC's.[26] The sulfur atom has been introduced adjacent to the carbon position on which the cationic charge is presumed to reside. If these analogues are cyclized by the OSC's, the carbocations should be stablised by the lone-pair of electrons on the adjacent sulfur.[27] It was anticipated that such stabilization might impart a sufficiently long life to the intermediate that a nearby nucleophilic amino acid residue in the active site might compete with the ring closure to give covalently bound substrate. The reactive residue was anticipated to be that which is

responsible for the stabilization of the presumptive cationic intermediate. Again a more thorough rationale is presented in Chapter 4.

B: Thesis organization

This thesis has been organized into five chapters. Chapters 2 and 3 describe two different methodologies for synthesis of ammonium ion analogues **35-42** outlined in Figure 1-12. The results of the biological evaluation of compounds **35** to **42** as inhibitors of fungal OSC's are presented in Chapter 3. Chapter 4 outlines the synthesis of vinyl sulfur substituted oxidosqualenes **43** and **44**. Chapter 5 describes a new strategy for the synthesis of 1,5-dienes of the type used in the preparation of the terpenoid chains required for the synthesis of the inhibitors described in Chapters 2-4.



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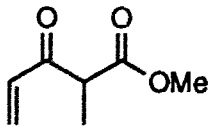

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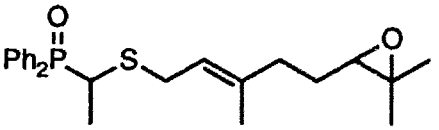

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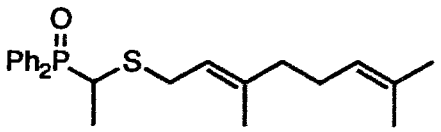

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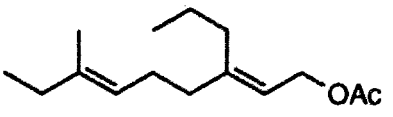

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Chapter 2 describes an efficient route to preparation of C-2,C-3 functionallized 4-piperidones. The initial step required the conjugate addition of higher order (H. O.) organocuprates to *N*-(carbobenzyloxy)-3-carbomethoxy-5,6-dihydro-4-pyridone (**45**) *on route* to the synthesis of required monocyclic *N*-methyl-2,3-substituted-4-piperidinols as analogues of intermediate **5**. A

through study on the conjugate addition of organocuprate to 5,6-dihydro-4-pyridones is presented along with the successful route to the 1,5-tetraene sidechain, which was appended to C-2 of the intermediate 4-piperidone. This approach was used to synthesize compounds **35-40**. This methodology could not be used to prepare compound **41** and its methiodide salt **42**, and a different strategy was conceived and executed.

In Chapter 3 the successful route to the synthesis of analogue **41** and **42** is presented. This methodology utilizes a modified Mannich-like cyclocondensation between imines and appropriately substituted γ,δ-unsaturated-β-keto esters to give C-2, C-3 substituted-4-piperidones, intermediates in the synthesis of mimic **41**. This chapter outlines the synthesis of some valuable terpenoid synthons as well as an improved route to γ,δ-unsaturated-β-keto esters. The reactivity of imines and γ,δ-unsaturated-β-keto esters is also discussed in this chapter. It was found that methyl 2-methyl-3-oxo-4-pentenoate (**46**) reacts with imines to give only <u>one</u> of the two possible diastereomers of the C-2, C-3 substituted *N*-methyl-4-piperidone. The possible explanation for the stereochemical outcome of the cycloaddition is discussed.

In Chapter 3, the results of the biological evaluation of the ammonium ions listed in Figure 1-12 (**35** to **42**) are also presented. These compounds were evaluated as inhibitors of 2,3-oxidosqualene-lanosterol cyclase of the yeast *Candida albicans*, both in intact-cell and in cell-free enzyme systems. A discussion of the results is presented.

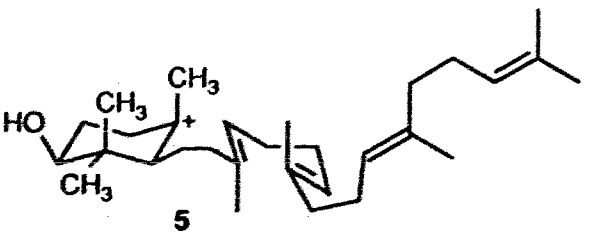
Chapter 4 outlines successful routes for the synthesis of the vinyl sulfur substituted 2,3-oxidosqualene **43** and **44**. These oxidosqualene analogues were prepared from farnesol and geraniol. The chemistry described in this chapter uses the classical Wittig-Horner olefination to give geometrically pure all *trans* vinyl sulfur substituted 2,3-oxidosqualenes. This work required the

synthesis of new Wittig-Horner type (α-thioterpenoidyl)diphenylphosphine oxide reagents (**47** and **48**). These were condensed with the appropriate aldehydes at low temperatures, the intermediate α-hydroxy diphenylphosphinoyl compounds isolated as the *erythro* and *threo* mixture and separated by chromatography on silica. The *erthro* compounds were then converted to *E* vinyl sulfur-substituted 2,3-oxidosqualenes **43** and **44**.

Chapter 5 describes the synthesis of 7-methyl-3-propyl-2(*E*),6(*E*)-nonadienyl acetate (**49**), a terpenoid compound isolated from the square-necked grain beetle, *Cathartus quadricollis* (Guér.). The methodology makes extensive use of organotin and organocuprate chemistry for the synthesis of geometrically pure trisubsubstituted 1,5-polyenes. This strategy was devised with the intention of using it for the synthesis of trisubstituted 1,5 polyenes required for the preparation of the inhibitors presented in Chapters 2, 3, 4. Due to the large quantities of starting materials required for the total synthesis of the inhibitors, more conventional chemistry chosen.

CHAPTER 2

This chapter outlines the synthesis of compounds **35**, **36a**, **36b**, **37**, **38**, **39** and **40** as analogues of presumptive intermediate **5** in the enzymatic cyclization of 2,3-oxidosqualene (**1**) to lanosterol (**2**). The syntheses presented in this chapter were accomplished by the conjugate addition of organocuprate reagents to *N*-(carbobenzyloxy)-3-carbomethoxy-5,6-dihydro-4-pyridone, **45**.



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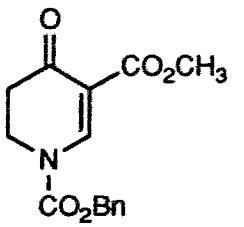

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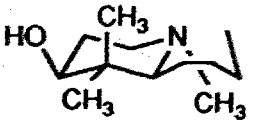

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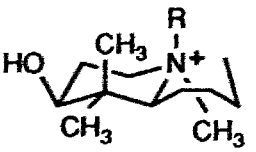

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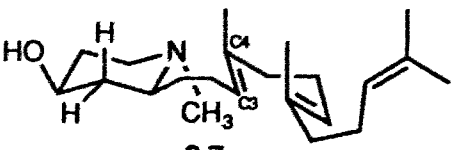
36b R=CH$_3$; X=I$^-$



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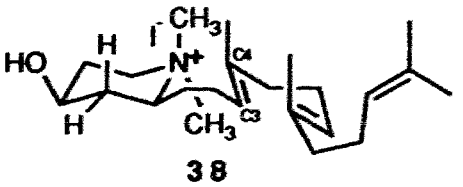

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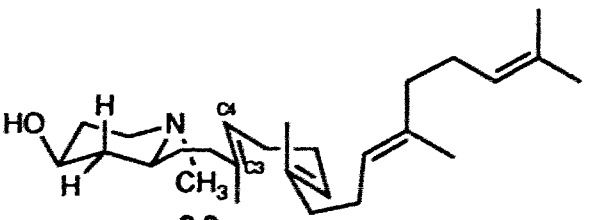

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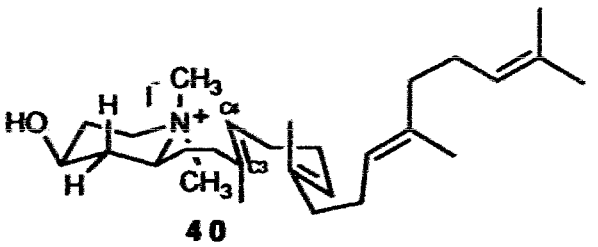

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I: Introduction

A: Inhibition of Enzymes Using Charged Heteroatom Substituted Analogues as Mimics of Presumed Carbocationic Intermediates

It is recognized[28] that if a molecule is structurally and electronically analogous to an intermediate produced during an enzyme catalysed reaction then the molecule may bind tightly with the site normally responsible for binding the intermediate. This is thought to be accomplished by exploiting the interactions active in the binding of the intermediate. The use of charged heteroatoms usually nitrogen and sulfur but also arsenic, to mimic carbocations postulated to be involved in biological transformations has been found to be an effective strategy in the design of inhibitors of enzymes operating in sterol[1,c19,20,23-25] and terpenoid[21,22] biosynthetic pathways.

The use of positively charged heteroatoms to mimic cationic carbon intermediates produced during enzymatic catalysis has been reviewed several times in the recent past.[1c,24g,h] Therefore, the background on this topic in this chapter has been limited to a few specific examples.

1: Inhibition of Δ^{24}-Methyl transferases

Enzymes that alkylate the Δ^{24}-unsaturation of sterols are found in yeast and plants but not in mammals. It has been postulated that the conversion of $\Delta^{24(-25)}$ sterols to $\Delta^{24(-28)}$ methylene sterols by Δ^{24}-sterol methyl transferases (24-SMT) involves at least two carbocation intermediates. The first step involves the nucleophilic attack by the Δ^{24}-π electrons of the sterol on the S-

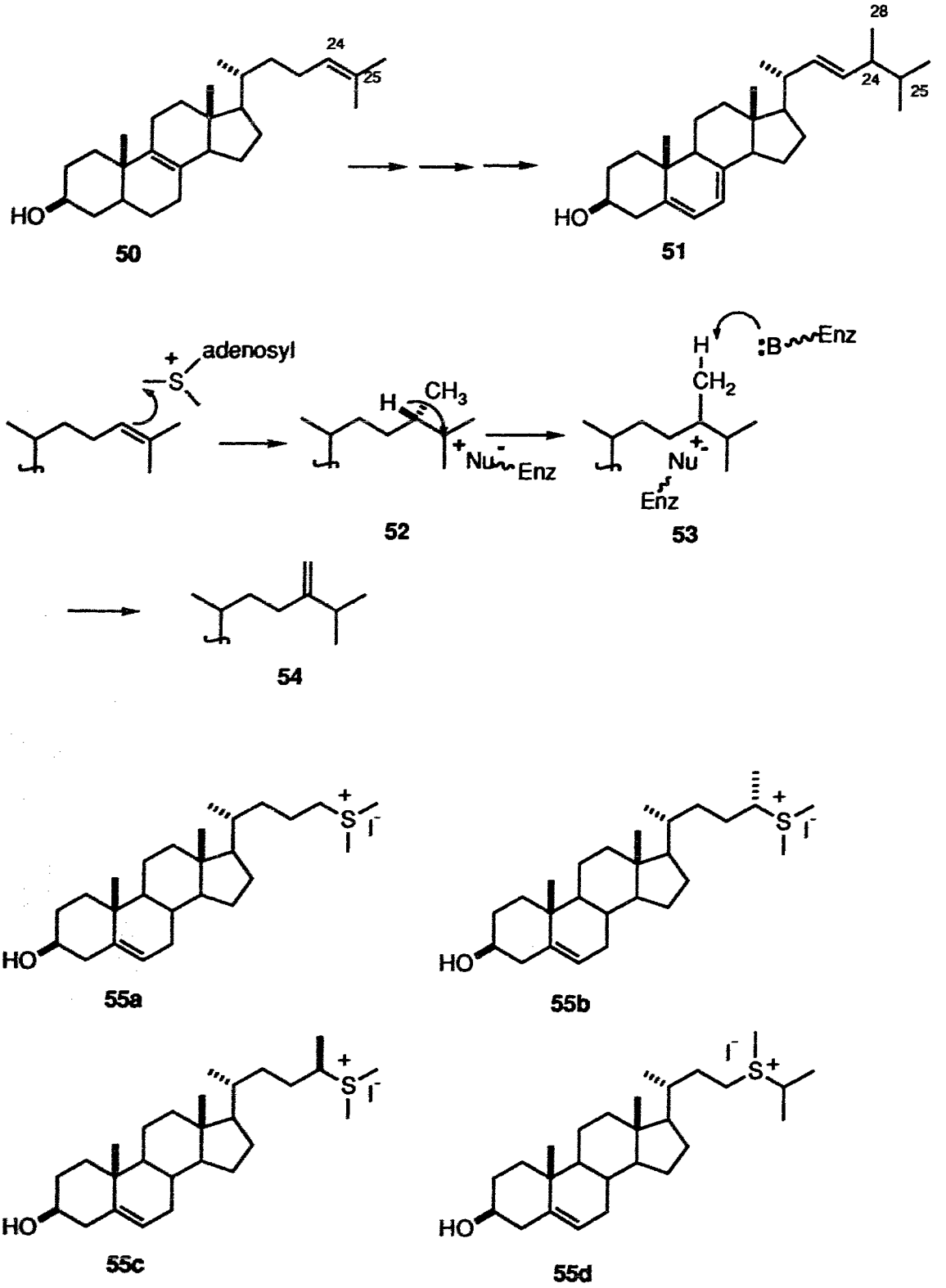
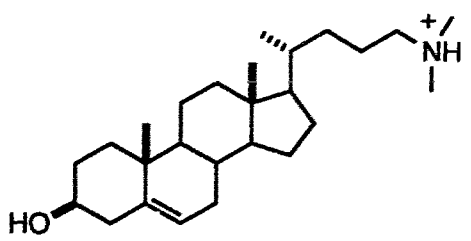
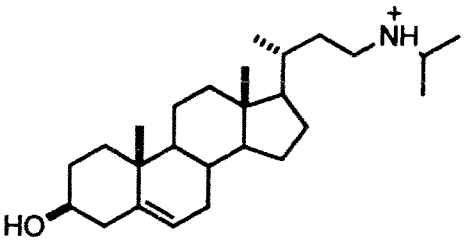


Figure 2-1: Inhibition of Δ^{24}-sterol methyl transferases by ammonium and sulfonium ion mimics.



55e



55f

Figure 2-1: Inhibition of Δ^{24}-sterol methyl transferases by ammonium and sulfonium ion mimics (cont.).

methyl group of the S-adenosyl-L-methionine. This generates an intermediate C-25 cation (**52**) containing a C-24 methyl. Hydrogen migration moves the positive charge from C-25 to C-24 (**53**). This is followed by the elimination of a hydrogen from the C-24 methyl to produce a 24-methylenesterol[29,19,23,24e,h] (**54**). Sulfonium ion[1c,20a,23c] (**55a**, **55b** **55c** and **55d**) and ammonium ion[1c, 23c] (**55e** and **55f**) analogues of presumptive cationic intermediates **52** and **53** involved in the alkylation of zymosterol, **50**, during biosynthesis of ergosterol, **51**, are potent inhibitors of yeast 24-SMT. In fact, **55a** bound to this enzyme 25,000 times more tightly than the natural substrate.

2: Inhibition of Squalene Synthase

Similarly, ammonium ion[1c,21,22a] (**61b**, **62a**, and **62b**) and sulfonium ion[1c,19e,20b] (**61a** and **63**) mimics of presumptive cationic intermediates involved in the biosynthesis of squalene (**10**) by squalene synthase are inhibitors of this enzyme. The transformations considered to be involved in the production of squalene by this enzyme are insertion of C-1 of one farnesyl pyrophosphate into the C-2, C-3 double bond of a second to generate

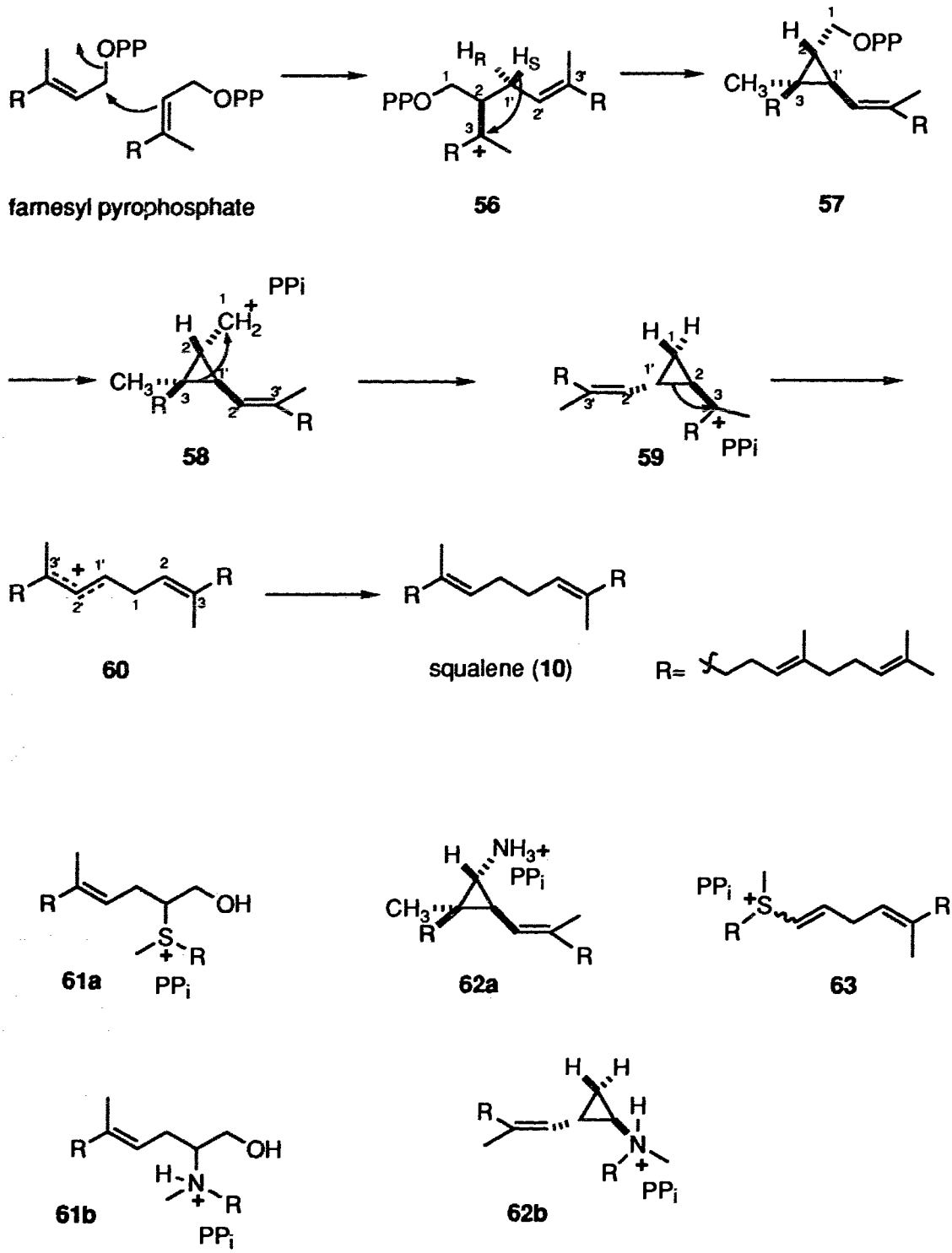


Figure 2-2: Inhibition of squalene syntase by ammonium and sulfonium ion mimics.

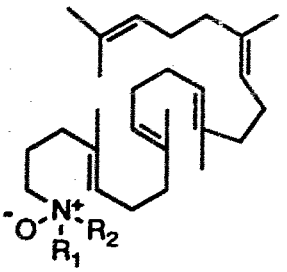
intermediate **56** which rearranges to presqualene pyrophosphate (**57**). This degrades to give ion pair intermediate **58**. Further conversion of **58** *via* a cyclopropylcarbinyl rearrangement yields intermediates **59** and **60**. Hydride reduction of latter completes the enzymatic formation of squalene, **10**.[30,1c]

The inhibitory activity of rationally designed intermediate mimics of sterol biosynthesis has in the past has played a significant role in the elucidation of the mechanisms by which many of these enzymes operate. For example, sulfonium ion mimic[1c,20] **55b** was found to bind the 24-SMT more strongly than **55c**, suggesting that the stereochemistry of alkylation was as illustrated in (Figure 2-1). Furthermore, in the mechanism for synthesis of squalene (**10**) from presqualene diphosphate **57** shown above (Figure 2-2), the involvement of inorganic pyrophosphate (PPi) as the counter anion of cations **58** and **59** was established by administrating ammonium ion mimics[21,22] **62a** and **62b**, respectively. It was found that **62a** or **62b** had significant inhibitory activity only in the presence of PP_i. Similar results have been observed for sulfonium ions **61a** and **63**.[20b]

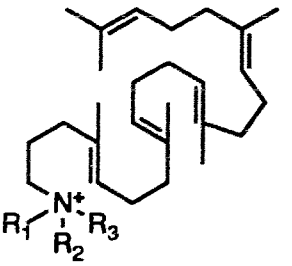
3: Inhibition of 2,3-Oxidosqualene cyclases

OSC[24-26,31-33,1C] is inhibited by both substrate,[25] and product analogues.[24h] Ammonium ion analogues of presumptive intermediates and amine oxide mimics of intermediates presumed to be formed from epoxide opening have proven to be good inhibitors.[24] Cattel's group[24h] has shown that ammonium ion mimics of intermediates **4** and **6** effectively inhibit the cyclases from animal, fungal and plant sources (Figure 2-3).

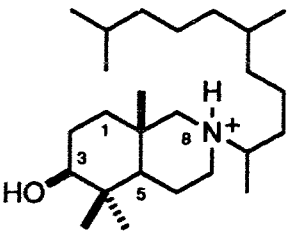
Systematic analysis of the inhibitory activity of the compounds revealed that the (i) most effective inhibitors are those that possess a positive charge at



64a. R_1=R_2=Me
64b. R_1=R_2=Et



65a. R_1=H; R_2=R_3=Me
65b. R_1=R_2=R_3=Me
65c. R_1=H; R_2=R_3=Et
65d. R_1=H; R_2=R_3=$(CH_2)_5$



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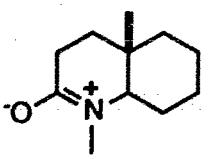

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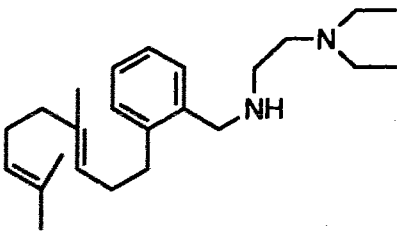

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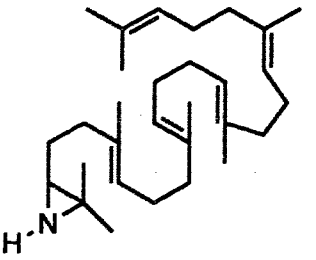

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Figure 2-3: Ammonium and *N*-oxide inhibitors of 2,3-oxidosqualene-lanosterol cyclase.

C-2 of the squalene skeleton (mimics of intermediate **4**); (ii) the *N*-oxides, **64a** and **64b** are more potent inhibitors than the corresponding amines; (iii) the cyclase will tolerate C-2 amine mimics with methyl (**65a** and **65b**), ethyl (**65c**) as well as cyclic (**65d**) substituents on the nitrogen; (iv) the structure of the major hydrocarbon tail attached to nitrogen is important for inhibition, removal of double bonds in the squalene moiety or its replacement by a shorter chain results in a decrease in activity; (v) the 8-aza-decalin (**66**) mimic of intermediate **6** was inhibitory only when squalene like side chains were present on the nitrogen, activity was lowered when the branch methyls of the side chain or the

3-β hydroxyl (steroid numbering) group were removed. It appears also that the binding site of the OSC's is quite sensitive to steric hindrance due to larger groups on the inhibitors.

Table I: Inhibition of SOC's using ammonium and amine oxide mimics of intermediates **4** and **6**.

	[a]IC_{50} (μM)			
	OSC source			
Compound	*C. albicans*	*S. cerevisiae*	Pea seedlings	Rat liver
64a [24f][b]	ND[c]	16	0.3	3.7
64b [24f]	ND	14	0.15	1.5
65a [24f]	ND	10	1.3	7.5
65b [24f]	ND	ND	1.1	5.1
65c [24f]	ND	14	0.55	3.2
65d [24f]	ND	ND	3.5	ND
66 [24g,h]	ND	ND	NI[d]	2.0
67 [24h]	ND	ND	ND	165
68 [33a]	2.0	ND	ND	21
69 [33a]	0.15	ND	ND	0.23

[a]IC_{50}, concentration of inhibitor required to reduce enzyme activity by 50% ; [b][ ], reference; [c]ND, not determined; [d]NI, no inhibition.

There are significant differences in the activity of a given inhibitor for OSC's derived from different sources (Table I). This is important in re-inforcing the observation that cyclases from different sources have different amino acid compositions at the active sites. Additionally these differences in inhibitory activity could possibly be manipulated to selectively target the OSC of a pathogenic yeast over the host in a therapeutic treatment. OSC has been targeted in development of chemotherapeutic treatments of yeast infections[33] and hypocholesterolemic agents.[32] This enzyme has been shown to be essential for growth of fungi.[34] Unfortunately, as antifungal agents, most

inhibitors shown above are more active towards animal OSC's than the fungal OSC's. Compound **68**, a presumed mimic of one of the cationic intermediates formed during the cyclization of 2,3-oxidosqualene, tested by Jolidon and colleagues at F. Hoffmann-La Roche, shows a 10-fold selectivity in inhibition of the fungal cyclase over the rat-liver cyclase.[33]

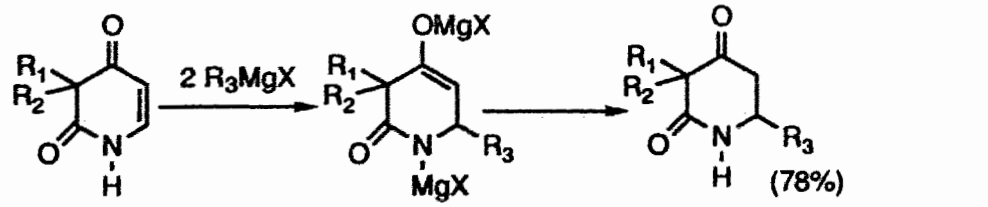
II: Results and Discussion

A: Rationale

We believed that the initial formation of ring A, leading to intermediate **5** was the slowest step in the enzymatic cyclization of 2,3-oxidosqualene (**1**) to protosterol (**3**), as observed by van Tamelen for the "biomimetic" cyclizations.[9b] The cyclases were postulated to exert much stricter control in the formation of ring A, concurrent with the formation of intermediate **5**, than at any other point in the sequence leading to the formation of protosterol **3**. Thus, inhibition of OSC's by heteroatom cationic mimics of **6** were expected to be significant.

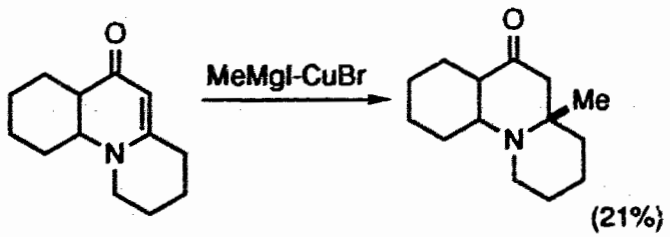
We chose to explore the existence of the presumptive intermediate **5** using azacyclic analogues as mimics of **5**.[35] We envisioned that these analogues would be easy to prepare in various substitution patterns. We retained as many of the structural features of the presumptive intermediate **5** in the mimics as possible. We have examined the requirements for double bonds, the length of the side chain at C-2 (piperidine ring), the position of the branch methyls of the side-chain , and C-3 substituents of the 4-piperidinol ring. Although these compounds are racemic mixtures, the *syn* relationship between the C-4 β-hydroxyl group and the C-2 side chain of the piperidinols has been maintained. In each case it is believed that the enantiomer that corresponds to the absolute stereochemistry of the biosynthetic intermediate will inhibit the

cyclase. The amines **35**, **37** and **39** are expected to be fully protonated at physiological pH and thus should mimic the charge characteristics of intermediate **5** to an extent similar to that of their quaternary ammonium salts **36a**, **36b**, **38** and **40**.



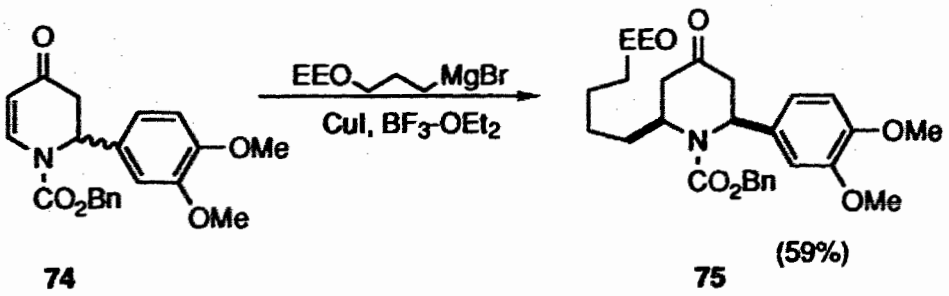
70 **71** $R_1=R_2=CH_3$, $R_3=C_6H_5$

[36a]Lutz, A. H. *et al. Helvetica Chimica Acta* **1956**, *39*, 81



72 **73**

[36b]Horri, Z.-I. *et al. Chem. Pharm. Bull.* **1969**, *17*, 846.



74 **75**

[36c]Comins, D. L. *et al. J. Am. Chem. Soc.* **1988**, *110*, 7445.

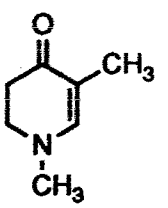
Figure 2-4: Examples of uncatalysed and Cu(I) mediated conjugate additions of Grignard reagents to 4-piperidones

It was envisioned that the required compounds could be prepared by the conjugate addition of organocuprates, derived from the appropriate Grignard or lithium species, to an α,β-unsaturated 4-piperidone. Vinylogous amides, including α,β-unsaturated 4-piperidones have been shown to undergo uncatalysed and Cu(I) mediated conjugate additions of Grignard reagents to produce C-2 substituted derivatives (Figure 2-4).[36] This approach would allow the introduction of various side chains at C-2 to the same basic skeleton without changing the overall synthetic strategy. After some exploratory work, we found *N*-(carbobenzyloxy)-3-carbomethoxy-5,6-dihydro-4-pyridone, **45**,[35] to be the ideal candidate.

B: Synthesis Section

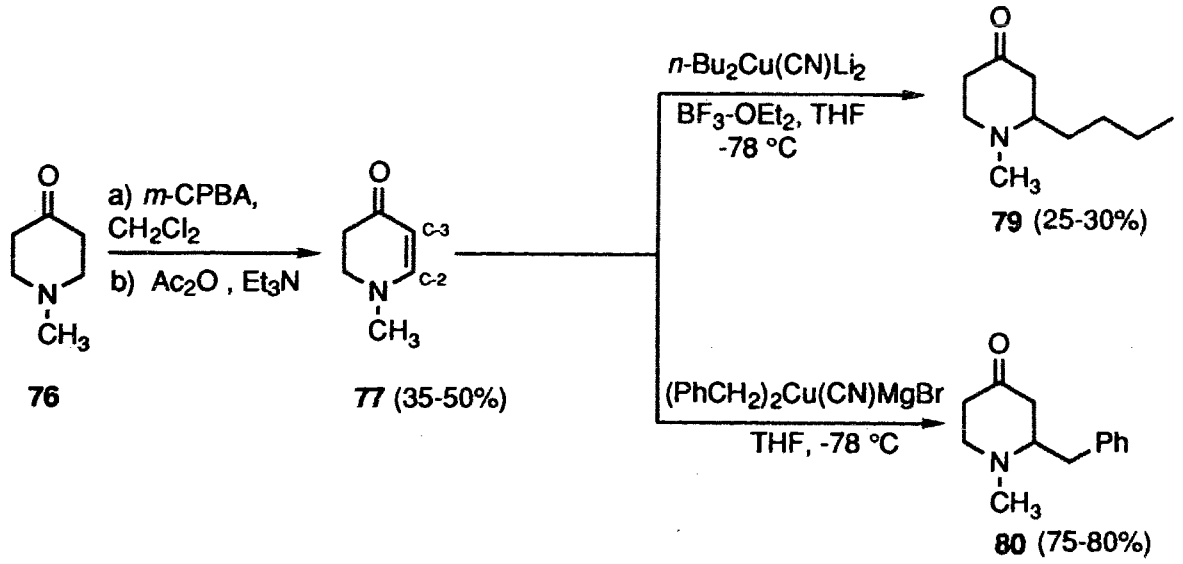
1: Conjugate Addition of Cuprates to 77

Since the target compounds are masked C-2, C-3 substituted *N*-methyl-4-piperidones, we initially thought it would be possible to use the known *N*-methyl-5,6-dihydro-4-pyridone[37] **77** as the substrate for cuprate additions. This reaction would give a C-2 substituted intermediate containing a copper enolate that could be trapped with MeI to give C-2 alkyl C-3 monomethylated-*N*-methyl-4-piperidones. We would then prepare *N*-methyl-3-methyl-5,6-dihydro-4-pyridone (**78**) and carry out the analogous experiment to give the C-3 gem dimethyl compounds. With this in mind we began the synthesis.



78

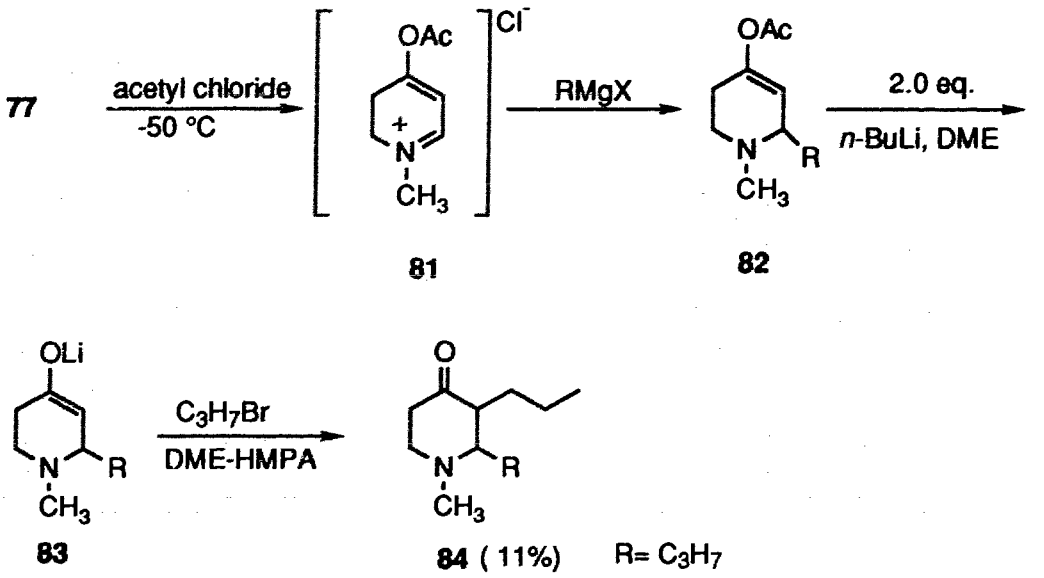
Scheme 1: Addition of cuprates to **77**.



76 **77** (35-50%) **79** (25-30%) **80** (75-80%)

Although *N*-methyl-5,6-dihydro-4-piperidone (**77**) (Scheme 1) was easy to prepare from commercially available *N*-methyl-4-piperidone (**76**) using reaction conditions described by Stutz and Stadler[37] (yield, 35-50%), it was found to be unstable and began to polymerize almost immediately after isolation. When **77** was treated with $(n\text{-Bu})_2Cu(CN)Li_2$ at -78 °C, none of the product (**79**) resulting from conjugate addition could be isolated. Inclusion of 1.2 equivalents of BF_3-Et_2O,[38] to activate the α,β-unsaturated system toward conjugate addition gave the C-2 substituted product (**79**) in yield of 25-30%. We were unable to increase this yield. Surprisingly magnesio-cuprate derived from 2.0 equivalents of $PhCH_2MgBr$ and 1.0 equivalent of CuCN gave the C-2 substituted 4-piperidone (**80**) in yields of >85%. The differential reactivity of the magnesio *vs* the lithium cuprate could be attributed to two factors: (i) the magnesium counter ion is a better Lewis acid than the lithium ion and, therefore, may activate the enone by complexing to the oxygen; (ii) Grignard reagents are less reactive and are softer nucleophiles than the alkyl lithiums. The corresponding magnesio-cuprate may form a better complex with the π-

system of the enone than the corrresponding lithiocuprates. Differential reactivity between the cuprates derived from alkyl lithium species and alkyl magnesium halides has previously been noted.[39]



[40] Husson, H.-P.; *et al. Tetrahedron Lett.* **1987**, *28*, 6457.

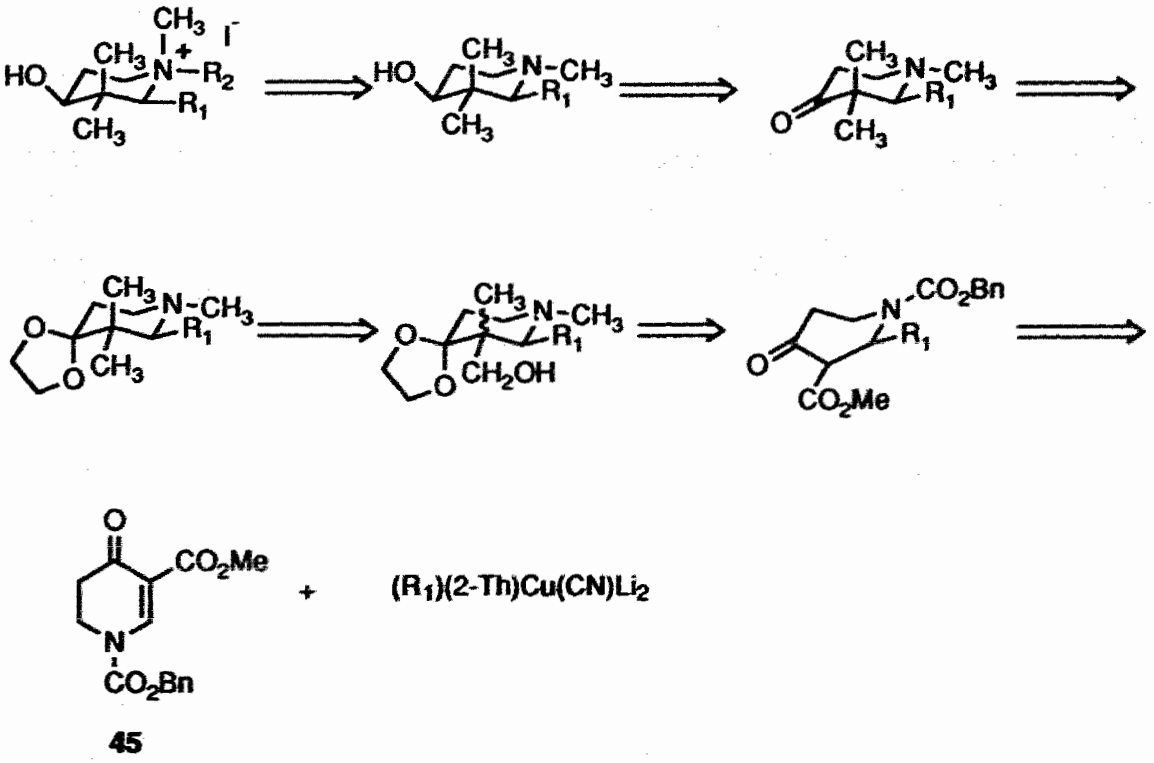
Figure 2-5: Additions of Grignard reagents to 4-acetoxy-5,6-dihydropyridinium chloride (**81**) and attempted alkylation of enolates of 4-piperidones.

While our study was in progress, Husson *et al.*[40] reported that lithium enolates of *N*-alkyl-4-piperidone do not alkylate well at C-3. Although no explanation for this lack of reactivity was given one can assume that alkylation of the lithium enolate **83** (Figure 2-5) could be retarded by the lone pair electrons of the nitrogen. Indeed, the lithium of the enolate could be chelated by both the enolate oxygen and the nitrogen, resulting in a less reactive enolate. Since we required the generation of lithio-cuprates to introduce the very fragile homoallylic terpenoid chains at C-2 of 4-piperidones and

lithiocuprates gave low yields in additions to **77**, we abandoned this synthon in favour of pyridone **45**.

The lone pair electrons on the vinylogous nitrogen of 4-piperidones is expected to interact significantly with the α,β-unsaturated system, and this should govern both their reactivity and stability. This property of **77** has been exploited by Husson[40] to prepare, *in situ*, 4-acetoxy-5,6-dihydropyridinium chloride **81** (Figure 2-5). This intemediate reacted with Grignard reagents to give C-2 substituted 4-piperidone enol acetates **82** in good yields. We suspected that if the nitrogen lone pair was delocalized into in an amide or a carbamate linkage, this might result in increased stability as well as modified reactivity. Amide protecting groups could be easily removed or transformed in a latter step.

Scheme 2: Retro-synthesis of the C-3 gem dimethyl substituted analogues of intermediate **5**.

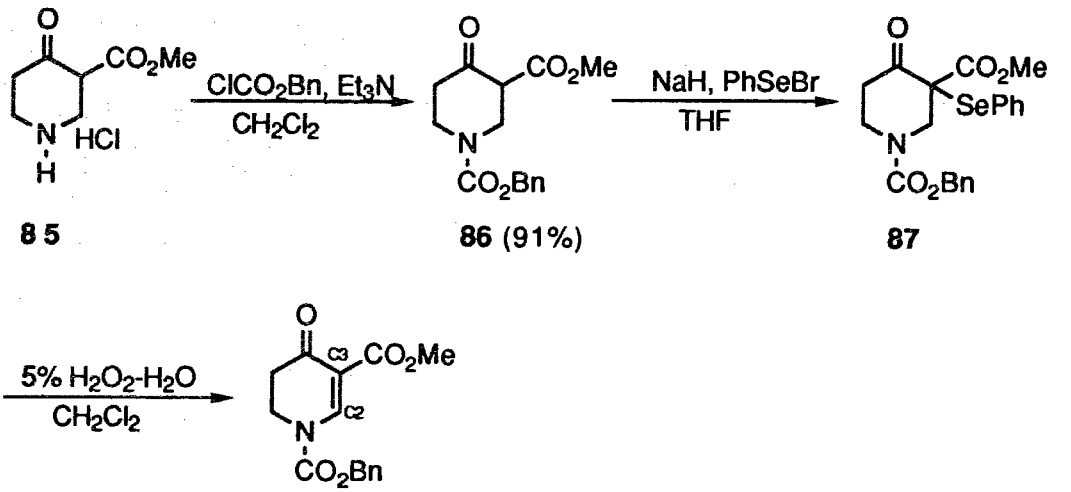


These requirements could be met with *N*-(carbobenzyloxy)-3-carbomethoxy-5,6-dihydro-4-pyridone, **45**.[35] We could introduce side chains at C-2 *via* cuprate addition, mono-methylate the β-ketoester activated C-3, and transform the ester functionality to the second required C-3 methyl group *via* a reduction, tosylation and hydride displacement sequence. In the process, we could reduce the benzyloxycarbamate protecting group to the required *N*-methyl substituent (Scheme 2).

2: Synthesis of 45

The *N*-(carbobenzyloxy)-3-carbomethoxy-5,6-dihydro-4-pyridone **45**, was obtained in three steps in 70-75% overall yield from commercially available methyl-4-oxo-piperidine-3-carboxylate hydrochloride **85** (Scheme 3).[41] The latter was protected as the benzyl carbamate derivative **86** and selenylated[42]

Scheme 3: Synthesis of **45**.



85 **86** (91%) **87**

45 (76% in two steps)

at C-3 to give **87** as a light yellow crystalline solid (70-80 %). Best results were obtained in the selenylation when the sodium salt of β-ketoester **86** was

generated with NaH in THF at 0 °C and then cooled to -50°C prior to addition of PhSeBr. The selenylated product **87** was easily purified by recrystallization from ethyl acetate-hexanes or treated as a crude isolate with 5% H_2O_2(aq) in CH_2Cl_2 at 0°-5 °C to give, after chromatography, the unsaturated piperidone, **45**.

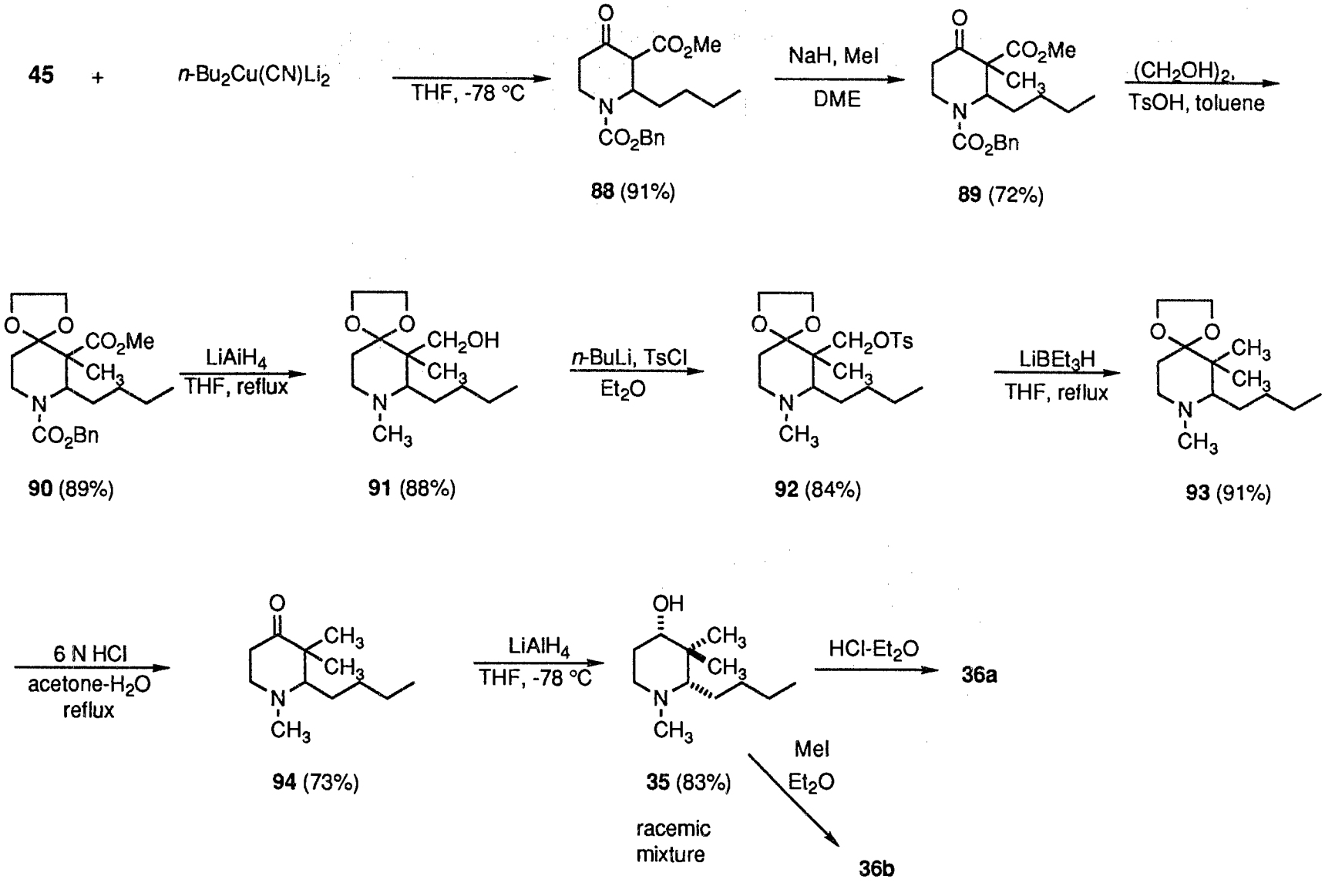
We found enone **45**[35] to be an excellent candidate for this strategy. It allowed introduction of various alkyl groups at C-2 as well introduction of geminal methyls at C-3. Cuprates prepared from alkyl lithium or Grignard reagents reacted with **45** to give high yields of conjugate addition products.

3. Synthesis of 35, 36a and 36b

Amine **35** and its salts **36a** and **36b** were synthesized as models to test the minimal structural requirements of the side chain of analogues of **5** for inhibition of OSC. Fabrication of this amine required the introduction of a relatively small carbon chain (C_4) at C-2 of **45** *via* conjugate addition of *n*-$Bu_2Cu(CN)Li_2$ and modification of C-3 to give geminal methyls.

The conjugate addition of (*n*-Bu)$_2$Cu(CN)Li$_2$ to **45** to give **88** was instantaneous and high yielding (87-91%) at -78 °C (Scheme 4). ^{1}H NMR spectral assignments of **88** were complicated due to restricted rotation about the nitrogen-carbon bond of the carbamate combined with enol-keto tautomerization of the β-keto ester. Methylation of **88** at room temperature was carried out in DME to give **89** in 72% yield. This reaction apparently gave a single diastereomer as verified by GC analysis as well as ^{1}H and ^{13}C NMR spectroscopy. The relative stereochemistry of **89** was not determined. Protection of the latter as the ethylene ketal (**90**, in 89% yield) followed by treatment with $LiAlH_4$ in refluxing THF reduced the methyl ester and the benzyl carbamate in 88% yield to a hydroxymethyl and a *N*-methyl, respectively (**91**).

Scheme 4: Synthesis of **35**, **36a** and **36b**.

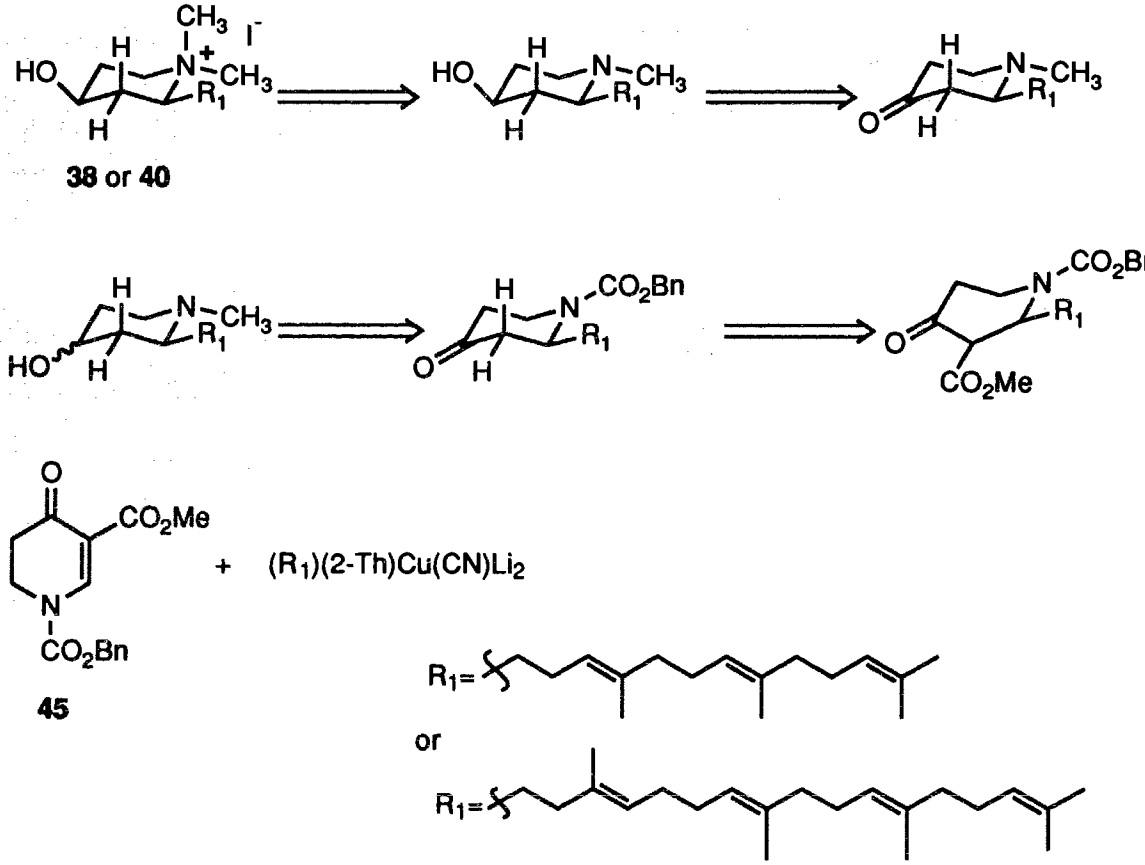


The structure of the product was confirmed by the absence of the ^{1}H NMR signal due to the hydrogens (δ 3.70) of the methyl ester as well as the appearance of the signal due to the hydrogens of the *N*-methyl (δ 2.25). The hydroxyl group of **91** was converted to a tosylate (**92**, in 84% yield) by generation of the lithium alkoxide with *n*-BuLi in ether at 0 °C followed by addition of *p*-TsCl. Other methods of tosylation such as pyridine/*p*-TsCl and KOH/*p*-TsCl, were unsuccessful. Tosylate **92** decomposed slowly upon storage, even at -20 °C, and was used within a day of preparation. Hydride displacement of the tosylate with $LiEt_3BH$[43] in refluxing THF gave the C-3 geminal dimethyl containing ketal **93** in 91% yield. Hydrolysis of **93** to give the 4-piperidone **94** required refluxing in 6N HCl (74% yield). The latter was reduced[44] at -78 °C with $LiAlH_4$ to give the equatorial alcohol as a racemic mixture **35**, in an overall yield of 24% in 9 steps, with >95% diastereoselectivety as verified by gas chromatographic analysis. The equatorial stereochemistry of the hydroxyl group (C-4) of **35** was confirmed by ^{1}H NMR which revealed a doublet of doublets at δ 3.20 (J =12.0, 5.0 Hz) for the axial methine hydrogen, 4-*H*, on the hydroxyl bearing carbon (C-4). Treatment of **35** with ether solutions of anhydrous HCl or MeI gave the respective ammonium salts **36a** and **36b** as white hygroscopic powders.

As noted above the hydrolysis of ketal **93** required extremely harsh conditions. We were unable to remove this protecting group with more milder conditions. For example, no ketone **94** was isolated when **93** was treated with either refluxing 50% acetic acid in methanol-water mixture, refluxing *p*-TsOH in acetone-water, or 50% trifluroacetic acid in water. Even with aqueous 6N HCl, refluxing for 6-10 hours was required for complete hydrolysis of ketal **93**. We have no explanation for the unusual stability of the ketal of this 4-piperidone. Others[45] have observed ethylene ketals of *N*-alkyl-4-piperidones to be stable to

acid. Since much less drastic conditions are required to remove ethylene ketals of *N*-acyl-4-piperidones,[46] the basic character of the *N*-alkyl nitrogen may be playing a role. Conformations of the respective piperidine rings may also control the hydrolytic ability of the ketal substituent.[46] Preliminary experiments indicated that the *E* trisubstituted unsaturation in the 1,5-terpenoid side chains of the proposed targets **37** and **39** and **41** would not survive the above conditions (6N HCl) for ketal removal. Thus, the synthesis of amines **37** and **39** was modified as presented below. A new strategy was devised for the synthesis of **41**, containing geminal dimethyls at C-3 and is presented in Chapter 3.

Scheme 5: Retro-synthesis of compounds **38** and **40**.



4: Synthesis of 37&38 and 39&40

Given the successful introduction of a butyl group at C-2 of **45** we turned our attention to the introduction of chains of medium and exact length corresponding to intermediate **5**. Additional modifications to the synthesis are as follows (Scheme 5): (i) the gem dimethyls at C-3 of the piperidine ring were not introduced; (ii) the carbomethoxy group at C-3 of the β-ketoester was removed by decarboxylation (iii) the resulting *N*-(carbobenzyloxy)-4-piperidones were reduced in a single step to give *N*-methyl-4-piperidinols in a 1:1 (axial:equatorial) diastereoismeric mixture; (iv) the alcohols were re-oxidized to *N*-methyl-4-piperidones and stereospecifically re-reduced to give C-2 substituted *N*-methyl-4-hydroxypiperidines, **37** and **39** with the 4-hydroxyl exclusively in the equatorial configuration.

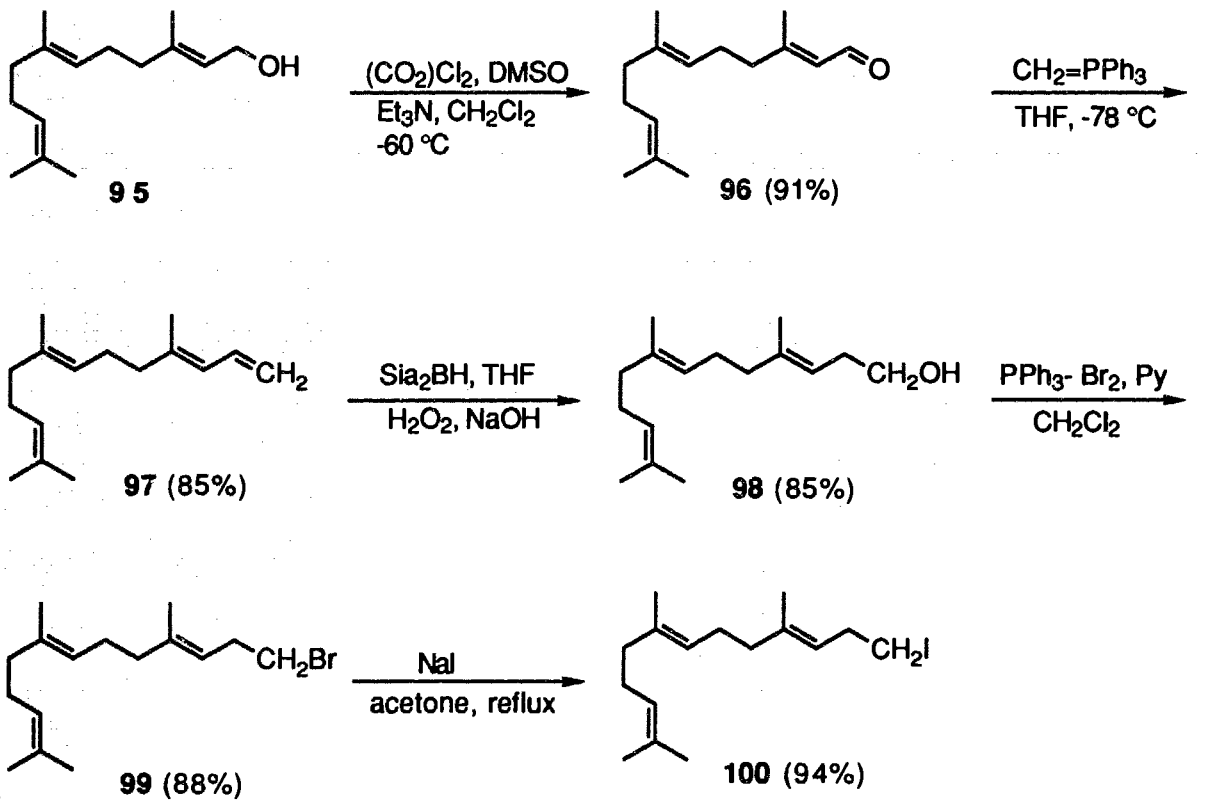
a: Synthesis of 37 and 38

Incorporation of a medium sized side chain containing branched methyls and unsaturation was initially pursued (Scheme 6). The homofarnesyl side chain was chosen, since it provided an opportunity to determine if the position of the branch methyls (side chain C-3 methyl moved to C-4, see **37** *vs* **39** on the chain affects inhibitory power. The change in the original strategy required omission of the C-3 gem dimethyls from the piperidine ring. This exclusion made the syntheses much simpler. Comparison of the inhibitory power of **37&38** with that of **39&40** should give some insight into the necessity of these methyls as "lipophilic" anchors, for proper binding.[7h,24f]

Homofarnesol was prepared from (*E*), (*E*)-farnesol (**95**) using the procedure described by Leopold[47a] for the synthesis of homogeraniol from geraniol. (*E*), (*E*)-Farnesol (**95**) was first converted to farnesal[48] (**96**) in 92% yield *via* a Swern oxidation.[49] The latter was reacted with

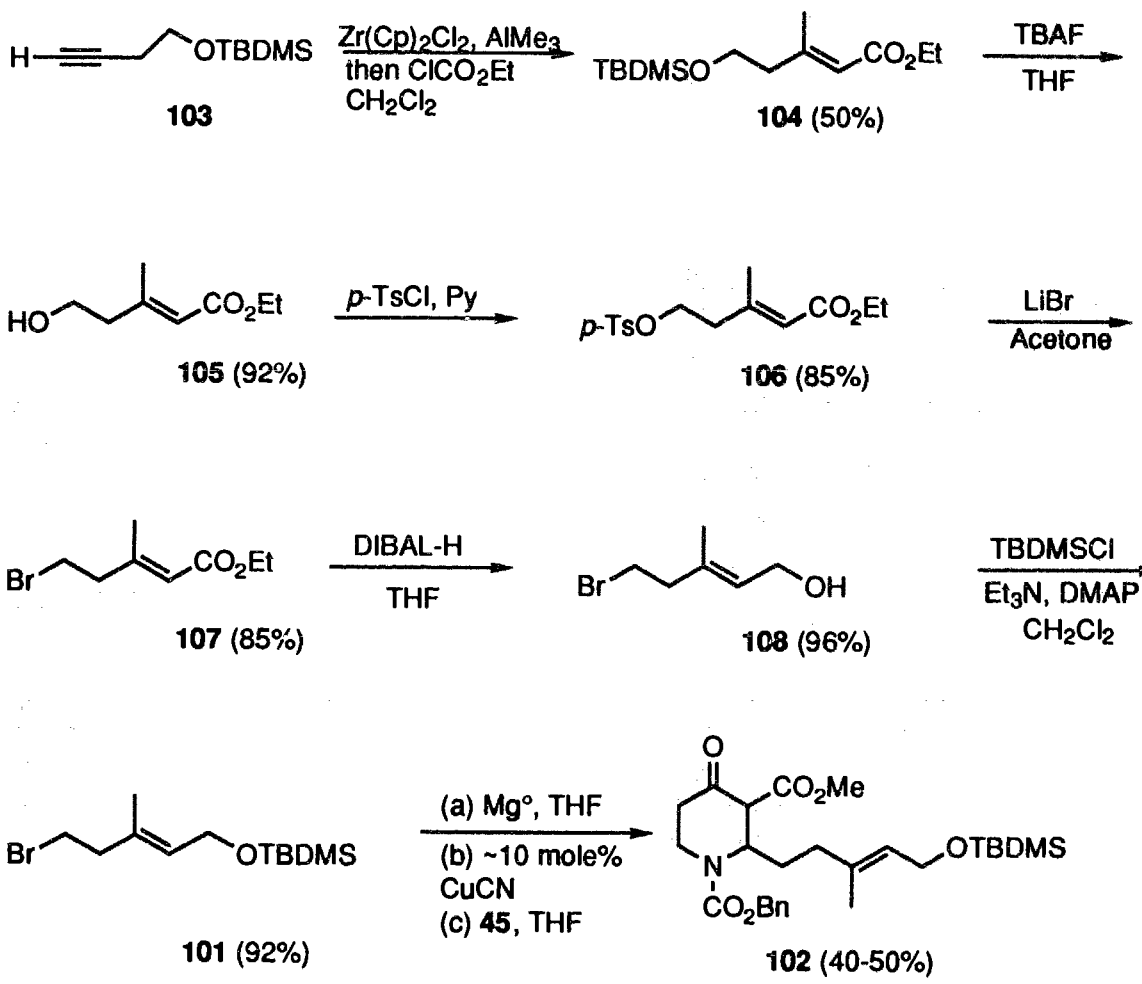
methylenetriphenylphosphorane, generated from methyltriphenylphosphonium iodide and PhLi in THF at -78 °C, to give the tetraene **97**[48] in 91%. Hydroboration of **97** with disiamyl borane[50] followed by oxidative work-up gave homofarnesol (**98**)[47b,c] in 81%. Treatment of **98** with bromotriphenylphosphonium bromide gave homofarnesyl bromide (**99**), which was converted to homofarnesyl iodide (**100**)[47c] by reaction with NaI in acetone.

Scheme 6: Synthesis of homofarnesyl iodide, **101**.



The method of choice for the cuprate mediated 1,4-conjugate introduction of homofarnesyl chain to C-2 of enone **45** involved generation of the higher order (H.O.)[51] lithio-cuprate, **109**, from homofarnesyl lithium produced from **100** with lithium 2-thienylcyanocuprate, (2-Th)Cu(CN)Li.[41]

Scheme 7: Synthesis of **102**.



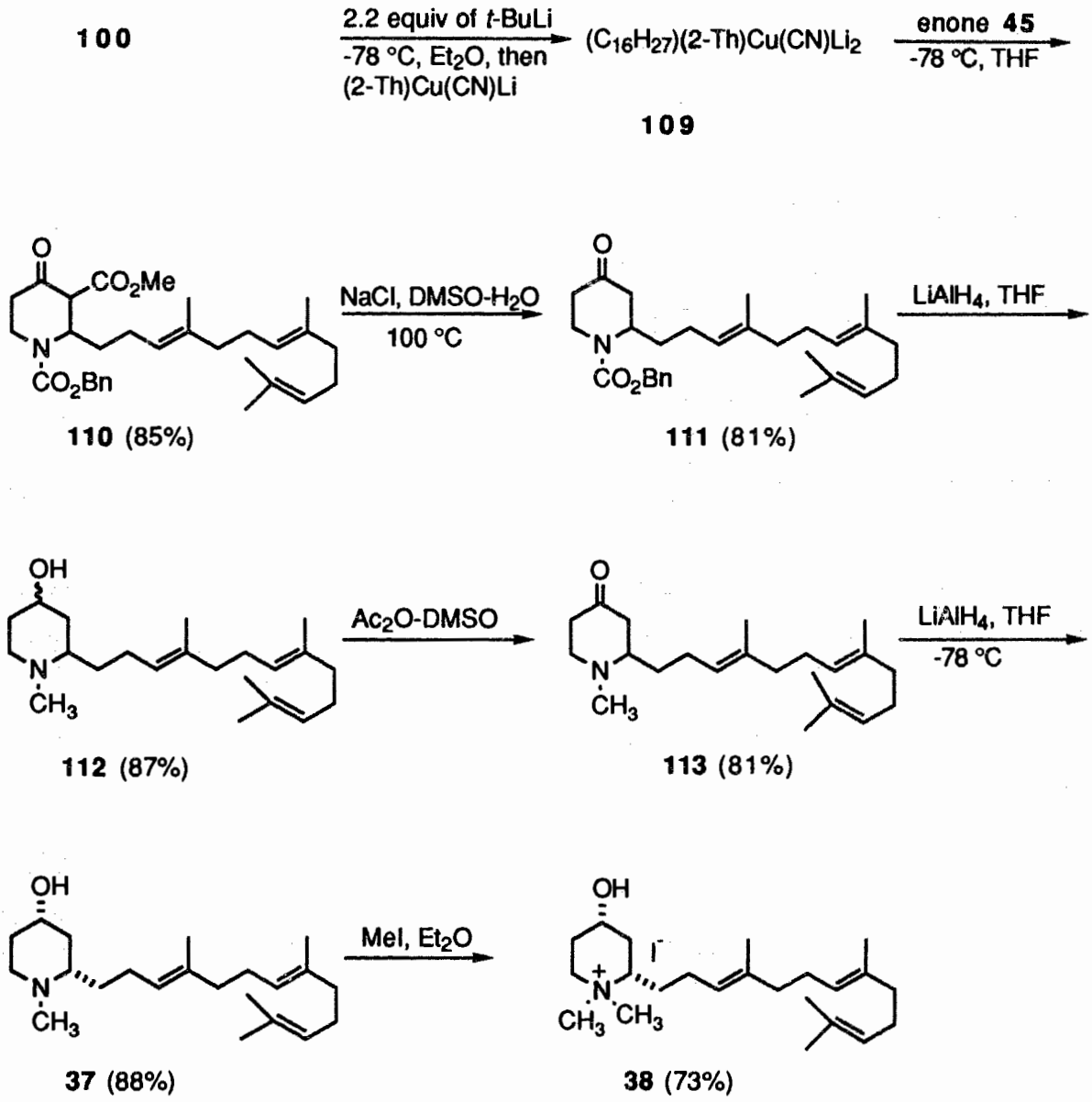
A preliminary study of formation of the Grignard reagent from homoallylic bromide **101** revealed that copper catalyzed conjugate addition (~10% CuCN) or addition of the Grignard derived cuprate (~50% CuCN) to **45** on a relatively small scale (<1 mmol) was troublesome, and the yields were consistently poor (yield of **102** in 40%-50%) (Scheme 7). Gas chromatographic analysis of the quenched homoallylic Grignard reagent revealed several species accompanied the protonated products, suggesting β-elimination and/or self coupling had taken place upon the formation of the Grignard. Substituted piperidone **102** was prepared in hopes that it could be used as an intermediate in the synthesis

of compounds **39** and **41**. Since a more convergent strategy was developed for these syntheses (presented in the following pages), this approach was abandoned.

The bifunctional intermediate **104**[52] was prepared by initial one pot carboalumination[53] of silyl protected homopropargyl alcohol **103** and trapping the vinyl alane with ethyl chloroformate in yields of 45-50% (in ~95% isomeric purity) (Scheme 7). Emulsions encountered during the usual aqueous work-up of $Zr(Cp)_2Cl_2$ catalysed carboalumination reactions were overcome by quenching the reaction with a minimum amount of water (~10 eq.) followed by addition of Celite and hexane. This provided metal salts that were easily filtered. Aqueous work-up of the filtrate and evaporation of volatile impurities under high vacuum resulted in relatively pure (~85% by GC) ester, **104**.[52] Exposure of **104** to a THF solution of tetrabutyl ammonium fluoride yielded hydroxy ester **105**. Tosylation of **105** using standard conditions (*p*-TsCl, pyridine) gave **106**, which was treated with LiBr in refluxing acetone to give bromo ester **107**. The latter was reduced to alcohol **108** using DIBAl-H in THF. Reaction of **108** with *tert*-butyldimethylsilyl chloride gave **101**. The Grignard reagent was prepared by the treatment of **101** with Mg° in THF. Then CuCN (catalytic amount, 5-10%) was added followed by the addition of enone **45** to give **102**, in yields of 40-50%.

By comparison, the conversion of homoallylic iodides **100** to the corresponding lithium species *via* lithium-iodine exchange using *t*-butyl lithium at -78 °C in diethyl ether[54] was very clean (Scheme 8). Lithium-iodine exchange presumably minimizes β-elimination as well as Wurtz-type coupling that occurs during the generation of the corresponding Grignard species.

Scheme 8: Synthesis of **37** and **38**.



Addition of lithium (2-thienyl)cyanocuprate to the homoallyl lithium species generated from **100** at -78 °C in THF gave the corresponding H. O. cuprate, **109**, as a brown solution. Reaction of the cuprate (**109**) with enone **45** produced **110** in 85% yield. The ^{1}H NMR of **110** revealed the presence of an enolic proton (δ 12.2), and the absence of a signal due to the vinyl hydrogen of **45** confirmed that transfer of the homofarnesyl group to C-2 of **45** had occurred (Scheme 8).

Decarboxylation of **110** using Krapcho[55] conditions of heating with NaCl in wet DMSO at 100 °C for 5-8 h, gave **111** in relatively good yields (75-80%). Reduction of ketone of **111** was accompanied by reduction of the carbamate to the *N*-methyl group. This treatment gave amino alcohol **112** as a nearly 1:1 (axial:equatorial) diastereoisomeric mixture. This was deduced from integration of the ^{1}H NMR ($CDCl_3$) signals due to the methine hydrogen, 4-*H*, on the hydroxy bearing carbons (C-4) [$\delta_{\text{axial H}}$ 3.60 (septet due to overlapping tt, J =11.0, 5.50 Hz); $\delta_{\text{equatorial H}}$ 4.07 (multiplet)] and the signal due to *N*-CH_3 hydrogens. The latter appeared as two signals at δ 2.32 and δ 2.24 due to the diastereoisomers containing an axial and equatorial hydroxyl group, respectively. The diastereoisomeric ratio was improved to >95% (equatorial alcohol), racemic alcohol **37** (45-50% in 4 steps), when the diastereoisomers were first oxidized to *N*-methyl-4-piperidone (**113**) and reduced using $LiAlH_4$ at -78 °C.[44] The oxidation was achieved with Ac_2O in DMSO (Albright-Goldman procedure)[56] to give ketone, **113**, in fairly good yields (76-81%) with only minor amounts of the O-acetylation. Treatment of racemic amino alcohol, **37**, with MeI in dry diethyl ether yielded the quaternary ammonium iodide, **38**, as a slightly yellow, hygroscopic semisolid.

b: Synthesis of 39 and 40

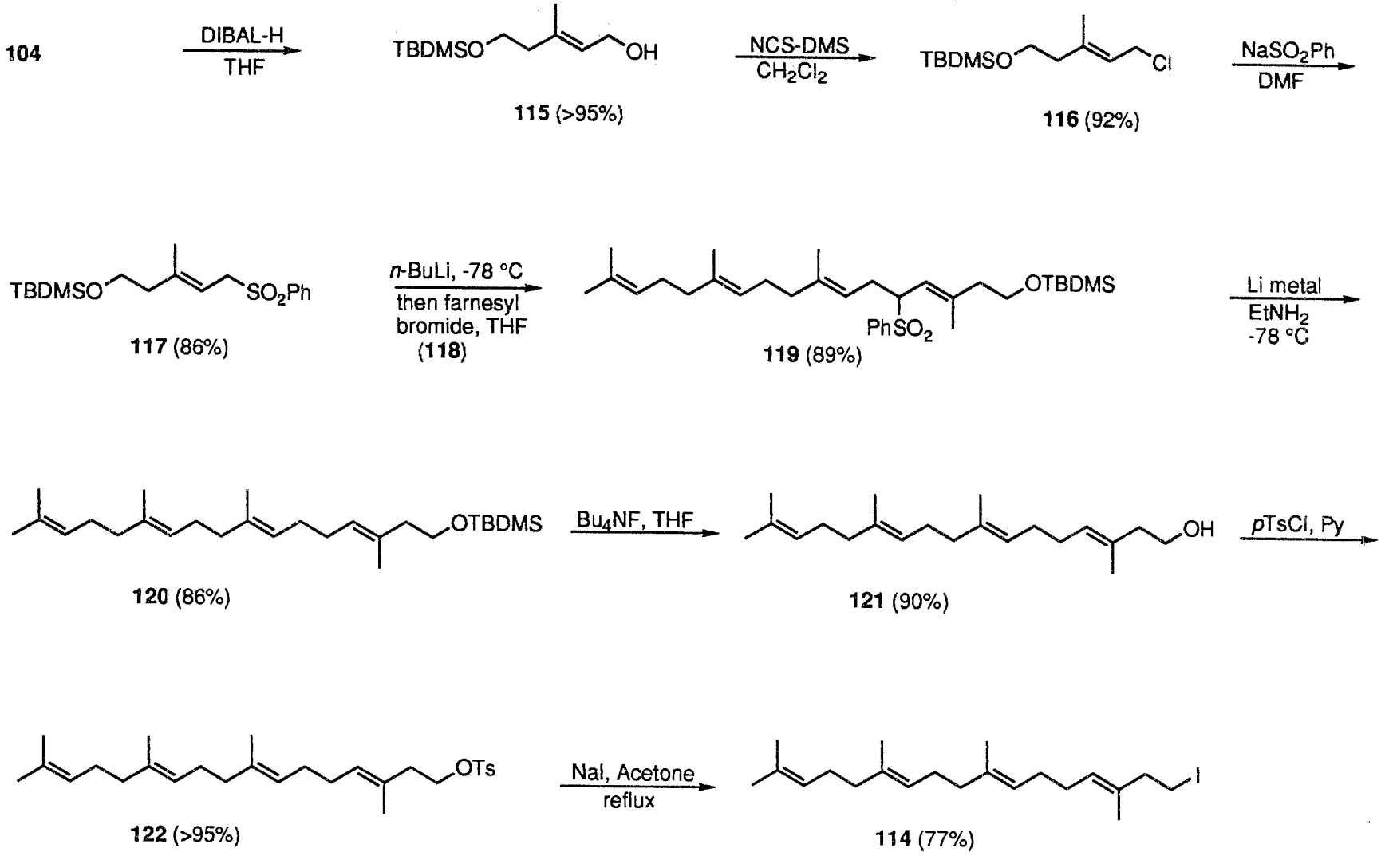
The tetraene iodide, **114**, possessing the actual tetraene side chain found in the presumptive intermediate **5**, was similarly coupled to C-2 of enone **45**. The synthesis of **114** required the preparation and coupling of intermediate **117** to farnesyl bromide (**118**) (Scheme 9).

Silyl ester, **104**, was reduced without purification by DIBAL-H to alcohol **115**[52] which was easily purified by flash chromatography. Conversion of **115** to the allylic chloride **116**[52] was accomplished, in nearly quantitative yield, by treatment of the alcohol with NCS-DMS complex.[57] Reaction of **116** with the sodium salt of benzenesulfinic acid in DMF[58] gave sulfone **117** in 84% over two steps. Generation of the allylic anion[58] of **117** with *n*-BuLi in THF at -78 °C followed by addition of farnesyl bromide (**118**) gave the coupled product **119** in 89%. Removal of the sulfone group[58] with Li in $EtNH_2$ at -78 °C yielded **120**, which was subsequently deprotected by treatment with tetrabutylammonium fluoride to give the free alcohol **121**. Tosylation of **121** with *p*-TsCl in pyridine gave **122**, which was converted to tetraene iodide **114** by reaction with NaI in refluxing acetone.

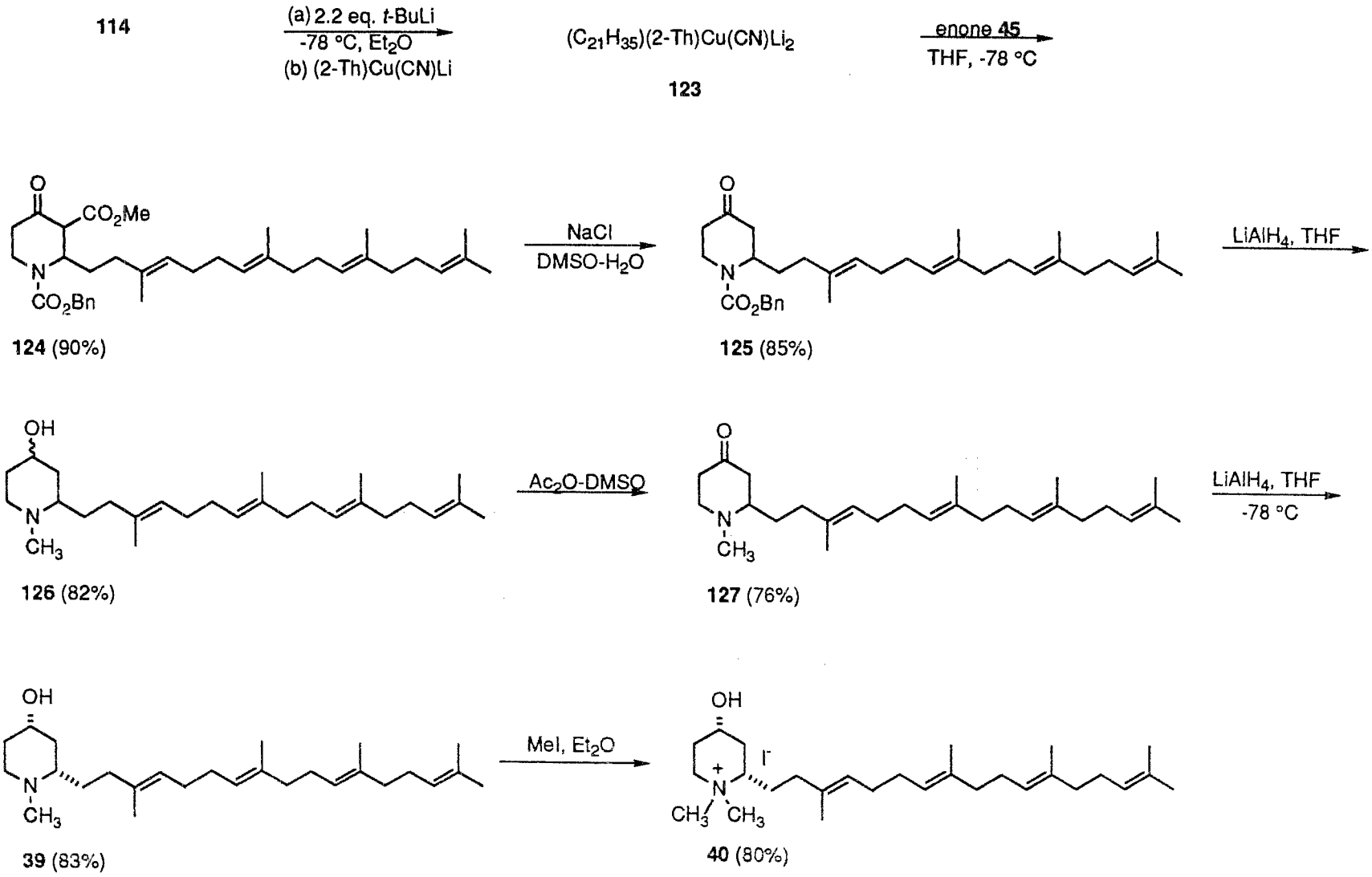
The corresponding H. O. cuprate, **123**, was generated by treatment of iodide **114** with 2.2 eq. of *t*-butyl lithium in Et_2O at -78 °C followed by addition of (2-Th)Cu(CN)Li in THF (Scheme 10). Reaction of the cuprate with **45** at -78 °C in THF gave the C-2 coupled product **124** in 90% yield (overall yield of 14% starting with **103**).

The sequence of steps in the preparation of inhibitor **39** and its methiodide salt **40** was accomplished by exactly the same procedure outlined for the synthesis of compounds **37** and its salt **38**. Decarboxylation of **124** with NaCl in DMSO-H_2O gave **125** in nearly 80% yield. Reduction of **125** gave, as a mixture of diastereoisomers of *N*-methyl amino alcohol **126** in 87% yield. The

Scheme 9: Synthesis of tetraene iodide **114**.



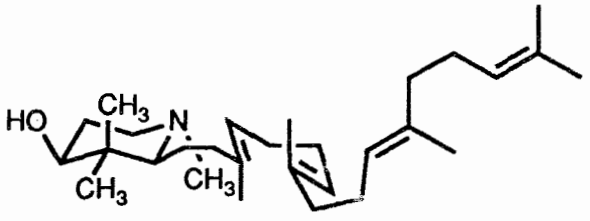
Scheme 10: Synthesis of **39** and **40**.



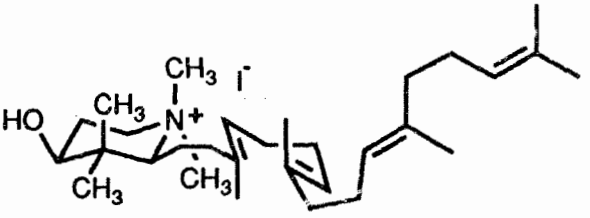
diastereoisomeric ratio was again improved to >95% (equatorial alcohol), using the two-step procedure of oxidation of **126** to *N*-methyl ketone **127** followed by stereospecific reduction using $LiAlH_4$ to give the equatorial alcohol **39** (83%yield; 50% yield in 4-steps). Treatment of amino alcohol, **39**, with MeI in dry diethyl ether yielded the quaternary ammonium iodides, **40**, as slightly yellow, hygroscopic semisolid.

Chapter 3

In this chapter synthesis of aza-analogues **41** and **42** is presented. The results of the biological evaluation of **41** and **42** and aza-analogues prepared in Chapter 2 (**35** to **40**) as inhibitors of OSC from *Candida albicans* are also presented. The present synthetic route starts with a cyclocondensation reaction between methyl 2-methyl-3-oxo-4-pentenoate (**46**) and imine **128** to give C-2, C-3 substituted *N*-methyl-4-piperidone **129**, as the key synthon.



41



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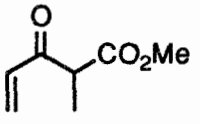
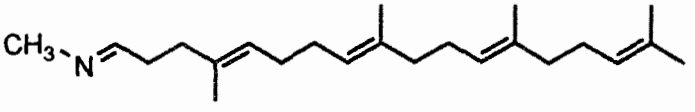


46



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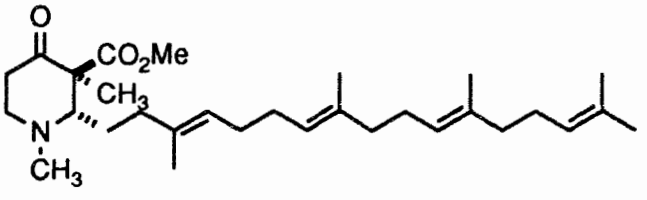


129

I: Introduction

A: Objective

Synthesis of ammonium ion mimics **41** (and **42**) required the introduction of C-3 gem dimethyls on the piperidine ring. These substitutions render the ammonium ion analogues structurally equivalent to the presumptive intermediate **5**. We wanted to determine the effect of the methyl substitution on inhibitory power. We were specifically looking for the ability of C-3 methyl substitution to influence the relative inhibitory power of OSC's of animals and OSC's of yeast origin. Substitutions has previously been noted to affect the ability of 2,3-oxidosqualenoids **20c**[7i,11b] and **21d**,[7h] as well as the ammonium ions,[24f,h,33] to inhibit differentially OSC's from these two sources. 2,3-Oxidosqualenoid **20c** is accepted as a substrate by yeast OSC, yet vertebrate OSC's are irreversibly inactivated by this compound. The opposite is true for 2,3-oxidosqualenoid, **21d**. This compound acts as a substrate for the vertebrate OSC's and as a irreversible inhibitor of the yeast OSC's. Because of these subtle differences in the active sites of vertebrate OSC's versus yeast OSC's, we felt that the preparation of mimics **41** and **42** was crucial for a rigorous biological study.

Although, we were able to introduce gem dimethyls at C-3 of **35** (and **36a** and **36b**) (presented in Chapter 2) starting from **45**, the same strategy could not be used to introduce gem dimethyls at C-3 of the piperidine ring on compounds **41** (and **42**) due to the delicate nature of the terpenoid side chains. The drastic conditions required to remove the ketal protecting group of **93** (see transformation **92** to **93** Scheme 4) would have certainly isomerized the all *E* unsaturations of the terpenoid side chain of **41** (and **42**). Although modifications to this strategy would have allowed for this transformation, we

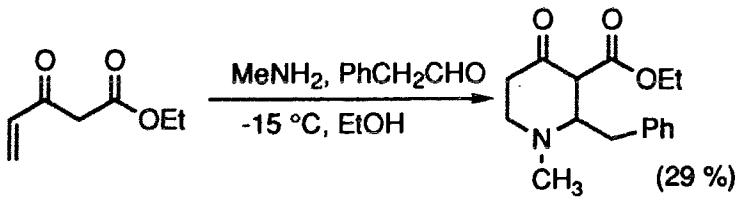
sought a shorter route. The new methodology utilizes the cyclocondensation of Nazarov-type γ,δ-unsaturated-β-ketoester with imines to generate C-2, C-3 substituted 4-piperidones. Piperidone **129** was used as the key intermediate for synthesis of mimic **41** (and **42**).

II: Results and Discussion

A: Background

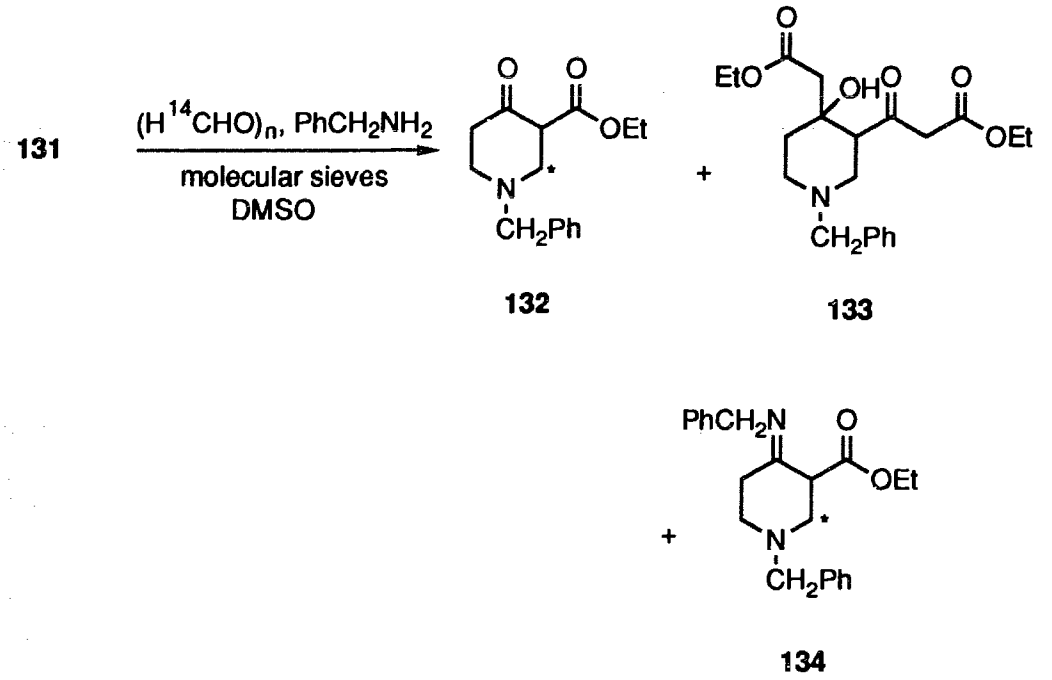
While 3-oxo-4-pentenoates (Nazarov's reagent)[59,60] have been established as excellent annulating agents in the synthesis of several terpenes and alkaloids,[61,62,63,64] of particular interest to us was the use of these reagents in the preparation of functionallized 4-piperidones.[62,63]

Hohenlohe-Oehringen[62a] had first reported the synthesis of 3-carboethoxy-2-benzyl-1-methyl-4-piperidone (**130**) in 29% yield from the reaction of phenyl acetaldehyde, methyl amine and ethyl-3-oxo-4-pentenoate (**131**). Presumably, the imine 2-phenylethylidene methyl amine, generated *in situ* reacts in a Michael fashion with **131**, generating an iminium intermediate that is trapped to give the cyclized product (**130**). More recently Nakatsuka and co-workers[62b] have used the same methodology to prepare ^{14}C labelled N-benzyl-4-piperidone (**132**) from paraformaldehyde and benzyl amine (Figure 3-1). Although the cyclization yield was considerably better than that for **130**, they also isolated **133** and **134**, arising from the large excess (4.0 equiv. of each) of benzylamine and **131** used. To our knowledge, these were the only reported examples, although there were several citations for the preparation of 4,5-dihydro-4-pyridones using **131** and cyclic imino ethers[63a,b] or cyclic thioimidates[63b] as Michael donors.



131 **130**

[62a]Hohenlohe-Oehringen, K. *Monatsch* **1962**, *93*, 576.



132 **133**

134

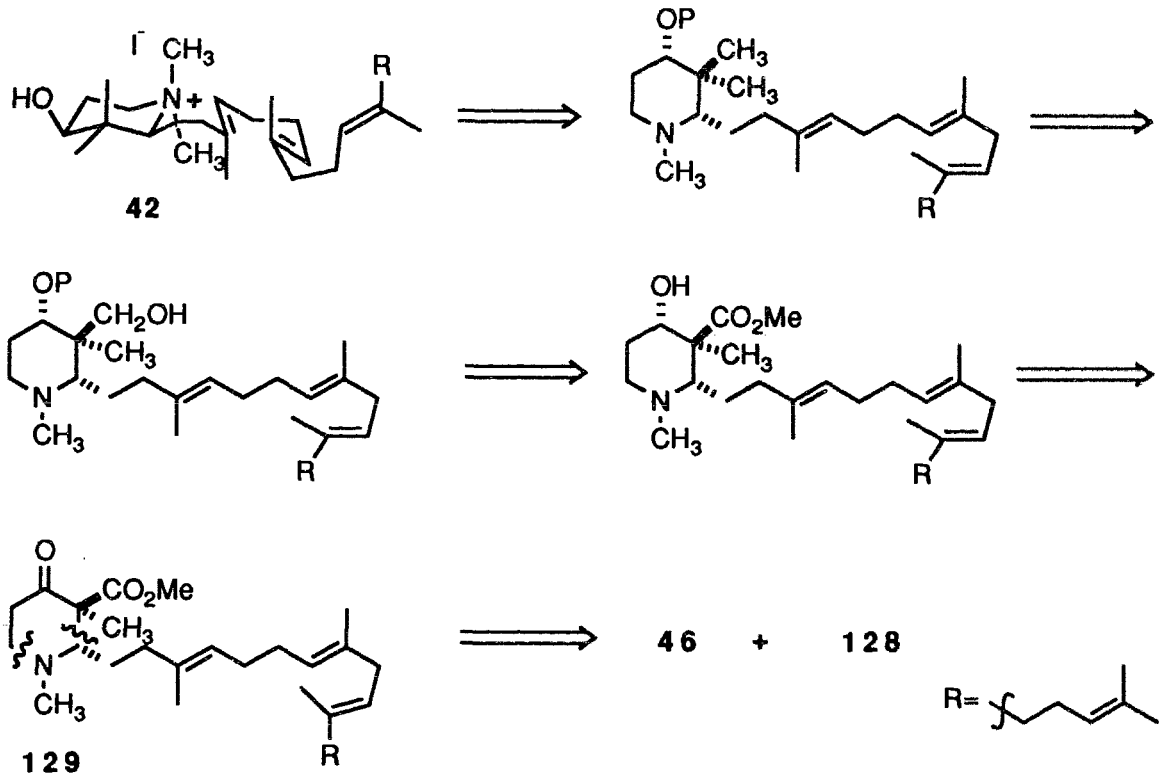
[62b]Nakatsuka, I.,*et al. J. Label Compound. Radiopharm.* **1979**, *16*, 407.

Figure 3-1: Synthesis of 4-piperidones *via* cyclocondensation using Nazarov's reagent,**131**.

B: Synthesis Section

The chosen strategy required that we prepare *N*-methyl-4-piperidone-3-carboxylate (**129**), substituted at C-2 with the appropriate tetraene side chain and at C-3 with a methyl group. We envisioned that the carbomethoxy at C-3 would then be transformed to the second C-3 methyl through a sequence of reduction, followed by tosylation and hydride displacement. This strategy

Scheme 11: Retro-synthesis of **42**.

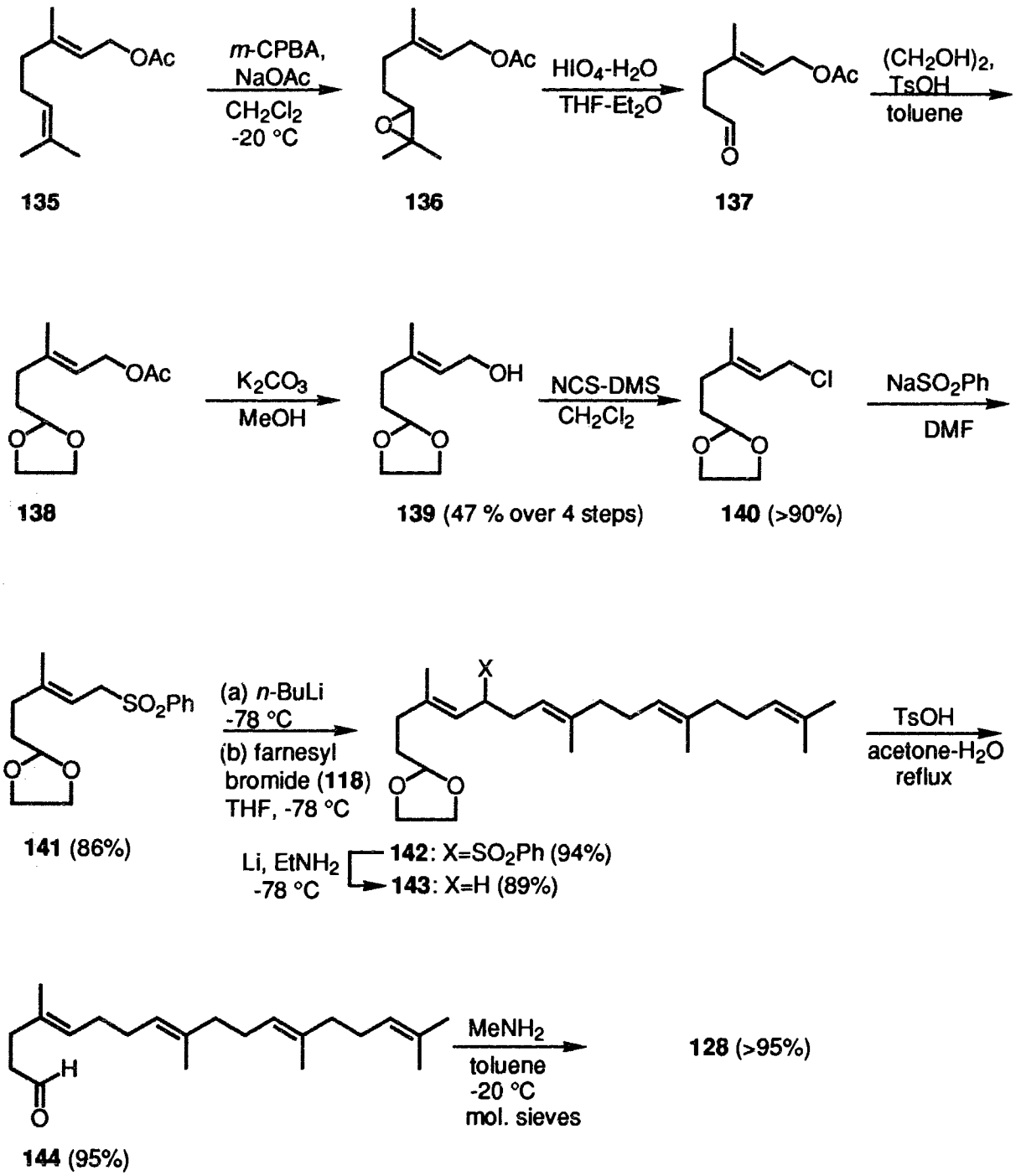


required preparation of C-2 methyl substituted γ,δ-unsaturated-β-ketoester, **46**, and its reaction with the methyl imine **128** of tetraene aldehyde,**144**.

1: Synthesis of Imine 128

We began synthesis of the imine **128** with selective epoxidation of geranyl acetate (**135**) using *m*-CPBA at -20 °C to give **136** in >90% yield (Scheme 12). This was followed by oxidative cleavage of the epoxide, **136**, with $HIO_4 \cdot H_2O$ to give aldehyde **137**. Attempted purification of **137** by distillation under reduced pressure resulted in polymerization, thereby reducing the isolated yield. Therefore,a crude mixture of **137** was treated with ethylene glycol and *p*-TsOH in toluene to give acetal, **138** which, in turn, was deacetylated ($MeOH/K_2CO_3$). Purification at this stage by chromatography on silica gave (pure) alcohol, **139** (47% over 4 steps).[64] The latter was then

Scheme 12: Synthesis of imine **128**.



converted to chloride **140** using NCS/DMS[57], and this was treated with $NaSO_2Ph$ in DMF[58] to give sulfone **141** in 86% yield over two steps. The sulfone, **141**, was then treated successively with *n*-BuLi and farnesyl bromide (**118**) at -78 °C in THF to give coupled product **142**, which was subsequently treated with Li/$EtNH_2$ to give protected tetraene, **143**, in a combined yield of 84%. The ethylene acetal of **143** was then removed by reluxing with *p*-TsOH in aqueous acetone to give aldehyde, **144**, in 90% yield.

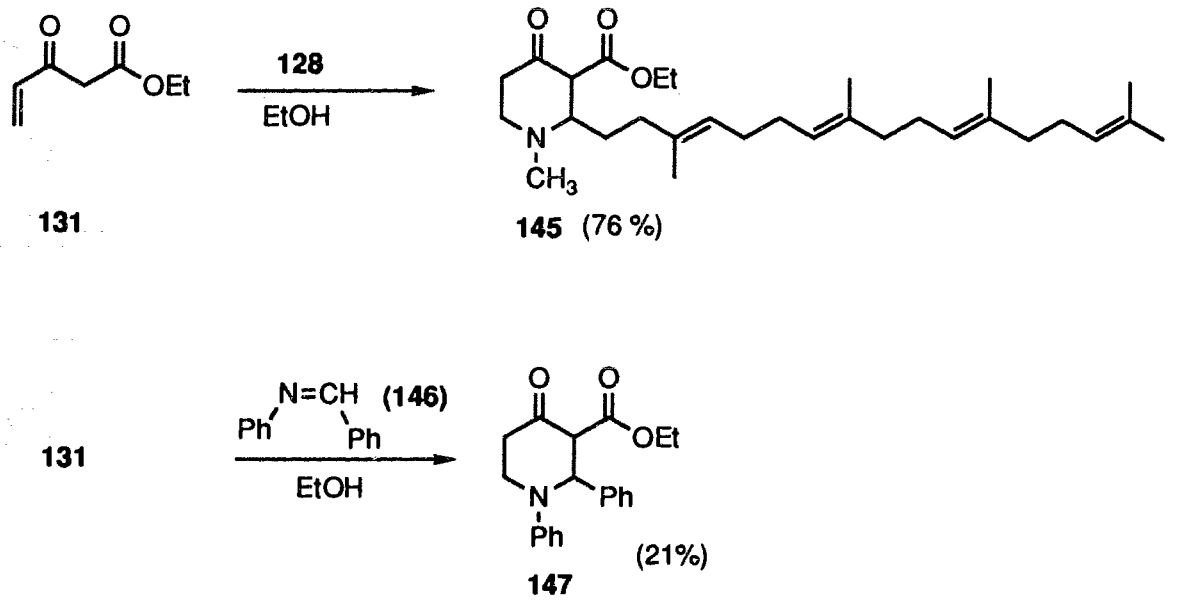
We realized that we might be able to increase the yields of the cyclizations if we could isolate the imine prior to its treatment with the γ,δ-unsaturated β-ketoesters, instead of using them *in situ* as previously described.[62] Imine, **128**, was therefore prepared, isolated and used immediately in the cyclization reaction. Preparation of **128** was accomplished, in nearly qualitative yield, by reaction of $MeNH_2$ (5-10 equivalents) with aldehyde **144** in a sealed tube over activated powdered 3 Å molecular sieves in dry toluene at -20 °C for 6-8 h.

2: Cyclocondensation of Nazarov's reagent (131) with 128 and 146

Cyclization with imine **128** was initially attempted with C-2 unsubstituted ethyl 3-oxo-4-pentenoate **131** (Nazarov's reagent).[59,60] The cyclized product, **145**, was isolated in ~60% yield over two steps using DMSO/THF (9/1) as the solvent (Scheme 13). We were able to increase the yield (**145**) to 76% when anhydrous EtOH was used as the solvent. Aside from enhancing the solubility of the imine, reactions in EtOH required much shorter times, and aqueous work-up was not needed. Several additional solvents[65] including THF, CH_2Cl_2 and CH_3CN as well combinations were examined. The imine was immisible in CH_3CN. The other solvents gave reasonable yields, except that longer reaction times were required than when EtOH was used. We attempted a cyclization

using a more stable imine, benzylidene aniline,[66,66c] **146**. The best yield of cyclization product, **147**, was 21% (from EtOH) obtained when 2 equivalents of **131** were used. The decreased yield in the latter reaction was attributed to the decreased nucleophilicity (Michael donor) of the nitrogen lone pair.[66]

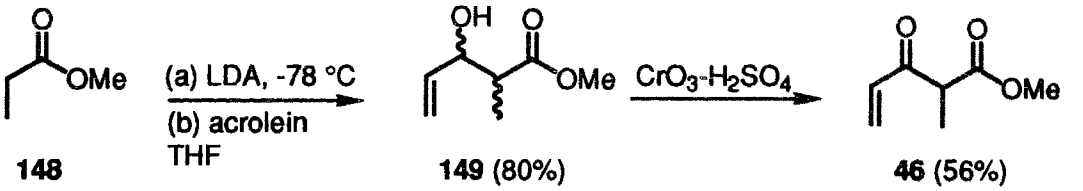
Scheme 13. Cyclocondensation of **131** with **128** and **146**.



3: Synthesis of 46

Having successfully accomplished the cyclization of **128** with **131**, we initiated synthesis of the required C-2 substituted methyl 2-methyl-3-oxo-4-pentenoate (**46**). This synthesis was easily accomplished using a method analogous to that described by Zibuck and Streiber[60a] for the preparation of 3-oxo-4-pentenoates. Treatment of methyl propionate (**148**) with LDA at -78 °C followed by the dropwise addition of acrolein gave hydroxy ester **149** as 1:1 mixture of diastereoisomers in nearly 80% yield (Scheme 14). Hydroxy ester **149** was then oxidized with Jones' reagent at 0 °C to give **46** in 55-60% yield.

Scheme 14: Synthesis of **46**.



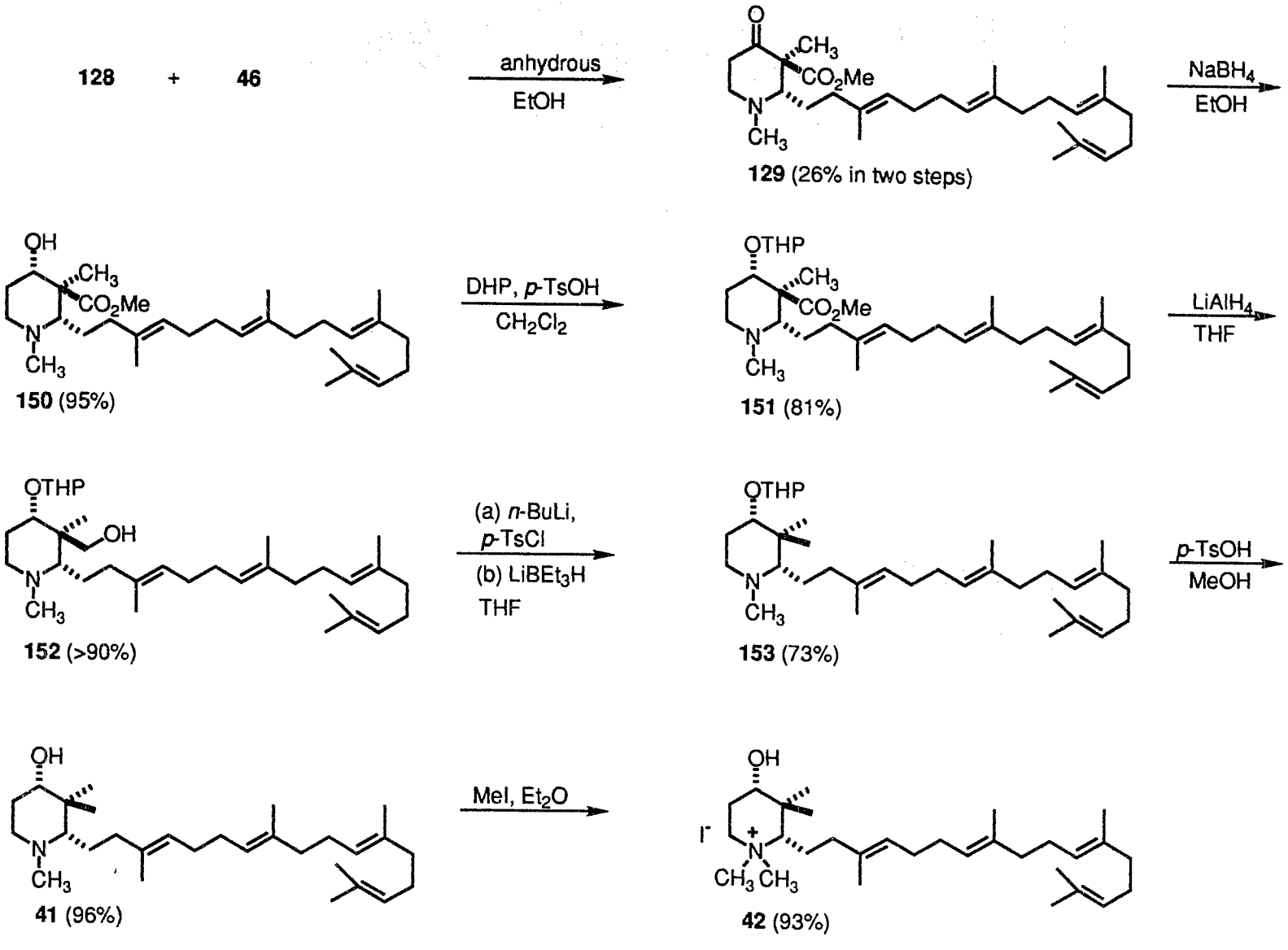
148 **149** (80%) **46** (56%)

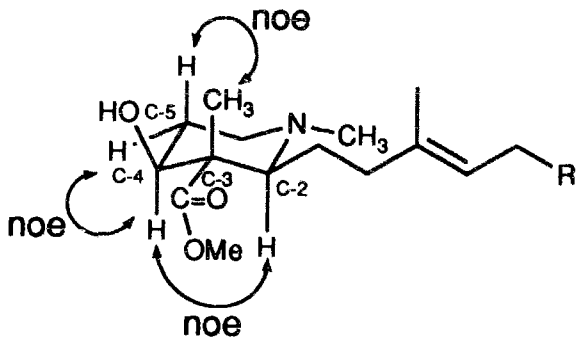
4: Synthesis of 41 and 42

Addition of 2.0 equivalents of methyl 2-methyl-3-oxo-4-pentenoate (**46**) to freshly prepared imine **128**, in EtOH gave the cyclized product, **129**, in 26-30% overall yield in two steps (Scheme 15). We were unable to increase the yield of this cyclization. The reaction gave several other very polar uncharacterized products. It appears that steric interference due to the CH_3 at C-2 of **46** is sufficiently large that the reactivity of the reagent is decreased. Cyclization failed completely with *N*-benzylideneaniline (**146**).

We were surprised to find that the reaction of **46** with **128** gave only one diastereomer (**129**). A NOESY analysis of the reduction product **150** showed a pattern that was indicative of the stereochemistry that is shown above (and Scheme 15). It appears that the CH_3 at C-3 and the side chain at C-2 have a *syn* relationship. NOe's were observed between the CH_3 hydrogen and the axial hydrogen at C-5 of the piperidine ring as well as between the axial hydrogen at C-4 and the axial hydrogens at C-2 and C-6. One explanation for the observed stereochemistry may be that reaction occurs *via* the *Z* enolic form of **46** [65] and a chair-like transition-state (Scheme 16).

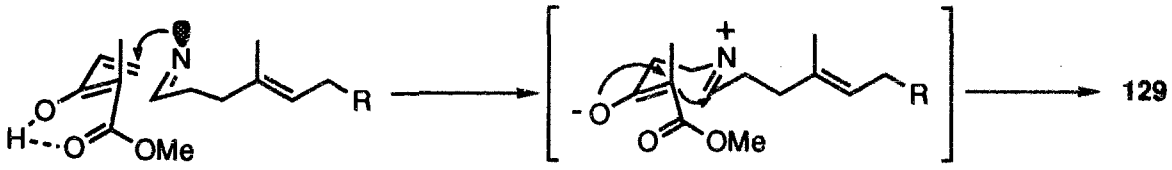
Scheme 15: Synthesis of **41** and **42**.





150

Scheme 16: Proposed transition-state for the synthesis of **129**.



46 + 128

Elaboration of the ring substituents began with the reduction ($NaBH_4$, EtOH) of ketone **129** to hydroxy derivative **150** in nearly quantitative yield. The equatorial alcohol at C-4 was the only diastereoisomer detected by ^1H NMR [C_6D_6/D_2O, $\delta_{4\text{-H axial}}$=3.80 (dd, J=11.5, 5.0 Hz)]. Alcohol **150** was protected without purification as THP ether **151**, which was obtained as mixture of diastereoisomers in 81% yield after chromatographic purification. Attempted purification of **150** by Silica gel chromatography led to much lower yields. Although the THP ether diastereoisomers (**151**) could be separated by flash chromatography, we elected to carry the mixture in the synthesis. The ester of **151** was next reduced to a hydroxy methyl (**152**) using $LiAlH_4$ in refluxing THF. Silica gel chromatography was avoided at this step since there was substantial loss of the product upon chromatographic separation. The hydroxy methyl group of **152** was then transformed to a methyl group in a one-pot procedure

via tosylation and hydride displacement. Thus, to **152** was added sequentially *n*-BuLi and *p*-TsCl to generate, *in situ*, the tosylated product. Subsequent treatment with $LiBEt_3H$ gave the gem dimethyl substituted product **153** in 73% yield. The THP protecting group of **153** was easily removed by treatment with *p*-TsOH in MeOH to give 85-90 % of the C-2, C-3 substituted racemic 4-hydroxy piperidine **41**. Only the signal due to equatorial hydroxy isomer was detected by ^1H NMR [$CDCl_3$, $\delta_{4\text{-}H\ axial}$= 3.16 (dd, J=11.5, 5.0 Hz), confirming the axial orientation of this hydrogen]. This was then converted to its methiodide salt **42** by exposure of **41** to MeI in Et_2O.

C: Biological Results

Amine analogues **37**, **39** and **41** and their methiodide salts **38** , **40**, and **42** , respectively, were found to be potent inhibitors of mammalian[67] and fungal OSC's. They inhibited OSC of the pathogenic yeast *C.albicans*[68,69] both in cell-free extracts and in whole cells. Preliminary studies of the inhibition on 2,3-oxidosqualene cyclase and antifungal activity results are presented in Table II (see experimental section also pp 97-98). These results were provided by Dr. N. H. Georgopapadakou at Hoffmann-La Roche Ltd in New Jersey, New York and by Drs. A.-M. Polak and P. G. Hartman at Hoffmann-La Roche & Co. in Basel, Switzerland.

We have found that for all mimics of intermediate **5** studied, the piperidine ring, even in the ammonium ion form is not sufficient for inhibition; the properly substituted, π-bond containing side chain is also required. Compounds **35**, **36a** and **36b**, containing the butyl side chain at C-2 of the piperidine ring all failed to inhibit the cyclase up to 50 µg/mL (~250 to 200 µM) in whole cells and consequently were not evaluated in the cell-free preparations of the enzyme. These results are not unexpected. The extremely

Table II. Biological results of the evaluation of compounds **35** to **42** as inhibitors of OSC.

	IC_{50} (μM)[a]		*In vitro* activity MIC (μg/mL)[b]				
	C. albicans cyclase						
Compounds	intact-cells	cell-free	*C. albicans*	*S. cerevisiae*	*H. capsulatum*	*A. fumigatus*	*T. mentagrophytes*
35	>250	ND[c]	100 (50)[d]	ND	ND	ND	ND
36a	>250	ND	100 (43)	ND	ND	ND	ND
36b	>250	ND	100 (30)	ND	ND	ND	ND
37	150	9.6	200 (60)	50 (15)[d]	100 (30)	10 (3)	10 (3)
38	10.5	4.2	200 (42)	200 (42)	100 (20)	100 (20)	100 (20)
39	12.5	0.67	200 (50)	50 (13)	10 (3)	>100 (25)	>100 (25)
40	3.7	5.53	50 (9)	50 (9)	100 (18)	>100 (18)	>100 (18)
41	23	0.23	>200 (47)	200 (45)	10 (2)	>100 (23)	>100 (23)
42	3.5	14	50 (9)	50 (9)	100 (18)	>100 (18)	>100 (18)

[a]IC_{50}: Concentration of inhibitor required to reduce enzyme activity by 50%.
[b]MIC: Minimum inhibitory growth concentration.
[c]ND: Not determined.
()[d]: Concentration in μM.

short side chain renders these ions hydrophilic. The natural substrate 2,3-Oxidosqualene (**1**), on the other hand is very hydrophobic. One can safely surmise that the active site of OSC's contains a hydrophobic substrate binding domain. Cattel *et al.*[24f,h] also found that ammonium ion mimics of the intermediate formed upon initial epoxide opening required "lipophilic" squalenoid chains to make them efficient inhibits of the OSC's.

Consider the IC_{50} (Table II) of free amine analogue **37** *vs* amines **39** and **41**. On the cell-free OSC, **37** is over 14-fold less potent than **39** and nearly 42-fold less potent than **41**. Amine **37** has a side chain shortened by five carbons, one olefinic bond omitted and a methyl at C-3 of the side chain relocated to C-4 and the gem-dimethyls at C-3 of the piperidine ring were also absent compared with the structure of the ion (**5**) it is presumed to be mimicking. Structural deviations of **39** from presumptive intermediate **5** are limited to omission of the gem-dimethyls at C-3 on the piperidine ring. In keeping with previous observations,[24f,h] the size of the side chain, the number of olefinic sites in the chain and the positioning of the methyls in the chains are important for inhibitory activity.

Analogue **41** meets all the requirements of presumptive intermediate **5** and should show the best interactions with the putative hydrophobic pocket on the enzyme. Free amine **41**, was indeed found to be the best inhibitor, being nearly 3-fold more potent than amine **39**. Thus, introduction of the gem-dimethyls at the C-3 of the piperidine (**39** *vs* **41**) clearly enhances the inhibitory activity.

At the pH of the assay medium (pH~7.0) free amines are expected to be fully protonated and should mimic the cationic intermediates. In despite of this, permanently charged ammonium ions have been found to be stronger inhibitors than the corresponding free amines. Surprisingly the methiodide salts **40** and

42 were much less inhibitory than expected in the cell-free system. There is a strong possibility that the salts were unstable in aqueous solution, as stock solutions of the methiodide salts tended to lose inhibitory activity with time. There is also the possibility that the methiodide salts are too sterically encumbered and thus cannot interact effectively with the OSC binding pocket, an observation also previously noted by Cattel for analogues of intermediate **4** as inhibitors of OSC's.[24f,h] The lower IC_{50} of **40** and **42** in the whole cells *versus* the cell-free OSC could be partially due to these salts acting as detergents to breach the integrity of the cell envelope.

The amines and their methiodide salts were also examined for their antifungal activity (MIC, minimum inhibitory concentration required to completely inhibit cell growth) against several pathogenic fungi (Table II). For a given compound, antifungal activity does not always correlate with inhibitory activity against OSC (IC_{50}). For instance, **41** has an IC_{50} of 23 μM against whole cells of*C. albicans* OSC, yet shows a MIC of 200 μg/mL against this organism. Compound **39** shows antifungal activity against *Aspergillus fumigatus* and *Trichophyton mentagrophytes* at 10 μg/mL. Analogue **39** displays a MIC of 200 μM against *C. albicans* while exhibiting an IC_{50} of 150 μM. Several explanations can be offered for the observed antifungal activity: the permeability of the cell envelope may be different for different compounds; the cyclase of different fungi may be inhibited with different potency by the compounds; the antifungal activity could be due in part to the ability of these compounds to act as detergents and interrupt the integrity of the cell membrane (those with MIC's >100μg/mL). we are unable to explain the lack of correlation between the IC_{50} of **41** in the whole cell assays and the observed MIC for *C. albicans*. Significantly, the compounds also showed activity against the gram-positive bacterium *S. aureus*, an organism that does not contain sterols.

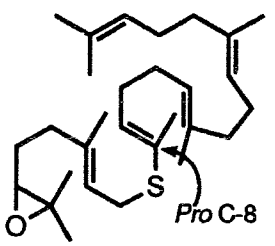
Compounds **35**, **36a** and **36b** showed no significant inhibitory activity against the OSC, yet displayed MIC's of 100 μM against *C. albicans*.

It should be noted that we have observed a 7-fold selectivity of compound **41** for cyclase of *C. albicans* over the pig-liver[67] OSC. The reverse was found for compound **37**, which shows a 16-fold selectivity for the pig-liver[67] OSC. This observation supports our initial rationale for the preparation of **41** and **42** and indeed selective inhibition of OSC from different sources is possible. Moreover, selective inhibition of the yeast OSC over the vertebrate OSC has been achieved, a criterion necessary for design of antifungal drugs. By "tweaking" the substituents on the inhibitors structures, it may be possible to enhance this selectivity even more.

The inhibition results are in agreement with the postulate that the enzymatic cyclization of 2,3-oxidosqualene to lanosterol by 2,3-oxidosqualene-lanosterol cyclase involves an intermediate such as **5**.[9] Compound **41**, a close structural analogue of **5** displays a IC_{50} that is comparable to 2,3-iminosqualene[25] (IC_{50} of 0.15 μM for *C. albicans*),[33] one of the most powerful inhibitors of *C. albicans* 2,3-oxidosqualene cyclase.

Chapter 4

The work presented in this chapter describes preparation of **43** and **44**, which are two of several possible vinyl sulfur substituted 2,3-oxidosqualenes that are expected to be mechanism-based inhibitors of the OSC.



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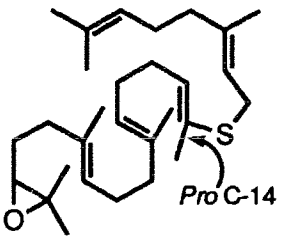


44

I: Introduction

A: Background.

Our approach of using vinyl substituted sulfur 2,3-oxidosqualenes as possible mechanism-based inhibitors to probe the existence of cationic intermediates **6**, and **28** stems from an earlier examination of a similar strategy used by Cattel, *et al.*[26a] These scientists prepared 22,23-dihydro-20-oxa-2,3-oxidosqualene, **154**, containing a vinyl ether linkage, as the possible mechanism-based interrupting group (Figure 4-1). This compound (**154**) was designed for the purpose of examining the existence of protosterol **3**. It was presumed by these workers that this substrate would be accepted and cyclized by rat-liver OSC to the presumptive C-20 (steroid numbering) carbocation intermediate **155**, an analogue of the natural protosterol **3**. This cationic intermediate was then expected to be stabilised by the adjacent oxygen in the form of an oxocarbenium ion intermediate (**156**). Nucleophilic attack on the

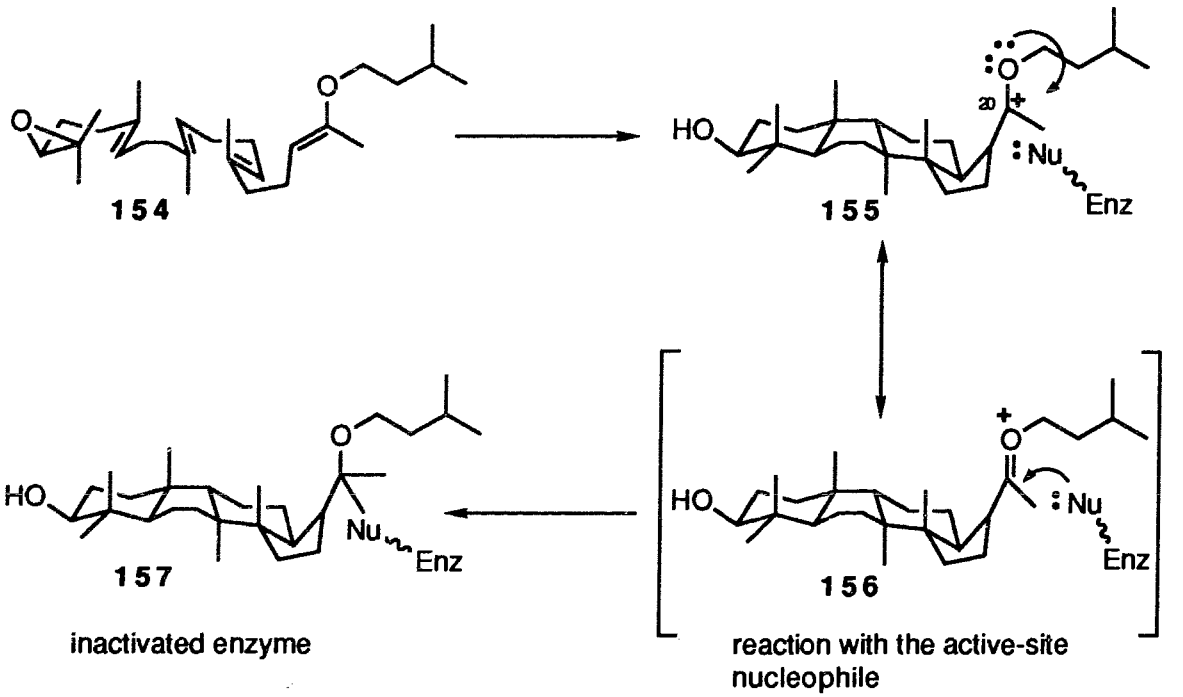


Figure 4-1: 22,23-Dihydro-18(*E*)-20-oxa 2,3-oxidosqualene (**154**), a possible mechanism-based inhibitor of OSC.

oxocarbenium carbon by a basic amino acid residue, responsible for the stabilisation of the normal protosterol **3**, on C-20 of **156** was expected to have lead to the inactivation of the enzyme through covalent bond formation. This was not the case. Oxa-2,3-oxidosqualanoid **154** was found to be a competitive inhibitor, not an irreversible "suicide" inhibitor as expected. Unfortunately these scientists did not determine if any cyclized product(s) had been formed from **154**.

Virgil and Corey,[7f] using an extension of Cattel's[26a] strategy, cyclized vinyl ether 20-oxa-2,3-oxidosqualene (**18**) (Figure 4-2) to demonstrate the initial stereochemistry of C-17 (steroid numbering) in the enzymatic cyclization of 2,3-oxidosqualene (**1**) to protosterol (**3**). Here also, vinyl ether **18** was found to be a weak competitive inhibitor of the yeast OSC. These scientists labelled **18** with a *pro* C-17 ^{3}H and, using this radioactive probe were able to

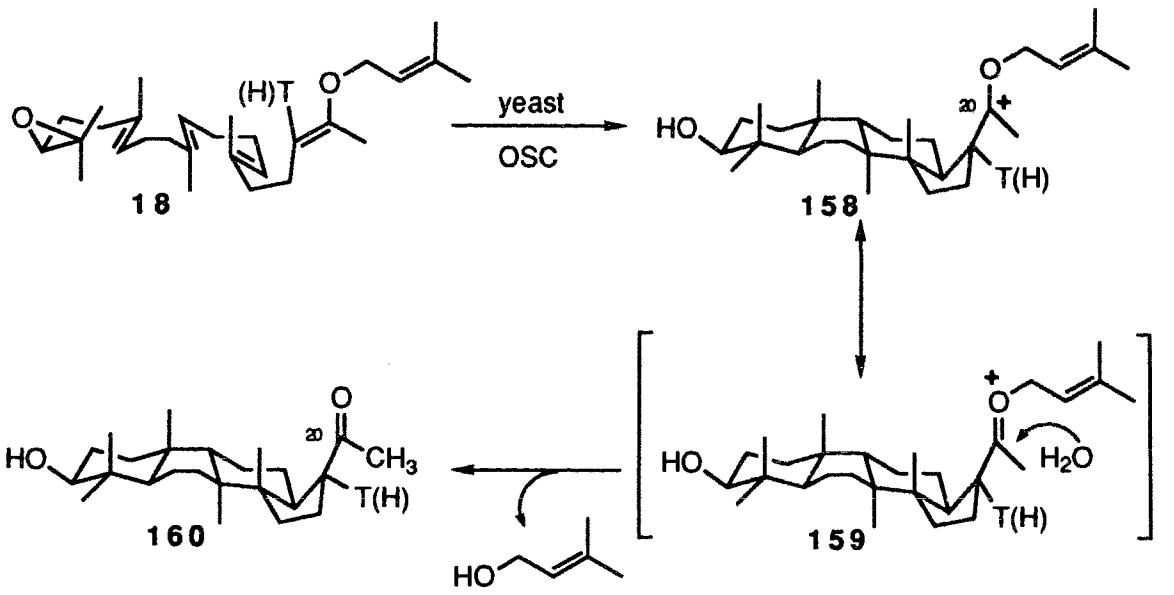


Figure 4-2: OSC mediated cyclization of 18 (*E*)-20-oxa-2,3-oxidosqualene (**18**); determination of the existence and orientation of protosterol intermediate (**3**).

determine that **18** was accepted by the OSC and cyclized to tetracyclic ketone **160**. This presumably arose through oxocarbenium ion intermediate **158** which, upon elimination from the cyclase then reacted with water to give ketone **160**. These results allowed determination of the folding of the side chain during the cyclization of 2,3-oxidosqualene (**1**) to lanosterol (**2**). The protosterol **3** was shown to have a β-oriented side chain at C-17. This is contrary to the previously held view that the cyclization resulted in an α-oriented side chain at C-17 of the C-20 cationic intermediate of protosterol (**3**). Previously it was considered that the C-20 cation underwent "covalent attachment"[4b,d] to an amino acid of the enzyme prior to hydrogen migration from C-17 to C-20. Although the existence of carbocationic intermediate at C-20 has been demonstrated by this work, the lack of irreversible inactivation of the cyclases by **18** suggests that covalent attachment by a nucleophile of the cyclase active site is not necessary. Recent work by Prestwich[7i, 11b] suggests that there seems to

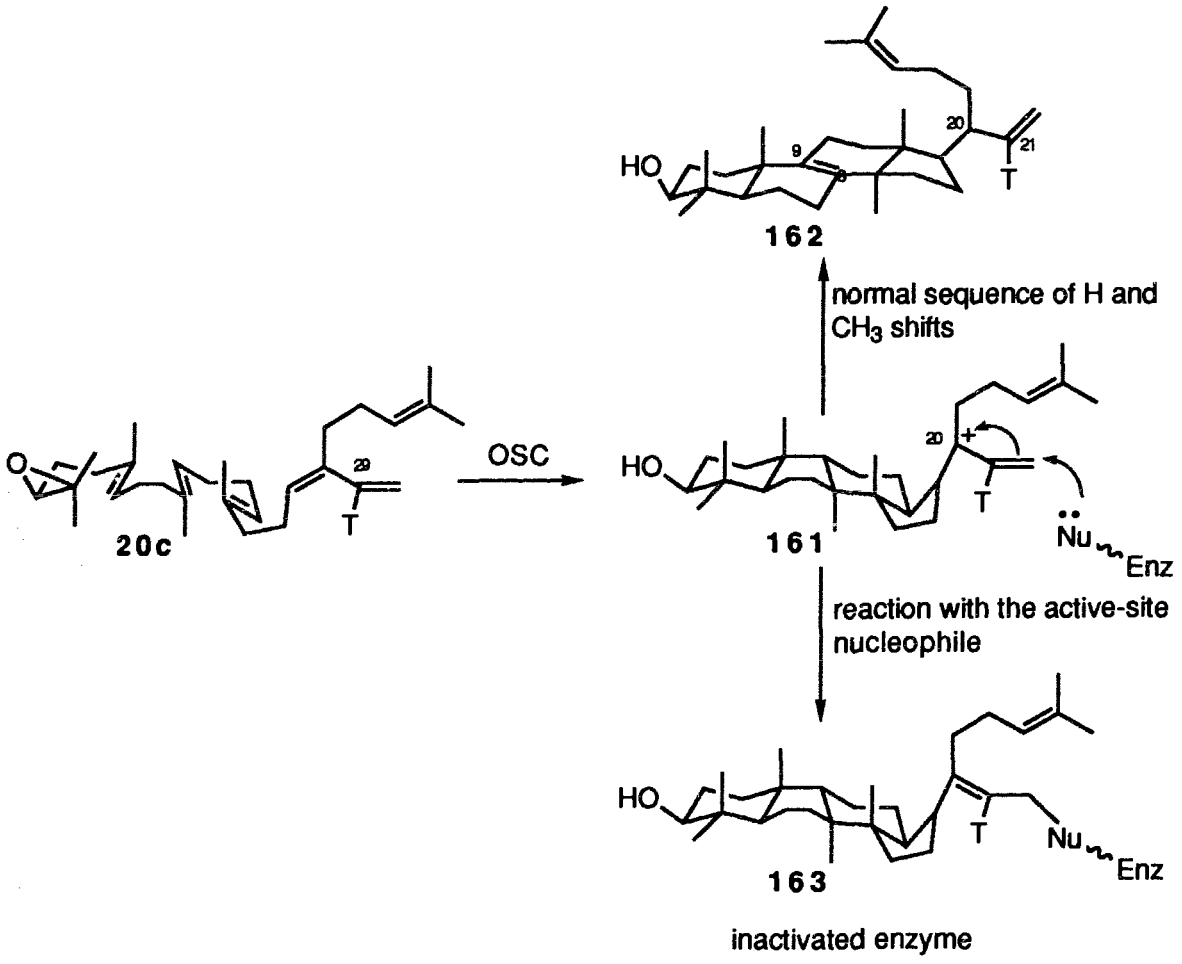


Figure 4-3: 29-Methylidene-2,3-oxidosqualene, **20c**, a mechanism-based inactivator of mammalian OSC.

be nucleophilic residues which may stabilize the C-20 cation through electrostatic interactions.

A slightly different approach was taken by Prestwich and Xiao[7i] to determine the existence of the C-20 carbocation of protosterol **3**. They prepared ^3H labelled 29-methylidene-2,3-oxidosqualene (**20c**) as a mechanism-based inactivator of OSC's (Figure 4-3). 2,3-Oxidosqualenoid **20c** was accepted as a substrate by the yeast cyclase and transformed to tetracyclic lanosterol-like product **162**. They found that **20c** was an irreversible inactivator of vertebrate OSC's.[11b] These scientists postulate that **20c** is cyclized by the

cyclase to the C-20 cationic intermediate **161**. This either undergoes the normal sequence of hydride and methyl migrations and proton loss to give the lanosterol analogue **162** or the C-20 cation was trapped by an active-site nucleophile to give the inactivated enzyme by the mode represented by **163**. The approach of using allylic substituents to stabilise presumptive carbocationic species in the biological system is analogous to the approach used by Johnson and colleagues[16] to increase the yields of tetracyclic products of biomimetic cyclizations (see Chapter 1, figure 1-8).

B: Rationale

Our rationale for the introduction of sulfur at the chosen positions in the oxidosqualene backbone stems from the ability of sulfur to stabilize radicals, carbanions and carbocations[27] α to the sulfur atom. Theoretical calculations[27a] on $^{+}CH_2SH$ have shown that sulfur is an efficient π-and σ-donor to electron deficient α-carbon. Specifically, sulfur forms a very strong π-bond to an adjacent cationic centre. The interaction can be thought of as resulting in thiocarbenium ions,[27b] much like oxocarbenium ions generated in the case of oxygen substituents adjacent to carbocationic centers, as in the case of the vinyl oxa-oxidosqualenoids inhibitors. We have situated the sulfur atoms adjacent to the carbons on which the cation is presumed to be generated. We envisioned the formation of stabilized cationic intermediates that could react with the nucleophilic residues of the active site to give covalently modified OSC (mechanism-based inactivation).

Thiocarbenium ions, if generated by the cyclase as the result of cyclization of **43** and **44**, should be as stable, if not more stable,[27a,c,d] than the oxocarbenium intermediates. This approach of using vinyl thioethers to probe for the existence of carbocyclic cationic intermediates should prove valid.

Chemically, vinyl thioethers are more stable to hydrolysis than vinyl ethers.[27c] It was hoped that the larger sulfur atom and the longer C-S (1.82 Å) bond as compared to C-C (1.54 Å) and C-O (1.43 Å) bonds, would not present a challenge to the OSC's. The acceptance by the OSC's of structurally modified substrates makes **43** and **44** attractive tools for this study. We have chosen to introduce sulfur at *pro* C-7 (**43**) and *pro* C-15 (**44**). These compounds, if found to be inhibitory would represent the very first mechanism-based inactivators of the OSC based on the "step-wise" cyclization involving partially cyclized carbocationic intermediates.

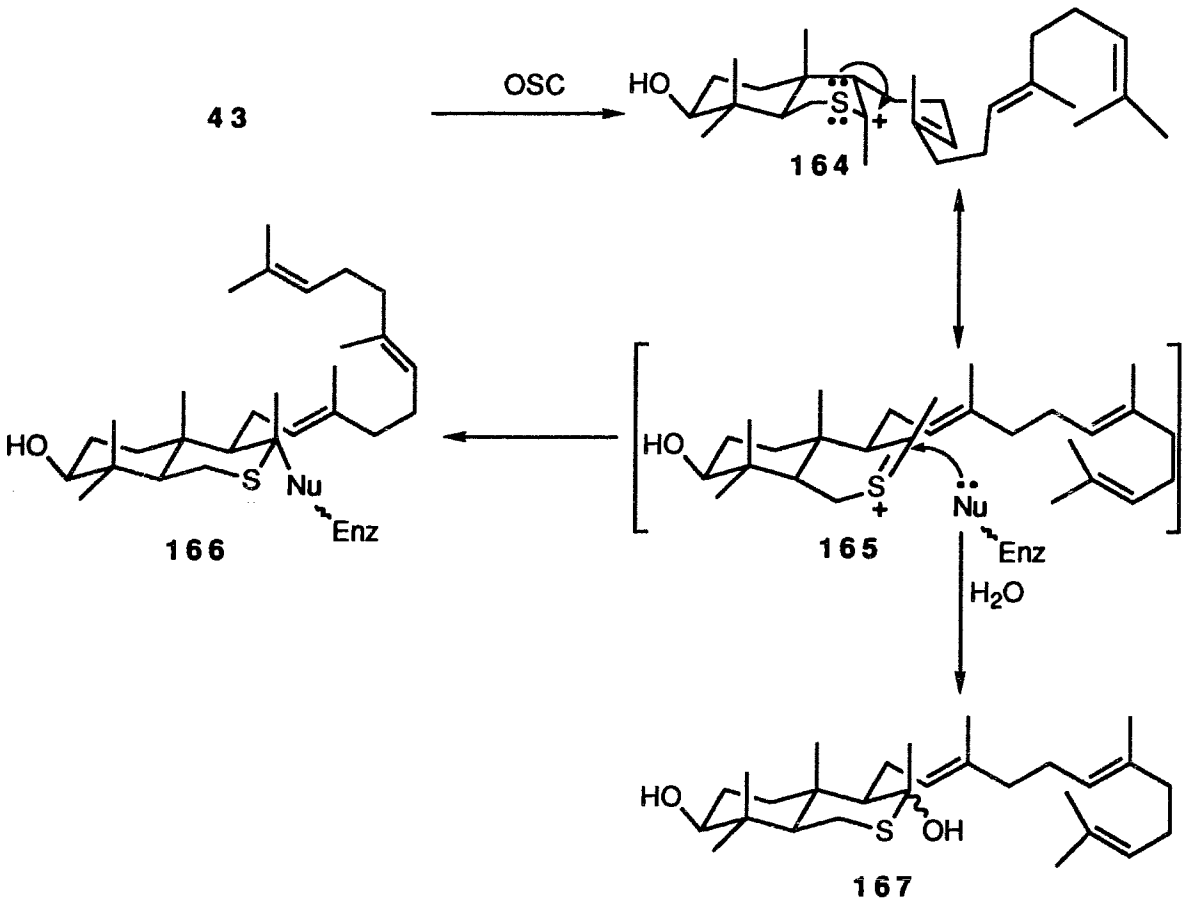


Figure 4-4: 9-Thia-2,3-oxidosqualene (**43**), a potential mechanism-based inhibitor of OSC.

The sulfur atom in 2,3-oxidosqualenoid **43** is adjacent to the *pro* C-8 position. The possible consequences in the placement of the sulfur in this position are outlined schematically in Figure 4-4. The sulfur atom falls in ring B, a crucial point of control in the enzymatic cyclization. Normally (Figure 1-2) ring B must be folded in a boat conformation before reaction can proceed to give cationic intermediate **6**. This reaction is followed by formation of ring C leading to the anti-Markovnikov intermediate **7** (Figure 1-2). Sulfur substitution at *pro* C-7 will hopefully stabilize the C-8 cationic intermediate and lead to the interruption of the cyclization. If the "stepwise" cyclization hypothesis is correct, we would expect to see mechanism based inactivation of the cyclase *via* covalent coupling to *pro* C-8 centre (steroid numbering) of the bicycle **165**, resulting in **166**. Hopefully the coupling will be to the nucleophilic amino acid residue normally responsible for the stabilisation of this cationic intermediate **6**. Alternatively intermediate **165** could be captured by water to give dihydroxylated product, **167**, sulfur substituted analogue of **26**.

The sulfur atom of substrate **44**, in *pro* C-15, is in a position of the squalene oxide that falls in the region of the five membered ring (ring D). This substrate may prove decisive in the elucidation of the mechanism of ring C formation. According to one hypothesis cationic intermediate **6** cyclizes by attack of the C-8 cationic intermediate by the Δ^{14} bond (2,3-oxidosqualene numbering) to give partially cyclized cationic intermediate **27** (Figure 1-9). This then can undergo equilibration between the five-membered Markovnikov stabilised intermediate **28** and the six-membered anti-Markovnikov intermediate **7**.[6a,b,7h,k,l] Products of cyclization are then determined by the substrate. If ring C formation proceeds through an equilibrium in which intermediate **28**[6a,b,7h,k,l] is formed prior to its rearrangement to anti-Markovnikov cationic intermediate **7** (see Figure 1-9), then the sulfur at *pro* C-

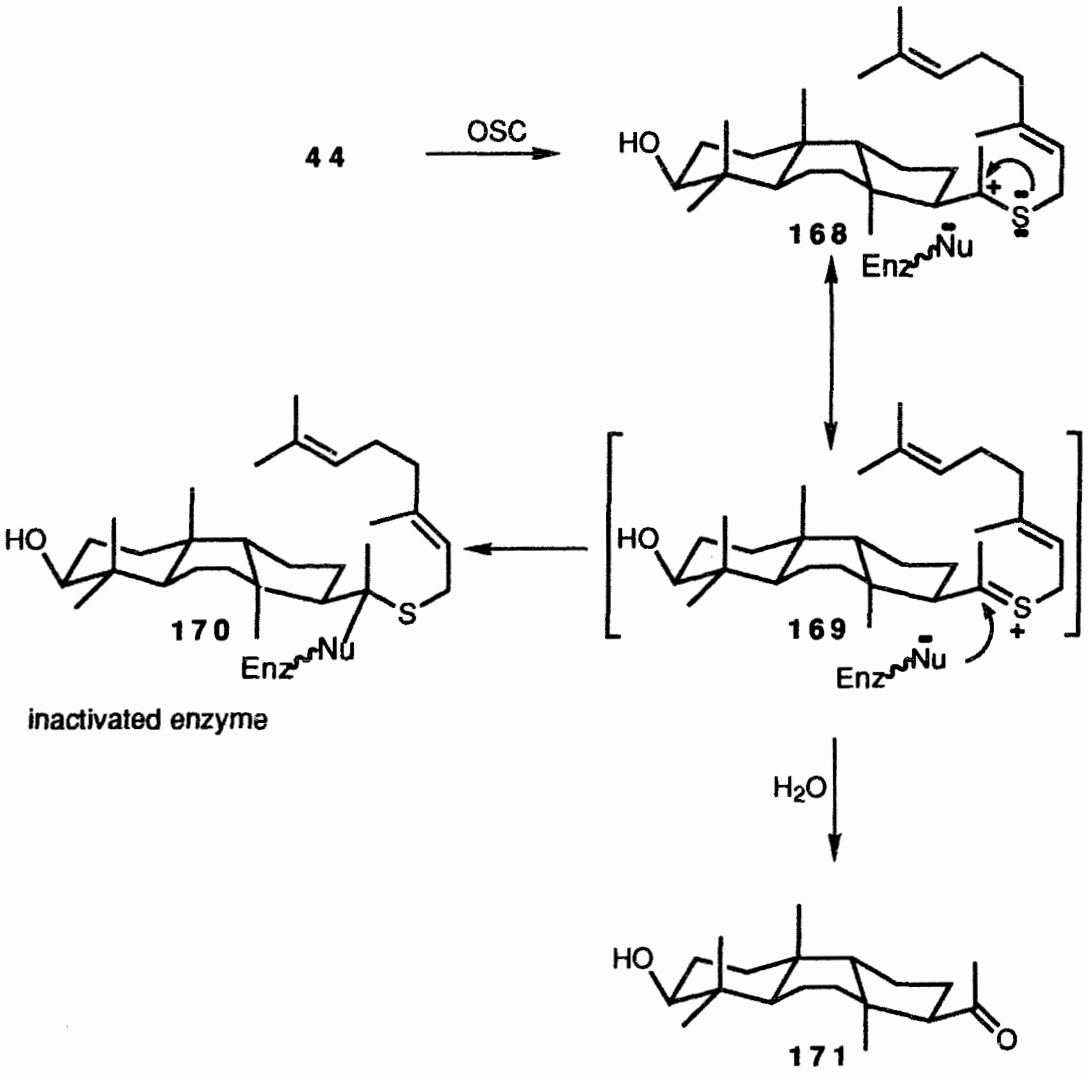
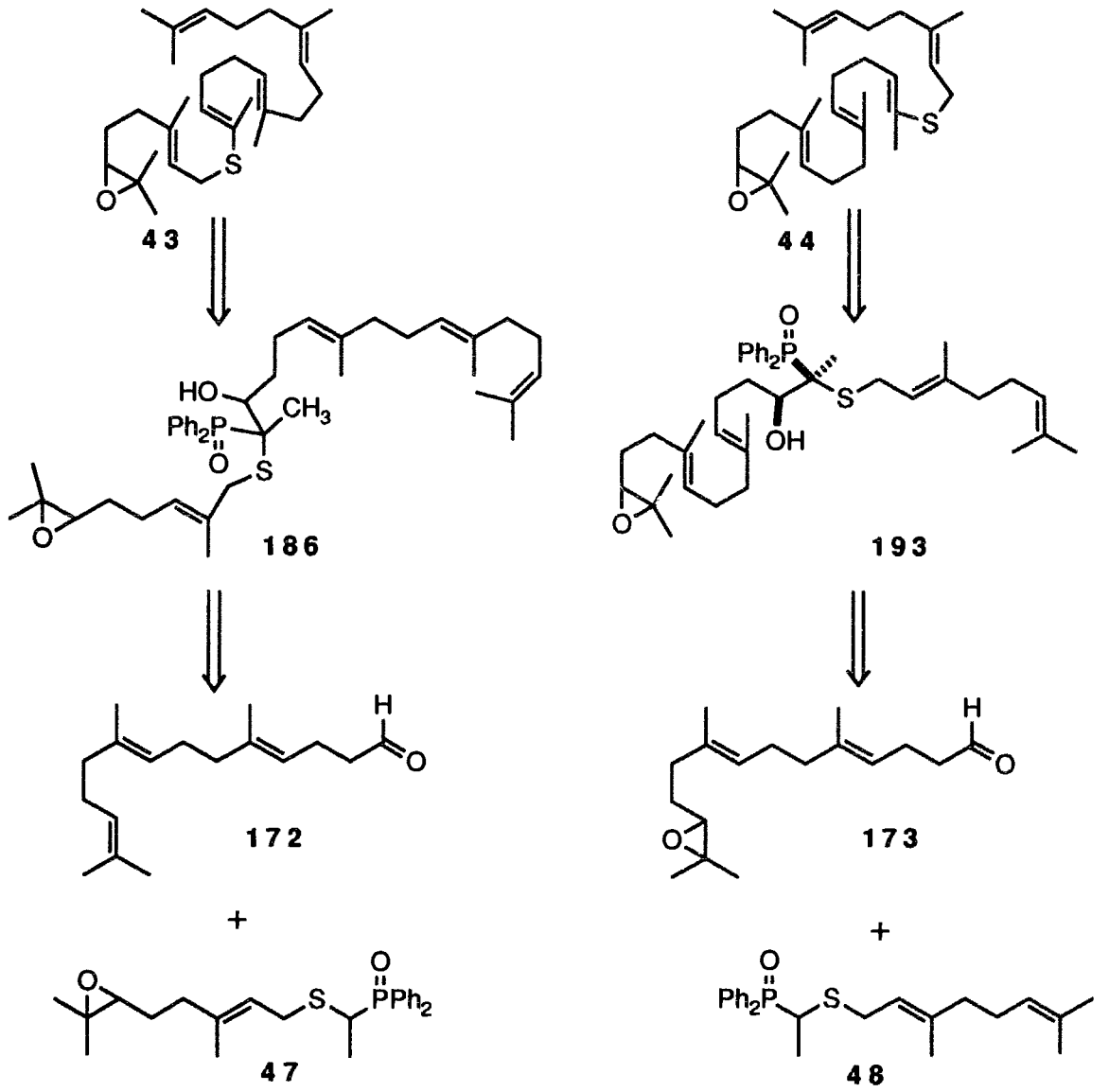


Figure 4-5: 16-Thia-2,3-oxidosqualene (**44**), a potential mechanism-based inhibitor of OSC.

15 should stabilise the cation at C-14. In this event, **44** would be expected to act as a mechanism-based inactivator of the OSC through covalent attachment to the appropriate nucleophilic residue, represented by **170** (Figure 4-5). If formation of ring C proceeds *via* the one-step anti-Markovnikov ring closure giving **7** directly, then the sulfur in **44** would not be expected to interfere in the formation of protosterol **3**, and one might expect to isolate some novel sulfur substituted steroids.

Scheme 17: Retro-synthesis of **43** and **44**.



II: Results and Discussion

A. Synthesis Section

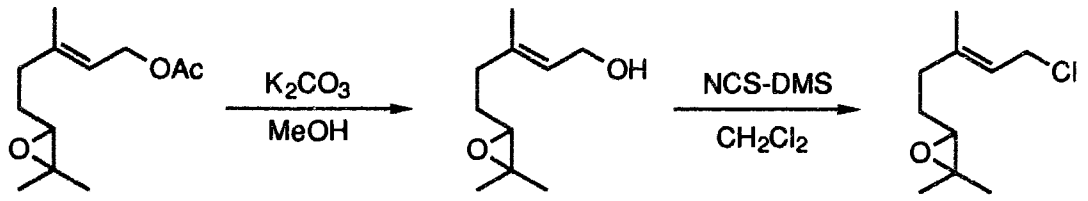
Retrosynthetic analysis of the proposed vinyl thioethers **43** and **44** showed that the *E* vinyl sulfide bond could be best introduced by a modified Wittig-Horner reaction in a strategy analogous to that used by Cattel[26a] and

Corey[71] for the synthesis of vinyl ethers **154** and **18**, respectively. It was envisioned that appropriate diphenyl(thioterpenoidyl)phosphine oxide reagents could be added to triene-aldehyde **172** and epoxy-triene-aldehyde **173** at low temperatures, the resulting α-hydroxy diphenylphosphinoyl oxidosqualenoid diastereoisomers separated and converted to the respective targets (Scheme 17).

1. Synthesis of 9-Thia-[10(*E*)/10(*Z*)]-2,3-oxidosqualene (43/174).

We began this project by preparation of 10*E*/10*Z* mixture (**43/174**) to establish the chemical shifts and the coupling constants of the newly introduced double bond (11-H_{vinyl}) and to test the viability of this approach. It had been established that both dialkyl (α-thioalkyl)phosphonate[70a-c] and diphenyl (α-thioalkyl)phosphine oxides[71] add to aldehydes to give *Z*/*E* mixtures of vinyl sulfides. The *E* isomer is always isolated as the major product (*Z*/*E* ratio of ~40/60). For the synthesis of *Z*/*E* , **43/174**, diethyl phosphonate **175** and aldehyde **172** were prepared.

Scheme 18: Synthesis of chloride **176**.

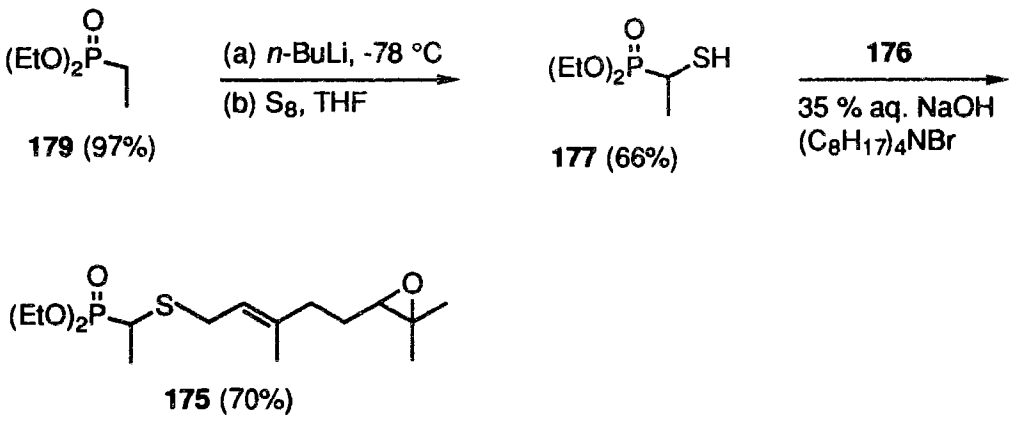


136 **178** (90%) **176** 80%)

Diethyl 1-(3,7-dimethyl-2(*E*)-6,7-oxidooctenylthio)phosphonate, **175**, was prepared from the reaction of 6,7-oxidogeranyl chloride (**176**) with diethyl

phosphorylethyl-1-thiol (**177**).[70c] Synthesis of 6,7-epoxy geranyl chloride was initiated by the treatment of previously prepared 6,7-epoxy geranyl acetate, **136**, with K_2CO_3 in MeOH to give epoxy alcohol **178** in 90 % yield (Scheme 18). The latter was then converted to chloride **176** in 80-85 %, by the method of Corey[58] using NCS-DMS complex in CH_2Cl_2. Phosphoryl thiol **177** was prepared, in 66%, by sequential treatment of diethyl ethylphosphonate **179** with *n*-BuLi followed by the addition of elemental sulfur (Scheme 19).[70c] Reaction of α-phosphoryl thiol **177** in 35 % aqueous NaOH with chloride **176** under phase transfer conditions[71d] gave **175** in 70%.

Scheme 19: Synthesis of phosphonate **175**.



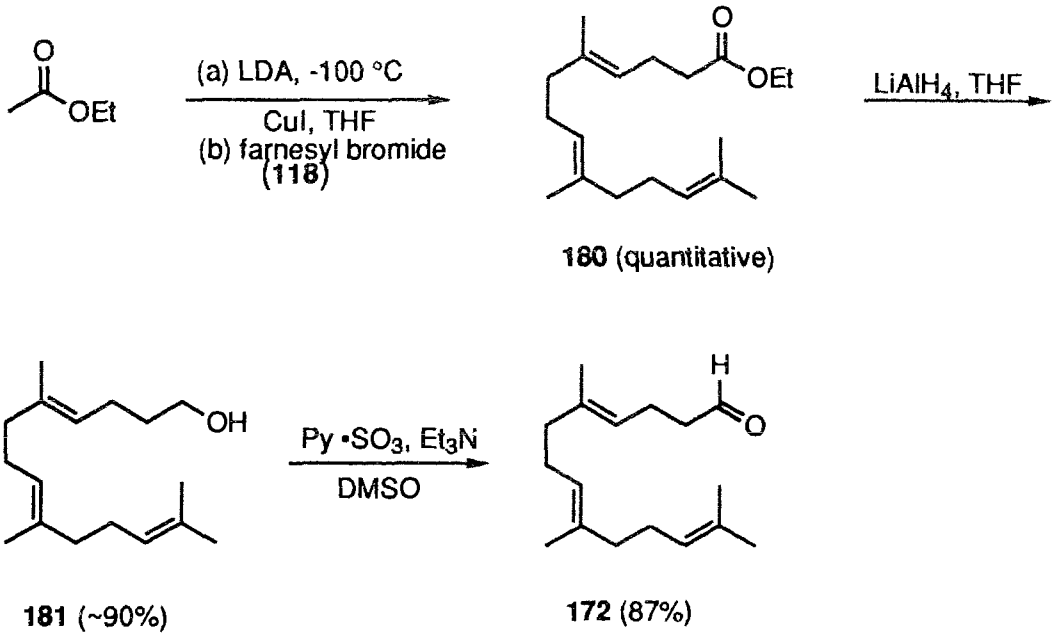
179 (97%)

177 (66%)

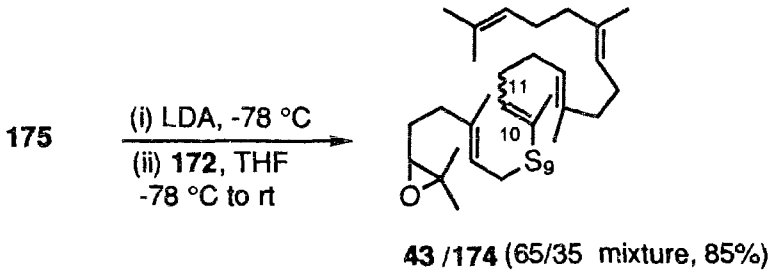
175 (70%)

Triene aldehyde **172** was prepared by the procedure of Coates *et al.*[73] Treatment of farnesyl bromide (**118**) with the Cu (I) enolate of ethyl acetate gave triene ethyl ester **180** in nearly quantitative yield (Scheme 20). This was then reduced to the corresponding alcohol **181** using $LiAlH_4$ again in high yields. Oxidation of **181** to aldehyde **172** was accomplished in 87 % using Py-SO_3 and Et_3N in DMSO.[7f]

Scheme 20: Synthesis of aldehyde **172**.



Scheme 21: Synthesis of **43/174** mixture.



Addition of aldehyde **172** to the lithium anion of α-thiophosphonate **175** at -78 °C and warming to room temperature gave **43/174** as a 65/35 mixture (Scheme 21). ^{1}H NMR ($CDCl_3$) shows the following chemical shifts and coupling constants of the 11-H_{vinyl} : 10 (*Z*) double bond, δ 5.52 (tq, *J*=6.7, 1.31 Hz); 10 (*E*) double bond, δ 5.38 (tq, *J*=6.7 , 1.19 Hz). The 11-*H* of 10 (*Z*), being *syn* to C-10 CH_3 hydrogens, shows a slightly larger coupling constant than the 11-*H* of 10 (*E*) bond, 1.31 Hz versus 1.19 Hz. NOEDS proved to be of little help

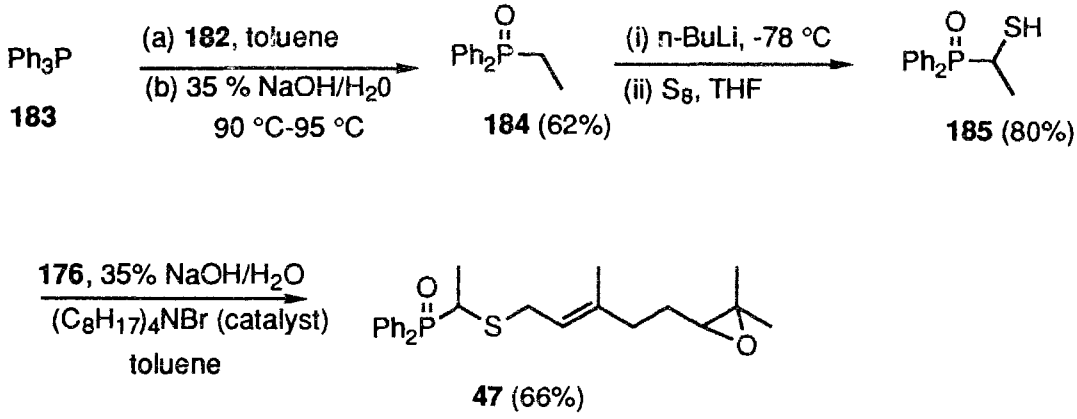
in determining the stereochemistry. The configurational assignment seem to be justified by the integration of the vinyl 11-*H* (*E*) which was larger than that of the vinyl 11-*H* (*Z*). In addition the difference between the chemical shifts and the coupling constants observed for the vinyl 11-*H* were consistent with the assignment. Corey,[70a] Mikolajczyk[70c] and Warren[71a] report that *E* thioethers compounds, in which the vinyl hydrogen is *syn* (or cis) to the sulfur of the vinyl sulfide (*E*), always resonates upfield (lower frequency) then that of the *Z* isomers. Compound **43**, exhibited a signal for the 11-*H* (*E*) at δ 5.38.

2: Synthesis of 10(*E*)-9-Thia-2,3-oxidosqualene (43).

Synthesis of isomerically pure **43** began with the preparation of diphenyl-1-[3,7-dimethyl-6,7-oxido-2(*E*)-hexenylthio]ethylphosphine oxide (**47**). Grayson and Warren[71] have reported the synthesis and addition of diphenyl α-thioalkyldiphenylphosphine oxides to carbonyl compounds to give vinyl sulfides. These authors limited their study to the synthesis of (α-thioalkyl) diphenylphosphine oxides containing thiomethyl and thiophenyl substituents, which could be prepared from reaction of the lithium anions of primary alkyldiphenylphosphine oxides with commercially available dimethyl and diphenyl disulfides. Since we required geometrically pure vinyl sulfides we centred our attention on preparation of thiophosphine oxides that, when reacted with carbonyls, would yield isolable α-hydroxy diphenylphosphinoyl condensation products. Our strategy was then to separate by chromatography the diastereoisomers and convert each diastereoisomer to a geometrically pure vinyl sulfide. The ability of Ph_2PO groups to give isolate-able intermediate hydroxy derivatives made these an exceptionally attractive reagents.[71b,c] Indeed *erythro/threo* diastereoisomers (relative stereochemistry based on L/D erythrose and L/D threose)[72] that are generated could be separated using flash

chromatography. Treatment of the *erythro* (the functional groups are *syn* to each other) adducts with a base lead to the *E* double (by *syn* elimination) bond whereas the *threo* (the functional groups are *anti* to each other) diastereoisomer was converted to the *Z* isomer.

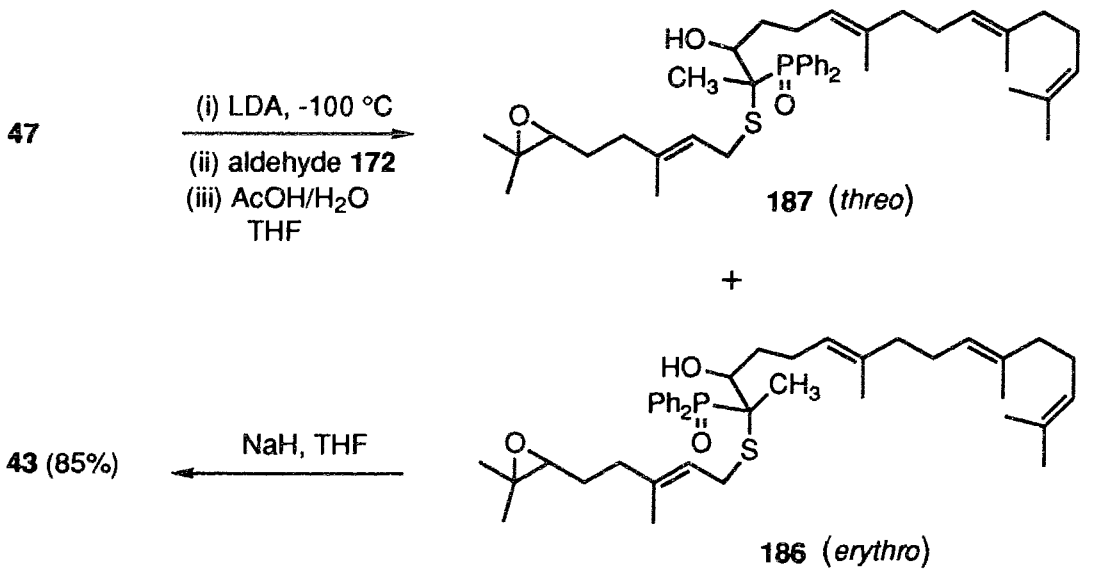
Scheme 22: Synthesis of **47**.



We found that diphenyl-1-[3,7-dimethyl-6,7-oxido-2(*E*)-hexenylthio]ethylphosphine oxide (**47**) could be easily prepared by a modified procedure of Mikolajczyk[70c] previously used for the synthesis of diethyl 1-(3,7-dimethyl--6,7-oxido-2(*E*)-octenylthio)phosphonate (**176**). Thus treatment of ethyl triphenylphosphonium bromide, prepared from ethyl bromide (**182**) and triphenylphosphine (**183**) with 35 % aqueous NaOH solution[71,74] at 100 °C for 5 h gave diphenyl ethylphosphine oxide (**184**). Sequential addition of *n*-BuLi at -78 °C followed by the addition of elemental sulfur to **184** gave diphenylphosphinoyl ethyl-1-thiol (**185**) in 80 % (Scheme 22). The alkylation of thiol **185** with 6,7-epoxy geranyl chloride, **176**, under phase transfer conditions, using 35% NaOH,[71d] gave **47** in 66% yield.

The addition of aldehyde **172** to the lithium anion of **47** at -100 °C, followed by an acetic acid quench, gave the hydroxy intermediates **186/187** as a ~40/60 (^{1}H NMR) mixture in 73% yield (Scheme 23). Partial separation of the

Scheme 23: Synthesis of 9-thia-2,3-oxidosqualene (**43**).

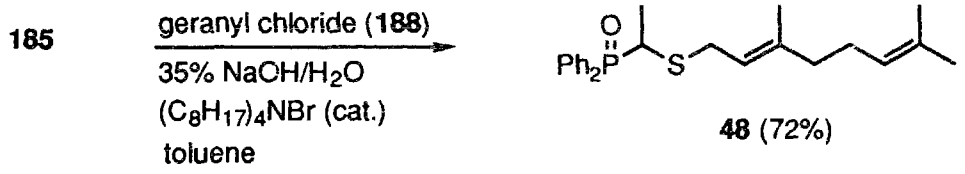


combined yield, **186** and **187**, 73%

diastereoisomers was achieved using two cycles of flash chromatography. This provided pure *erythro* diastereoisomer **187**, which eluted first, and then *threo* diastereoisomer **186** contaminated with ~10 % of *erythro* diastereoisomer. The treatment of **187** with NaH in THF gave pure 10(*E*)-9-thia-2,3-oxidosqualene (**43**). ^{1}H NMR ($CDCl_3$) shows a signal for vinyl 11-*H* at δ 5.38 (1H, tq, *J*=6.70, 1.19 Hz). A nOe of 2.9% was observed between 11-*H* and 8-*H*, confirming the assigned stereochemistry. Prolonged storage of the thia-oxidosqualenes resulted in decomposition.

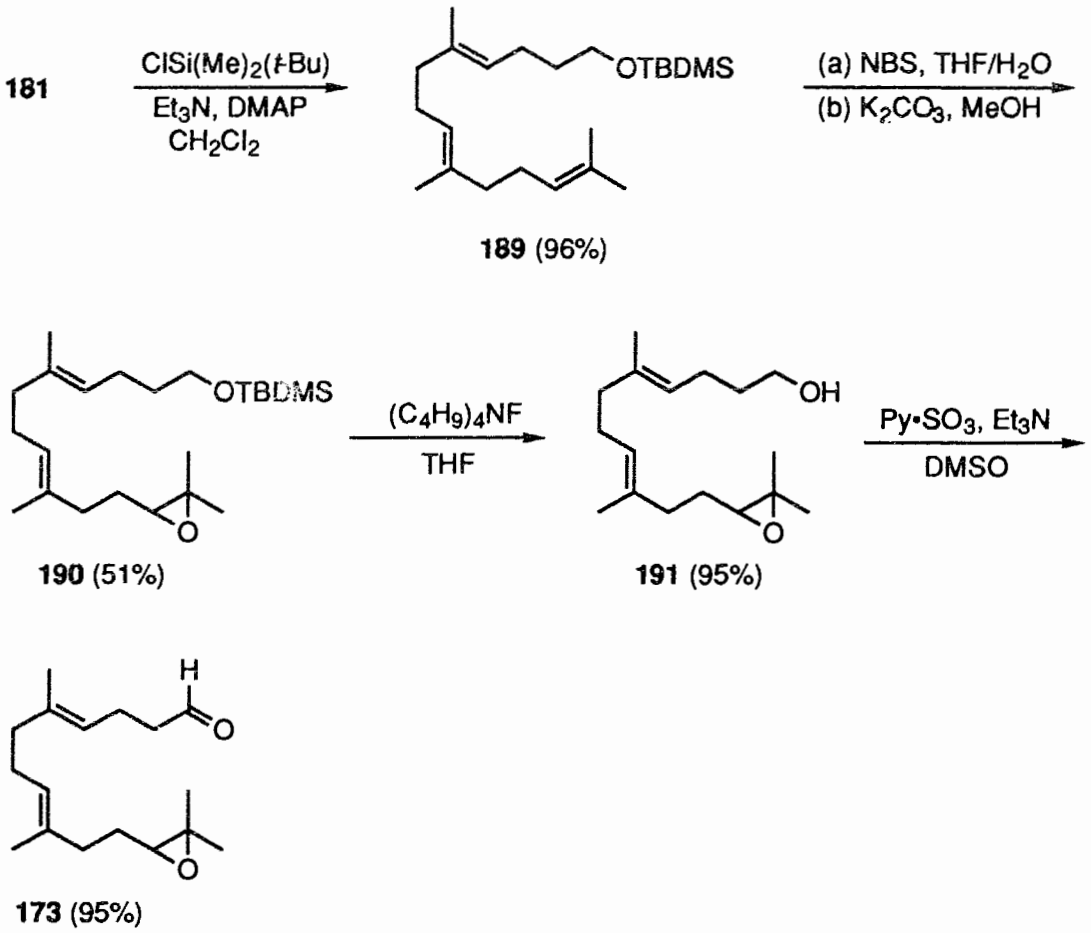
3: Synthesis of 14(*E*)-16-Thia-2,3-oxidosqualene (44). Synthesis of 14(*E*)-16-thia-2,3-oxidosqualene (**44**) was similarly accomplished by the reaction of diphenyl-1-(thio-3,6-dimethyl-2(*E*),6(*E*)-octadienyl)ethylphosphine oxide (**48**) with 12,13-epoxy-5,9,13-trimethyl-4(*E*),8(*E*)-tetradecatrien-1-al (**173**). Reaction of geranyl chloride, **188**,[75] with diphenylphosphinoyl ethyl-1-thiol, **185**, under phase-transfer[72] conditions using 35% NaOH gave **48** in 72% yield (Scheme 24).

Scheme 24: Synthesis of **48**.



Synthesis of 12,13-epoxy aldehyde (**173**) began with the initial protection of alcohol **181** as a silyl ether **189** in 96% yield (Scheme 25). Reaction of silyl[7f] ether **189** in H_2O/THF (40/60) with NBS followed by treatment with K_2CO_3 in MeOH gave epoxide **190** in 51% yield in two steps. When alcohol **181** was protected as the acetate then treated with NBS in H_2O/*t*-BuOH[76] and then K_2CO_3 in MeOH, a much lower yield in terminal epoxide, **191**, (18 % in two steps) was obtained. Removal of the silyl ether, **190**, to give alcohol **191** in 95% yield was facilitated with tetrabutylammonium fluoride. Oxidation of **191** using Py·SO_3 in DMSO in the presence of Et_3N gave epoxy aldehyde **173** in nearly quantitative yield.

Scheme 25: Synthesis of aldehyde **173**.

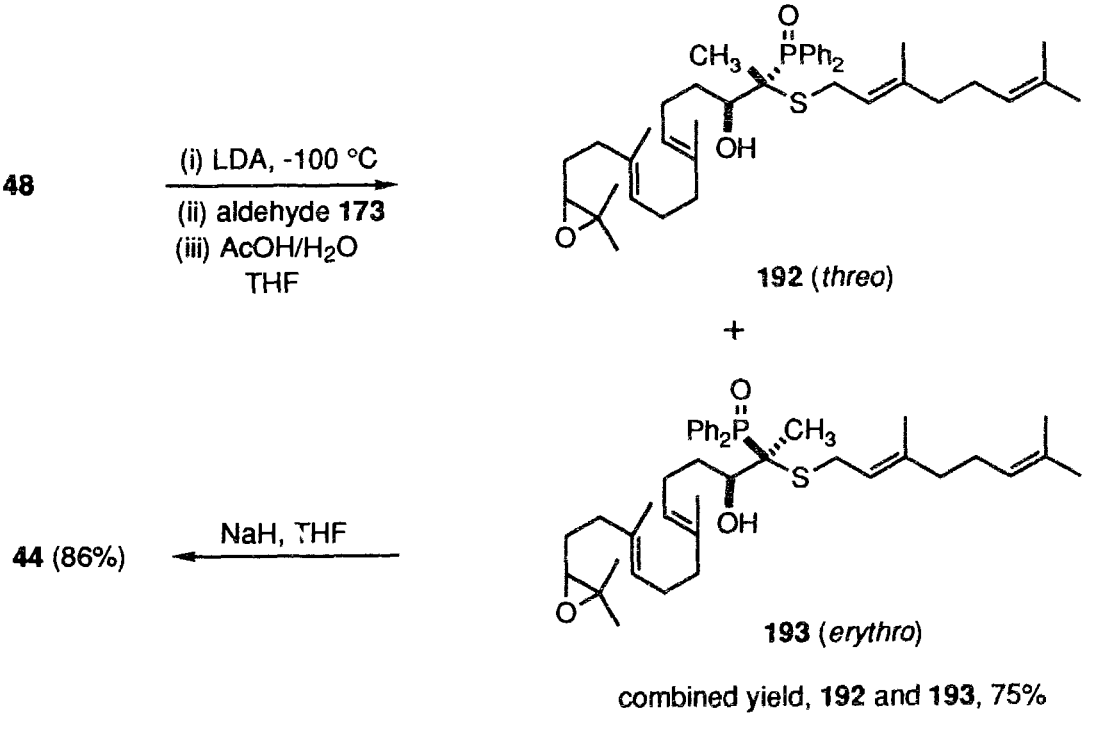


Addition of aldehyde **173** to the lithio anion of **48** at -100 °C gave, after acetic acid quench, α-hydroxy diphenylphosphinoyl product **192/193** as a ~35/65 mixture (by ^{1}H NMR) in ~75% yield (Scheme 26). Thin layer chromatographic analysis showed two overlapping spots. The darker spot (assumed to be the major *erythro* diastereoisomer) again eluted more rapidly. Two cycles of flash chromatography gave pure *erythro* diastereomer **193** in 36% yield. The remainder of the reaction product was eluted as a mixture of the minor diastereoisomer **192** and **193**. Treatment of **193** with NaH in THF gave pure 14(*E*)-16-thia-2,3-oxidosqualene, **44**, in 86% yield. ^{1}H NMR ($CDCl_3$) revealed a signal for 14-*H* at **δ 5.38** (1H, tq, *J*=7.0, 1.20 Hz). Treatment of the

mixture of the minor diastereoisomer **192** and **193** with NaH in THF gave, as the major product 14(*Z*)-16-thia-2,3-oxidosqualene. This gave a ^{1}H NMR signal for *Z* vinyl 14-*H* at δ 5.53 (tq, *J*=7.00, 1.30 Hz). The ^{1}H NMR spectrum of **44** was identical to compound **43**. This again confirmed the assigned stereochemistry for the geometrical isomers.

2,3-Oxidosqualenoids **43** and **44** have yet not been evaluated as inhibitors of OSC's.

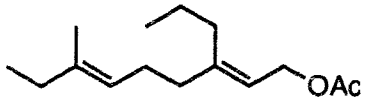
Scheme 26: Synthesis of 16-thia-2,3-oxidosqualene (**44**).



combined yield, **192** and **193**, 75%

Chapter 5

This chapter describes a methodology for the preparation of synthons for the synthesis of trisubstituted 1,5-polyene. Application of this methodology is exemplified by the synthesis of **49**, a terpenoid isolated from the square-necked grain beetle, *Cathartus quadricollis* (Guér.).[77]

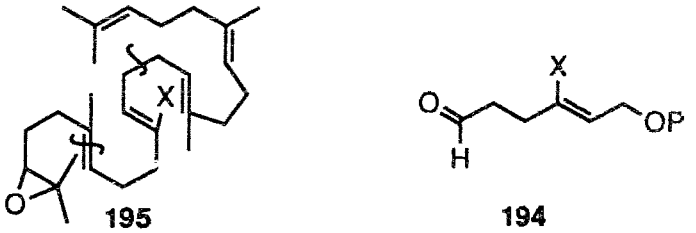


49

I: Introduction

A: Background

In our ongoing investigation on the synthesis of modified 2,3-oxidosqualenes as both mechanism-based inhibitors of OSC's and possible substrates for chemico-enzymatic synthesis of novel steroids by this enzyme, we required intermediates of type **194a-d** for the synthesis of 2,3-oxidosqualenoids **195a-d** (Figure 6-1). It was envisioned that these synthons (**194a-d**) could be readily prepared using stannylcuprate chemistry being investigated in our laboratory.[78-81] Before initiating a multistep synthesis of 2,3-oxidosqualenoids **195a-d**, we first examined the feasibility of this approach by synthesis of a structurally similar but less complex terpenoid **49**.

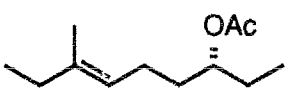


195 194

(a) X= $CH{=}CH_2$
(b) X= CH_2OCH_3
(c) X= CH_2SCH_3
(d) X= $CH{=}C{=}CH_2$

Figure 6-1: Proposed synthesis of modified 2,3-oxidosqualenes (**195a-d**), as possible substrates for OSC, using **194a-d** as the key synthons.

In the investigation of the square-necked grain beetle, *Cathartus quadricollis* (Guér.) in our laboratories, Dr. Pierce and colleagues identified (3*R*)-7-methyl-6(*E*)-nonen-3-yl acetate (quadrilure, **196**) as an aggregation pheromone produced by males.[77] The male beetles also produced a compound with 1,5-diene acetate assigned the structure 7-methyl-3-propyl-2,6(*E*)-nonadienyl acetate on the basis of a detailed analysis of its decoupled ^1H NMR spectrum. However, the ^1H NMR spectra data did not permit an assignment of the geometry of the Δ^2 double bond. Although this compound was apparently inactive in the laboratory bioassays, determination of its effects on the behavior of the beetles was difficult due to the small amount of the natural compound produced. The synthesis of **49** was undertaken to establish its structure and to provide sufficient material for testing.



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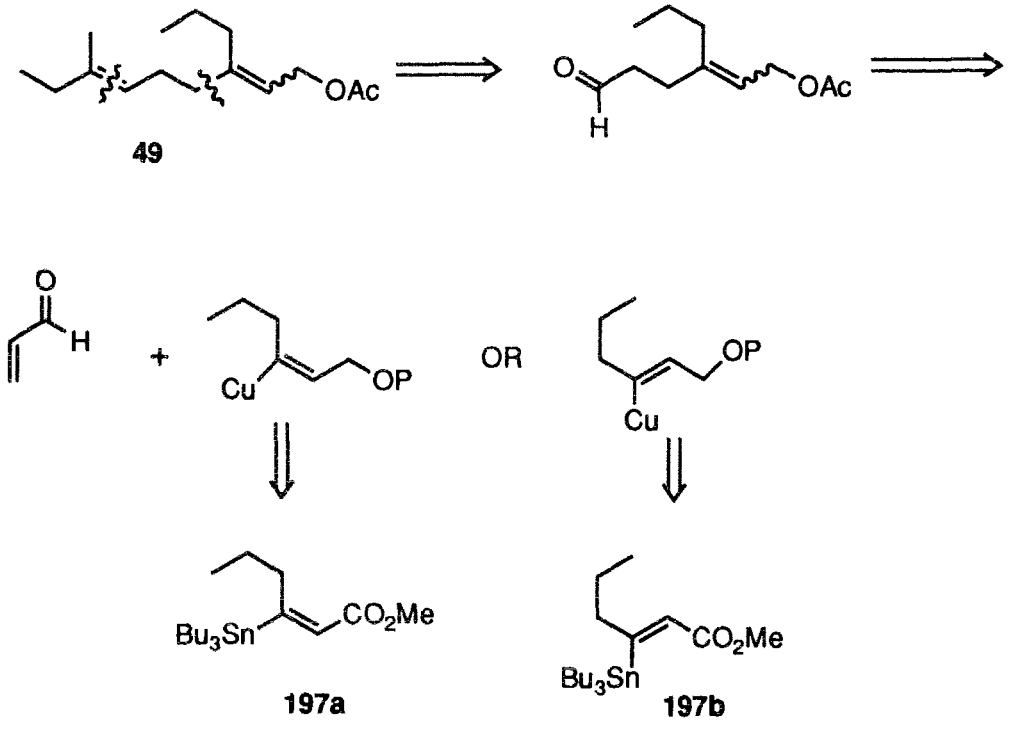
II. Results and Discussion

A: Synthesis Section

Retrosynthetic analysis **49** revealed that both the 2(*E*) and the 2(*Z*) isomers could be readily synthesised by the application stannylcuprate chemistry. As outlined in Scheme 27, the appropriately-protected vinyl stannyl allylic alcohols, prepared from fragments **197a** or **197b** could be transmetallated to their corresponding vinyl cuprates and added to acrolein in a conjugate fashion, giving the respective 2(*E*) and 2(*Z*) unsaturation. The resulting intermediate aldehydes would then serve as the template for the introduction of the second double bond at C-6-C-7.

Scheme 27: Retro-synthesis of 2(*E*) and 2(*Z*) isomers of **49**.

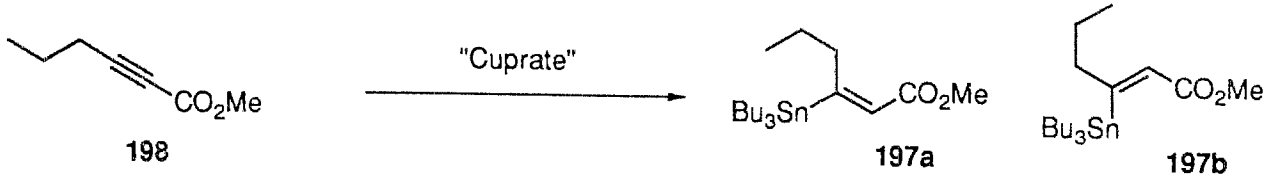


Prior experience with terpenoid insect pheromones lead us to believe there was a greater probability that **49** possessed a 2(*E*), 6(*E*) rather than the 2(*Z*),6(*E*) or 2(*Z*),6(*Z*) configuration. Though both methyl 3-tributylstannyl-2(*E*)- and methyl 3-tributylstannyl-2(*Z*)-hexenoate, (**197a**) and (**197b**), respectively, were prepared, **197a** was utilized first in the reaction sequence leading to the synthesis of appropriately substituted 2(*E*), 6(*E*)-nonadienyl acetate (**49**).

1: Synthesis of 197a and 197b

The preparation of methyl 3-stannyl-2(*E*)- hexenoate (**197a**), was straightforward using the method of Piers *et al.*[80] The addition of methyl 2-hexynoate (**198**) to Bu_3SnCu (prepared form CuBr-DMS complex) at -78 °C in THF followed by MeOH quench, gave the required α,β-unsaturated ester **197a**, in 93 % yield with geometrical purity of >98%. Although **197b** could be prepared by the addition of $Bu_3SnCu(SPh)Li$ to **199**, as also described by Piers,[80] we found that the higher order (H. O.) mixed stannyl cuprates[78,81] $Bu_3Sn(R)Cu(CN)Li_2$ also added to α, β-unsaturated acetylenic esters to give the predominantly the 3-stannyl-2(*Z*)-α,β-unsaturated esters (Table III). Of the three cuprates tried (R=*n*-butyl, R=2-thienyl, and R=*N*-imidazole), the reagent with a *N*-lithio-imidazole[82] ligand gave the desired **197b** in both the highest yields (>85%) and the greatest geometrical purity (>95%, *Z*). Therefore, treatment of **198** with $Bu_3Sn(N\text{-Imid})Cu(CN)Li_2$[82] at -78 °C and warming the reaction to ambient temperature gave a mixture of **197a** and **197b** in a ratio of 4/96 in nearly 90 % yield. The trace amount of **197a** was easily removed by flash chromatography.

Table III. Reaction of mixed H.O. stannyl cuprates to methyl 2-hexynoate (**198**)



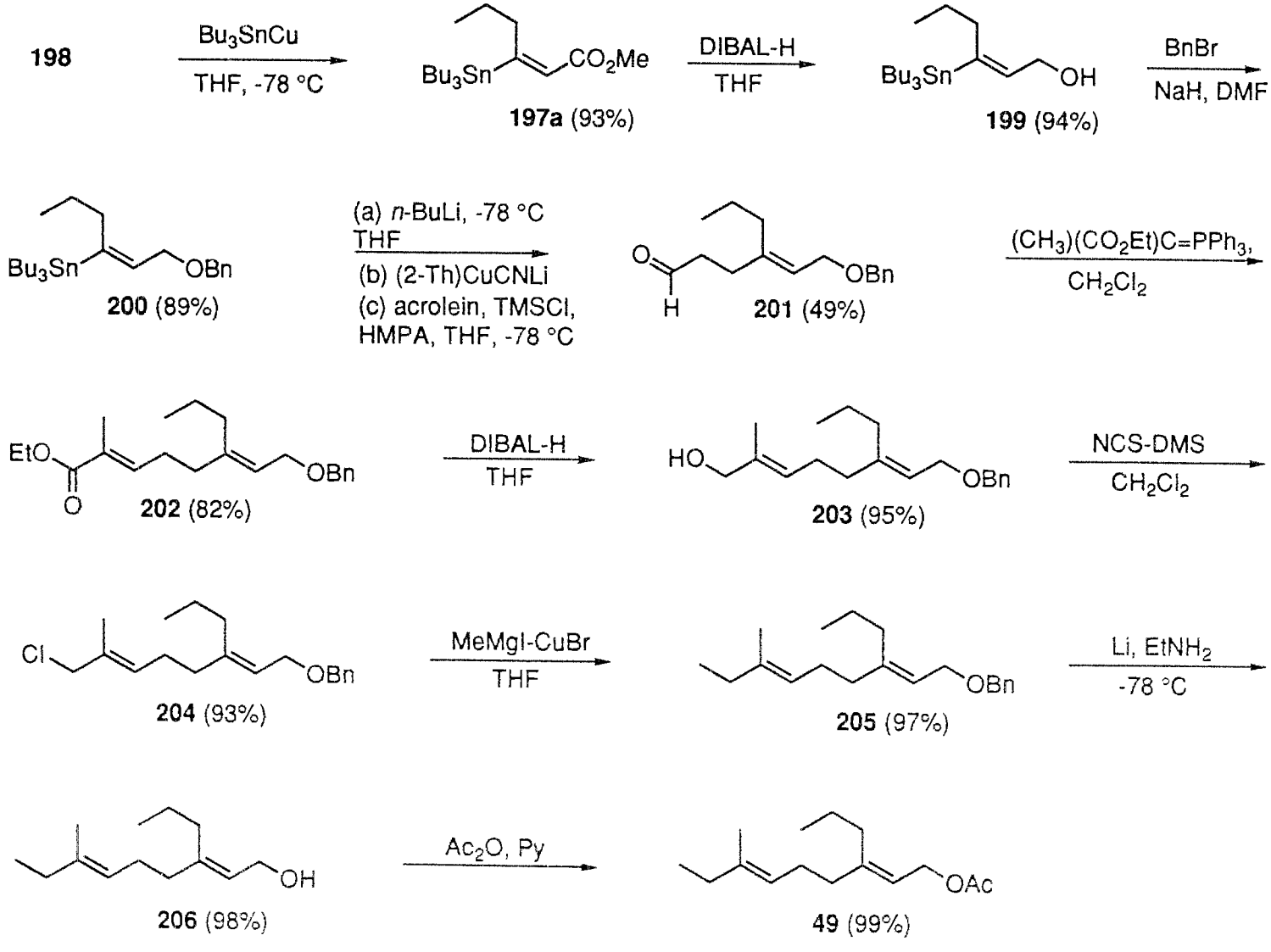
198 **197a** **197b**

Entry	"Cuprate"	Reaction conditions	**197a:197b**[a]	Yield (%)[b]
1	$(Bu_3Sn)(n\text{-}Bu)Cu(CN)Li_2$	-50 °C, 2hr., sat. NH_4Cl/NH_4OH	15:85	80-85
2	$(Bu_3Sn)(2\text{-}Th)Cu(CN)Li_2$	rt, 2hr., sat. NH_4Cl/NH_4OH	10:90	80-85
3	$(Bu_3Sn)(N\text{-imid})Cu(CN)Li_2$	rt, 2 hr., sat. NH_4Cl/ NH_4OH	4:96	>85

[a]ratios were determined using GC analysis.

[b]yields are of isolated, purified products.

Scheme 28: Synthesis of **49**.



2: Synthesis of 49

Methyl 3-tributylstannyl-2(*E*)-hexenoate (**197a**) was reduced to alcohol **199** with DIBAL-H and protected as its benzyl ether **200** in a combined yield of 84% (Scheme 28). The key reaction in this synthesis involved the coupling of **200** with acrolein. Transmetallation of the vinyl tributylstannyl group of **200** to the vinyl lithium species by treatment with *n*-BuLi at -78 °C and its conversion to the H. O. thienyl cyanocuprate[51] went smoothly. The most problematic reaction encountered was the conjugate addition of the cuprate to acrolein. The best yields of **201** (40-50%) were obtained when trimethylsilylchloride (TMSCl)-HMPA[83] was used as activating agent of acrolein. Repeated attempts to increase the yield of this reaction met with failure.

Aldehyde **201** was treated with (carbethoxyethylidene)triphenyl phosphorane in refluxing CH_2Cl_2 to give α, β-unsaturated ester **202** (>95% *E*), in 82% yield. A trace amount of the *Z* isomer was removed by flash chromatography. Ester **202** was then reduced to alcohol **203** with DIBAL-H, which was converted to chloride **204** by treatment with NCS-DMS[57] complex in CH_2Cl_2 in 88% yield over 2 steps. The allylic chloride **204** was reacted with the cuprate reagent derived from 2.0 eq. of MeMgI and CuBr-DMS, at -78 °C to give **205** in 97% yield. When **204** was reacted with $Me_2Cu(CN)Li_2$ at -78 °C, GC analysis of the reaction mixture revealed the presence of several additional products of similar molecular weights. Although this reaction was not repeated, it was assumed that a slight excess of MeLi may have been present and this lead to the elimination of chloride ion as well as some S_N2' addition. The less reactive magnesio-cuprate[39] gave only the expected S_N2 product, **205**.

The benzyl protecting group of **205** was removed using Li metal in $EtNH_2$ at -78 °C to give alcohol **206** (98% yield) which was acetylated (Py/Ac_2O) to give **49** almost quantitatively. Acetate , **49**, was found to be

identical to the natural compound by comparison of GC retention times, ^{1}H NMR, and mass spectral data. The synthesis of the 2(*Z*),6(*E*) isomer was, therefore, abandoned.

Successful preparation of aldehyde **201** has proven the usefulness of the methodology. The synthesis **195a-d** and other modified 2,3-oxidosqualenes using this method is currently being explored by others in this laboratory.

Chapter 6

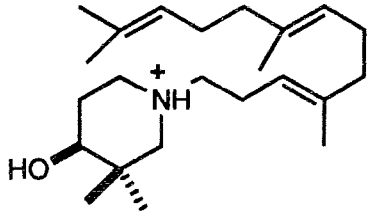
I: Conclusion

We have achieved inhibition of 2,3-oxidosqualene-lanosterol cyclases (OSC) by administering ammonium ion mimics of cationic intermediate **5**, which is suspected to be formed during cyclization of 2,3-oxidosqualene (**1**) to lanosterol (**2**) according to the "step-wise" cyclization hypothesis. These ammonium ion mimics, in particular compounds **39** and **41**, displayed micromolar IC_{50} values for OSC's from the fungus *Candida albicans* and pig-liver.[67] Aside from the mechanistic implications, the biological results obtained may have much broader impact.

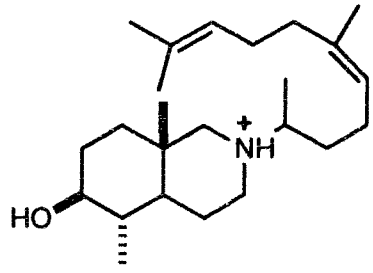
2,3-Oxidosqualene-lanosterol cyclase (OSC) has been targeted for the development of hypocholesterolemic[32] and antifungal agents.[34] In mammals, the cyclase represents an excellent target for potential serum cholesterol lowering drugs. Compactin, which blocks HMG-CoA reductase, is the current drug of choice for lowering serum cholesterol.[85] Unfortunately prolonged administration of this drug has been found to cause blockage of DNA replication leading to inhibition of cell growth and cell division.[86] This deleterious side effect is a result of the inhibition of mevalonic acid biosynthesis by this drug. Mevalonic acid aside from being a precursor to cholesterol, is also required for DNA replication.[86a] Inhibition of 2,3-oxidosqualene-lanosterol cyclase would result in selective blockage in the biosynthesis of lanosterol the direct precursor of cholesterol. Recent studies have shown 2-aza-2,3-dihydrosqualene (**65a**) to be a powerful inhibitor of cholesterol biosynthesis in Swiss 3T3 fibroblasts.[87] An IC_{50} of 0.3 μM was measured for **65a**. The inhibition of lanosterol production also results in the accumulation of 2,3:22,23-dioxidosqualene, a

precursor of 24,25-epoxycholesterol, a known repressor of HMG-CoA reductase. This leads to further decrease cholesterol biosynthesis.

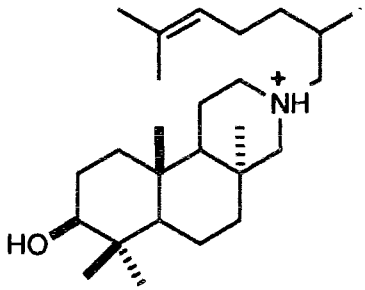
In fungi, lanosterol and its metabolite ergosterol, have been found to be essential for cell growth and maintenance of the cell envelope integrity.[34] Several fungi, including for example *C. albicans*, cannot utilise exogenous sterols.[33,68,69] If lanosterol production is inhibited cell arrestment or cell death is imminent. In an animal host, selective inhibition of fungal OSC may prove to be a useful form of antifungal therapy. The observation that the active sites of the OSC's from mammalian and fungi are sufficiently different, suggests that selective inhibition of pathogenic fungi over the mammalian or plant hosts is possible. Our biological results have shown compound **41** to be 7 -fold more selective in inhibition of the OSC from *C. albicans* versus the OSC from pig-liver.[67] Furthermore, some of our mimics show selective antifungal properties (MIC values, Table II) against various human pathogenic fungi.



207



208



209

As for the deduction of the mechanism of cyclization of 2,3-oxidosqualene (**1**) to protosterol (**3**), it now appears that this cyclization may in fact proceed *via* several distinct, partially cyclized cationic intermediates, involving the formation of intermediate **5** in the rate-limiting step as proposed by van Tamelen.[4c,9] Taton *et al.*[88] have recently published the results of a biological study (published after the completion of this thesis) on the inhibition of OSC's from various sources by monocyclic analogue **207**, bicyclic analogue **208**, and tricyclic analogue **209** of intermediates **5**, **6**, and **7** respectively. They found that monocyclic *N*-alkyl-4-hydroxypiperidine **207**, mimic of intermediate **5** (similar to the mimics of **5** prepared in this thesis) to be the strongest inhibitor of the series. This displayed an IC_{50} of 1.0 μM on 2,3-oxidosqualene-cycloartenol cyclase from maize embryos.

This led these scientists to the same conclusion reached by van Tamelen. The formation of intermediate **5** may be the rate-limiting step (Figure 1-2) as it is in the biomimetic polycyclization.[6,9] Tighter binding of monocyclic mimic **207** to the active site of the OSC's compared with the other mimics may be the result of better electrostatic interaction with a nucleophilic residue of the active site responsible for the formation and the stabilisation of intermediate **5**.

Bicyclic analogue **208** was also found to be an effective inhibitor of the OSC's tested, displaying an IC_{50} of 4.0 μM for 2,3-oxidosqualene-cycloartenol cyclase. This stands to reason, since cationic intermediate **6** is thought to play an important role during the anti-Markovnikov annellation *via* π-complexed intermediate **27** (Figure 1-9) to give intermediate **7**. This must require a certain amount of enzymatic stablisation. As well, the isolation of **26** (Figure 1-8), which has apparently arisen from the capture of **6** by a H_2O molecule, also suggests the presence of enzymatically stabilised intermediate **6**. The 13-aza-

tricyclic analogue **209** failed to inhibit the 2,3-oxidosqualene-cycloartenol-, lanosterol-, and β(α)-amyrin-cyclases.

It is believed that for efficient inhibition of the OSC, the inhibitors must show a conformational flexibility similar to the natural substrate 2,3-oxidosqualene (**1**), such that upon initial binding of these compounds in their ground state, a rapid conformational change of the active site is triggered, bringing the putative anionic counterpart into the vicinity of the ammonium ion centre. This may explain the trend of increasing biological activity as the size and the number of double bonds in the side chain of aza-cyclic mimics **35** to **42** (see Table II) were increased. The lack of conformational flexibility of 13-aza-tricyclic analogue **209** could explain the lack of its biological activity.

It is with this belief that we anticipate that 10(*E*)-9-thia-2,3-oxidosqualenoid (**43**) and 14(E)-16-thia-2,3-oxidosqualenoid (**44**) will be effective "irreversible" mechanism-based inhibitors of OSC. The binding of these substrates by OSC is expected to induce their cyclization concurrent with the conformational change in the enzyme active-site. The generation of the appropriate thiocarbenium ion should result in both an increased life-time of the cationic species as well as significant amount conformational deviation from the naturally occurring cationic species. The result is expected to be the covalent modification of the enzyme by coupling of the inhibitor with the putative basic amino acid residue normally responsible for ion pairing with the cationic intermediate.

Chapter 7

I: Experimental Section

A: Spectroscopic Analysis

NMR, Nuclear magnetic resonance, spectra were recorded on a Bruker AMX-400 spectrometer or Bruker WM-400 operating at 400.13, and 100.62 MHz for ^{1}H and $^{13}C\{^{1}H\}$ spectra, respectively. ^{1}H chemical shifts are reported in parts per million (ppm, δ) and relative to TMS (0.00 ppm). $^{13}C\{^{1}H\}$ are referenced to $CDCl_3$ (77.0 ppm). IR spectra were recorded on Perkin Elmer Model 599B and FT 1605 spectrophotometers calibrated with polystyrene (reference 1601 cm^{-1}). IR spectra of oils were obtained as films between NaCl plates; spectra of solids were obtained from KBr disks. Low-resolution mass spectra were obtained on a Hewlett-Packard 5985B GC/MS equipped with a DB-1 capillary column (30 mm X 0.32 mm ID; with 0.25 μm) system operating at 70 eV for electron impact (EI) ionization. Chemical ionization (CI) was performed using isobutane as the proton source. Gas chromatographic analyses were performed on Hewlett-Packard 5880A and 5890 instruments equipped with a flame ionization detector and a J/W fused silica DB-1 capillary column (15 m X 0.25 mm ID; with 0.25 μm film). High resolution mass spectra were recorded on Kratos/AEI MS 50 spectrometer at the University of British Columbia. Elemental analysis were performed at Simon Fraser University by Mr. M. Yang using a Carlo Erba Model-1106 Elemental Analyzer. All flash chromatography[84] was performed on Silica Gel 60 (230-400 mesh, E Merck, Darmstadt). Thin layer chromatography (TLC) was performed on aluminum backed plates precoated with Merck silica gel 60F-254 as the adsorbent and

visualized by treatment with an acidic solution of $Ce(SO_4)_2$ (1%) and molybdic acid (1.4%) followed by gentle heating on a hot plate.

B: Chemical purifications and General Procedures

Tetrahydofuran (THF), diethyl ether (Et_2O) and dimethoxyethane (DME) were freshly distilled from sodium benzophenone-ketyl; hexamethylphosphosphoramide (HMPA). Diisopropylamine and triethylamine and trimethylsilyl chloride (TMSCl) were distilled from CaH_2 and stored under argon; dimethylsulfide (DMS), dichloromethane (CH_2Cl_2), and toluene were freshly distilled from CaH_2 prior to use. Ethanol (EtOH) was distilled from Mg filings and stored under argon. *N*-chlorosuccinimide (NCS) and *N*-bromosuccinimide were recrystallized from glacial acetic acid, washed with ice water and dried under high vacuum; *para*-toluenesulfonyl chloride (*p*-TsCl) was purified by distillation under reduced vacuum (0.5 mmHg); acrolein was distilled, first at atmospheric pressure and then under vacuum (0.05 mmHg) from a round bottom flask cooled to -30 °C and condensed at -78 °C (Dry ice/acetone) and used immediately. Unless otherwise stated, chemicals obtained from commercial sources were used without further purification. All moisture and air sensitive reactions were conducted under a positive pressure of argon in glassware that was flame dried under vacuum. A nitrogen glove bag was used to weigh all the moisture and oxygen sensitive compounds. Syringes and cannulas were used to transfer oxygen and water sensitive liquid reagents. Unless specifically stated standard work-up refers to the combined organic extracts being washed with ice-cold brine, dried over $MgSO_4$ (or anhydrous K_2CO_3 for amine) and the solvent removed using a rotary evaporator. Chromatography refers to flash chromatography.[84]

C: Biological Procedures

1: *In vitro* antifungal activity

The minimum inhibitory concentrations (MIC values, concentration of inhibitor required to completely inhibit growth of the organism *in vitro*) of the inhibitors were measured on Rowley agar against standard strains of *Candida albicans* and *Aspergillus fumigatus* after two days incubation and *Histoplasma capsulatum* and *Trichophyton mentagrophytes* after seven days.

2: Sterol Biosynthesis Inhibition Assays in Whole Cells

Procedure as previously described in reference 69. *C. albicans* was grown at 37 °C in a 10 mL of YECD broth (0.5% yeast extract, 0.5% Casitone, and 0.5% glucose) and supplemented with 0.01 mM [^{14}C]acetate (1μCi) and the appropriate concentrations of inhibitor until late-log phase (monitored by optical density of 660 nm, 1.3). Cells were harvested after 8 h by centrifugation, washed once with cold 5% trichloroacetic acid, and extracted once with 1.5 mL of methanol followed by 1 mL of a 1:1 mixture of methanol-benzene. The extracts were spotted on silica gel TLC plates and developed with heptane/acetic acid/isopropyl ether (60/4/35) as the eluant. The ergosterol/oxidosqualenes (sum of 2,3-oxido and 2,3,22,23-dioxidosqualene) was established by scraping the respective band or bands into scintillation vials, diluting with 5 mL of Aquasol and the counting the radioactivity. The inhibition of 2,3-oxidosqualene cyclases results in the accumulation of 2,3-oxidosqualene and the production of 2,3,22,23-dioxidosqualene and the decrease in ergosterol production in the cells (see reference 33). IC_{50} was then

determined at the concentration of the inhibitor that reduced the ratio of ergosterol/oxidosqualenes to 50 % of the control.

3: Cell-Free Enzyme Inhibition Assays

As previously described in reference 33. IC_{50} values (the concentration of the inhibitor required to decrease the activity of the enzyme by 50%) were measured using a cell-free preparation of *Candida albicans*. Cells were collected from an 8 h culture in TYG medium and were digested for 30 min with Zymolase 100T (Seikagaku Kogyo, Japan). For a gram of cell mass were used 1.0 mg of Zymolase, 12.5 µl of 2-mercaptoethanol and 5.0 mL of a digestion buffer (50 mM phosphate pH 7.4 containing 1.0 M mannitol). The resulting protoplasts were collected by centrifugation and lysed in 100 mM phosphate buffer pH 6.9. The supernatant after centrifugation at 15000 *g* is the cell-free extract which retains full cyclase activity shown by a 42% incorporation of racemic ^{14}C-2,3-oxidosqualene in the presence of the non-ionic detergent Decyl Poe (n-decylpentaoxyethlene, Bacem, Switzerland). This detergent inhibits the further metabolisation of lanosterol to fungal sterols by the cell-free preparation, and thus allows an accurate measurement of the inhibitory activity of the test compounds. The non-saponifiable lipids were extracted and applied to TLC plates (silica gel F-254, Merck, Germany) which were developed twice in dichloromethane. The radiolabelled spots, in this case only oxidosqualene and lanosterol, were quantified with an automatic TLC scanner (Rita 3200, Raytest, Germany). The % activity was plotted against log inhibitor concentration to determine the IC_{50}.

II: Experimental Procedures and Spectral Data

A: Chapter 2

***N*-Methyl-5,6-dihydro-4-pyridone (77).** This compound was prepared as described in reference 37. To a stirred solution of **76** (5.0 g, 44.2 mmol) in CH_2Cl_2 (50 mL) at -40 °C was added dropwise a solution of *m*-CPBA (10.0 g, 48.6 mmol, 85%) in CH_2Cl_2 (150 mL) over 1 h. This was stirred for an additional 2 h and Et_3N (31 mL, 220 mmol) was added followed by Ac_2O (4.6 mL, 48.6 mmol). The reaction mixture was allowed to warm to 0 °C and stirred for 3 h, poured into a separatory funnel and washed with ice cold 15% NaOH solution (3 X 150 mL) and ice cold brine (1 X 50) and dried over anhydrous K_2CO_3. Distillation under reduced pressure gave **77** (1.6 g, 32%): bp 108-109 °C @ 0.50 mmHg, lit.[37]110°C@1.0 mmHg. ^1H NMR ($CDCl_3$, ppm) 6.94 (1H, d, *J*=8.0 Hz), 4.92 (1H, d, *J*=8.0 Hz), 3.42 (2H, t, *J*=8.0 Hz), 3.01 (3H, s), 2.47 (2H, t, *J*=8.0 Hz).

2-*n*-Butyl-*N*-methyl-4-piperidone (79). To a stirred slurry of CuCN (242 mg, 2.7 mmol) in THF (5 mL) under argon at -78 °C was added dropwise *n*-BuLi (2.16 mL, 5.4 mmol 2.5 M in hexanes). This was allowed to react for 30 min. To the light yellow cuprate solution was added *via* syringe, BF_3-OEt_2 (0.25 mL, 2.0 mmol) followed by the dropwise addition of enone **77** (200 mg, 1.8 mmol) in THF (2.0 mL). After 30 min, the reaction was terminated by the addition of a solution of 1% NH_4OH (5 mL). This was warmed to 0 °C and extracted with ether (5 X 15 mL) and dried over anhydrous K_2CO_3. Chromatographic purification using ethyl acetate/MeOH/Et_3N (85/10/5) as the eluant gave **79** (75 mg, 24 %). IR (film) 2970, 2870, 2799, 1725, 1470, 1420,

and 1280 cm-1; mass spectrum, CI *m/z* (isobutane, rel intensity) 170 (M^++1, 100); ^{1}H NMR ($CDCl_3$, ppm) 3.12 (1H, m), 2.58 (1H, m) 2.50 (2H, m), 2.40 (3H, m), 2.30 (1H, m), 1.58 (1H, m), 1.42 (1H, m), 1.30 (6H, bm), 0.92 (3H, t, *J*=7.0 Hz); ^{13}C NMR ($CDCl_3$, ppm) 209.2, 62.9, 54.3, 45.0, 40.4, 32.3, 30.8, 27.2, 22.8, 13.9.

2-Benzyl-*N*-methyl-4-piperidone (80). To a stirred slurry of CuCN (121 mg, 1.4 mmol) in THF (5 mL) under argon at -78 °C, was added benzyl magnesium bromide (2.8 mL, 2.8 mmol, 1M in Et_2O). After 30 min, **77** (100 mg, 0.9 mmol) in THF (2 mL) was added dropwise. Reaction was terminated after 30 min by the addition of 1% aqueous NH_4OH solution (5 mL), extracted with ether (5 X 10 mL) and the combined extracts dried over K_2CO_3. Chromatography using ethyl acetate/MeOH/Et_3N (95/3/2) as the eluant gave **80**, as an oil (160 mg, 87%). IR (film) 2995, 2840, 1725, 1600, 1437, 1397, 1337, 1257, and 1097 cm^{-1}; mass spectrum, CI *m/z* (isobutane, rel intensity) 204 (M^++1, 100); ^{1}H NMR ($CDCl_3$, ppm) 7.28 (2H, m), 7.20 (1H, m), 7.13 (2H, m), 3.12 (2H, m), 2.80 (1H, m), 2.67 (1H, m), 2.55 (3H, s), 2.50 (2H, m), 2.38 (1H, m), 2.22 (2H, m); 13C NMR (CDCl3, ppm) 208.7, 138.2, 129.3 (2C), 128.5 (2C), 126.4, 64.3, 53.4, 44.5, 41.3, 40.4, 38.3. Anal. calcd. for $C_{13}H_{17}NO$: C, 76.81; H, 8.43; N, 6.89. Found: C, 76.88; H, 8.42; C, 6.98.

***N*-(Carbobenzyloxy)-3-carbomethoxy-4-piperidone (86):** To a slurry of methyl-4-piperidone-3-carboxylate hydrochloride (**85**) (5.0 g, 25.8 mmol) in 50 mL of CH_2Cl_2 and Et_3N (9.0 mL, 65.0 mmol) at 0 °C, was added benzyl chloroformate (5.0 g, 28.0 mmol) over 20 min. This was stirred at 0 °C for 0.5 h and at room temperature for 1 h. The reaction mixture was poured into an ice cold 2N HCl solution (100 mL) and extracted with CH_2Cl_2 (2 X 50

mL). The extracts were combined, washed with ice cold saturated $NaHCO_3$ solution Standard work-up gave **86** (6.7 g, 91%), as the only product detected by ^1H NMR and TLC, as an oil. An analytical sample was prepared by chromatography with hexane/ethyl acetate (7/3) as the eluant. IR (film) 2200-3700 (b), 1740, 1600 cm^{-1}; mass spectrum, CI *m/z* (isobutane, rel. intensity) 292 (M^++1, 100);.^1H NMR ($CDCl_3$, ppm) 12.00 (~1H, s, exchangeable/D_2O), 7.36 (5H, m), 5.16 (2H , s), 4.13 (2H, bs), 3.78 (3H, s), 3.64 (2H, m), 2.40 (2H, bs); ^{13}C NMR [218K, $CDCl_3$, ppm (major/minor rotomers)] 163.4/163.2, 154.5/154.4, 151.7, 140.7-126.4 (6C, m, aromatic), 115.5/115.0, 70.4/67.4 & 63.3/64.6 (OCH_2Ph), 52.0, 41.6/41.8, 39.0/39.4, 28.4/28.7. Anal. calcd. for $C_{15}H_{17}NO_5$: C, 61.85; H, 5.90; N, 3.94. Found: C, 61.80; H, 5.84; N, 4.18.

***N*-(Carbobenzyloxy)-3-carbomethoxy-3-phenylselenyl-4-piperidone (87)**: To a stirred slurry of NaH (1.7 g, 42.5 mmol, 60% in oil) washed free of oil with pentane (4 X 10 mL), in THF (250 mL) at 0 °C under argon was added dropwise a solution of **86** (10.0 g, 35.0 mmol) in THF (30 mL) over 20 min. The solution was stirred at room temperature for 45 min. and then cooled to -50 °C. To this was added dropwise a solution of phenylselenyl bromide (9.25 g, 39.2 mmol) in 50 mL of THF over 50 min. The mixture was stirred at -50 °C for 2 h and room temperature for 1 h, poured into ice cold saturated K_2CO_3 solution (200 mL) and extracted with Et_2O (3 X 50 mL). Standard work-up gave **87** (15.2 g, crude) as a yellow oil that crystallized on standing. A small sample was recrystallized from CH_2Cl_2/hexane to give light yellow crystals, m.p. 108-109° C. IR (KBr pellet) 2950 (b), 1700(b), 1410, and 1260 cm^{-1}; mass spectrum, CI *m/z* (isobutane, rel. intensity) 448 (M^++1, 100); ^1H NMR ($CDCl_3$, ppm) 7.40 (10H, bm), 5.10 (2H, s), 4.60 (1H, bm), 4.10 (1H, bs), 3.62 (3H, s), 3.38 (1H, bs), 3.55 (1H, bs), 2.66 (2H, bs); ^{13}C NMR [243K,

$CDCl_3$, ppm (major/minor rotomers)] 200.8, 167.8, 154.3, 138.0-123.0 (m, 12C) 62.5/67.7, 60.6, 53.3, 51.5, 43.3, 40.2/40.0. Anal. calcd. for $C_{21}H_{20}NO_5Se$: C, 56.51; H, 4.74; N, 3.14. Found: C, 56.71; H, 4.62; N, 2.95.

***N*-(Carbobenzyloxy)-3-carbomethoxy-5,6-dihydro-4-pyridone (45):** To a vigorously stirred solution of **87** (15.0 g, crude) in CH_2Cl_2 (150 mL) at 0 °C was added dropwise a solution of H_2O_2 (7.8 g, 30% by weight in H_2O) in distilled water (40 mL) while monitoring the disappearance of **87** by TLC. The reaction was warmed to room temperature over 30 min, poured into ice cold saturated K_2CO_3 (100 mL), the organic layer separated and the aqueous layer extracted with CH_2Cl_2 (2 X 50 mL). Standard work-up followed by chromatography with ethyl acetate/hexane (7/3) as the eluant yielded **45** (7.7g, 76% in two steps) as an oil that crystallized on standing, m. p. 74-75 °C. IR (film) 2950, 1700, 1760, 1400, and 1250 cm^{-1}; mass spectrum, CI *m/z* (isobutane, rel intensity) 290(M^++1, 100); ^1H NMR ($CDCl_3$, ppm) 8.83 (1H, s), 7.40 (5H, bs), 5.30 (2H, s), 4.05 (2H, t, J=7.0 Hz), 3.82 (3H, s), 2.63 (2H, t, J=7.0 Hz); ^{13}C NMR [218K, $CDCl_3$, ppm (major/minor rotomers)] 189.2, 164.6/164.0, 151.8/152.0, 151.4, 133.6/133.4, 129.1, 128.9, 128.7, 107.5/108.0, 69.9, 52.4/52.2, 42.1/42.5, 35.6. Anal. calcd. for $C_{15}H_{15}O_5N$: C, 62.28; H, 5.23; N, 4.84. Found: C, 62.14; H, 5.31; N, 4.76.

2-*n*-Butyl-*N*-(carbobenzyloxy)-3-carbomethoxy-4-piperidone (88): To a slurry of CuCN (1.07 g, 12.0 mmol) in THF (70 mL), under argon at -78°C, was added dropwise *n*-BuLi (9.60 mL, 24.0 mmol, 2.5 M solution in hexane) and the reaction was stirred for 30 min. To the cuprate, at -78 °C, was added dropwise a solution of enone **45** (2.9 g, 10.0 mmol) in THF (25 mL) over 15 min. The resulting yellow solution was stirred for an additional 30 min. at

which time saturated NH_4Cl/NH_4OH (50 mL, pH~8) was added. The slurry was stirred while warming to 0 °C then extracted with Et_2O (4 X 40 mL). Standard work-up followed by chromatography using hexane/ethyl acetate (9/1) as the eluant afforded **88** (3.2 g, 91%) as an oil that crystallized on standing, m.p. 60-61°C. IR (KBr) 2950 (b), 1650, 1700 (b) and 1430 cm^{-1}; mass spectrum, *m/z* (rel. intensity) 347 (M^+, 20), 316 (37), 290 (46), 214 (100); ^1H NMR ($CDCl_3$, ppm) 12.50 (enolic H, bs), 7.10 (5H, bm), 5.10 (2H, bm), 4.00 (1H, bs), 3.31 (3H, bs), 2.80 (1H, bm), 2.26 (1H, bs), 1.81 (1H, bm), 1.61 (1H, bs), 1.31 (6H, bm), 0.81 (3H, bm); ^{13}C NMR [213K, $CDCl_3$, ppm (major/minor rotomers)] 170.9, 169.6/170.3, 154.9, 135.9, 128.2-127.7 (5C, m) 100.6/101.1, 66.9, 51.7, 48.8, 34.3/34.8, 33.1/32.8, 28.2, 27.9, 22.1, 14.0. Anal. calcd. for $C_{19}H_{25}NO_5$: C, 65.67; H, 7.25; N, 4.03. Found: C, 65.36; H, 7.43; N, 4.04.

2-*n*-Butyl-*N*-(carbobenzyloxy)-3-carbomethoxy-3-methyl-4-piperidone (89): To a slurry of NaH (427.0 mg, 10.0 mmol, 60% in oil) washed free of oil with dry pentane, in freshly distilled 1,2-dimethoxyethane (DME) (50 mL), under argon at 0 °C, was added dropwise over 20 min. a solution of **88** (3.0 g, 9.0 mmol), in DME (25 mL). The mixture warmed to room temperature and stirred for 45 min. MeI (1.7 mL, 27.0 mmol) was then added and the reaction mixture stirred at room temperature for 48-50 h. The reaction was poured into saturated NH_4Cl solution. (50 mL), extracted with Et_2O (3 X 50 mL). Standard work-up followed by chromatography using hexanes/ethyl acetate (8/2) as the eluant gave the methyl product **89** (2.7 g, 72%) as a single diastereoisomer, an oil that crystallized on standing, m.p. 55-57 °C. IR (KBr) 3400, 2950, 1700 (b), 1690, 1720, 1760 and 1425 cm^{-1}; mass spectrum, CI *m/z* (isobutane, rel. intensity) 362 (M^++1, 100); ^1H NMR ($CDCl_3$, ppm) 7.35 (5H, m), 5.17 (2H, s), 4.47 (2H, m), 3.70 (3H, s), 3.00 (1H, bm), 2.70 (1H, bs), 2.45 (1H,

bs), 1.60 (2H, bs), 1.45 (2H, bs), 1.20 (5H, bm), 0.78 (3H, s); ^{13}C NMR [213K, $CDCl_3$, ppm (major/minor rotomers)] 206.2, 170.7/170.9, 155.6, 135.4, 128.3-127.7 (5C, m), 67.4, 60.0/60.2, 58.3/58.2, 52.5, 36.7/37.0, 35.9/36.0, 28.3/28.5, 27.1/27.2, 22.1, 22.0, 14.0. Anal. calcd. for $C_{20}H_{27}NO_5$: C, 66.47; H, 7.53; N, 3.88. Found: C, 66.20; H, 7.59; N, 3.78.

2-*n*-Butyl-*N*-(carbobenzyloxy)-3-carbomethoxy-3-methyl-4-piperidone Ethylene Ketal (90): Compound **89** (2.0 g, 5.5 mmol) in 50 mL of toluene containing ethylene glycol (1.0 g, 16.7 mmol) and 50.0 mg of *p*-TsOH were refluxed for 20 h in a 100 mL flask fitted with Dean-Stark collector. The cooled reaction mixture was poured into ice-cold saturated $NaHCO_3$ (50 mL) and extracted with ether (3 X 30 mL). Standard work-up followed by chromatography using hexanes/ethyl acetate (7/3) as the eluant gave **90** (2.0 g, 89%) as an oil that crystallized on standing, m.p. 80-81°C. IR (KBr) 2945, 1770, 1685 and 1420 cm^{-1}; mass spectrum, CI *m/z* (isobutane, rel. intensity) 406 (M^++1, 100); ^1H NMR ($CDCl_3$, ppm) 7.30 (5H, m), 5.17 (2H, m), 4.60/4.40 (1H, rotomers, d), 4.25 (1H, bs), 4.20 (2H, m), 3.96 (2H, m), 3.70 (3H, s), 3.10 (1H, m), 1.83 (1H, m), 1.35 (3H, m), 1.17 (6H, bm), 0.78 (3H, m); ^{13}C NMR [213K, $CDCl_3$, ppm (major/minor rotomers)] 172.6, 155.9/155.8, 136.2/136.0, 127.7-128.2 (5C, m), 108.7, 66.9, 65.3, 64.0, 58.5/58.3, 52.1/52.2, 51.7/51.6, 35.9/36.1, 30.7/31.3, 28.6/28.5, 27.8, 22.2/22.4, 21.4/21.7, 14.2. Anal. calcd. for $C_{22}H_{31}NO_6$: C, 65.21; H, 7.70; N, 3.46. Found: C, 64.94; H, 7.61; N, 3.29.

2-*n*-Butyl-*N*-(carbobenzyloxy)-3-hydroxymethyl-3-methyl-4-piperidone Ethylene Ketal (91): To a slurry of $LiAlH_4$ (1.0 g, 26.0 mmol) in 30 mL THF, under an atmosphere of argon, was added dropwise a solution of ketal **90** (2.5 g, 6.1 mmol) in 15 mL of THF. The reaction was refluxed for 3 h, at

which time excess $LiAlH_4$ was destroyed at 0 °C by slow addition of 1.0 g of water followed by 1.0 g of 15% NaOH followed by 3.0 g of water. The solids were filtered and rinsed thoroughly with small portions of Et_2O (5 X 25 mL). Standard work-up followed by chromatography using with ethyl acetate/MeOH/Et_3N (95/3/2) as the eluant afforded **91** (1.5 g, 88%) as an oil. IR (neat) 3600-3100(b), 2800-3000(b), 1675 and 1450 cm^{-1}; mass spectrum, CI *m/z* (isobutane, rel intensity) 258 (M^++1, 100); ^1H NMR ($CDCl_3$, ppm) 3.90 (4H, m), 3.77 (2H, s), 2.80 (1H, m), 2.40 (2H, m), 2.25 (3H, s), 2.17 (1H, m), 1.70 (2H, m), 1.47 (2H, m), 1.30 (4H, m), 0.88 (3H, t, *J* =7.0 Hz), 0.70 (3H, s); ^{13}C NMR ($CDCl_3$, ppm) 110.3, 70.8, 66.6, 65.0, 64.6, 54.2, 45.2, 42.9, 32.8, 32.5, 28.5, 23.0, 15.2, 13.8. Anal. calcd. for $C_{14}H_{27}NO_3$: C, 65.34; H, 10.58; H, 5.45. Found: C, 65.15; H, 10.60; N, 5.63.

2-*n*-Butyl-1,3-dimethyl-3-hydroxymethyl-*p*-toluenesulfonyl-4-piperidone Ethylene Ketal (92): To a solution of **91** (1.75 g, 7.0 mmol) in dry Et_2O (25 mL), under positive pressure of argon at 0 °C, was added *n*-BuLi (3.35 mL, 8.4 mmol, 2.5 M in hexanes) and the reaction was stirred for 10 min. A solution of *p*-toluenesulfonyl chloride (1.90 g, 10.0 mmol) in Et_2O (10 mL) was added dropwise and the mixture stirred at 0 °C for 5 h. The reaction was then diluted with 15% NaOH solution (25 mL) and extracted with Et_2O (3 X 30 mL). Standard work-up followed by chromatography using diethyl ether/ethyl acetate/triethylamine (95/3/2) as the eluant gave the tosylate **92** (2.41g, 84%) as a crystalline solid (unstable to prolonged storage, no m. p. obtained). IR (KBr) 3400 (b), 2940, 2940, 1600 and 1350 cm^{-1}; mass spectrum, CI *m/z* (isobutane, rel. intensity) 240 (M^++1-$C_7H_7O_2S$, 100); ^1H NMR ($CDCl_3$, ppm) 7.78 (2H, m), 7.33 (2H, m), 4.10-3.85 (6H, bm), 2.60 (1H, m), 2.43 (3H, s), 2.25 (3H, s), 1.85 (2H, m), 1.60 (2H, bm), 1.30-1.20 (6H, bm), 1.03 (3H, s), 0.87 (3H,

m); ^{13}C NMR ($CDCl_3$, ppm) 144.4, 138.7, 128.4 (2C), 125.8 (2C), 110.0, 78.4, 65.7, 65.5, 58.6, 46.7, 44.5, 29.8, 27.3, 24.9, 22.8, 21.1, 13.6, 10.2. Anal. calcd. for $C_{21}H_{33}NO_5S$: C, 61.25; H, 8.08; N, 3.40. Found: C, 61.01; H, 8.08; N, 3.48.

2-*n*-Butyl-1,3,3-trimethyl-4-piperidone Ethylene Ketal (93): To a solution of **92** (2.0 g, 5.0 mmol) in THF (30 mL) under argon, was added $LiBEt_3H$ (10.0 mL, 10.0 mmol, 1.0 M in THF) and the solution was refluxed for 3 h. The reaction was cooled to room temperature then poured into ice-cold 15% NaOH solution (25 mL), stirred for 30 min. and extracted with ether (4 X 30 mL). Standard work-up followed by chromatography with hexanes/ethyl acetate/triethyl amine (70/27/3) as the eluant gave **93** (1.1 g, 91%) as an oil. IR (film) 2950 (b), 2870, 2790 and 1455 (b) cm^{-1}; mass spectrum, EI*m/z* (rel intensity) 241 (M^+, 4), 212 (13), 198 (10), 184 (100), 98 (44); ^1H NMR ($CDCl_3$, ppm) 3.90 (4H, m), 2.70 (1H, ddd, *J*=12.0, 4.5, 3.0 Hz), 2.25 (1H, td, *J*=12.0, 3.0 Hz) 2.21 (3H, s), 1.91 (1H, dt, *J* =13.0, 4.5 Hz), 1.77 (1H, m), 1.45 (3H, bm), 1.24 (4H, m), 0.99 (3H, s), 0.86 (3H, t, *J* =7.0 Hz), 0.82 (3H, s); ^{13}C NMR ($CDCl_3$, ppm) 110.5, 70.7, 65.0, 64.8, 54.3, 44.2, 44.1, 43.7, 33.8, 31.4, 30.3, 24.2, 20.5, 19.3, 15.2. HRMS cacld. for $C_{14}H_{27}NO$: 241.2043, found 241.2017.

2-*n*-Butyl-1,3,3-trimethyl-4-piperidone (94): A solution of ketal **93** (1.0 g, 4.0 mmol) in acetone (25 mL) and 6N HCl (10 mL) was refluxed for 8 h. The cooled reaction mixture was diluted with ice water (25 mL), neutralized with solid $NaHCO_3$ and extracted with ether (5 X 30 mL). Standard work-up followed by chromatography using ethyl acetate/methanol/triethylamine (95/3/2) as the eluant afforded the 4-piperidone **94** (600 mg, 73 %) as a an oil. IR (film) 2950, 2790, and 1710 cm^{-1}; mass spectrum *m/z* (rel intensity).197 (M^+, 38), 168 (33), 140 (69), 126 (13), 112 (100), 98 (85), 84 (15), 70 (15), 57 (25); ^1H NMR

($CDCl_3$, ppm) 2.92 (1H, overlapping dt, J=13.0, 5.0 Hz), 2.50 (3H, m), 2.37 (3H, s), 2.10 (1H, m), 1.42 (1H, m), 1.30 (5H, m), 1.20 (3H, s), 1.07 (3H, s), 0.90 (3H, t, J =7.0 Hz); ^{13}C NMR ($CDCl_3$, ppm) 213.8, 72.2, 52.3, 50.0, 42.6, 37.7, 32.6, 26.4, 23.6, 22.9, 20.8, 13.8. HRMS calcd. for $C_{12}H_{23}NO$: 197.1781, found 197.1786.

2-*n*-Butyl-1,3,3-trimethyl-4-hydroxypiperidine (35): To a slurry of $LiAlH_4$ (100 mg, 2.6 mmol) in THF (15 mL) at -78 °C, under a positive pressure of argon, was added dropwise a solution of piperidone, **94** (50 mg, 2.8 mmol) in THF (10 mL). Excess $LiAlH_4$ was destroyed after 30 min by the addition of 0.1 g of H_2O followed by 0.1 g of 15% NaOH solution followed by 0.3 g of H_2O at 0 °C. The resulting solid was filtered and rinsed thoroughly with Et_2O (5 X 25 mL). Standard work-up followed by chromatography using ethyl acetate/methanol/triethylamine(90/7/3) as the eluant gave the 4-piperidinol **35** (460 mg, 83%) which was >95% the equatorial diastereoisomer, as a clear viscous oil. IR (film) 3100-3500 (b), 2950, and 1450 cm^{-1}; mass spectrum, EI *m/z* (rel intensity) 199 (M^+,45), 182 (20), 142 (100), 98 (38), 57 (33); ^1H NMR ($CDCl_3$, ppm) 3.20 (1H, dd, J =12.0, 5.0 Hz), 2.83 (1H, td, J =12.0, 4.0 Hz), 2.20 (3H, s), 2.03 (1H, dt, J =12.0, 4.0 Hz), 1.75 (1H, dq, J =12.0, 4.0 Hz), 1.70 (1H, m), 1.55 (1H, m), 1.30 (6H, bm), 0.95 (3H, s), 0.90 (3H, t, J =7.0 Hz), 0.85 (3H, s); ^{13}C NMR ($CDCl_3$, ppm) 76.9, 72.8, 55.6, 43.6, 39.9, 33.0, 30.5, 29.4, 24.1, 23.1, 13.9, 13.5. Anal. calcd. $C_{12}H_{25}NO$: C, 72.31; H, 12.64; N, 7.03. Found: C, 71.99; H, 12.48; N, 6.83. HRMS calcd. for $C_{12}H_{25}NO$: 199.1936, found 199.1898.

2-*n*-Butyl-1,3,3-trimethyl-4-hydroxypiperidinium Chloride (36a): To a solution of **35** (50 mg, 0.25 mmol) in 1 mL of dry Et_2O in a tapered

test tube was added 3 drops of 1 N HCl in Et_2O. The amine hydrochloride salt precipitated as white flakes. The ether was evaporated under a stream of nitrogen and the hydrochloride salt rinsed with dry Et_2O (3 X 1 mL). The residual solvent was removed under reduced pressure to give the hydrochloride salt **36a** (40 mg) as white flakes, m. p. 150-155 °C. IR (KBr) 3600-3200 (b), 2930, 2700 and 1410 cm^{-1}; mass spectrum, FAB *m/z* (Xenon/glycerol, rel intensity) 200 (M^+-Cl^-, 100); ^1H NMR (CD_3OD, ppm) 3.40 (2H, m), 3.10 (1H, m), 2.84 (3H, s), 2.73 (1H, bs), 1.87 (3H, bm), 1.58 (1H, bs), 1.47 (1H, bm), 1.40 (4H, m), 1.11 (3H, s), 0.96 (3H, t, J =7.5 Hz), 0.96 (3H, s); ^{13}C NMR (218K, CD_3OD, ppm) 73.2 (2C), 54.9, 42.2, 41.6, 34.1, 28.8, 28.6, 24.0, 23.8, 14.5, 12.2.

2-*n*-Butyl-1,1,3,3-tetramethyl-4-hydroxypiperidinium Iodide (36b): To a solution of **35** (100 mg, 0.5 mmol) in 5.0 mL of dry Et_2O in a tapered screw cap test tube was added MeI (0.2 mL). The reaction was placed in the dark at room temperature for 24 h. The ether was evaporated under a flow of nitrogen and the crystals rinsed with dry Et_2O (3 X 2 mL). The residual solvent was removed under vacuum to give the salt **36b** (120 mg) as a white powder, m.p. 161-163°C. IR (KBr) 3400 (b), 2975 and 1470 cm^{-1}; mass spectrum, FAB *m/z* (Xenon/glycerol, rel. intensity) 214 (M^+-I^-, 100); ^1H NMR (CD_3OD, ppm) 3.35 (2H, m), 3.12 (1H, m), 3.10 (3H, s), 3.00 (3H, s), 2.10 (1H, bm), 1.93 (1H, bm), 1.76 (2H, bm), 1.60 (1H, bm), 1.44 (4H, bm), 1.09 (3H, s), 1.04 (3H, s), 0.97 (3H, t, J =7.5 Hz); ^{13}C NMR (CD_3OD, ppm) 81.5, 74.1, 65.1, 56.5, 45.2, 42.6, 35.2, 27.1, 27.0, 26.7, 23.6, 15.3, 13.9. Anal. calcd. for $C_{13}H_{28}NOI$: C, 45.75; H, 8.27; N, 4.10. Found C, 45.67; H, 8.50; N, 3.93.

3,7,11-Trimethyl-2(*E*),6(*E*),10-dodecatriene-1-al (Farnesal)[48] (96): This was prepared by Swern oxidation[49] of farnesol (**95**) (10.0 g, 45.0 mmol) according to the procedure of Leopold *et al.*[47a] for the oxidation of geraniol to geranial. Chromatography using ethyl acetate/hexanes (10/90) gave **96** in 91% yield. ^{1}H NMR ($CDCl_3$, ppm) 9.98 (1H, d, J =8.1 Hz), 5.88 (1H, d, J =8.1 Hz), 5.07 (3H, m), 2.23 (4H, m), 2.15 (3H, s), 2.05 (2H, m), 1.97 (2H, m), 1.67 (3H, s), 1.60 (3H, s); ^{13}C NMR ($CDCl_3$, ppm) 191.0, 163.5, 136.4, 131.3, 127.3, 124.0, 122.6, 40.4, 39.5(2C), 25.5, 24.7 (2C), 17.5, 17.4, 15.9.

4,8,12-Trimethyl-1,3(*E*),7(*E*),11-tridecatetraene (97):[48] This was also prepared in as analogous fashion according to the procedure of Leopold *et al.*[47a], in 85% yield starting with **96** (9.0g, 40 mmol) and 1.1 equivalent methylenetriphenylphosphorane, generated from methyltriphenylphosphonium iodide and PhLi. ^{1}H NMR ($CDCl_3$, ppm) 6.55 (1H, dt, J =17.0, 10.0 Hz), 5.85 (1H, d, J =10.0 Hz), 5.09 (3H, m), 4.97(1H, dd, J =10.0, 1.0 Hz), 2.00 (8H, m), 1.17 (3H, s), 1.67 (3H, s), 1.57 (6H, app s).

4,8,12-Trimethyl-3(*E*),7(*E*),11-tridecatriene-1-ol (Homofarnesol) (98): Hydroboration of tetraene **97** (6.5 g, 30.0 mmol) with 1.1 eq. disiamyl borane gave **98** in 85% yield according to the procedure as described by Leopold *et al.*[47a] ^{1}H ($CDCl_3$, ppm) 5.08 (3H, m), 3.60 (2H, t, J =6.5 Hz), 2.27 (2H, q, J =6.7 Hz), 2.15-1.90 (8H, m), 1.67 (3H, s), 1.64 (3H, s), 1.59 (6H, app s); ^{13}C NMR ($CDCl_3$, ppm) 138.8, 135.3, 131.3, 124.4, 121.0, 119.9, 63.4, 39.8, 39.7, 31.5, 26.8, 26.5, 25.6, 17.6, 16.2, 16.0; mass spectrum, EI *m/z* (rel intensity) 236 (M^+, 1), 193 (4), 136 (15), 123 (14), 107 (10), 93 (12), 81 (51), 69 (100). The spectra are in agreement with those reported in reference 47c.

1-Bromo-4,8,12-trimethyl-3(*E*),7(*E*),11-tridecatriene (Homofarnesyl bromide) (99): To a solution of PPh_3 (7.3 g, 28.0 mmol) in CH_2Cl_2 (100 mL) under argon, at 0 °C, Br_2 was added dropwise until a yellow color persisted. A few crystals of PPh_3 were added to consume the excess Br_2. Pyridine (2.9 mL, 35.0 mmol) was added followed by the dropwise addition of homofarnesol (**98**) (5.5 g, 23.3 mmol) in CH_2Cl_2 (20 mL). The reaction was stirred for 5 h. The solvent was evaporated *in vacuo*, the precipitate diluted with hexane (50 mL) and filtered through a pad of Celite. The precipitate was rinsed well with hexane. Standard work-up followed by filtration through a small silica gel column using hexane as the eluant gave bromide, **99** (6.1 g, 88%) as an oil. ^1H NMR ($CDCl_3$, ppm) 5.10 (3H, m), 3.33 (2H, t, J =7.3 Hz), 2.57 (2H, dt, J =7.3, 7.0 Hz), 2.20-1.95 (8H, m), 1.67 (3H, s), 1.62 (3H, s), 1.60 (6H, app s); ^{13}C NMR ($CDCl_3$, ppm) 138.5, 135.2, 131.2, 124.4, 123.9, 120.9, 39.7, 39.6, 32.9, 31.7, 26.8, 26.4, 25.6, 17.6, 16.2, 16.0; mass spectrum, EI *m/z* (rel intensity) 257/255 (M^+, 2), 189/187 (3), 136 (20), 121 (11), 95 (15), 93 (10), 91 (90), 81 (63), 69 (100), 67 (40), 55 (18), 53 (15).

1-Iodo-4,8,12-trimethyl-3(*E*),7(*E*),11-tridecatriene (Homofarnesyl Iodide) (100): To a solution of **99** (6.0 g, 20.0 mmol) in acetone (50 mL) was added NaI (5.0 g, 33.3 mmol) and the mixture stirred for 6 h at room temperature then refluxed for 2 h. Most of the solvent was evaporated *in vacuo,* then the residue was diluted with water (50 mL) and extracted with ether (3 X 50 mL). The extracts were combined and washed with 5% aqueous sodium thiosulfate (1 X 20 mL). Standard work-up followed by chromatography using ethyl acetate/hexanes (2/98) as the eluant afforded the iodide **100** (6.5 g, 94%) as an oil. ^1H NMR ($CDCl_3$, ppm) 5.09 (3H, m), 3.10 (2H, t, J =7.4 Hz), 2.6

(2H, dt, J =7.5, 7.0 Hz), 2.1-1.9 (8H, m), 1.67 (3H, s), 1.61 (3H, s), 1.60 (6H, app s); ^{13}C NMR ($CDCl_3$, ppm) 138.1, 135.1, 131.2, 124.4, 123.9, 123.0, 39.7, 39.6, 32.4, 26.8, 26.4, 25.7, 17.7, 16.3, 16.0, 5.8; mass spectrum, EI *m/z* (rel intensity) 346 (M^+,1), 303 (4), 137 (9) 136 (28), 123 (11), 121 (11), 109 (6), 107 (8), 95 (20), 93 (10), 91 (8), 82 (10), 81 (59), 79 (15), 77 (8), 69 (100), 67 (40), 55 (10). The spectra are in agreement with those reported in reference 47c.

Ethyl-5-[(*tert*-butyldimethylsilyl)oxy]-3-methyl-2(*E*)-pentenoate (104): To a slurry of $ZrCp_2Cl_2$ (29.0 g, 100 mmol) in dry CH_2Cl_2 (250 mL) under argon was added dropwise $AlMe_3$ (20.0 mL, 200 mmol) over 10 min. 1-[(*tert*-butyldimethylsilyl)oxy]-4-butyne **103** (19.0 g, 100 mmol) in CH_2Cl_2 (25.0 mL) was then added and the reaction stirred for 42 h. The resulting vinyl alane was cooled to 0 °C then freshly distilled ethyl chloroformate (19.1 mL, 200 mmol) was added dropwise. After 3 h at room temperature, excess $AlMe_3$ was destroyed (*caution*!!) by the addition of 11 mL of distilled water under a stream of argon at 0 °C. The slurry was diluted with 200 mL of hexanes then 10 g of Celite was added and the salts filtered. The Celite pad was rinsed thoroughly with hexane (~200 mL). The filtrate was concentrated *in vacuo*, diluted with 150 mL of hexanes and washed with distilled water. Standard work-up followed by the removal of volatile impurities under high vacuum (0.05 mm Hg) gave the unsaturated ester **104**, (15.5 g), >95% *E* isomer (80-85% pure, as judged by GC analysis). A small sample was chromatographed using hexane/ethyl acetate (9/1) as the eluant. IR (film) 2940 (b), 1710, 1650, 1470 and 1390 cm^{-1}; mass spectrum, EI *m/z* (rel intensity) 257 (M^+-CH_3, 3), 227 (19), 216 (14), 215 (90), 169 (20), 133 (20), 125 (13), 103 (100), 95 (33), 89 (55), 75 (75), 57 (20); ^1H NMR ($CDCl_3$, ppm) 5.65 (1H, m), 4.14 (2H, q, J =7.0 Hz), 3.73 (2H, t, J =7.0 Hz), 2.36 (2H, t, J =7.0 Hz), 2.17 (3H, d, J =1.2 Hz), 1.26 (3H, t, J =7.0 Hz), 0.87

(9H, s), 0.032 (6H, s); ^{13}C NMR ($CDCl_3$, ppm) 166.6, 156.7, 117.3, 61.3, 59.4, 44.0, 25.8 (3C), 19.1, 18.2, 14.3, -5.4 (2C). Anal. calcd. for $C_{14}H_{27}O_3Si$: C, 61.95; H, 10.02. Found: C, 61.67; H, 10.21.

Ethyl 5-hydroxyl-3-methyl-2(*E*)-pentenoate (105): Silyl ether **104** (1.5 g, 5.5 mmol) was dissolved in a stirred 1.0 M solution of tetrabutylammonium fluoride (10 mL) in THF. After 1 h, water (50 mL) was added and the aqueous layer extracted with Et_2O (4 X 25 mL). Standard work-up followed by chromatography using ethyl acetate/hexanes (25/75) as the eluant gave **105** (0.80 g, 92%). IR (film) 3360 (b), 2910, 1710, 1690, 1640, and 1370 cm^{-1}; mass spectrum, EI *m/z* (rel. intensity) 158 (M^+,10), 140 (36), 128 (52), 113 (100), 112 (88), 111 (55), 100 (31), 82 (55), 67 (29), 55 (17), 43 (12); ^1H NMR ($CDCl_3$, ppm) 5.73 (1H, q, *J*=1.0 Hz), 4.14 (2H, q, *J*=7.0 Hz), 3.78 (2H, t, *J*=7.0 Hz), 2.39 (2H, t, *J*=7.0 Hz), 2.18 (3H, d, *J*=1.0 Hz), 1.27 (3H, t, *J*=7.0 Hz); ^{13}C NMR ($CDCl_3$, ppm) 166.3, 155.9, 117.8, 60.1, 59.5, 43.6, 18.6, 14.2. Anal. calcd. for $C_8H_{14}O_3$: C, 60.75; H, 8.92. Found: C, 60.66; H, 8.89.

Ethyl 3-methyl-5-(*p*-toluenesulfonyl)-2(*E*)-penteneoate (106): To a stirred solution of **105** (1.6 g, 10 mmol) in CH_2Cl_2 (25 mL) at 0°C was added pyridine (5 mL) followed by *p*-TsCl (1.90 g, 10 mmol). After 10 h, the reaction was poured into ice water (50 mL) and extracted with Et_2O (4 X 35 mL). The combined extracts were washed with 1N HCl (2 X 50 mL) and with saturated $NaHCO_3$ solution (1 X 50 mL). Standard work-up followed by chromatography using ethyl acetate/hexanes (25/75) as the eluant to give **106** (2.5 g, 85%) as an oil. IR (film) 2980, 1710, 1660, 1600, 1450, and 1360 cm^{-1}; mass spectrum, EI *m/z* (rel intensity) 267 (M^+-C_2H_5O, 29), 155 (86), 140 (100), 112 (81), 95 (33), 91 (81), 82 (19), 65 (24); ^1H NMR ($CDCl_3$, ppm) 7.77 (2H, d,

J=8.3 Hz), 7.34 (2H, d, *J*=8.3 Hz), 5.60 (1H, q, *J*=1.0 Hz), 4.14 (2H, q, *J*=7.0 Hz), 4.12 (2H, t, *J*=7.0 Hz), 2.46 (2H, t, *J*=7.0 Hz), 2.40 (3H, s), 2.05 (3H, d, *J*=1.0 Hz), 1.27 (3H, t, *J*=7.0 Hz); ^{13}C NMR ($CDCl_3$, ppm) 166.0, 152.9, 145.0, 133.0, 129.9 (2C), 127.9 (2C), 118.5, 67.4, 59.7, 39.6, 21.6, 18.4, 14.3. Anal. calcd. for $C_{15}H_{20}O_5S$: C, 57.68; H, 6.46. Found: C, 57.64; H, 6.55.

Ethyl 5-bromo-3-methyl-2(*E*)-penteneoate (107): To a stirred solution of **106** (2.5, 8.5 mmol) in acetone (30 mL) was added LiBr (1.15 g, 12.8 mmol). After 12 h at room temperature the mixture was refluxed for 30 min, diluted with ice water (50 mL) and extracted with Et_2O (4 X 35 mL). Standard work-up followed by chromatography using ethyl acetate/hexanes (10/90) as the eluant gave **107** (1.6 g, 85%) as a clear oil. IR (film) 2970, 2930, 1710, 1650, 1440, and 1370 cm^{-1}; mass spectrum, EI *m/z* (rel intensity) 222/221 (M^++1, 15), 177/176 (67), 141 (54), 113 (100), 95 (54), 67 (49); ^1H NMR ($CDCl_3$, ppm) 5.71 (1H, q, *J*=1.0 Hz), 4.15 (2H, q, *J*=7.0 Hz), 3.49 (2H, t, *J*=7.0 Hz), 2.70 (2H, t, *J*=7.0 Hz), 2.18 (3H, d, *J*=1.0 Hz), 1.28 (3H, t, *J*=7.0 Hz); ^{13}C NMR ($CDCl_3$, ppm) 166.3, 154.9, 126.6, 118.1, 59.7, 43.4, 29.2, 18.2, 14.2.

5-Bromo-3-methyl-2(*E*)-pentenol (108): To a stirred solution of **107** (5.0 g, 22.5 mmol) in THF (50 mL), under argon at -78 °C, was added dropwise *via* a syringe, neat DIBAL-H (10.03 mL, 56.3 mmol). The reaction was warmed to -30 °C over 3 h and poured into stirring 50% aqueous solution of tartaric acid (200 mL). This was extracted with Et_2O (5 X 100 mL). Standard work-up gave **108** (3.9 g, 96%) as a light yellow oil. IR (film) 3300 (b), 2930, 2870, 1700, 1650, 1440, and 1390 cm^{-1}; mass spectrum, EI *m/z* (rel. intensity) 180/178 (M+, 33), 165/163 (21), 152/150 (12), 199 (36), 71 (100); ^1H NMR ($CDCl_3$, ppm) 5.49 (1H, t, *J*=7.0 Hz), 4.17 (2H, d, *J*=7.0 Hz), 3.48 (2H, t, *J*=7.0

Hz), 2.58 (2H, t, *J*=7.0 Hz), 1.69 (3H, s); ^{13}C NMR ($CDCl_3$, ppm) 135.4, 126.5, 58.9, 42.3, 30.8, 15.7.

1-Bromo-5-[(*tert*-butyldimethylsilyl)oxy]-3-methyl-3(*E*)-pentene (101): To a stirred solution of **108** (3.50 g, 19.6 mmol) and Et_3N (4.2 mL, 30 mmol) in CH_2Cl_2 (50 mL) was added sequentially, *tert*-butyldimethylsilyl chloride (3.3 g, 22 mmol) and DMAP (~25 mg, catalyst). After 7 h, the reaction mixture was poured into ice water (50 mL) and the organic layer separated. The aqueous layer was extracted with CH_2Cl_2 (2 X 50 mL). Standard work-up followed by chromatography using ethyl acetate/hexanes (5/95) as the eluant gave **101** (5.0 g, 92%) as a clear oil. IR (film) 2930, 2850, 1720, 1660, 1470, 1390, 1260, 1110, 1060, and 840 cm^{-1}; mass spectrum EI *m/z* (rel intensity) 237/235 (M^+-C_4H_9, 25), 139/137 (14), 81 (100), 79 (28), 75 (72), 73 (17), 57 (12); ^1H NMR ($CDCl_3$, ppm) 5.38 (1H, tq, *J*=6.5, 1.0 Hz), 4.19 (2H, d, *J*=6.5 Hz), 4.34 (2H, t, *J*=8.0 Hz), 2.55 (2H, t, *J*=8.0 Hz), 1.64 (3H, s), 0.90 (9H, s), 0.067 (6H, s); ^{13}C NMR ($CDCl_3$, ppm) 133.5, 127.7, 60.1, 42.6, 30.8, 26.0 (3C), 18.4, 16.0, -5.1 (2C). Anal. cacld. for $C_{12}H_{25}O$: C, 49.14; H, 8.63. Found: C, 49.26; H, 8.63.

***N*-(Carbobenzyloxy)-3-carbomethoxy-2-[3-methyl-5-[(*tert*-butyldimethylsilyl)oxy]-3(*E*)-pentenyl]-4-piperidone (102)**: To a stirred mixture of Mg turnings (0.13 g, 5.4 mmol) in THF (10 mL), at room temperature under argon, were added a 3 drops of 1,2-ethylenedibromide. After the bubbling had stopped, a solution of bromide **101** (1.0 g, 3.6 mmol) in THF (5 mL) was added dropwise, *via* a cunnula over 10 min. After 2 h at room temperature, a sample from the reaction mixture was quenched (saturated NH_4Cl solution) and analysed by GC, which showed complete consumption of

the **101**, but only ~60% of the GC integration was due to the protonated Grignard of **101**. The grey solution was transferred *via* a cannula to another round bottomed flask (under argon) containing CuCN (20 mg, 10% based on 60% conversion of **101** to Grignard) and this cooled to -78 °C. The resulting slurry was warmed to 0 °C and then recooled to -50 °C. Enone **45** (1.1 g, 3.6 mmol) dissolved in THF (10 mL) was then added dropwise. The reaction was warmed to 0 °C over 2 h, diluted with saturated NH_4Cl/NH_4OH (9/1) (50 mL) and extracted with Et_2O (4 X 35 mL). Standard work-up followed by chromatography using ethyl acetate/hexanes (20/80) as the eluant gave **102** (0.73 g, 41%) as a viscous oil. ^1H NMR of **102** was found to be difficult to interpret. In $CDCl_3$, **102** exists a 1/1 mixture of enol/keto forms. The spectrum was further complicated by the restricted rotation about the carbamate linkage. IR (film) 2935 (m), 2856, 1703, 1660, 1618, 1443, 1429, 1360-1304 (m), 1248, 1208, 1109, 1068, 836 and 776 cm^{-1}; mass spectrum, EI *m/z* (rel intensity) 489 (M+, trace amount), 412 (trace amount), 338 (15), 290 (70), 214 (75), 91 (100); ^1H NMR ($CDCl_3$, characteristic peaks, ppm) 9.20 (~0.5 H, d, enolic H), 7.35 (5H, m, aromatic), 5.5-5.0 (3.5 H, m, OCH_2 + vinyl H and ketonic H), 4.30/4.15 (2H, m), 3.78 (3H, s, OMe), 3.12 (1H, m), 2.50 (1H, m), 2.20 (1H, m), 2.00 (2H, m), 1.60 (2H, m), 1.56 (3H, m), 0.90 (6H, s), 0.06 (6H, s); ^{13}C NMR ($CDCl_3$, characteristic peaks, rotomers, ppm) 170.9 (m, C=O), 170.2 (C=O), 155.2 (C=O), 137-124 (7C, vinyl and aromatics), 102/103(C-4, enolic form), 68.0 (OCH_2), 60.2/60, 51.6, 49.6 (OCH_3), 39.0/38.3, 35.9, 35.6/35.2, 32.0, 29.8, 28.6/28.2, 26.0 (3C), 18.4, 16.5, -5.1 (2C). Anal. calcd. for $C_{27}H_{41}NO_6Si$: C, 64.38; H, 8.20; N, 2.78. Found: C, 64.59; H, 8.29; N, 2.90.

***N*-(Carbobenzyloxy)-3-carbomethoxy-2-[4,8,12-trimethyl-3(*E*),7(*E*),11-tridecatrienyl]-4-piperidone (110)**: To iodide **100** (1.73 g,

5.0 mmol), deoxygenated under high vacuum and purged with argon, was added dry Et_2O (50 mL) and cooled to -78 °C. *t*-BuLi (6.2 mL, 11.0 mmol, 1.7 M in pentane) was added dropwise over 15 min and the yellow solution was stirred for 30 min. Lithium (2-thienyl)(cyano)cuprate (20.2 mL, 5.05 mmol, 0.25 M in THF) was added to the alkyl lithium over 10 min. The resulting brown suspension was warmed to -30 °C for 30 min. to solubilise the reagent. The clear solution of H. O. cuprate (**109**) solution obtained was recooled to -78 °C and **45** (1.5 g, 5.0 mmol) in THF (25 mL) was added dropwise to this. The reaction was stirred for an additional 45 min and quenched with saturated NH_4Cl/NH_4OH solution (50 mL, pH~8). The slurry was warmed to 0 °C and extracted with Et_2O (4 X 50 mL). Standard work-up followed by chromatography with ethyl acetate/hexanes (15/85) gave **110** (2.2 g, 85%) as an oil. IR (film) 2917, 2855, 1703, 1659, 1617, 1440, 1384, 1359, 1303, 1245, 1212, 1110, 1068, 1011 and 821 cm^{-1}; mass spectrum, CI *m/z* (isobutane, rel intensity) 510 (M^++1, 8), 466 (6), 376 (11), 374 (6), 348 (5), 153 (100), 127 (33), 125 (40); ^1H NMR [$CDCl_3$, ppm (major/minor rotomers)] 12.20 (~1H, enolic proton, m), 7.40 (5H, m), 5.30-5.05 (5H, m), 5.00/4.85 (1H, dm, J=7.0 Hz), 4.30/4.15 (1H, dd, J=10.0, 7.0), 3.8 (3H, s), 3.21-3.10 (1H, m), 2.55-2.42 (1H, m), 2.2 (1H, m), 2.10-1.90 (10H, m), 1.68 (3H, s), 1.60 (6H, app s), 1.57 (3H, s), 1.50 (2H, m); ^{13}C NMR [$CDCl_3$, 243K, ppm (major/minor rotomers)] 171.3/171.2, 170.9/170.1, 155.2, 136.5, 135.5/135.4, 134.8, 131.3, 128.6-127.9 (3C), 124.3, 124.1, 123.6/123.3, 101.3/100.9, 67.3, 51.7, 49.5, 39.7, 39.6, 35.4/35.0, 34.0/33.6, 28.7/28.3, 26.6, 26.5, 25.9, 24.9, 17.8, 16.1(2C). Anal. calcd. for $C_{31}H_{43}NO_5$: C, 73.06; H, 8.50; N, 2.75. Found: C, 72.81; H, 8.49; N, 2.94.

***N*-(Carbobenzyloxy)-2-[4,8,12-trimethyl-3(*E*),7(*E*),11-tridecatrienyl]-4-piperidone (111):** A DMSO (20 mL) solution of **110** (1.0

g, 2.0 mmol), H_2O (150 mg, 8.0 mmol) and powdered NaCl (230 mg, 4.0 mmol) was heated at 100-110 °C for 6 h under an argon atmosphere. The reaction was cooled to room temperature, diluted with ice water and extracted with ether (4 X 50 mL). Standard work-up followed by chromatography using ethyl acetate/hexanes (3/7) as the eluant gave **111** (735 mg, 81%) as an oil. IR (film) 2965, 2917, 2855, 1702, 1423, 1381, 1351, 1347, 1309, 1233, 1177, 111 and 1010 cm^{-1}; mass spectrum, CI *m/z* (isobutane, rel intensity) 452 (M^++1, 100), 360 (73), 316 (40), 232 (60), 142 (50), 136 (30); ^1H NMR ($CDCl_3$, ppm) 7.40 (5H, m), 5.20 (2H,s), 5.10 (3H, m), 4.70 (1H, bs), 4.40 (1H, bs), 3.20 (1H, tm, *J* =12 Hz), 2.70 (1H, m), 2.50 (1H, bs), 2.30 (2H, m), 2.10 (4H, m), 2.00 (6H, app m), 1.70 (3H, s), 1.60 (3H, s), 1.58 (3H, s), 1.56 (3H, s), 1.55-1.48 (2H, m); ^{13}C NMR ($CDCl_3$, ppm) 207.4, 155.3, 136.4, 136.3, 135.0, 131.2, 128.6, 128.2, 128.0, 124.4, 124.1, 122.6, 67.6, 52.2, 45.4, 40.6, 39.7, 39.6, 38.5, 32.5, 26.8, 26.6, 25.7, 24.2, 17.7, 16.0 (2C). Anal. calcd. for $C_{29}H_{41}NO_3$: C, 77.12; H, 9.15; N, 3.10. Found: C, 76.86; H, 8.99; N, 3.07.

***N*-Methyl-2-[4,8,12-trimethyl-3(*E*),7(*E*),11-tridecatrienyl]-4-piperidone (113)**: A solution of piperidone **111** (1.3 g, 2.8 mmol) in THF (10 mL) was added dropwise to a slurry of $LiAlH_4$ (530 mg, 14 mmol) in THF (20 mL) under an argon atmosphere. The mixture was refluxed for 2 h, cooled to 0°C and the excess hydride destroyed by the addition of 0.55 mL of H_2O, followed by 0.55 mL of 15% NaOH solution followed by 1.65 mL of H_2O. The salts were filtered and rinsed thoroughly with Et_2O (5 X 25 mL). The organic washes were combined, dried (anhyd. K_2CO_3) and the solvent evaporated *in vacuo*. Chromatography using ethyl acetate/methanol/triethylamine (95/3/2) as the eluant gave **112** (800 mg, 87%) as a mixture of (axial/equatorial) alcohols (~50/50). ^1H and ^{13}C NMR spectra of the product showed two sets of signals

for the diastereoisomers. The ratio was determined by the integration of the signals due to the methine hydrogen (δ_{axial} 3.37, tt, J= 11.0, 4.5 Hz,*vs* $\delta_{equatorial}$ 3.80, m, C_6D_6) of the hydroxy bearing carbon (C-4) and the integration of the two signals due to the *N*-methyl ($\delta_{axial\ OH}$ 2.30, $\delta_{equatorial\ OH}$ 2.25, $CDCl_3$).

The pair of diastereoisomers was oxidized to ketone **113** using Albright-Goldman conditions. The diastereoisomeric mixture **112** (400 mg, 1.2 mmol) in DMSO (4 mL), was added to DMSO-Ac_2O complex prepared from acetic anhydride (6.0 mL) and DMSO (30 mL). After stirring overnight (~10 h) at room temperature the reaction mixture was poured into ice-cold saturated $NaHCO_3$ (20 mL), stirred for 1h and extracted with Et_2O (5 X 50 mL). Standard work-up followed by chromatography using CH_2Cl_2/Et_2O (1/1) as the eluant gave ketone **113** (320 mg, 81%) as a slightly yellow oil as well as some of the O-acetylated product. IR (film) 2923, 2854, 2790, 1723, 1450, 1374, 1275, 1242, 1133 and 1010 cm-1; mass spectrum, EI *m/z* (rel intensity) 331 (M^+, 7), 262 (18), 194 (23), 174 (7), 138 (16), 125 (14), 119 (5), 112 (100), 96 (31), 91 (6), 81 (6), 68 (40), 69 (27); ^1H NMR ($CDCl_3$, ppm) 5.08 (3H, m), 3.12 (1H, dtm, J =12.0, 4.0 Hz), 2.65-2.47 (3H, m), 2.40 (3H, s), 2.45-2.25 (3H, m), 2.45-2.27 (10H, m), 1.68 (3H, s), 1.60 (9H, app s), 1.50 (2H, m); ^{13}C ($CDCl_3$, ppm) 209.2, 135.8, 135.0, 131.2, 124.3, 124.1, 123.4, 62.3, 54.1, 44.8, 40.3, 40.2, 39.7, 39.6, 32.6, 26.7, 26.5, 25.6, 23.4, 17.0, 16.0, 15.9. HRMS calcd. for $C_{22}H_{37}NO$ 331.2877, found 331.2880.

***N*-Methyl-2-[4,8,12-trimethyl-3(*E*),7(*E*),11-tridecatrienyl]-4-hydroxypiperidine (37)**: To a slurry of $LiAlH_4$ (50 mg, 1.5 mmol) in THF (10 mL) at -78 °C under an argon atmosphere was added dropwise piperidone **113** (250 mg, 0.75 mmol) in THF (5.0 mL). After stirring for 30 min. the reaction was warmed to 0 °C, 15% NaOH solution (10 mL) was added and the mixture

extracted with Et_2O (5 X 25 mL). Standard work-up followed by chromatography using ethyl acetate/methanol/triethylamine (95/3/2) as the eluant afforded **37** (220 mg, 88%) as an oil (>95% equatorial). IR (film) 3330 (b), 2924, 2855, 2770, 1066, 1450, 1370, 1273, 1110, 1080 and 990 cm^{-1}; mass spectrum, CI *m/z* (isobutane, rel intensity) 334 (M^++1, 100); ^1H NMR (C_6D_6, ppm) 5.30 (3H, m), 3.37 (1H, tt (septet), *J* =11.0, 5.5 Hz), 2.67 (1H, dt, *J* =12.0, 3.5 Hz), 2.25 (6H, bm), 2.16 (4H, m), 2.12 (3H, s), 2.05 (1H, bm), 1.92 (1H, td, *J* = 12.0, 2.5 Hz), 1.77 (2H, bm), 1.72 (3H, s), 1.66 (6H, app s), 1.61 (3H, s), 1.60-1.53 (3H, m), 1.38 (1H, q, *J*= 12.0 Hz); ^{13}C NMR ($CDCl_3$, ppm) 135.2, 134.8, 131.1, 124.3, 124.1, 124.0, 69.0, 61.6, 55.3, 41.6, 39.7, 39.6, 39.5, 33.5, 32.6, 26.7, 26.5, 25.6, 23.4, 17.6, 16.0, 15.9. Anal. calcd. for $C_{22}H_{39}NO$: C, 79.22; H, 11.99; N, 4.20. Found: C, 79.01; H, 11.78; N, 3.97.

***N,N*-Dimethyl-2-[4,8,12-trimethyl-3(*E*),7(*E*),11-tridecatrienyl]-4-hydroxypiperidinium Iodide (38)**: To a solution of **37** (25 mg, 0.072 mmol) in dry Et_2O (1 mL), in a screw-cap vial, was added MeI (0.10 mL). The mixture was placed in the dark for 15 h. Excess MeI and ether were evaporated under a gentle stream of argon and the yellow paste was washed with small portions of dry Et_2O (4 X 2 mL). The residual solvent was removed under reduced pressure (0.1 mmHg) to give salt **38** (25 mg, 73%) as light yellow, hygroscopic, semisolid. IR (KBr) 3386 (b), 2923, 1450, 1381, 1072 (m) cm^{-1}; mass spectrum, FAB *m/z* (xenon, Noba, rel. intensity) 348 (M^+-I^-, 100); ^1H NMR ($CDCl_3$, ppm) 5.1 (3H, m), 4.15 (1H, m), 4.08 (1H, bm), 3.35 (1H, td, *J* =13.0, 3.0 Hz), 3.68 (1H, t, *J* =12 Hz), 3.36 (3H, s), 3.17 (3H, s), 2.40-1.90 (14H, bm), 1.70 (3H, s), 1.60 (3H, s), 1.58 (6H, app s), 1.45-1.55 (2H, m); ^{13}C NMR ($CDCl_3$, ppm) 138.2, 135.2, 131.3, 124.2, 123.8, 121.1, 71.2, 64.74, 64.3, 53.3, 43.41, 39.7, 39.6, 34.3, 29.8, 29.3, 26.7, 26.6, 25.6, 24.5, 17.7, 16.4, 16.0.

5-[(*tert*-Butyldimethylsilyl)oxy}-3-methyl-2(*E*)-penten-1-ol (115):[56] To a THF (100 mL) solution of ester **104** (15.0 g, ~80% pure) at -78°C, under a positive pressure of argon, was added neat diisobutylaluminium hydride (DIBAL-H) (16.0 mL, 90 mmol). The reaction was warmed to 0°C and stirred for 2 h. Excess reagent was destroyed by the addition of EtOAc (5 mL) and poured into a vigorously stirred ice-cold 25% aqueous solution of tartaric acid (150 mL). The mixture was extracted with Et_2O (4 X 50 mL) and the combined extracts were washed with ice-cold saturated $NaHCO_3$ solution. Standard work-up followed by chromatography using ethyl acetate/hexanes (15/85) as the eluant gave the allylic alcohol, **115** (9.0 g, 38% in two steps) as an oil. Mass spectrum, EI *m/z* (rel intensity) 173 (M^+-C_4H_9, 6), 155 (6), 105 (100), 89 (9), 81 (10), 75 (95), 73 (22); ^1H NMR ($CDCl_3$, ppm) 5.42 (1H, t, J=7.0 Hz), 4.14 (2H, d, J=7.0 Hz), 3.69 (2H, t, J=7.0 Hz), 2.24 (2H, t, J=7.0 Hz), 1.69 (3H, s), 0.88 (9H, s), 0.04 (6H, s); ^{13}C NMR ($CDCl_3$, ppm) 136.6, 125.3, 62.1, 59.2, 42.8, 25.9 (3C), 18.72, 16.62, -5.34 (2C).

5-[(*tert*-Butyldimethylsilyl)oxy]-1-chloro-3-methyl-2(*E*)-pentene (116):[56] To a solution of *N*-chlorosuccinimide (NCS) (1.07 g, 8.0 mmol) in dry CH_2Cl_2 (45 mL) at 0 °C, under an argon atmosphere, was added dimethyl sulfide (DMS) (0.74 mL, 10.0 mmol) and the slurry cooled to -25 °C. To the cooled NCS-DMS complex was added dropwise a solution of **115** (1.0 g, 4.4 mmol) in CH_2Cl_2 (10 mL). The mixture was warmed to 0 °C and stirred for 8 h. The reaction was poured into ice cold distilled water (50 mL) and extracted with hexanes (4 X 30 mL). Standard work-up gave **116** (1.0 g, 92 %) as an oil. Mass spectrum, EI *m/z* (rel intensity) 192/190 (M^+-C_4H_9, 6, 16), 155 (10), 145 (10), 125 (65), 123 (100), 95 (61), 93 (94), 81 (90), 75 (30), 73 (45), 57

(20); ^{1}H NMR ($CDCl_3$, ppm) 5.47 (1H, t, *J* =8 Hz), 4.09 (2H, d, *J* =8 Hz), 3.69 (2H, t, *J* =7 Hz), 2.26 (2H, t, *J* =7 Hz), 1.74 (3H, d, *J* =1 Hz), 0.88 (9H, s), 0.04 (6H, s); ^{13}C NMR ($CDCl_3$, ppm) 139.9, 122.1, 101.7, 42.6, 40.7, 25.9 (3C), 18.2, 16.4, -5.4 (2C).

1-(Benzenesulfonyl)-5-[(*tert*-butyldimethylsilyl)oxy]-3-methyl-2(*E*)-pentene (117): To a solution of **116** (1.0 g, 4.0 mmol) in dry DMF (15 mL) at rt, under an argon atmosphere, was added $NaSO_2Ph$ (1.0 g, 6.0 mmol) and the mixture stirred overnight (~10 h). The contents of the flask were poured into distilled (50 mL) water and extracted with hexane (4 X 30 mL). Standard work-up followed by chromatography using ethyl acetate/hexanes (15/85) as the eluant afforded the sulfone **117** (1.2 g, 86%) as an oil. IR (film) 2925, 2856, 1472, 1447, 1318, 1253, 1132 and 1086 cm^{-1}; mass spectrum, EI*m/z* (rel intensity) 241 (M^+-$C_6H_{15}Si$, 3), 217 (68), 199 (100), 135 (71), 89 (45), 81 (55), 73 (52); ^{1}H NMR ($CDCl_3$, ppm) 7.87 (2H, d, *J* =7.0 Hz), 7.64 (1H, t, *J* =7.0 Hz), 7.53 (2H, t, *J* =7.0 Hz), 5.22 (1H, tq, *J* =7.5, 1.0 Hz), 3.80 (2H, d, *J* =7.5), 3.60 (2H, t, *J* =7.0 Hz), 2.2 (2H, t, *J* =7.0 Hz), 1.32 (3H, d, *J* =1 Hz), 0.88 (9H, s), 0.04 (6H, s); ^{13}C NMR ($CDCl_3$, ppm) 143.6, 138.6, 133.5, 128.9 (2C), 128.5 (2C), 112.0, 61.7, 56.0, 42.8, 25.8 (3C), 18.2, 16.5, -5.4 (2C). Anal. calcd. for $C_{18}H_{30}O_3SiS$: C, 60.98; H, 8.53. Found: C, 60.90; H, 8.65.

5-(Benzenesulfonyl)-1-[(*tert*-butyldimethylsilyl)oxy]-3,8,12,16-tetramethyl-3(*E*),7(*E*),11*E*),15-heptadecatetraene (119): To sulfone **117** (1.0 g, 2.8 mmol), deoxygenated under high vacuum, under a positive pressure of argon in dry THF (20 mL) at -78 °C was added dropwise *n*-BuLi (1.20 mL, 3.1 mmol, 2.5 M in hexanes). The yellow solution was stirred for 1 h and a solution of farnesyl bromide (**118**) (900 mg, 3.1 mmol) in THF (10 mL) was added dropwise. After stirring the reaction for 5 h at -78°C, MeOH (3 mL)

was added followed by water (50 mL). The mixture was warmed to 0 °C and extracted with Et_2O (3 X 40 mL). Standard work-up followed by chromatography using ethyl acetate/hexanes (1/9) as the eluant yielded **119** (1.4 g, 89 %) as an oil. IR (film) 2930, 1664, 1446, 1383, 1305, 1253, 1147 and 1086 cm^{-1}; mass spectrum, EI *m/z* (rel intensity) 559 (M^+, 15), 419 (6), 418 (17), 417 (53), 286 (18), 285 (86), 259 (15), 257 (20), 217 (14), 191 (15), 143 (100), 133 (14), 123 (26); ^1H NMR ($CDCl_3$, ppm) 7.85 (2H, d, *J* =8.0 Hz), 7.60 (1H, t, *J* =8.0 Hz), 7.50 (2H, t, *J* =8.0 Hz), 5.00 (4H, m), 3.72 (1H, td, *J* =10.0, 3.5 Hz), 3.52 (2H, td, *J* =7.5, 2.0 Hz), 2.88 (1H, ddd, *J* =14.5, 7.5, 3.0 Hz), 2.35 (1H, ddd, *J* =14.5, 7.5, 3.0 Hz), 2.16 (2H, t, *J* =7.0 Hz), 2.0 (8H, bm), 1.67 (3H, s), 1.60 (3H, s), 1.59 (3H, s), 1.56 (3H, s), 1.22 (3H, s), 0.88 (9H, s), 0.04 (6H, s); ^{13}C NMR ($CDCl_3$, ppm) 142.4, 138.6, 138.3, 135.1, 133.2, 131.1, 129.1 (2C), 128.6 (2C), 124.2, 123.9, 118.7, 118.6, 64.8, 61.9, 43.0, 39.7 (2C), 26.7, 26.6, 26.5, 25.9 (3C), 25.6, 18.2, 17.6, 16.9, 16.3, 15.9, -5.4 (2C). Anal. calcd. for $C_{33}H_{54}O_3SiS$: C, 70.91; H, 9.74. Found: C, 70.88; H, 9.76.

1-[(*tert*-Butyldimethylsilyl)oxy]-3,8,12,16-tetramethyl-3*E*),7*E*),11(*E*),15-heptadecatetraene (120): To $EtNH_2$ (~25 mL) at -78 °C, under argon, was added sulfone **119** (500 mg, 1.0 mmol) in 4 mL of THF, followed by small pieces of lithium wire (~50 mg, excess). This was stirred until the solution became dark blue (35 min). Solid NH_4Cl (500 mg) was added and the excess lithium was removed with forceps. The mixture was diluted with water (25 mL) and extracted with ether (4 X 40 mL). Standard work-up followed by chromatography using ethyl acetate/hexanes (5/95) as the eluant gave **120** (360 mg, 86%) as an oil. IR (film) 2925, 2856, 1666, 1446, 1382, 1254, 1096 and 836 cm^{-1}; mass spectrum, CI *m/z* (isobutane, rel intensity) 419 (M^++1, 10), 316 (13), 288 (14), 287 (63), 285 (14), 261 (25), 231 (13), 217 (12), 205 (37),

203 (20), 193 (14), 191 (26), 179 (15), 177 (31), 165 (13), 163 (45), 161 (62), 159 (96), 151 (26), 149 (38), 137 (100), 135 (20), 133 (20), 125 (13), 123 (35); ^{1}H NMR ($CDCl_3$, ppm) 5.13 (m, 4H), 3.65 (2H, t, J =7.1 Hz), 2.19 (2H, t, J =7.1 Hz), 2.00 (12H, bm), 1.68 (3H, s), 1.61 (3H, s), 1.60 (9H, app s), 0.88 (9H, s), 0.04 (6H, s); ^{13}C NMR ($CDCl_3$, ppm) 135.1, 134.8, 132.1, 131.1, 126.2, 124.4, 124.3, 124.2, 62.5, 43.1, 37.6 (2C), 28.3, 28.2, 26.8, 26.7, 25.9 (3C), 25.6, 18.3, 17.6, 16.4, 16.0, 15.9, -5.3 (2C). Anal. calcd. for $C_{27}H_{50}OSi$: C, 77.44; H, 12.03. Found: C, 77.41; H, 12.26.

3,8,12,16-Tetramethyl-3(*E*),7(*E*),11(*E*),15-heptadecatetraen-1-ol (121): Silyl ether **120** (2.2 g, 5.4 mmol) was dissolved in 30 mL of 1.0 M solution of tetrabutylammonium fluoride in THF and stirred for 4 h at room temperature The solution was diluted with water (100 mL) and extracted with ether (4 X 40 mL). Standard work-up followed by chromatography using ethyl acetate/hexanes (2/8) as the eluant yielded **121** (1.5 g, 90%) as an oil. Mass spectrum, CI *m/z* (isobutane, rel intensity) 305 (M^++1, 60), 287 (16), 219 (30), 205 (21), 193 (28), 191 (20), 179 (20), 177 (20), 163 (25), 153 (25), 151 (36), 149 (320, 137 (100), 136 (20), 135 (20), 125 (21), 123 (41); ^{1}H NMR ($CDCl_3$, ppm) 5.24 (1H, m), 5.10 (3H, m), 3.64 (2H, t, J =6.0 Hz), 2.24 (2H, t, J =6.0 Hz), 2.05 (8H, m), 1.98 (4H, m), 1.68 (3H, s), 1.62 (3H, s), 1.59 (9H, app s); ^{13}C NMR ($CDCl_3$, ppm) 135.6, 135.0, 131.3, 131.2, 127.9, 124.4, 124.2, 124.0, 60.1, 42.7, 39.7 (2C), 28.3, 28.1, 26.8, 26.7, 25.7, 17.7, 16.1, 16.0, 15.8. Anal. calcd. for $C_{21}H_{36}O$: C, 82.83; H, 11.90. Found: C, 83.04; H, 12.17.

1-[(*p*-Toluenesulfonyl)oxy]-3,8,12,16-tetramethyl-3(*E*),7(*E*),11(*E*),15-heptadecatetraene (122): To a stirred solution of **121** (1.5 g, 5.1 mmol) in pyridine (10 mL) and CH_2Cl_2 (25 mL) at 0°C, was

added *p*-TsCl (1.07 g, 5.6 mmol). The reaction was stirred for 10 h at 0°C, poured into ice-water (50 mL) and extracted with ether (3 X 50 mL). The extracts were combined, washed with ice cold 2N HCl solution followed by ice cold saturated $NaHCO_3$. Standard work-up gave tosylate **122** (2.4 g) as an oil. An analytical sample was purified by chromatography using ethyl acetate/hexanes (5/95) as the eluant. IR (film) 2922, 1598, 1448, 1363, 1188, 1177, 1098 and 964 cm^{-1}; mass spectrum, EI *m/z* (rel intensity) 458 (M^+, 1), 229 (4), 192 (3), 161 (3), 155 (4), 149 (10), 147 (4), 137 (10), 136 (15), 123 (9), 121 (15), 107 (15), 95 (18), 94 (15), 93 (15), 93 (20), 91 (30), 82 (23), 81 (100), 79 (20), 69 (83), 68 (22), 67 (27), 55 (10), 53 (9); ^1H NMR ($CDCl_3$, ppm) 7.78 (2H, d, J =8.3 Hz), 7.33 (2H, d, J =8.3 Hz), 5.01 (4H, m), 4.06 (2H, t, J =7.0 Hz), 2.44 (3H, s), 2.3 (2H, t, J =7.0 Hz), 2.05 (4H, m), 1.98 (8H, m), 1.67 (3H, s), 1.60 (6H, app s), 1.58 (3H, s), 1.51 (3H, s); ^{13}C NMR ($CDCl_3$, ppm) 144.5, 135.4, 134.9, 133.4, 131.4, 131.1, 129.7 (2C), 129.4, 128.0, 127.8 (2C), 124.3, 124.2, 123.9, 69.0, 39.7 (2C), 38.7, 28.2, 27.9, 26.7, 26.6, 25.6, 21.5, 17.6, 15.9 (2C). Anal. calcd. for $C_{28}H_{42}O_3S$: C, 73.32; H, 9.23. Found: C, 73.33; H, 9.20.

1-Iodo-3,8,12,16-tetramethyl-3(*E*),7(*E*),11(*E*),15-heptadecatetraene (114): To a solution of tosylate **122** (2.3 g, crude, 5.0 mmol) in acetone (50 mL) was added powdered NaI (1.1 g, 7.3 mmol) and the mixture was refluxed for 4 h. The cooled reaction mixture was poured into ice water (50 mL) and extracted with diethyl ether (3 X 40 mL). The combined extracts were washed with 5% aqueous sodium thiosulfate (1 X 20 mL). Standard work-up followed by chromatography using ethyl acetate/hexanes (5/95) as the eluant afforded iodide **114** (1.6 g, 77% in two steps) as an oil. IR (film) 2964, 2923, 2854, 1666, 1444, 1381, 1243, 1168 cm^{-1}; mass spectrum, CI *m/z* (isobutane, rel intensity) 415 (M^++1, 60), 413 (29), 333 (18), 287 (20), 269

(15), 205 920), 193 (54), 191 (27), 177 (18), 165 (15), 163 (21), 151 (30), 149 (26), 143 (22), 137 (100), 136 (23), 135 (23), 125 (30), 123 (50); ^{1}H NMR ($CDCl_3$, ppm) 5.22 (1H, m), 5.14 (3H, m), 3.21 (2H, t, J =7.5 Hz), 2.52 (2H, t, J =7.5 Hz), 2.02 (12H, m), 1.69 (3H, s), 1.6 (12H, app s); ^{13}C NMR ($CDCl_3$, ppm) 135.2, 134.8, 133.6, 131.1, 127.4, 124.4, 124.2, 124.0, 43.9, 39.7 (2C), 28.3, 27.9, 26.8, 26.7, 25.7, 17.67, 16.1, 160, 15.4, 4.71. Anal. calcd. for $C_{21}H_{35}I$: C, 60.87; H, 8.51. Found: C, 60.94; H, 8.44.

***N*-(Carbobenzyloxy)-3-carbomethoxy-2-[3,8,12,16-tetramethyl-3(E),7(*E*),8(*E*),11(*E*),15-heptadecatetraenyl]-4-piperidone (124)**: This compound was prepared using the same conditions as for preparation of **110.** Reaction of **114** (700 mg, 1.7 mmol) in dry Et_2O (25 mL) with *t*-BuLi (2.2 mL, 3.75 mmol, 1.7 M in pentane) followed by lithium (2-thienyl)cyanocuprate (7.2 mL, 1.8 mmol, 0.25 M in THF) and enone **45** (540 mg, 1.9 mmol) in THF (10.0 mL), respectively, gave **124** (900 mg, 90%) as an oil, after chromatography using ethyl acetate/hexanes (1/9) as the eluant. IR (film) 2920, 2855, 1705, 1660, 1617, 1440, 1385, 1359, 1302, 1245, 1212, 1110, 1068, 1011 and 820 cm^{-1}; mass spectrum, CI *m/z* (isobutane, rel intensity) 578 (M^++1, 62), 534 (33), 444 (33), 306 (35), 290 (100), 200 (40), 156 (38), 137 (37), 123 (31); ^{1}H NMR [$CDCl_3$, ppm (major/minor rotomers)] 12.20 (~1H, m, enolic proton), 7.30 (5H, m), 5.25-5.05 (6H, bm), 4.97/4.84 (1H, dm, J =10.0 Hz), 4.27/4.14 (1H, dd, J =10.0, 7.0 Hz), 3.78 (3H, s), 3.15 (1H, m), 2.52 (1H, m), 2.20 (1H, m), 2.10 (4H, bm), 2.00 (10H, bm), 1.70 (3H, s), 1.60 (12H, app s), 1.55 (2H, m); ^{13}C NMR [$CDCl_3$, ppm (major/minor rotomers)] 171.3/171.1, 170.7/170.0, 155.2, 136.7, 135.1, 134.9, 134.4, 131.0, 129-127 (m, 4C), 124.5, 124.4, 124.2, 101.3/100.9, 67.3, 51.6, 49.7, 39.6 (2C), 35.9, 35.2, 34.7, 31.9/31.8, 28.5, 28.2,

28.1, 26.5, 25.8 (2C), 17.7, 16.4, 16.0, 15.9. Anal. calcd. for $C_{36}H_{51}NO_5$: C, 74.84; H, 8.90; N, 2.42. Found C, 75.02; H, 8.84; N, 2.30.

***N*-(Carbobenzyloxy)-2-[3,8,12,16-tetramethyl-3(*E*),7(*E*),11(*E*),15-heptadecatetraenyl]-4-piperidone (125)**: Piperidone **125** was prepared from **124** (650 mg, 1.13 mmol), DMSO (7.0 mL) containing H_2O (82 mg, 4.0 mmol) and powdered NaCl (132 mg, 2.0 mmol) in the same manner as described for **111**. Chromatography using ethyl acetate/hexanes (2/8) as the eluant gave **125** (520 mg, 85 %) as an oil. IR (film) 2917, 1703 (b), 1422, 1343, 1309, 1233 and 1113 cm^{-1}; mass spectrum, CI *m/z* (isobutane, rel intensity) 520 (M^++1, 25), 476 (15), 428 (20), 386 (100), 151 (29), 137 (55), 123 (55); ^1H NMR ($CDCl_3$, ppm) 7.37 (5H, bs), 5.18 (2H, s), 5.10 (4H, bm), 4.60 (1H, bs), 4.40 (1H, bs) 3.23 (1H, tm, J =12.0 Hz), 2.67 (1H, bm), 2.47 (1H, bs), 2.35 (1H, bs), 2.30 (2H, bs), 2.06 (4H, bm), 1.98 (10H, bm), 1.68 (3H, s), 1.59 (9H, app s), 1.55 (3H, s), 1.54 (2H, bs); ^{13}C NMR ($CDCl_3$, ppm) 207.4, 155.3, 136.4, 135.2, 134.9, 133.5, 131.1, 128.5 (2C), 128.1, 128.0, 127.9 (2C), 125.2, 124.4, 124.2, 124.1, 67.6, 52.2, 45.2, 40.6, 39.7 (2C), 38.5, 35.6, 30.8, 28.3, 28.2, 28.1, 26.7, 26.6, 25.6, 17.64, 16.0, 15.9 (2C). Anal. calcd. for $C_{34}H_{49}NO_3$: C, 78.57; H, 9.50; N, 2.69. Found: C, 78.41; H, 9.30; N, 2.43.

***N*-Methyl-2-[3,8,12,16-tetramethyl-3(*E*),7(*E*),11(*E*),15-heptadecatetraenyl]-4-piperidone (127):** This compound was prepared in the same manner as **113.** Ketone **125** (500 mg, 1.0 mmol) and $LiAlH_4$ (200 mg, 5.0 mmol) gave **126** (320 mg, 82%) as a ~1/1 mixture of diastereoisomeric alcohols. Oxidation of **126** (250 mg, 0.62 mmol) was performed using a solution of acetic anhydride (3.0 mL) and DMSO (20 mL) at room temperature, for 16 h. Chromatography using Et_2O/CH_2Cl_2 (1/1) as the eluant gave **127**

(190 mg, 76%) as a yellow oil. IR (film) 2917, 1703 (b), 1422, 1343, 1309, 1233 and 1113 cm^{-1}; mass spectrum, CI *m/z* (isobutane, rel intensity) 400 (M^++1, 100); ^1H NMR ($CDCl_3$, ppm) 5.12 (2H, m), 3.12 (1H, ddd, *J* =12.0, 10.0, 4.0 Hz), 2.60 (1H, m), 2.50 (1H, m), 2.39 (3H, s), 2.39-2.25 (4H, m), 2.05 (4H, m), 2.00 (10H, m), 1.68 (3H, s), 1.59 (12H, app s), 1.50 (2H, m); ^{13}C NMR ($CDCl_3$, ppm) 209.1, 135.2, 134.9, 134.3, 131.2, 124.8, 124.4, 124.2, 124.1, 62.3, 54.0, 44.8, 40.3, 40.2, 39.7 (2C), 34.8, 30.8, 28.2, 28.1, 26.8, 26.6, 25.6, 17.63, 16.0 (3C). Anal. calcd. for $C_{27}H_{45}NO$: C, 81.15; H, 11.35; N, 3.51. Found: C, 81.10; H, 11.46; N, 3.53.

***N*-Methyl-2-[3,8,12,16-tetramethyl-3(*E*),7(*E*),11(*E*),15-heptadecatetraenyl]-4-hydroxypiperidine (39):** This was prepared in the same manner as **37**. Reaction of $LiAlH_4$ (27 mg, 0.7 mmol) and piperidone **127** (120 mg, 0.30 mmol) at -78°C followed by chromatographic purification using ethyl acetate/methanol/triethylamine (95/3/2) as the eluant gave amino-alcohol, **39** (100 mg, 83%, >95% equatorial). IR (film) 3330 (b), 2926, 2854, 1666, 1449, 1375 and 1081 cm^{-1}; mass spectrum, EI *m/z* (rel intensity) 401 (M^+, 2), 264 (5), 196 (6), 127 (11), 115 (7), 114 (100), 96 (10), 70 (11), 69 (13); ^1H NMR (C_6D_6, ppm) 5.30 (4H, m), 3.37 (1H, tt (septet), *J* =11.0, 5.5 Hz), 2.67 (1H, dt, *J* =12.0, 3.5 Hz), 2.25 (14H, bm), 2.12 (3H, s), 2.05 (1H, bm), 1.92 (1H, td, *J* = 12.0, 2.5 Hz), 1.77 (2H, bm), 1.72 (3H, s), 1.66 (9H, app s), 1.61 (3H, s), 1.60-1.53 (3H, m), 1.38 (1H, q, *J* = 12.0 Hz); ^{13}C NMR ($CDCl_3$, ppm) 135.0, 134.9, 134.7, 131.0, 124.3 (2C), 124.2, 124.1, 69.0, 61.5, 55.3, 41.7, 39.7, 39.6, 34.8, 31.8, 28.2, 28.1, 26.7, 26.6, 25.6, 17.6, 16.0 (3C). Anal. calcd. for $C_{27}H_{47}NO$: C, 80.74; H, 11.79; N, 3.49. Found: C, 80.86; H, 11.92; N, 3.31.

***N,N*-Dimethyl-2-[3,8,12,16-tetramethyl-3(*E*),7(*E*),11(*E*),15-heptadecatetraenyl]-4-hydroxypiperidinium Iodide (40)**: This was prepared in the same manner as **38** in 80% yield, starting with **39**. IR (KBr) exactly as reported for **38**; mass spectrum, FAB *m/z* (Xenon, Noba) 416 (M^+-I^-); ^1H NMR ($CDCl_3$, ppm) 5.20 (1H, bm), 5.10 (3H, bs), 4.14-4.0 (2H, bm), 3.83 (1H, td, J=13.0, 3.0 Hz), 3.62 (1H, t, J = 11.0 Hz), 3.36 (3H, s), 3.17 (3H, s), 2.40-2.10 (2H, m), 2.10-1.85 (16H, bm), 1.66 (3H, s), 1.60 (3H, s), 1.57 (9H, app s), 1.56-1.45 (2H, m); ^{13}C NMR ($CDCl_3$, ppm) 135.5, 134.9, 132.3, 131.2, 126.9, 124.3, 124.1, 123.8, 71.4, 64.8, 64.4, 53.3, 43.5, 39.7 (2C), 35.8, 34.3, 29.8, 28.3, 28.1, 27.5, 26.7, 26.6, 25.6, 17.6, 16.0 (3C).

B: Chapter 3

Ethyl 3-oxo-4-pentenoate (131). This was prepared by the procedure described by Zibuck and Streiber.[60a] To a solution of LDA at -100 °C [prepared from diisopropyl amine (15.5 mL, 110 mmol) and *n*-BuLi (44 mL, 2.5 M in hexanes) at -78 °C, in THF (300 mL)] was added dropwise dry ethyl acetate (8.8 g, 100 mmol) in THF (25 mL) over 15 min. This was followed by the addition of freshly distilled acrolein (6.7 mL, 100 mmol) over a 5 min. After stirring for 10 min, a solution of saturated NH_4Cl (25 mL) was added followed by Et_2O (200 mL). The resulting slurry was poured into a separatory funnel and the aqueous layer drained and the organic layer washed with saturated NaCl solution (2 X 100 mL). The organic layer was dried ($MgSO_4$) and distilled under reduced pressure to give ethyl 3-hydroxy-4-pentenoate as an oil (11.7 g, 80%): bp 63-64 °C/0.10 mmHg (Kugelrohr oven temperature) lit.[60a] bp 75 °C/0.60 mmHg.

Ethyl 3-hydroxy-4-pentenoate (7.2 g, 50 mmol) was diluted with acetone (200 mL) and cooled to 0 °C. To this was added dropwise (~30 min), with stirring, a solution of Jones' reagent, prepared from concentrated H_2SO_4 (5.5 mL) and CrO_3 (5.5 g, 55 mmol) and poured into water to make up a 55 mL solution. After 1 h, MeOH (20 mL) was added and the reaction was stirred for an additional 20 min, poured into a separatory funnel and extracted with Et_2O (3 X 100 mL). The extracts were combined and washed with ice-cold water (2 X 100 mL) and ice cold saturated NaCl solution (2X100 mL), dried over $MgSO_4$, and distilled under reduced pressure to give **131** (4.0 g, 55 %): bp 43-46 °C/0.7 mmHg (Kugelrohr temperature), lit[66a] bp 45 °C/0.60 mmHg. IR (film) 2983, 1740, 1659, 1590, 1423, 1242, 1150, 1039 and 812 cm^{-1}; ^1H NMR ($CDCl_3$, ppm) exactly as reported in ref. 66a: 11.80 (enol H, s), 6.43-5.90 (2H,

m), 5.50 (1H, app t), 5.05 (~1H, s), 4.30-4.10 (2H, m), 3.60 (ketonic H, bs), 1.30-1.20 (3H, overlaping t).

6, 7-Epoxy geranyl acetate (136). To a mechanically stirred solution of geranyl acetate (**135**) (32.0 g, 163 mmol) in CH_2Cl_2 (500 mL) at -35 °C to -20 °C containing NaOAc (13.5 g, 165 mmol) was added *m*-CPBA (37.0 g, 165 mmol, 80% by weight) in 3.0 g portions over 1.5 h. The mixture was warmed to 0 °C and stirred for an additional 2 h, and poured into 500 mL of saturated $NaHCO_3$ and the organic layer separated. The aqueous layer was extracted with CH_2Cl_2 (2 X 50 mL). The extracts were combined, washed with ice cold 1N NaOH (100 mL). Standard work-up gave almost pure epoxide **136** (34.0 g, 98%) as an clear oil. Mass spectrum CI *m/z* (isobutane, rel. intensity) 213 (M^++1, trace amount), 153 (100, M^+-OAc), 135(24); ^1H NMR ($CDCl_3$, ppm) 5.37 (1H, tq, *J*=7.5, 1.2 Hz), 4.57 (2H, d, *J*= 7.0 Hz), 2.68 (1H, t, *J*=6.0 Hz), 2.15 (2H, m), 2.03 (3H, s), 1.72 (3H, s), 1.65 (2H, m), 1.28 3H, s), 1.23 (3H, s); ^{13}C NMR ($CDCl_3$, ppm) 170.6, 140.9, 118.9, 63.6, 60.9, 58.0, 36.0, 26.9, 24.6, 20.7, 18.5, 16.2.

6-Acetoxy-4-methyl-4(*E*)-hexenal (137).[64] To a mechanically stirred solution of epoxide **136** (20.0 g, 94 mmol) in Et_2O (300 mL) at 0 °C was added dropwise $HIO_4 \cdot 2H_2O$ (23.0 g, 100 mmol) dissolved in THF (200 mL) over 2 h. The slurry was stirred for an additional 0.5 h, poured into H_2O (250 mL), the layers partitioned and the aqueous layer extracted with Et_2O (3 X 50 mL). The combined extracts were washed with saturated $NaHCO_3$ (2 X 50 mL). standard work-up gave **137** (15.4 g, 82% crude), which was used without purification in the next step. Mass spectrum EI *m/z* (rel intensty) 126 (M^+-C_2H_3O, 26), 110 (M^+-$C_2H_3O_2$, 39), 95 (10), 84 (100), 83 (12), 82 (25), 81 (52),

79 (28), 77(10), 68 (26), 67 (48), 55 (27), 54 (20); ^{1}H NMR ($CDCl_3$, ppm) 9.80 (1H, t, *J*=1.80 Hz), 5.52 (1H, tq, *J*=7.0, 1.0 Hz), 4.55 (2H, d, *J*=7.0 Hz), 2.60 (2H, td, *J*=7.5, 1.8 Hz), 2.35 (2H, t, *J*=7.5 Hz), 2.00 (3H, s), 1.70 (3H, s); ^{13}C NMR ($CDCl_3$, ppm) 201.3, 170.6, 139.7, 118.5, 60.7, 39.2, 31.2, 20.6, 16.36.

6-Ethylenedioxy-1-acetoxy-3-methyl-2(*E*)-hexene (138)[64]. A solution of **137** (15.0 g, 75 mmol, crude), $(CH_2OH)_2$ (8.0 g, 132 mmol) and *p*-TsOH (300 mg) in toluene (200 mL) were refluxed for 5 h under a nitrogen atmosphere using a Dean-Stark separator to remove the water. A solution of saturated $NaHCO_3$ (100 mL) was added, the organic layer separated and the aqueous layer extracted with Et_2O (2 X 50 mL). Standard work-up gave **138** (15.5 g, 96%, crude), which was used without purification in the next step. Mass spectrum CI *m/z* (isobutane, rel intensity) 215 (M^++1, trace amount), 155 (100); ^{1}H NMR ($CDCl_3$, ppm) 5.37 (1H, tq, *J*=7.0, 1.0 Hz), 4.85 (1H, t, *J*=4.5 Hz), 4.55 (2H, d, *J*=7.0 Hz), 3.95 (2H, m), 3.85 (2H, m), 2.15 (2H, m), 2.04 (3H, s), 1.77 (2H, m)1.70 (3H, s); ^{13}C NMR (CDCl3, ppm) 170.6, 141.0, 118.3, 103.7, 64.5 (2C), 60.9, 33.3, 31.7, 20.6, 16.0.

6-Ethylenedioxy-3-methyl-2(*E*)-hexenol (139).[64] To a solution of **138** (15.5 g, 72 mmol, crude) in MeOH (500 mL) was added K_2CO_3 (2.0 g). After stirring for 10 h, most of the MeOH was removed *in vacuo*, the resulting was slurry diluted with H_2O (100 mL) and extracted with Et_2O (3 X 50 mL). Standard workup followed by chromatograhy using ethyl acetate/ hexanes (50/50) as the eluant gave **139** (7.7 g, 65 %; 47 % yield over 3 steps) as a clear oil. IR (film) 3408, 2953-2883 (m), 1669, 1445, 1410, 1140, 1032 and 897 cm^{-1}; mass spectrum CI *m/z* (isobutane, rel intensity) 171 (M^++1, trace amount), 155 (100); ^{1}H NMR ($CDCl_3$, ppm) 5.43 (1H, tq, *J*=6.5, 1.3 Hz), 4.85 (1H, t, *J*=5.0 Hz),

4.13 (2H, d, *J*=7.0 Hz), 3.95 (2H, m), 3.83 (2H, m), 2.13 (2H, t, *J*=7.5 Hz), 1.77 (2H, m), 1.68 (3H, s); ^{13}C NMR ($CDCl_3$, ppm) 137.8, 123.7, 104.0, 64.6 (2C), 58.77, 33.4, 31.84, 16.0.

6-Ethylenedioxy-1-chloro-3-methyl-2(*E*)-hexene (140). Alcohol **139** (2.5 g, 14.6 mmol) in 25 mL of CH_2Cl_2 was added dropwise to NCS-DMS complex, prepared from NCS (3.0 g, 22.5 mmol) and DMS (2.2 mL, 30 mmol) at 0 °C according to the procedure of Corey,[57] in CH_2Cl_2 (50 mL) at -20 °C. The cloudy mixture was stirred at 0 °C for 6 hr, poured into water (50 mL) and the organic layer separated. The aquous layer was extracted with CH_2Cl_2 (3 X 25 mL). Standard work-up gave chloride **140** (2.70 g, 97 % pure by GC) as a yellow oil. IR (film) 2955.4, 2883.6, 1721.6, 1662.7, 1448.8, 1386.5, 1254.5, 1140.1, 1036.7, and 944 cm^{-1}; mass spectrum CI *m/z* (isobutane, rel intensity, major isotope) 191 (M^++1, 34), 155 (93), 131 (32), 129 (100); ^1H NMR ($CDCl_3$, ppm) 5.45 (1H, tq, *J*=7.5, 1.2 Hz), 4.83 (1H, t, *J*=4.5 Hz), 4.05 (2H, d, *J*=7.5 Hz), 3.93 (2H, m), 3.82 (2H, m), 2.15 (2H, t, *J*=7.5 Hz), 1.75 (2H, m), 1.70 (3H, s); ^{13}C NMR ($CDCl_3$, ppm) 140.8, 120.4, 103.8, 64.7 (2C), 40.8, 33.4, 31.8, 15.9.

6-Ethylenedioxy-1-(benzenesulfonyl)-3-methyl-2(*E*)-hexene (141). To a solution of **140** (2.5 g, 13 mmol, crude) in DMF (20 mL) at 0 °C was added $NaSO_2Ph$ (2.35 g, 14.3 mmol). After stirring for 5 hr, water (50 mL) was added and the aqueous layer extracted with Et_2O (5 X 25 mL). Standard work-up followed by chromatography using ethyl acetate/hexanes (1/1) gave the sulfone **141** (3.10 g, 86 %) as an oil. IR (film) 2955, 2886, 1663, 1447, 1407, 1305, 1240, 1150, 1085, 1034, 902, 741and 690 cm^{-1}; mass spectrum CI *m/z* (isobutane, rel intensity) 297 (M^++1, 100), 235 (33), 157 (15), 155 (73), 143 (62); ^1H NMR ($CDCl_3$, ppm) 7.87 (2H, d, *J*=7.0 Hz), 7.64 (1H, t, *J*=7.0 Hz) 7.53

(2H, t, J =7.0 Hz), 5.20 (1H, tq, J=7.5, 1.2 Hz), 4.80 (1H, t, J=4.5 Hz), 3.93 (2H, m), 3.83 (2H, m), 3.78 (2H, d, J=8.0 Hz), 2.10 (2H, t, J=7.5 Hz), 1.65 (2H, m), 1.30 (3H, s); ^{13}C NMR ($CDCl_3$, ppm) 145.5, 138.5, 133.5, 128.9 (2C), 128.4 (2C), 110.6, 103.7, 64.8 (2C), 55.9, 33.6, 31.8, 16.0. Anal cald. for $C_{15}H_{20}O_4S$: C, 60.79; H, 6.80. Found: C, 60.62; H, 6.51.

1-Ethylenedioxy-6-(benzenesufonyl)-4,9,13,17-tetramethyl-4(*E*),8(*E*),12(*E*),16-octadecatetraene (142). To a solution of sulfone **135** (3.0 g, 10.0 mmol) in THF (30 mL) under argon at -78 °C was added *n*-BuLi (4.4 mL, 11.0 mmol, 2.5 M in hexanes). After stirring for 1.5 h, farnesyl bromide (**118**) (3.1 g, 11 mmol) in THF (10 mL) was added dropwise and this was stirred for 5hat -78 °C. MeOH (2 mL) was added followed by H_2O (50 mL), the resulting was slurry warmed to room temperature and extracted with Et_2O (4 X 50 mL). Standard work-up followed by chromatography gave **142** (4.7 g, 94 %) as an oil. IR (film) 2923 (m), 1665, 1585, 1446, 1383, 1304, 1146, 1085, 1036, 896 and 741 cm^{-1}; mass spectrum EI *m/z* (rel intensity) 500 (M^+, trace amount), 359 (6), 221 (10), 297 (9), 159(10), 147 (13), 136/137 (15), 125 (12), 121 (15), 119 (15), 109 (15), 107/105 (13), 99 (31), 93 (37), 91 (13), 81(60), 79 (15), 77 (14), 73 (43), 69 (100), 55 (12); ^1H NMR ($CDCl_3$, ppm) 7.87 (2H, d, J =7.0 Hz), 7.64 (1H, t, J =7.0 Hz) 7.53 (2H, t, J =7.0 Hz), 5.05 (3H, m), 4.95 (1H, t, J=7.5 Hz), 4.77 (1H, t, J=4.5 Hz), 3.95 (2H, m), 3.85 (2H, m), 3.70 (1H, td, J=10.0, 3.0 Hz), 2.85 (1H, ddd, J=14.5 , 7.5, 3.0 Hz), 2.35 (1H, overlaping ddd, J=14.5, 7.5, 3.0 Hz), 2.10-1.90 (10H, m), 1.67 (3H, s), 1.65-1.60 (2H, m), 1.60 (3H, s), 1.57 (3H, s), 1.55 (3H, s), 1.18 (3H, d, J=1.2 Hz); ^{13}C NMR ($CDCl_3$, ppm) 144.2, 138.5, 138.0, 135.0, 133.2, 131.0, 128.9 (2C), 128.6 (2C), 124.2, 123.7, 118.4, 117.3, 103.6, 64.8 (2C), 64.6, 39.6 (2C), 33.7, 31.8, 26.6, 26.4, 26.3, 25.5, 17.5,

16.3, 16.2, 15.8. Anal. calcd. for $C_{30}H_{44}O_4S$: C, 71.96; H, 8.86. Found: C, 71.82; H, 8.72.

1-Ethylenedioxy-4,9,13,17-tetramethyl-4(*E*),8(*E*),12(*E*),16-octadecatetraene (143). To $EtNH_2$ (35 mL) at -78 °C under an argon atmosphere were added small pieces of Li wire (500 mg, 70 mmol) followed by a solution of **142** (4.7 g, 9.4 mmol) in THF (10 mL). This was stirred until the solution became blue at which time solid NH_4Cl (5 g) was added and the excess Li metal removed with forceps. Water (50 mL) was added and this extracted with Et_2O (4 X 40 mL). Standard work-up followed by chromatography using ethyl acetate/ hexanes (5/95) as the eluant gave **143** (3.0 g, 89 %) as an oil. Mass spectrum CI, *m/z* (isobutane, rel intensity) 361/360 (M^++1, 60/15), 300/299 (23/100), 229 (9), 218/217 (7/37), 205 (10), 203 (15), 193 (10), 192 (10), 191 (18), 189 (20), 175 (25); ^1H NMR ($CDCl_3$, ppm) 5.20-5.05 (4H, m), 4.84 (1H, t, *J*=4.5 Hz), 3.95 (2H, m), 3.85 (2H, m), 2.2-1.92 (14H, m), 1.75 (2H, m), 1.67 (3H, d, *J*=1.2 Hz), 1.62 (3H, s), 1.58 (9H, app s); ^{13}C NMR ($CDCl_3$, ppm) 135.2, 134.8, 134.2, 131.2, 124.6, 124.5, 124.3, 124.2, 104.4, 64.8 (2C), 39.7 (2C), 33.9, 32.5, 28.3, 28.2, 26.8, 26.7, 25.6, 17.6, 16.0 (3C). Anal. calcd. for $C_{24}H_{40}O_2$: C, 79.95; H, 11.18. Found: C, 79.84; H, 10.82.

4,9,13,17-Tetramethyl-4(*E*),8(*E*),12(*E*),16-octadecatetraenal (144). A solution of acetal **143** (3.0 g, 8.3 mmol) and *p*-TsOH (200 mg), in acetone/H_2O (85/15, 100 mL), was refluxed for 12 hr. Most of the acetone was removed *in vacuo* and the concentrate diluted with H_2O (50 mL) and extracted with Et_2O (5 X 25 mL). Standard work-up followed by chromatography using ethyl acetate/hexanes (5/95) as the eluant gave **144** (2.5 g, 95 %) as an oil. IR (film) 2924 (bm), 1727, 1443, 1382, and 1126 cm^{-1}; mass spectrum CI *m/z*

(isobutane, rel intensity) 317 (M^++1, 22), 299 (23), 235 (7), 217 (25)205 (8), 203 (9), 193 (30), 191 (16), 189 (14), 179 (11), 175 (14), 165 (14), 163 (19), 161 (13), 153 (11), 151 (22), 149 (49), 147 (11), 139 (10), 138 (10), 137 (100), 136 (28), 135 (21); ^{1}H NMR ($CDCl_3$, ppm) 9.80 (1H, t, *J*=1.8 Hz), 5.00-5.30 (4H, m), 2.50 (2H, td, *J*=7.5, 1.8 Hz), 2.30 (2H, t, *J*=7.5 Hz), 2.1-1.95 (12H, bm), 1.68 (3H, s), 1.61 (3H, s), 1.60 (9H, app s); ^{13}C NMR ($CDCl_3$, ppm) 197.8, 130.8, 130.3, 128.5, 126.6, 120.9, 119.9, 119.7, 119.4, 37.6, 35.2 (2C), 27.3, 23.6, 23.5, 22.2, 22.1, 21.113.1, 11.5, 11.4 (2C). Anal. calcd. for $C_{22}H_{36}O$: C, 83.48; H, 11.46. Found: C, 83.53; H, 11.36.

3-Carboethoxy-1-methyl-2-[3,8,12,16-tetramethyl-3(*E*),7(*E*),11(*E*),15-heptadecatetraenyl]-4-piperidone (145). To a suspension of freshly activated powdered 3A molecular sieves (2.5 g) in dry toluene (15 mL), in a sealed tube, was added aldehyde **144** (0.550 g, 1.6 mmol) and the mixture was cooled to -78 °C. Into the tube was then condensed a pre-weighed amount of $MeNH_2$ (0.51 g, 16 mmol). The system was sealed and stirred at -20 °C for 6 hr. Excess $MeNH_2$ was evaporated and the solution was filtered and transfered *via* canuula into a round bottom flask. The molecular sieves were rinsed well with dry Et_2O (5 X 10 ml). The organic washes were combined and concentrated *in vacuo*, using a vacuum pump, (0.5 mmHg) to give imine **128** as an oil (almost quantative conversion as judged by GC analysis).

Imine **128** was dissolved in anhydrous EtOH (10 mL) and cooled to 0 °C. To this was added dropwise, ethyl-3-oxo-4-pentenoate (**131**) (0.50 g, 3.52 mmol) in EtOH (5 mL). After stirring for 7 h, the solvent was removed *in vacuo* and the oil chromatographed using ethyl acetate/hexanes (25/75) as the eluant to give **145** (0.57g, 76 %) as a mixture of enol-keto tautomers. IR (film) 3406

(b), 2929 (m), 1744, 1720, 1650, 1600, 1446, 1379, 1299, 1223, 1195, 1061, and 830 cm^{-1}; mass spectrum EI *m/z* (rel intensity) 471 (M+, trace amount), 426 (trace amount), 344 (2), 220 (7), 197 (13), 185 (13), 184 (100), 151 (7), 149 (11), 139 (9), 138 977), 136 (8), 125 (8), 112 (10), 95 (11), 93 (11), 85 (10), 84 (11), 81 (19), 71 (11), 69 (44), 67 (12), 57 (15), 55 (20); ^1H NMR ($CDCl_3$, ppm) 12.20 (enolic H, s), 5.20-5.05 (4H, m), 4.30-4.10 (2H, m), 3.35-3.03 (~2.5H, contains ketonic H, m), 2.75 (1H, m), 2.50 (1H, m), 2.35 (3H, s), 2.20-1.90 (15H, m), 1.68 (3H, s), 1.58 (14H, app s), 1.30 (3H, m). Anal. calcd. for $C_{30}H_{49}NO_3$: C, 76.39; H, 10.47; N, 2.97. Found: C, 76.11; H, 10.61; N, 3.03.

3-Carboethoxy-1,2-diphenyl-4-piperidone (**147**). To a solution of *N*-benzylideneaniline (**146**) (181.0 mg, 1.0 mmol) in EtOH or THF (5 mL) was added dropwise ethyl-3-oxo-4-pentenoate (**131**) (284 mg, 2.0 mmol) in EtOH or THF (5 mL). After stirring for 24 h, the solvent was removed *in vacuo* and the residual oil chromatorgaphed using ethyl acetate/ hexanes (5/95) as the eluant to give **147** as a solid essentially in enol form (^1H NMR, $CDCl_3$). EtOH as the solvent gave **147**, 70 mg (21%) while use of THF gave **147**, 60 mg (19%). Mass spectrum, EI *m/z* (rel intensity) 323 (M+, 5), 246 (5), 218 (7), 200 (18), 181 (87), 180 (100), 152 (5), 131 (7), 106 912), 105 (8), 104 (21), 103 (6), 89 (8), 84 (10), 78 (18), 78 (92), 51 (26); ^1H NMR ($CDCl_3$, ppm) 12.50 (enolic H, s), 7.20 (7H, m), 7.00 (2H, d, *J*=6.3 Hz), 6 35 (1H, t, *J*=6.3 Hz), 4.15 (2H, t, J=7.0 Hz), 3.50 (1H, app dd, J=14.5, 6.5 Hz), 3.25 (1H, ddd, *J*=14.5, 11.5, 4.5 Hz), 2.65 (1H, ddd, *J*=18.0, 11.5, 6.5 Hz), 2.25 (1H, ddd, *J*=18.0, 4.5, 2.5 Hz), 1.10 (3H, t, *J*=7.0 Hz); ^{13}C NMR ($CDCl_3$, ppm) 172.1, 171.0, 149.4, 141.6, 129.2-128.7 (5C), 127.9, 127.8, 127.0, 119.4, 116.6, 99.4, 60.4, 57.4, 39.8, 27.0, 14.0. Anal. calcd. for $C_{20}H_{21}NO_3$: C, 74.28; H, 6.55; N, 4.33. Found: C, 74.60; H, 6.24; N, 4.33.

Methyl 3-hydroxy-2-methyl-4-pentenoate (149). To a solution of LDA (0.11 mmol) in THF (300 mL) at -78 °C under an argon atmosphere was added dropwise, *via* a syringe, neat methyl propionate (**148**) (9.63 mL, 100 mmol) over 15 min. After stirring for 0.5 h, neat acrolein (6.60 ml, 100 mmol) was added dropwise over 5 min and stirring continued for an additional 5 min. Saturated NH_4Cl (50 mL) was then added to produce a slurry that was poured into a separatory funnel containing Et_2O (200 mL), this shaken vigorously and the layers separated. The aqueous layer was extracted again with Et_2O (100 mL) and the organic extracts were combined. Standard work-up followed by bulb to bulb distillation (58-62 °C @ 0.5 mmHg) gave **149** (11.43 g, 80 %) as 1/1 mixture of diastereoisomers, as an oil. IR (film) 3464(b), 2984, 2952, 2883, 1738, 1460, 1436, 1354, 1259, 1201, 1175, 1048, 929; mass spectrum EI *m/z* (rel intensity) 144 (M+, trace amount), 113 (5), 88 (100), 57 (76), 56 (25), 55 (20); ^1H NMR ($CDCl_3$, ppm) 5.88-5.75 (1H, m), 5.35-5.25 (1H, dm, *J*=17 Hz), 5.22-5.15 (1H, dm, *J*=11 Hz), 4.40/4.17 (1H, diastereotopic, d 4.40, bm; d 4.17, t, *J*=7.0 Hz), 3.70 (3H, s), 2.70-2.55 (2H, bm), 1.16 (3H, d, *J*=7.0 Hz); ^{13}C NMR ($CDCl_3$, ppm, diastereoisomers) 175.7/175.4, 137.9/137.5, 116.7/115.9, 74.6/73.0, 51.6, 45.2/44.7, 13.6/11.2.

Methyl-2-methyl-3-oxo-4-pentenoate (46). To a mechanically stirred solution of hydroxy ester **149** (7.2 g, 50 mmol) in acetone (200 mL) at 0 °C was added dropwise Jones' reagent [55 mmol, prepared from CrO_3 (5.5 g, 55 mmol) and conc. H_2SO_4 (5.5 mL) and added to water to make up a 55 mL solution]. After stirring for 2 h, MeOH (5 mL) was added and the stirring maintained for an additional 20 min. The contents were poured into ice-cold water (250 mL) and Et_2O (250 mL). The organic layer was partitioned and the

aqueous layer extracted with Et_2O (3 X 50 mL). The combined extracts were washed with ice-water (3 X 100 mL) and saturated NaCl solution (100 mL), treated with anhydrous $MgSO_4$ and the solvent removed by simple distillation. Bulb to bulb distillation (receiving bulb cooled to -78 °C with dry ice, bp ~43-46 °C @ 0.5 mmHg) gave keto-ester **46** (4.0 g, 56 %), mixture of enol-keto tautomers, as an oil. IR (film) 3540 (b), 2950 (m), 1745, 1702, 1655, 1614, 1578, 1440, 1402, 1353, 1246, 1074, 1040, 982, 861, and 822 cm^{-1}; mass spectrum EI *m/z* (rel intensity) 142 (M+, trace amount), 114 (15), 55 (100); ^1H NMR ($CDCl_3$, ppm, enol/keto tautomers) 12.40 (enol H, s), 6.65-5.55 (2H, enol/keto tautomers), 3.80 (ketonic H, q, *J*=7.0 Hz), 3.78/3.70 (3H, s), 1.83/1.35 [(CH_3); d1.53 (s), d1.35 (d, *J*=7.0 Hz)]. ^{13}C NMR ($CDCl_3$, ppm, keto tautomer) 195.0, 174.0 134.2, 129.5, 52.2, 50.0, 12.7.

3-Carbomethoxy-1,3-dimethyl-2-[3,8,12,16-tetramethyl-3(*E*),7(*E*),11(*E*),15-heptadecatetraenyl]-4-piperidone (129). Imine **128** was prepared as described for the synthesis of **145**. Reaction of aldehyde **144** (1.71 g, 5.4 mmol) in toluene (20 mL), containing powdered 3A molecular sieves (5.0 g) and $MeNH_2$ (1.10 g, 32 mmol) in a sealed tube at -20 °C gave imine **128** in quantative yield.

Imine, **128**, was dissolved in anhydrous EtOH (25 mL), cooled to 0 °C and unsaturated β-keto ester **46** (1.9 g, 13.4 mmol) dissolved in EtOH (5 mL) was added dropwise. After stirring at room temperature for 12 hr, the solvent was evaporated *in vacuo* and the oil chromatographed using ethyl acetate/hexanes (20/80) as the eluant to give cyclized product **129** (0.67 g, 26 % in two steps) and substantial amount of polymeric material. The cyclized product was deduced by ^1H NMR to be a single diastereoisomer. IR (film) 2926, 2853, 1715 (b), 1448, 1377, 1255, 1207, 1116, and 1077 cm^{-1}; mass spectrum

EI *m/z* (rel intensity) 471 (M+, 3), 402 (6), 185 (8), 184 (59), 136 (12), 95 (20), 98 (23), 199 (40), 149 (12), 142 (31), 124 (14), 121 (10), 111 (12), 109 (10), 85 (10), 82 (13), 81(50), 70 (23), 69 (100), 68 (14), 67 (20), 57 (21), 55 (32); ^{1}H NMR (C_6D_6, ppm) 5.30 (4H, bm), 3.42 (1H, t, *J*=5.0 Hz), 3.40 (3H, s), 2.90 (1H, overlapping ddd, *J*=14.5, 10.0, 7.5 Hz), 2.53 (1H, app td, *J*=12, 5 Hz), 2.35 (1H, tm, *J*=10 Hz), 2.20-2.00 (18H, m), 1.72 (3H, s), 1.68 (6H, app s), 1.60 (3H, s), 1.58 (3H, s), 1.50 (1H, bm), 1.42 (3H, s), 1.25 (1H, bm); ^{13}C NMR ($CDCl_3$, ppm) 207.0, 173.6, 135.2, 134.8, 134.2, 131.0, 125.2, 124.3, 124.1, 124.0, 67.2, 62.6, 52.2, 48.8, 42.5, 39.6 (2C), 38.6, 36.6, 28.2, 28.0, 26.7, 26.6, 25.5, 22.6, 17.5, 17.1, 15.6 (3H). Anal. calcd. for $C_{30}H_{49}NO_3$: C, 76.39; H, 10.47; N, 2.97. Found C, 76.22; H, 10.23; N, 2.69. HRMS calcd for $C_{30}H_{49}NO_3$: 471.3712. Found; 471.3705.

3-Carbomethoxy-1,3-dimethyl-2-[3,8,12,16-tetramethyl-3(*E*),7(*E*),11(*E*),15-heptadecatetraenyl]-4-hydroxypiperidine (150). To a solution of ketone **129** (315 mg, 0.67 mmol) in EtOH (5 mL) at 0 °C was added $NaBH_4$ (60 mg, 1.5 mmol). After stirring for 1 h, 15 % aqueous NaOH (10 mL) was added and the resulting slurry extracted with Et_2O (5 X 25 mL). Standard work-up gave the reduced product **150** (300 mg, 95 %) as a single diastereoisomer. Chromatography was avoided at this step, since it substantially decreased the yield. IR (film) 3406, 2926, 2854, 1725, 1446, 1377, 1257, 1220, 1137, 1102, and 1035 cm^{-1}; mass spectrum EI *m/z* (rel intensity) 473 (M+, 4), 404 (4), 336 (11), 288 (4), 268 (20), 186 (100), 85 (15), 69 (30); ^{1}H NMR ($CDCl_3/D_2O$, ppm) 5.10 (4H, m), 3.90 (1H, dd, *J*=11.0, 6.0 Hz), 3.70 (3H, s), 2.80 (1H, dt, *J*=12.0, 4.0 Hz), 2.25 (3H, s), 2.15 (1H, m), 2.05 (6H, bm), 2.00 (8H, bm), 1.88 (1H, td, *J*=12.0, 6.0 Hz), 1.70 (2H, m), 1.68 (3H, s), 1.59 (6H, app s), 1.56 (6H, app s), 1.45 (1H, m), 1.35 (1H, m), 1.20 (3H, s); ^{13}C NMR ($CDCl_3$,

ppm) 176.4, 135.1, 134.8 (2C), 131.1, 124.6, 124.4, 124.2, 124.1, 73.9, 67.7, 55.1, 54.3, 51.9, 42.8, 39.7 (2C), 30.4, 29.4, 28.2, 28.1, 26.7, 26.6, 25.6, 17.6, 16.0, 15.9, 15.8, 9.1, missing one carbon. Anal. calcd. for $C_{30}H_{51}NO_3$: C, 76.06; H, 10.85; N, 2.96. Found: C, 75.70; H, 10.61; N, 3.04. HRMS cacld. 473.3868: found 473.3869.

3-Carbomethoxy-1,3-dimethyl-4-[(tetrahydropyranyl)-2-oxy]-2-[3,8,12,16-tetramethyl-3(*E*),7(*E*),11(*E*),15-heptadecatetraenyl]-piperidine (151). A solution of alcohol **150** (300 mg, 0.67 mmol), 5,6-dihydropyran (DHP) (100 mg, 1.2 mmol) and *p*-TsOH (150 mg, 0.74 mmol) in CH_2Cl_2 (5 mL) was stirred for 6 h, treated with 15 % aqueous NaOH (10 ml), and extracted with CH_2Cl_2 (5 X 20 mL). Standard work-up followed by chromatography using ethyl acetate/ hexanes (15/85) as the eluant gave **151** (310 mg, 81 %) as mixture of separable diastereoisomers (not separated but carried as a mixture through the synthesis). IR (film) 2946, 2851, 2782, 1739, 1442, 1379, 1281, 1260, 1220, 1136, 1078, 1029, and 978 cm^{-1}; mass spectrum EI *m/z* (rel intensity) 557 (M+, trace amount), 456 (80), 352 (5), 270 (100), 186 (270), 170 (73), 85 (67), 69 (50); ^1H NMR ($CDCl_3$, ppm, major diastereoisomer) 5.50 (4H, m), 4.75 (1H, t, *J*=2.6 Hz), 3.98 (1H, dd, *J*=11.5, 4.6 Hz), 3.70 (3H, s), 3.62 (1H, m), 3.42 (1H, m), 2.90 (1H, m), 2.28 (3H, s), 2.20 (1H, bm), 2.10 (7H, bm), 2.00 (8H, bm), 1.90-1.65 (3H, m), 1.68 (2H, s), 1.68-1.3 (20H, overlapping multiplets), 1.25 (3H, s). Anal. calcd. for $C_{35}H_{59}NO_4$: C, 75.36; H, 10.66; N, 2.51. Found: C, 75.27; H, 10.61; N, 2.74. HRMS calcd 557.4447; found 557.4444.

1,3-Dimethyl-3-hydroxymethyl-4-[(tetrahydropyranyl)-2-oxy]-2-[3,8,12,16-tetramethyl-3(*E*),7(*E*),11(*E*),15-heptadecatetraenyl]

piperidine (152). To a solution of the diastereoisomers of ester **151** (230 mg, 0.41 mmol) in THF (5 mL) under argon was added LAH (100 mg, 2.6 mmol) and the mixture refluxed for 1 hr. The mixture was cooled to 0 °C, diluted with Et_2O (20 mL) and treated successively with H_2O (0.10 mL) followed by aqueous 15 % NaOH solution (0.10 mL) followed by H_2O (0.30 mL). The solids were filtered, rinsed well with Et_2O (5 X 10 mL), dried over anhydrous K_2CO_3 and the solvent removed *in vacuo* to give alcohol **152** (230 mg, crude) as a mixture of diastereoisomers. Chromatography was avoided at this step since it is accompanied by a decrease in yield. IR (film) 3456 (bs), 2937 (m), 1443, 1379, 1275, 1167, 1133, 1075, 1026 and 980 cm^{-1}; mass spectrum EI *m/z* (rel intensity) 530 (M^+, 2), 428 (17), 242 (72), 158 (3), 142 (12), 124 (3), 111 (5), 110 (9), 101 (12), 97 (7), 95 (6), 87 (17), 85 (100), 84(12), 81 (10), 70 (17), 69 (47), 67 914), 57 (15), 55 (12); ^1H NMR ($CDCl_3$, ppm, characteristic peaks) 5.15 (4H, m), 4.70-4.45 (anomeric H, m), 4.00-3.30 (5H, m), 2.60/2.40 (3H, s), 2.20-1.30 (~41H, m), 0.88/0.78 (3H, s). HRMS calcd for $C_{34}H_{59}NO_3$, 529.4494; found 529.4506.

4-[(Tetrahydropyranyl)-2-oxy]-2-[3,8,12,16-tetramethyl-3(*E*),7(*E*),11(*E*),15-heptadecatetraenyl]-1,3,3-trimethylpiperidine (153). A solution of **152** (130 mg, 0.25 mmol) in THF (5 mL) at 0 °C, under an argon atmosphere, was treated with MeLi (0.35 mL, 0.5 mmol, 1.4 M in Et_2O), followed by dropwise addition of freshly distilled *p*-TsCl (150 mg, 0.79 mmol) in THF (3 mL) and the mixture stirred for 4 h. To the cloudy mixture was then added $LiBEt_3H$ (2.5 mL, 2.5 mmol, 1 M in THF) and this refluxed for 1 hr, cooled to 0 °C and diluted with 15 % aqueous NaOH (20 mL). Standard workup using Et_2O (5 X 25 mL) followed by chromatography using Et_3N/ethyl acetate/hexanes (1/19/80) as the eluant gave **153** (95 mg, 73 %) as a mixture

of diastereoisomers. IR (film) 2938 (m), 1442, 1382, 1360, 1188, 1118, 1077, 1027, 980, and 817 cm^{-1}; mass spectrum EI *m/z* (rel intensity) 514 (M+, 3), 413 (19), 412 (53), 227 (14), 226 (100), 142 (5), 138 (9), 126 (11), 124 (5), 98 (6), 85 (22), 83 (7), 70 (20), 69 (25), 67 (7), 55 (77); ^1H NMR ($CDCl_3$, ppm, mixture of diastereomers) 5.12 (4H, bm), 4.75/4.58 (anomeric H, app t, *J*=2.5/4.0 Hz), 3.90 (1H, m), 3.45 (1H, m), 3.20/3.00 (1H, dd, *J*=11.5, 4.5 Hz; app t, *J*=12.0 Hz), 2.85 (1H, m), 2.25 (3H, bs), 2.20-1.30 (~41H, overlapping m), 1.02/0.92 & 0.90/0.87 (6H, s). Anal. calcd. for $C_{34}H_{59}NO_2$: C, 79.48; H, 11.57; N, 2.73. Found: C, 79.18; H, 11.55; N, 2.90. HRMS calcd. 513.4545; found 513.4551.

2-[3,8,12,16-Tetramethyl-3(*E*),7(*E*),11(*E*),15-heptadecatetraenyl]-1,3,3-trimethyl-4-hydroxypiperidine (**41**). A mixture of **153** (95 mg, 0.184 mmol) and *p*-TsOH (50 mg) in MeOH (5 mL) was stirred for 5h and most of the solvent removed *in vacuo*. The slurry was diluted with saturated $NaHCO_3$ (10 mL) and extracted with Et_2O (5 X 10 mL), dried over anhydrous K_2CO_3 and the solvent evaporated *in vacuo*. Chromatography on a small column using MeOH/ethyl acetate/hexanes (5/35/60) as the eluant gave racemic amino-alcohol **41** (76 mg, 96 %). IR (film) 3388, 2930 (m), 1445, 1374, 1275, 1171, 1082, and 990 cm-1; mass spectrum EI *m/z* (rel intensity) 429 (M+, trace amount), 412 (M-H_2O, trace amount), 155 (5), 142 (100), 98 (10), 81 (5), 69 (19); ^1H NMR ($CDCl_3/D_2O$, ppm) 5.15 (4H, m), 3.16 (1H, dd, *J*=11.5, 5.0 Hz), 2.82 (1H, dt, *J*=12.0, 3.5 Hz), 2.22 (3H, s), 2.15-1.90 (16H, bm), 1.75 (1H, app dq, *J*=12.0, 5.0 Hz), 1.68 (3H, s), 1.64 (1H, m), 1.60 (9H, m), 1.57 (3H, s), 1.35 (2H, m), 0.95 (3H, s), 0.87 (3H, s); ^{13}C NMR ($CDCl_3$, ppm) 135.2, 135.1, 134.9, 131.2, 124.6, 124.4, 124.3, 124.2, 76.8, 72.3, 55.5, 43.6, 40.7, 40.0, 39.7 (2C), 30.4, 28.4, 28.2 (2C), 26.8, 26.7, 25.6, 24.0, 17.6, 16.1, 16.0, 15.9, 13.6. Anal.

calcd. for $C_{29}H_{51}NO$: C, 81.06; H, 11.96; N, 3.26. Found: C, 80.98; H, 12.03; N, 3.36. HRMS calcd. 429.3970; found 429.3963.

2-[3,8,12,16-Tetramethyl-3(*E*),7(*E*),11(*E*),15-heptadecatetraenyl]-1,3,3-trimethyl-4-hydroxypiperidinium Iodide (42). To a solution of amino alcohol **41** (20 mg, 0.047 mmol) in dry Et_2O (1 mL) in a tappered screw capped centrufuge tube was added MeI (0.10 mL) and the mixture was left undisturbed in a dark place. After 24 hr, the solvent and excess MeI was evaporated under a gentle stream of argon to give a white paste. Dry pentane (1 mL) added, the mixture was vortexed, then centrifuged and the pentane decanted. This cycle was repeated 3 times, the residual solvent evaporated under high vacuum to give salt **42** (26 mg, 93 %) as a hygroscopic solid. IR (KBr) 3385 (b), 2925, 1450, 1380, 1072 (m) cm^{-1}; mass spectrum FAB *m/z* (Xenon/noba, rel intensity) 444 (M^+-I^-, 100); ^1H NMR ($CDCl_3$, ppm) 5.20 (1H, bm), 5.10 (3H, m), 4.15 (1H,app td, *J*=13.0, 4.0 Hz), 4.00 (2H, bs), 3.72 (1H, bm), 3.38 (3H, s), 3.12 (3H, s), 2.40-2.15 (4H, bm), 2.10-1.91 (12H, m), 1.90-1.80 (2H, m), 1.67 (3H, s), 1.58 (12H, bs), 1.18 (3H, s), 1.15 (3H, s); ^{13}C NMR ($CDCl_3$, ppm) 135.4, 134.9, 133.2, 131.2, 126.3, 124.4, 124.2, 123.9, 78.5, 71.8, 63.5, 55.4, 45.6, 41.5, 41.0, 39.7 (2C), 28.3, 28.0, 26.8, 26.7 (2C), 26.3, 25.7, 25.4, 17.7, 16.2, 16.1, 16.0, 15.8.

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3,7-Dimethyl-6,7-oxido-2(*E*)-hexen-1-ol (6,7-epoxygeraniol) (178). To a stirred solution of 6,7-epoxy geraniol acetate (**136**) (10.6 g, 50 mmol) in MeOH (200 mL) was added K_2CO_3 (2.0 g, excess). After 5 h, most of the MeOH was removed *in vacuo* and the slurry diluted with H_2O (50 mL) and extracted with Et_2O (4 X 30 mL). Standard work-up followed by chromatography using ethyl acetate/hexanes (3/7) as the eluant gave **178** (7.6 g, 90%) as a clear oil. IR (film) 3406 (b), 2961, 2925, 1668, 1451, 1379, 1323, 1250, 1117, 1003 and 871 cm^{-1} ; mass spectrum, CI *m/z* (isobutane, rel intensity) 169 (M^++, 1), ,153 (100), 135 (20), 123 (4), 109 (trace); ^1H NMR ($CDCl_3$, ppm) 5.43 (1H, tq, *J*=7.0, 1.2 Hz), 4.13 (2H, d, *J*=7.0 Hz), 2.70 (1H, t, *J*=6.0 Hz), 2.15 (2H, overlapping ddt), 1.68 (3H, s), 1.64-1.60 (2H, m), 1.28 (3H, s), 1.24 (3H, s); ^{13}C NMR ($CDCl_3$, ppm) 137.9, 124.2, 64.0, 59.0, 58.3, 36.2, 27.0, 24.7, 18.6, 16.11.

1-Chloro-3-Methyl-7,6-Oxido-2(*E*)-hexene (6,7-epoxygeraniol chloride) (176) To a stirred solution of NCS (6.0 g, 45 mmol) in CH_2Cl_2 (100 mL) at 0 °C was added DMS (5.51 mL, 75 mmol). The resulting slurry was cooled to -20 °C and to this was added dropwise **178** (5.0g, 30 mmol) in CH_2Cl_2 (25 mL). After 5h at 0 °C, the clear reaction mixture was diluted with ice cold H_2O (100 mL), the organic layer separated and the aqueous layer extracted with CH_2Cl_2 (2 X 50 mL). Standard work-up followed by chromatography using ethyl acetate/hexanes (1/9) as the eluant gave **176** (4.5 g, 80 %) as an oil. IR (film) 2981, 2926, 1662, 1451, 1378, 1253, 1121 and 875 cm^{-1}; mass spectrum CI *m/z* (isobutane, rel intensity) 189 (M^++1, 51), 171 (40), 153 (100), 135 (25), 125 (5); ^1H NMR ($CDCl_3$, ppm) 5.54 (1H, tq, *J*=8.0, 1.20

Hz), 4.09 (2H, d, J=8.0 Hz), 2.69 (1H, t, J=6.5 Hz), 2.20 (2H, overlapping ddt), 1.74 (3H, s), 1.65 (2H, t, J=7.0 Hz), 1.30 (3H, s), 1.25 (3H, s); ^{13}C NMR ($CDCl_3$, ppm) 141.6, 120.9, 63.7, 58.2, 40.7, 36.1, 27.0, 24.7, 18.7, 16.0. Anal. calcd. for $C_{10}H_{17}OCl$: C, 63.65; H, 9.08. Found: C, 63.83; H, 9.18.

Diethylphosphonate ethane-1-thiol (177): Ethyl bromide, **182**, (7.84 mL, 105 mmol) was heated with neat triethylphosphite (22.8 mL, 110 mmol) at 150 °C in a sealed tube for 10 h. Distillation of the resulting crude oil at 40 °C @ 0.50 mmHg gave diethyl ethylphosphosphonate, **179**, (17.0 g, 97 %).[70c]

To a solution of **179** (5.0 g, 30 mmol) in THF (50 mL) at -78 °C, under argon was added *n*-BuLi (12.7 mL, 32 mmol, 2.5 M in hexanes). After 40 min, elemental sulfur (S_8) (1.03 g, 32 mmol), dried in a desiccator over P_2O_5, was added in one portion and the mixture warmed to -20 °C over 4 h. The reaction was terminated by the addition of aqueous 5% NaOH (20 mL). Most of the THF was removed *in vacuo* and the aqueous solution extracted with CH_2Cl_2 (3X30 mL) and organic extracts discarded. The aqueous layer was then acidified to pH ~5 with concentrated HCl and the turbid mixture re-extracted with CH_2Cl_2 (4X30 mL). The combined extracts were washed with ice water (50 mL), saturated NaCl (50 mL) and dried ($MgSO_4$) and the solvent removed *in vacuo* to give **177** (4.2 g, 66 %) as an oil. ^1H NMR ($CDCl_3$, ppm) 4.15 (4H, overlapping dq, J_{H-H}=7.0 Hz, J_{P-H}=7.1), 3.10 (1H, m), 2.02 (1H, d, J=8.3 Hz), 1.49 (3H, dd, J_{P-H}=17.7 Hz, J_{H-H}=7.7 Hz), 1.35 (6H, t, J=7.0 Hz); ^1H NMR spectrum is identical to that reported by Mikolajczyk, *et al.*[70c]

Diethyl ethyl-1-[3,7-dimethyl-6,7-oxido-2(*E*)-octenylthio)phosphonate (175). This was prepared by reaction of

(diethylphosphoryl)ethyl-1-thiol (**177**)[70c] with 6,7-epoxy geranyl chloride (**176**) under phase transfer conditions.[72] To a solution of (diethylphosphoryl) ethyl-1-thiol (**177**) (1.0 g, 5.05 mmol) in toluene (25 mL) and 35% aqueous NaOH (20 mL) was added 6,7-epoxy geranyl chloride (**176**) (1.0 g, 5.3 mmol) and phase transfer catalyst, tetraoctylammonium bromide, (100 mg). The mixture was stirred vigorously for 10 h and extracted with Et_2O (4X30 mL). The combined extracts were washed with ice water (2X25 mL) and saturated solution of NaCl (1 X 25) and dried ($MgSO_4$). Chromatography using ethyl acetate/hexanes (1/1) as the eluant gave **175** (1.2 g, 70%) as an oil. IR (film) 3406, 2933, 2234, 1712, 1448, 1389, 1233, 1164, 1055, 1024, 962, 797 and 732 cm^{-1}; mass spectrum, CI *m/z* (isobutane, rel intensity) 351 (M^++1, 82), 199 (60), 166 (25), 153 (100), 135 (30); ^1H NMR ($CDCl_3$, ppm) 5.30 (1H, tm,*J*= 7.0 Hz), 4.20 (4H, m), 3.50/3.30 (2H, m, diastereoisomeric H's), 2.78 (1H, m), 2.68 (1H, td, *J*=6.0, 1.0 Hz), 2.18 (2H, m), 1.70 (3H, s), 1.63 (2H, t, *J*=7.0 Hz), 1.45 (3H, ddd, J_{P-H}=17.0 Hz; J_{H-H}=7.5, 2.2 Hz), 1.33 (6H, td, J_{H-H}=7.5 Hz; J_{P-H}=3.0 Hz); ^{13}C NMR ($CDCl_3$, ppm) 139.2, 120.1, 63.7, 62.8/ 62.3(J_{P-H}), 58.0, 36.2, 33.9/32.4 (J_{P-H}), 29.7, 27.1, 24.8, 18.6, 16.3, 16.1, 16.0, 15.9. Anal. calcd. for $C_{16}H_{31}O_4SP$: C, 54.84; H, 8.92. Found: C, 54.66; H, 8.80.

5,9,13-Trimethyl-4(*E*),8(*E*),12-tetradecatrien-1-al (172). This was prepared as according to the procedure of Coates, *et al.*[73] To a slurry of CuI (7.61 g, 40 mmol) and ethyl acetate (1.95 mL, 20 mmol) in THF (100 mL) at -100 °C under argon was added dropwise, LDA [20 mmol, prepared from diisopropyl amine (2.8 mL, 20 mmol) and *n*-BuLi (8.0 mL, 20 mmol, 2.5 M in hexane) at -78 °C] in THF (25 mL). After 45 min, a solution of farnesyl bromide (**118**) (2.71 mL, 10 mmol) in THF (15 mL) was added dropwise. The mixture was further stirred for 1 h at -100 °C and allowed to warm to 0 °C over 3 h.

Saturated NH_4Cl/NH_4OH (9/1) (100 mL) was added, the mixture stirred for 30 min open to air and extracted with Et_2O (4 X 50 mL). Standard work-up gave coupled ester **180** in nearly quantitative yield (3.0 g), which was a single spot by thin layer chromatographic analysis. IR (film) 2977, 2924, 1738, 1447, 1375, 1347, 1252, 1179, 1098 and 1042 cm^{-1}; ^1H NMR ($CDCl_3$, ppm) 5.10 (3H, m), 4.13 (2H, q, J=8.0 Hz), 2.23 (2H, t, J=3.0 Hz), 2.00 (10 H, bm), 1.69 (3H, s), 1.67 (3H, s), 1.60 (6H, app s), 1.26 (3H, t, J=8.0 Hz). ^1H NMR spectrum is in agreement with that given in reference 73.

A solution of **180** (3.0 g, 10 mmol) in THF (25 mL) was added to a stirring slurry of $LiAlH_4$ (1.0 g, 26 mmol) in THF (35 mL) under argon. After 2 h, H_2O (1.0 g) was added followed by 15 % NaOH solution (1.0 g) and then by H_2O (3.0 g). The resulting slurry was stirred for 0.5 h, diluted with hexanes (50 mL) and filtered through a pad of Celite/$MgSO_4$ (3/1). The salts were rinsed with portions of hexanes (3 X 25 mL). The filtrate was reduced *in vacuo* to give the reduced alcohol **181** as a slightly cloudy oil. This was filtered through a small column of silica using Et_2O as the eluant to give a clear oil (2.3 g), single spot on TLC using ethyl acetate/hexanes (2/8). IR (film) 3346, 2927 (m), 1668, 1448, 1381, 1058 and 833 cm^{-1}; ^1H NMR ($CDCl_3$, ppm) 5.29-4.59 (3H, m), 3.66 (2H, t, 6.0 Hz), 2.28-1.90 (12H, m), 1.69 (3H, s), 1.62 (3H, s), 1.60 (6H, app s).

Alcohol **181** (1.5 g, 6.0 mmol) was dissolved in dry DMSO (35 mL) containing Et_3N (8.3 mL, 60 mmol). To this stirred solution was added dropwise, a solution of Py·SO_3 (2.9 g, 18 mmol) in DMSO (20 mL). This was stirred for 10 h and poured into ice-cold saturated NaCl (100 mL) and extracted with Et_2O (4 X 30 mL). Standard work-up followed by chromatography using ethyl acetate/hexanes (1/9) as the eluant gave aldehyde **172** (1.3 g, 87 %). IR (film) 2921, 2717, 1727, 1448, 1383 and 1108; ^1H NMR ($CDCl_3$, ppm) 9.78 (1H, t, J=1.6 Hz), 5.06 (3H, m), 2.46 (2H, m), 2.34 (2H, m), 2.00 (8H, m), 1.69 (3H, s),

1.67 (3H, s), 1.62 (3H, s), 1.60 (3H, s); ^{13}C NMR ($CDCl_3$, ppm) 202.5, 136.8, 135.1, 131.2, 124.3, 123.9, 122.0, 43.9, 39.7, 39.6, 26.7, 26.4, 25.6, 20.8, 17.6, 16.0, 15.9. ^1H NMR spectrum is in agreement with that reported in reference 73.

Synthesis of 174/43 as 10(*Z*/*E*) Mixture. To a solution of LDA [1.1 mmol, prepared from diisopropyl amine (0.17 mL, 1.2 mmol) and n-BuLi (0.45 mL, 1.1 mmol, 2.5 M in hexanes) at -78 °C] in THF (5 mL) at -78 °C, under argon was added dropwise a solution of phosphonate **175** (0.350 g, 1.0 mmol) in THF (3.0 mL). The solution was stirred for 0.5 h and to this was added dropwise a solution of aldehyde **172** (0.250 g, 1.0 mmol) in THF (3.0 mL). The mixture was warmed to room temperature over 2 h and stirred at room temperature for 3 h. Ice water (25 mL) was added and this was extracted with Et_2O (3 X 25 mL). Standard work-up followed by chromatography using ethyl acetate/hexanes (5/95) as the eluant gave inseparable 10*Z*/*E* (35/65) mixture of **174/43** (0.378 g, 85%). IR (film) 2920 (m), 1665, 1629, 1444, 1377, 1248, 1118, and 838 cm^{-1}; mass spectrum CI *m/z* (rel intensity) 445 (M^++1, 7), 135 (100), 135 (7); shows ^1H NMR ($CDCl_3$) shows the following chemical shifts and integrations of 11-*H* vinyl hydrogen: 10(*Z*) (**174**) , δ 5.52 (tq, *J*=6.70, 1.31 Hz); 10(*E*) (**43**), δ 5.38 (tq, *J*=6.70 , 1.19 Hz). HRMS calcd. for $C_{29}H_{48}OS$: 444.3426. Found: 444.3421.

Ethyl diphenylphosphine oxide (**184**): To an aqueous solution of 35% NaOH (50 mL) was added triphenyl ethylphosphonium bromide (10.0 g, 27 mmol) [prepared from Ph_3P, **183**, and bromoethane, **182**, in toluene] and the mixture heated at ~90 °C for 5 h. The slurry was cooled to room temperature and extracted with CH_2Cl_2 (4 X 50 mL). Standard workup followed by recrystallization from CH_2Cl_2/hexanes gave **184** as white crystals, mp 122

°C. IR (KBr) 2995 (m), 1440, 1179, 1123, 1024, 742, 721, 698, 672, 545 and 502 cm-1; mass spectrum, CI *m/z* (isobutane, rel intensity) 231 (M^++1); ^{1}H NMR ($CDCl_3$, ppm) 7.75 (6H, m), 7.50 (6H, m), 2.30 (2H, overlapping dq, J_{H-H}=7.5 Hz; J_{P-H}=11.5 Hz), 1.20 (3H, overlapping dt, J_{H-H}=7.5 Hz; J_{P-H}=17.5 Hz). Anal. calcd. for $C_{14}H_{15}OP$: C, 73.02; H, 6.57. Found: C, 73.24; H, 6.62.

Diphenylphosphinoyl ethane-1-thiol (185): To a suspension of ethyl diphenylphosphine oxide, **184**, (5.75 g, 25 mmol), in THF (150 mL) at -78 °C under argon was added dropwise *n*-BuLi (11.0 mL, 27.5 mmol, 2.5 M in hexanes). After 30 min, to the resulting clear red solution was added in one portion, S_8 powder (0.89 g, 27.5 mmol). The yellow solution was warmed to -40 °C and stirred at this temperature for 3 h. Then 15% NaOH solution (5.0 mL) was added and most of the THF removed *in vacuo*. The slurry (~25 mL) was diluted with ice water (50 mL) and extracted with CH_2Cl_2 (3 X 30 mL) and the organic extracts discarded. The aqueous layer was acidified to pH ~5 with concentrated HCl and the turbid mixture was extracted with CH_2Cl_2 (4 X 30 mL). The combined extracts were washed with ice water (2 X 25 mL), ice cold NaCl (25 mL) and dried ($MgSO_4$) to give **185** (5.23 g, 80 %) as a light yellow solid, mp 138-141 °C. IR (KBr) 3053, 2921, 1618, 1438, 1182, 1119, 1071, 1027, 997, 740, 722, 697, 630, and 531 cm^{-1}; mass spectrum CI *m/z* (isobutane, rel intensity) 263 (M^++1, 100); ^{1}H NMR ($CDCl_3$, ppm) 7.90 (4H, m), 7.50 (6H, m), 3.45 (1H, m), 2.07 (1H, dd, J_{H-H}=7.0 Hz; J_{P-H}=10.0 Hz), 1.50 (3H, dd,J_{H-H}=7.5 Hz; J_{P-H}=15.0 Hz), ^{13}C NMR ($CDCl_3$, ppm) 128.5-131.9 (12C, m), 31.5/30.9 (J_{P-C}), 18.7. Satisfactory combustion analysis could not be obtained due to disulfide contaminant.

Diphenylphosphinoyl 1-[3,7-dimethyl-6,7-oxido-2(*E*)-hexenylthio]ethane (**47**). To a solution of diphenylphosphinoyl ethane-1-thiol (**185**) (0.525 g, 2.0 mmol), at room temperature, in toluene (25 mL) was added 35 % NaOH (10 mL) followed by the addition of 6,7-epoxygeranyl chloride (**176**) (0.376, 2.0 mmol) in toluene (5 mL) and the phase transfer catalyst tetraoctylammonium bromide (50 mg). The mixture was stirred for 10 h, diluted with H_2O (25 mL) and Et_2O (50 mL). The layers were separated and the aqueous layer extracted with Et_2O (3 X 25 mL). Standard work-up followed by chromatography using ethyl acetate/hexane (7/3) as the eluant gave **47** (0.550 g, 66%) as an oil. IR (film) 3024, 2963, 2925, 1737, 1661, 1591, 1437, 1378, 1322, 1248, 1190, 1118, 1072, 1027, 998, 873, 741, 722 and 699 cm^{-1}; mass spectrum, CI *m/z* (isobutane, rel intensity) 417 (M^++2, 27), 416 (M^++1, 100), 263 (30), 231 (21), 230 (32), 229(18), 153 (22), 135 (10); ^1H NMR ($CDCl_3$, ppm) 7.85 (4H, m), 7.50 (6H, m), 5.15 (1H, m), 3.27 (1H, dq, J_{H-H}=7.5 Hz; J_{P-H}=11.0 Hz), 3.15 (2H, overlapping dd), 2.66 (1H, tm, *J*=6.0 Hz), 2.15 (2H, m), 1.60-1.50 (8H, m), 1.28 (3H, s), 1.24/1.23 (3H,s); ^{13}C NMR ($CDCl_3$, ppm) 139.6, 132.6-128.3 (12C, m), 119.9, 63.9, 58.2, 37.0/36.3 (J_{P-C}) , 29.7, 27.3, 24.8, 18.8, 16.1. Anal. cacld. for $C_{24}H_{31}O_2SP$: C, 69.54; H, 7.54. Found: C, 69.80; H, 7.44.

9-Thia-10(*E*)-2,3-oxidosqualene (**43**) To a solution of LDA (0.60 mmol), prepared from diisopropyl amine (0.90 mL, 0.65 mmol) and *n*-BuLi (0.375 mL, 0.60 mmol, 2.5 M in hexanes) at -78 °C, in THF (5.0 mL) at -100 °C was added dropwise **47** (0.207 g, 0.50 mmol) in THF (2 mL). After 20 min, to the dark orange solution was added aldehyde **172** (0.15 g, 0.60 mmol) and the reaction stirred for 30 min. Acetic acid (100 mg) and H_2O (100 mg) were added sequentially and most of the THF was removed *in vacuo*. The gelatinous oil was diluted with Et_2O and filtered through a small column of silica gel using

ethyl acetate as the eluant to give a diastereoisomeric mixture of **186/187** (240 mg, 73 %). Thin layer chromatographic analysis using repeated developements with increasingly polar mixtures of ethyl acetate and hexanes (1/9 to 1/1) revealed two closely migrating spots, the major diastereoisomer (darker component), assumed to be the *erythro* diastereoisomer, migrated higher than the minor diastereoisomer. ^{1}H NMR of the mixture showed a ratio of ~35/65, **186/187**. The oil was rechromatographed in two cycles using ethyl acetate/hexanes (2/8 to 1/1) as the eluant to give the major diastereomer (*erythro*) (88 mg, pure) and the rest as a mixture of the two diastereomers. Major diasteroisomer (**187**): IR (film) 3314, 2923 (m), 1690, 1437, 1378, 1324, 1566, 1112, 910, 856, 723 and 697 cm^{-1}; ^{1}H NMR ($CDCl_3$, ppm) 8.40 (2H, m), 8.10 (2H, m), 7.58 (6H, m), 5.67 (1H, bs), 5.09 (3H, bm), 5.00 (1H, t, *J*=8.5 Hz), 4.07 (1H, t, *J*=8.5 Hz), 2.87 (1H, tm, *J*=8.5 Hz), 2.65 (1H, t, *J*=6.0 Hz), 2.30 (2H, bm), 2.20-1.90 (14H, bm), 1.68 (3H, s), 1.64 (3H, s), 1.59 (6H, app s), 1.57 (3H, s), 1.55 (3H, s), centred at 1.40 (3H, d, J_{P-H}=16.0 Hz), 1.30 (3H, d, *J*=2.0 Hz), 1.25 (3H, s).

The major diastereoisomer, **187**, (88 mg, 0.133 mmol) was dissolved in THF (5 mL). To this was added NaH (10 mg, 0.266 mmol, 60 % in oil). The mixture was stirred at room temperature, under argon, for 6 h ar which point H_2O (100 mg) was added. The solvent was removed *in vacuo* and the slurry chromatographed using ethyl acetate/hexanes (1/9) as the eluant to give pure **43** (50 mg, 85 %). This compound was found to be unstable to prolonged storage, even at -30 °C under an argon atmosphere. IR (film) 2921(m), 1665, 1630, 1444, 1377, 1248, 1118 and 838 cm^{-1}; mass spectrum, CI *m/z* (isobutane, rel intensity) 445 (M^++1, trace), 427 (trace), 277 (9), 259 (15), 187 (5), 177 (7), 169 (16), 167 (5), 155 (12), 154 (15), 153 (100), 137 (15), 135 (28); ^{1}H NMR ($CDCl_3$, ppm) 5.38 (1H, tq, *J*=6.70, 1.19 Hz), 5.30 (1H, tq, *J*=7.5, 1.20 Hz), 5.10

(3H, s), 3.32 (2H, d, J=7.5 Hz), 2.69 (1H, t, J=6.0 Hz), 2.20-1.94 (16H, bm), 1.87 (3H, s), 1.69 (3H, s), 1.68 (3H, s), 1.60 (9H, bs), 1.30 (3H, s), 1.26 (3H, s); ^{13}C NMR ($CDCl_3$, ppm) 138.2, 135.7, 135.0, 131.2, 129.7, 127.0, 124.4, 124.2, 123.7, 120.2, 63.9, 58.2, 39.7 (2C), 36.2, 29.7, 29.2, 27.9, 27.4, 26.8, 26.7, 25.6, 24.8, 18.7, 18.1, 17.6, 16.1, 16.0, 15.9. Anal. calcd. for $C_{29}H_{48}OS$: C, 78.32; H, 10.88. Found: C, 78.50; H, 11.14.

Diphenylphosphinoyl-1-(3,6-dimethyl-2(*E*),6(*E*)-octadienylthio)ethane (48). To a stirred solution of diphenylphosphinoyl ethyl-1-thiol, **185**, (0.525 g, 2.0 mmol) in toluene (25 mL) was added 35% aqueous solution of NaOH (10 mL) and tetraoctylammonium bromide (50 mg) as the phase transfer catalyst followed by geranyl chloride (**188**) (0.345 g, 2.0 mmol). The mixture was stirred at room temperature for 10 h, diluted with H_2O (25 mL) and extracted with Et_2O (4 X 25 mL). Standard work-up followed by chromatography using ethyl acetate/hexanes (7/3) as the eluant gave **48** (0.57g, 72 %) as an oil. IR (film) 3024, 2924, 2967, 2930, 1437, 1376, 1190, 1118, 1072, 741, 723 and 697 cm^{-1}; mass spectrum, CI *m/z* (isobutane, rel intensity) 400/399 (M^++1, 26/100), 397(13), 230 (31), 229 (78), 137 (25); ^1H NMR ($CDCl_3$, ppm) 7.87 (4H, m), 7.50 (6H, m), 5.05 (2H, m), 3.27 (1H,, J_{pH}=11.0 Hz; J_{H-H}=7.50 Hz), 3.12 (2H, dd, J=8.0 Hz, 3.5 Hz), 2.10-1.98 (4H, m), 1.67 (3H, s), 1.58 (3H, s), 1.56 (6H, app s), 1.54 (3H, dd, J_{P-H}=15.0 Hz; J_{H-H}=7.5 Hz); ^{13}C NMR ($CDCl_3$, ppm) 140.5, 132.2-128.2 (13C), 123.8, 119.3, 39.6, 36.8/36.1 (J_{P-C}), 29.9, 26.5, 25.6, 17.7, 16.1, 16.0. Anal. calcd. for $C_{24}H_{31}OSP$: C, 72.33; H, 7.84. Found: C, 72.00; H, 7.67.

1-[(*tert*-Butyldimethylsilyl)oxy]-5,9,13-trimethyl-4(E),8(E),12-tetradecatriene (189) To a solution of alcohol **181** (1.0 g, 4.0 mmol) in

CH_2Cl_2 (25 mL), was added Et_3N (0.83 mL, 6.0 mmol) and *tert*-butyldimethylsilyl chloride (0.670 g, 4.8 mmol) and DMAP (50 mg, catalyst). The mixture was stirred for 10 h, diluted with water and extracted with Et_2O (3 X 35 mL). Standard work-up followed by chromatography using ethyl acetate/hexanes (5/95) as the eluant gave silyl ether **189** (1.4 g, 96%). IR (film) 2928, 2851, 1444, 1383, 1255, 1099, 836 and 775 cm^{-1}; mass spectrum, CI *m/z* (isobutane, rel intensity) 365 (M^++1), 307 (6), 234 (18), 233 (100), 231 (24); ^1H NMR ($CDCl_3$, ppm) 5.12 (3H, m), 3.60 (2H, t, *J*=6.0 Hz), 2.10-1.95 (10 H, bm), 1.68 (3H, s), 1.60 (9H, app s), 1.55 (2H, bm), 0.90 (9H, s), 0.05 (6H, s); ^{13}C NMR ($CDCl_3$, ppm) 135.3, 134.9, 124.5, 124.3, 124.1, 62.7, 39.7 (2C), 33.1, 26.8, 26.7, 26.0 (3C), 24.6, 24.2, 18.3, 17.6, 16.0, -5.3 (2C).

1-[(*tert*-Butyldimethylsilyl)oxy]-12,13-epoxy-5,9,13-trimethyl-4(*E*),8(*E*)-tetradecatriene (190) To a stirred solution of silyl ether (**189**) (1.0 g, 2.7 mmol) in a THF/H_2O (60/40) (25 mL) at 0 °C was added dropwise a solution of NBS (0.508 g, 2.83 mmol) in THF/H_2O (60/40) (25 mL) over 30 min. The mixture was stirred for an additional 1 h, diluted with H_2O (50 mL) and extracted with Et_2O (4X50 mL). Standard work-up gave the assumed intermediate 12-bromo-13-hydroxy derivative contaminated with the starting material.

The crude mixture was dissolved in MeOH (100 mL) and to this was added K_2CO_3 (1.0 g, 7.25 mmol). The mixture was stirred for 10 h. Most of the solvent was evaporated *in vacuo* (~20 mL remained), the slurry was diluted with ice-water (50 mL) and extracted with Et_2O (4X30 mL). Standard work-up followed by chromatography using ethyl acetate/hexanes (1/9) as the eluant gave epoxide **190** (0.52 g, 51 % in two steps) and unreacted triene **189** (0.300 g). IR (film) 2928, 2856, 1462, 1378, 1253, 1100, 1006, 836 and 775 cm^{-1};

mass spectrum, CI *m/z* (isobutane, rel intensity) 382/381 (M^++1, 12/41), 363 (15), 250 (10), 249 (53), 232 (17), 231 (100); ^{1}H NMR ($CDCl_3$, ppm) 5.15 (2H, m), 3.58 (2H, t, *J*=6.5 Hz), 2.70 (1H, t, *J*=6.0 Hz), 2.15-1.96 (10H, m), 1.60 (3H, s), 1.59 (3H, s), 1.54 (2H, m), 1.29 (3H, s), 1.25 (3H, s), 0.95 (9H, s), 0.05 (6H, s); ^{13}C NMR ($CDCl_3$, ppm) 135.2, 134.0, 124.9, 124.2, 64.1, 62.7, 58.2, 39.6, 36.3, 33.0, 27.5, 26.7, 25.9 (3C), 24.9, 24.2, 18.7, 18.3, 16.0, 15.9, -5.4 (2C). Anal. calcd. for $C_{23}H_{44}O_2Si$: C, 72.57; H, 11.65. Found: C, 72.53; H, 11.53.

5,9,13-Trimethyl-4(*E*),8(*E*)-12,13-Epoxytetradecatrien-1-ol (191) Epoxy silyl ether **190** (0.50 g, 1.32 mmol) was dissolved in a 1.0 M solution of tetrabutylammonium fluoride in THF (20 mL) and stirred for 4 h. Ice water was added and this extracted with Et_2O (4 X 25 mL). Standard work-up followed by chromatography using ethyl acetate/hexanes (3/7) as the eluant gave epoxy alcohol **191** (0.330 g, 95 %) as an oil. IR (film) 3432, 2926, 1447, 1379, 1250, 1122, 1059 and 873 cm^{-1}; mass spectrum, CI *m/z* (isobutane, rel intensity) 268/267 (M^++1, 19/100), 250 (17), 249 (95), 153 (30), 137 (11), 135 (27), 127 (16), 123 (15), 111 (16), 109 (27); ^{1}H NMR ($CDCl_3$, ppm) 5.13 (2H, m), 3.63 (2H, t, *J*=6.5 Hz), 2.70 (1H, t, *J*=6.5 Hz), 2.18-1.98 (10 H, bm), 1.60 (8H, m), 1.29 (3H, s), 1.25 (3H, s); ^{13}C NMR ($CDCl_3$, ppm) 135.6, 134.1, 124.8, 123.9, 64.2, 62.7, 58.3, 39.6, 36.3, 32.8, 27.5, 26.5, 24.8, 24.2, 18.7, 16.0, 15.9. Anal. calcd. for $C_{17}H_{30}O_2$: C, 76.64; H, 11.35. Found: C, 76.36; H, 11.18.

5,9,13-Trimethyl-4(*E*),8(*E*)-12,13-epoxytetradecatrien-1-al (173). To a stirred solution of epoxy alcohol **191** (0.730 g, 0.00274 mmol) and Et_3N (3.8 mL, 27.4 mmol) in DMSO (25 mL) under argon was added dropwise a solution of Py·SO_3 (1.54 g, 9.7 mmol) in DMSO (10 mL). The mixture was stirred at room temperature for 3.5 h, diluted with ice-cold saturated NaCl (50

mL) and extracted with Et_2O (4 X 50 mL). The combined extracts were washed with ice water (1 X 50 mL). Standard work-up followed by chromatography using ethyl acetate/hexanes (1/9) as the eluant gave **173** (0.700 g, 95 %) as an oil. IR (film) 2923, 2717, 1725, 1687, 1450, 1378, 1249, 1122, 1054 and 874 cm^{-1}; mass spectrum, CI *m/z* (isobutane, rel intensity) 266/265), (M^++1,14/79), 248 (17), 247 (100), 230 (8), 229 (48), 165 (9), 163 (9), 161 (29), 153 (43), 149 (11), 137 (13), 135 (32), 127 (10), 125 (15), 121 (11), 109 (16), 107 (30); ^1H NMR ($CDCl_3$, ppm) 9.75 (1H, t, *J*=1.8 Hz), 5.10 (2H, m), 2.70 (1H, t, *J*=6.0 Hz), 2.45 (2H, td, *J*=7.0, 1.8 Hz), 2.32 (2H, app q, *J*=7.0 Hz), 2.20-1.90 (8H, m), 1.60 (6H, m), 1.29 (3H, s), 1.24 (3H, s); ^{13}C NMR ($CDCl_3$, ppm) 202.3, 136.7, 134.2, 124.6, 120.9, 64.1, 58.2, 43.9, 39.6, 36.3, 26.6, 26.4, 24.8, 20.9, 18.7, 16.0, 15.9. Anal. calcd. for $C_{17}H_{28}O_2$: C, 77.21; H, 10.68. Found: C, 77.40; H, 10.73.

16-Thia-14(*E*)-2,3-oxidosqualene (44). A solution of phosphine oxide **48** (0.303 g, 0.80 mmol) in THF (5 mL) was added dropwise to a stirred solution of LDA [1.0 mmol, prepared from diisopropyl amine (0.14 mL, 1.0 mmol) and *n*-BuLi (0.40 mL, 1.0 mmol, 2.5 M in hexanes at -78 °C] in THF (5 mL) under argon, at -100 °C. After 20 min, to the orange reaction mixture was added dropwise aldehyde **173** (0.200 g, 0.75 mmol) in THF (5.0, mL). The resulting light yellow solution was stirred for 30 min and treated with acetic acid (100 mg) and H_2O (100 mg). The solvent was evaporated *in vacuo* and the slurry filtered through a small column of silica gel using ethyl acetate/hexanes (7/3) as the eluant to give **192/193** (~35/65) (0.375 g, 75 %). Thin layer chromatographic analysis on silica gel revealed two components. The major spot elutied faster (using repeated elutions with solvents of increasing polarity (acetate/hexanes). The major diastereoisomer (*erythro*), **193**, was purified by column chromatography on silica gel in two cycles using ethyl acetate/hexanes

(2/8 to 1/1) as the eluant. This gave pure **193** (0.180 g, 36%) and **192**, *threo*, ((150 mg), contaminated with ~10% of **193**. Major diastereoisomer, **193**: IR (film) 3314, 2925, 1739, 1664, 1590, 1437, 1377, 1323, 1245, 1166, 1111, 1087, 855, 748, 750 and 698 cm^{-1}; ^1H NMR ($CDCl_3$, ppm) 8.40 (2H, overlapping dm, *J*=9.0 Hz), 8.10 (2H, overlapping dm, *J*=9.0 Hz), 7.50 (6H, m), 5.54 (1H, d, 3.5 Hz), 5.13 (2H, m), 5.03 (1H, tm, *J*=6.5 Hz), 4.94 (1H, t, *J*=8.0 Hz), 4.07 (1H, bt, *J*=8.0 Hz), 2.85 (1H, dd, *J*=11.0, 8.0 Hz), 2.68 (1H, t, *J*=6.0 Hz), 2.33 (2H, m), 2.20-1.90 (15H, m), 1.68 (3H, s), 1.63 (3H, s), 1.60 (3H, s), 1.58 (3H, s), 1.52 (3H, s), 1.40 (3H, d, J_{P-H}, 16.0 Hz), 1.29 (3H, s), 1.24 (3H, s).

The major diastereoisomer **193** (0.175 g, 0.265 mmol) was dissolved in THF (5 mL). To this was added NaH (21.5 mg, 0.53 mmol, 60 % in oil). The mixture was stirred for 6 h under argon and the reaction was terminated by the addition of water (100 mg). The solvent was removed *in vacuo* and the slurry diluted with Et_2O (1 mL) and filtered through a small silica gel column using Et_2O. Chromatography using ethyl acetate/hexanes (5/95) as the eluant gave **44** (0.103 g, 86 %) as an oil. IR (film) 2962, 2923, 2854, 1714, 1665, 1626, 1447, 1377, 1248, 1119 and 841 cm^{-1}; mass spectrum, CI *m/z* (isobutane, rel intensity) 446/445 (M^++1, 3/10), 309 (15), 307 (7), 291 (14), 275 (31), 257 (11), 169 (10), 138 (18), 137 (100), 135 (14), 127 (11), 125 (10), 123 (10); ^1H NMR ($CDCl_3$, ppm) 5.38 (1H, tq, *J*=7.0, 1.20 Hz), 5.25 (1H, tq, *J*=7.5, 1.20 Hz), 5.14 (2H, m), 5.07 (1H, tm, *J*=7.5 Hz), 3.31 (2H, d, *J*=7.5 Hz), 2.70 (1H, t, *J*=6.0 Hz), 2.10-1.97 (16 H, bm), 1.87 (3H, bs), 1.68 (6H, app s), 1.62 (3H, s), 1.60 (6H, bs), 1.30 (3H, s), 1.26 (3H, s); ^{13}C NMR ($CDCl_3$, ppm) 139.1, 135.5, 134.1, 131.6, 129.8, 126.9, 124.9, 124.0, 123.8, 119.5, 64.2, 58.2, 39.7, 39.6, 36.3, 29.9, 29.2, 28.0, 27.6, 26.7, 26.6, 25.6, 24.9, 18.7, 16.1, 17.7, 16.1, 16.0, 15.9. Anal. calcd.. for $C_{29}H_{48}OS$: C, 78.32; H, 10.89. Found: C, 78.19; H, 10.68. HRMS calcd for $C_{29}H_{48}OS$, 444.3426. Found 444.3434.

D: Chapter 5

Methyl 3-tributylstannyl-2(*E*)-hexenoate (197a). To a solution of LDA (26.0 mmol) at -78 °C in THF (50 mL) under an argon atmosphere was added neat *n*-Bu_3SnH (6.72 mL, 25.0 mmol). After stirring for 1.5 h, CuBr-DMS complex (5.13 g, 25 mmol) was added in one portion. The brown solution was stirred at -78 °C for 1 h, and to it was added dropwise methyl 2-hexynoate, **198**, (3.15 g, 25 mmol) in THF (25 mL). The temperature was maintained at -78 °C for an additional 2 h and the reaction was terminated with MeOH (10 mL), treated with 100 mL of saturated NH_4Cl/NH_4OH (9/1), stirred for 30 min and extracted with Et_2O (4 X 50 mL). Standard work-up followed by chromatography using ethyl acetate/hexanes (5/95) as the eluant gave **197a** (9.70 g, 93%) as clear oil. IR (film) 2958 (m), 1723.5, 1591, 1462, 1431, 1351, 1168, 1072, 1043, and 883 cm^{-1}; mass spectrum, CI *m/z* (isobutane, rel intensity, major isotopes) 419/417(M^++1, 100/75), 387/385(27/22), 361/359 (100/80); ^1H NMR ($CDCl_3$, ppm) 5.94 (1H, t, *J*=1.1 Hz; J_{Sn-H}=67.0 Hz), 3.68 (3H, s), 2.84 (2H, tq, *J*=8.0, 1.1 Hz; J_{Sn-H}=56.0 Hz), 1.53-1.38 (8H, bm), 1.30 (6H,sex, *J*=7.5 Hz), 0.90 (18 H, bm); ^{13}C NMR ($CDCl_3$, ppm) 174.0, 164.4, 127.4, 50.5, 37.2, 28.9 (3C), 27.3 (3C), 22.9, 14.0, 13.5 (3C), 10.0 (3C). Anal. calcd. for $C_{19}H_{38}O_2Sn$: C, 54.70; H, 9.18. Found: C, 54.68; H, 9.20.

Procedure for the preparation of methyl 3-(tributylstannyl)-2(*Z*)-hexenoate (197b).

From Reaction with Bu_3Sn(*N*-imid)$Cu(CN)Li_2$. A solution of (*N*-imidid)Cu(CN)Li [prepared from freshly sublimed imidazole (0.17 g, 2.5 mmol), which was treated with *n*-BuLi (1.0 mL, 2.5 mmol, 2.5 M in hexanes) at -78 °C in

THF (15 mL) and after 1 h, CuCN (0.23 g, 2.5 mmol)] was warmed to room temperature and stirred for an additional 1 h. The resulting cloudy green solution of (*N*-imid)Cu(CN)Li was further diluted by addition of THF (10 mL) and then added dropwise *via* canula to Bu_3SnLi (2.5 mmol) in THF (10 mL) at -78 °C, prepared from Bu_3SnH (0.672 mL, 2.5 mmol) and LDA (2.52 mmol) as described above for **197a**.

To the clear light yellow solution of Bu_3Sn(*N*-imid)Cu(CN)Li_2 was added dropwise **198** (0.315 g, 2.5 mmol) in THF (5 mL). After 0.5 h at -78 °C, the reaction was warmed to room temperature and stirred at this temperature for 2 h. Addition of saturated NH_4Cl/NH_4OH (9/1) (50 mL) and extraction of the aqueous layer with Et_2O (4 X 50 mL), followed by standard work-up gave a mixture of **197a** and **197b** (4/96 by GC). The isomers were easily separated by chromatography using ethyl acetate/hexanes (5/95) as the eluant. Isomer **197b** (0.87 g) eluted first and followed eluation of isomer **197a** (trace amount) in combined yield of 89 %.

From reaction with Bu_3Sn(*n*-Bu)Cu(CN)Li_2. To a solution of Bu_3SnLi (2.5 mmol), prepared from Bu_3SnH (0.672 mL, 2.5 mmol) and LDA (2.5 mmol) as described above, in THF (10 mL) at -78 °C was added quickly, *via* canula, *n*-BuCu(CN)Li (2.6 mmol) prepared from CuCN (0.24 g, 2.6 mmol) and *n*-BuLi (1.04 mL, 2.6 mmol, 2.5 M in hexanes) at -78 °C in THF (20 mL). A solution of Bu_3Sn(*n*-Bu)Cu(CN)Li_2 was stirred for 0.5h and **198** (0.315 g, 2.5 mmol) in THF (5 mL) was added dropwise. After stirring for an additional 2 h at -50 °C, saturated NH_4Cl/NH_4OH (50 mL) was added and the mixture warmed to 0 °C then extracted with Et_2O (4 X 50 mL). Standard work-up gave the crude product as a mixture of **197b** and **197a** (15/85 by GC analysis).

Chromatography using ethyl acetate/hexanes (5/95) as the eluant gave **197b** (0.76g, 87%) and **197a** (0.11 g, 13%), in combined yield of 83%.

Reaction with $Bu_3Sn(2\text{-}Th)Cu(CN)Li_2$. To solution of Bu_3SnLi (2.5 mmol), prepared from Bu_3SnH (0.67 ml, 2.5 mmol) and LDA (2.5 mmol) at -78 °C in THF (20 mL) was added (2-Th)Cu(CN)Li (10.5 mL, 2.6 mmol, 0.25 M in THF) and this was stirred for 0.5 h. To $Bu_3Sn(2\text{-}Th)Cu(CN)Li_2$ was added dropwise **198** (0.315 g, 2.5 mmol) in THF (5 mL), and this reaction was stirred for 0.5 h . The reaction was then warmed to room temperature and stirred for 2 h. Saturated NH_4Cl/NH_4OH (50 mL) was added and the mixture was extracted with Et_2O (4 X 50 mL). Standard work-up gave the crude product as a mixture of **197a** and **197b** (10/90, by GC analysis). Chromatography using ethyl acetate/hexanes (5/95) as the eluant gave **197b** (0.73g, 90%) and **197a** (0.084 g, 10%) in combined yield of 81%.

197b: IR (film) 2855-2871 (m), 1709, 1596, 1463, 1434, 1327, 1199 and 1061 cm^{-1}; mass spectrum CI *m/z* (isobutane, rel intensity, major isotopes) 417/419 (M^++1,trace amount), 361/359 (100/74); ^1H NMR ($CDCl_3$, ppm) 6.35 (1H, t, J=1.5 Hz, $J_{Sn\text{-}H}$=108.0 Hz), 3.72 (3H, s), 2.35 (2H, td, J=7.5, 1.5 Hz; $J_{Sn\text{-}H}$=44.0 Hz), 1.50-1.35 (8H, bm), 1.35-1.23 (6H, sex, J=7.5 Hz), 0.98-0.84 (18H, m); ^{13}C NMR ($CDCl_3$, ppm) 176.0, 168.2, 128.1, 51.3, 42.6, 29.2 (3C), 27.4 (3C), 22.4, 13.7, 13.6 (3C), 11.1(3C).

3-*n*-Tributylstannyl-2(*E*)-hexen-1-ol (199). To a solution of **197a** (9.5 g, 22.7 mmol) in THF (50 mL) under an argon atmosphere at -40 °C was added dropwise neat DIBAL-H (9.0 mL, 50 mmol). The reaction was warm to 0°C over 2 h , poured into a 25 % aqueous solution of tartaric acid (250 mL) and extracted with Et_2O (4 X 50 mL). Standard work-up, followed by

chromatography using ethyl acetate/hexanes (2/8) as the eluant gave **199** (8.3 g, 94%) as clear oil. IR (film) 3302 (b), 2955 (b), 1464, 1376, 1072, 1019, 960, 873; mass spectrum, CI *m/z* (isobutane, rel intensity, major isotopes) 391/389 (M^++1, trace amount), 373/371 (trace amount), 333/331 (30/25), 291/289 (100/80); ^{1}H NMR ($CDCl_3$, ppm) 5.75 (1H, bt, *J*=6.5 Hz; J_{Sn-H}=68.0 Hz), 4.23 (2H, t, *J*=6.5 Hz), 2.24 (2H, t, *J*=7.5 Hz; J_{Sn-H}=58.0 Hz), 1.60-1.40 (6H, bm), 1.4-1.26 (8H, bm), 0.93-0.84 (18H, bm); ^{13}C NMR ($CDCl_3$, ppm) 148.0, 139.4, 58.9, 35.6, 29.1(3C), 27.3 (3C), 23.4, 13.8, 13.5 (3C), 9.7 (3C). Anal. calcd. for $C_{18}H_{38}OSn$: C, 55.55; H, 9.84. Found: C, 55.74; H, 9.92.

1-(Benzyloxy)-3-(tributylstannyl)-2(*E*)-hexene (200). To a suspension of NaH (0.865g, 21.6 mmol, 60% in oil, washed free of oil with pentane (3 X 10 ml)) in DMF (25 mL) at 0 °C, under argon was added dropwise, **199** (7.0 g, 18.0 mmol), in DMF (10 mL) followed by neat benzyl bromide (2.25 ml, 18.9 mmol) *via* syringe. The mixture was stirred for 10 h at rt, poured into water (200 mL) and extracted with Et_2O (4 X 50 mL). Standard work-up followed by chromatography using ethyl acetate/hexanes (3/97) as the eluant gave **200** (7.66 g, 89%) as a clear oil. IR (film) 2930 (b), 1454, 1376, 1357, 1097, 1071 cm^{-1}; mass spectrum, CI *m/z* (isobutane, rel intensity, major isotopes) 479/477 (M^++1)trace amount), 423/421 (trace amount) 291/289 (100/80); ^{1}H NMR ($CDCl_3$, ppm) 7.30 (5H, bm), 5.74 (1H, tt, *J*=6.0, 1.0 Hz, *J*Sn-H=68.0 Hz), 4.50 (2H, s), 4.13 (2H, d, *J*=7.5 Hz), 2.22 (2H, t, *J*=7.5, J_{Sn-H}=58.0 Hz), 1.53-1.43 (6H, bm), 1.32 (8H, bm), 0.88 (18H, m); ^{13}C NMR ($CDCl_3$, ppm) 148.9, 138.6, 136.9, 128.3 (2C), 127.8 (2C), 127.5, 72.0, 66.3, 35.8, 29.1 (2C), 27.4 (2C), 23.4, 13..9, 13.6 (2C), 9.7 (2C). Anal. calcd. for $C_{25}H_{44}OSn$: C, 62.65; H, 9.25 Found: C, 62.85; H, 9.32.

6-(Benzyloxy)-4-propyl-4(*E*)-hexenal (201). Stannane **200** (4.85 g, 10.0 mmol) under argon atmosphere, deoxygenated by two cycles of evacuation of the flask with oil pump vacuum and purging with argon, was dissolved in THF (50 mL) and cooled to -78 °C. *n*-BuLi (4.86 mL, 11 mmol, 2.5 M in hexane) was added dropwise and after 2 h (2-thienyl)CuCNLi (46.0 mL, 11.5 mmol, 0.25 M in THF) was added *via* a syringe over a 10 min. The brown cuprate solution was stirred for 30 min and HMPA (3.5 mL, 20 mmol) was added followed by the dropwise addition of a solution of acrolein (1.0 mL, 15 mmol) and TMSCl (1.9 mL, 15 mmol) in THF (10 mL). After 2 h the reaction mixture was poured into mixture of ice-cold 0.25 N aqueous HCl (50 mL) and Et_2O (50 mL) and stirred for 0.5 h. The Et_2O layer was seperated and the aquous layer extracted with Et_2O (3 X 50 mL). Standard work-up followed by chromatography using ethyl acetate/hexanes (1/9) as the eluant gave **201** (1.2 g, 49 %) as a slightly yellow oil. IR (film) 2930, 2868, 2720, 1723, 1665, 1454, 1354, 1090, 737, 698; mass spectrum CI, *m/z* (isobutane, rel intensity) 247 (M^++1, 20), 245 (33), 229 (20) 202 (100), 155 (30); ^{1}H NMR ($CDCl_3$, ppm) 9.78 (1H, t, *J*=1.2 Hz), 7.30 (5H, m), 5.38 (1H, t, *J*=7.5 Hz), 4.50 (2H, s), 4.02 (2H, d, *J*=7.5 Hz), 2.55 (2H, tm, *J*=7.5 Hz), 2.35 (2H, t, *J*=7.5 Hz), 2.00 (2H, t, *J*=7.0 Hz), 1.37 (2H, sex, *J*=7.5 Hz), 0.86 (3H, t, *J*=7.0 Hz); ^{13}C NMR ($CDCl_3$, ppm) 201.4, 141.8, 138.2, 128.0 (2C), 127.5 (2C), 127.2, 122.0, 71.9, 66.0, 41.7, 32.6, 28.5, 21.3, 13.6. Anal. calcd. for $C_{16}H_{22}O_2$: C, 77.01; H, 9.01. Found: C, 77.27; H, 8.97.

Ethyl 8-benzyloxy-2-methyl-6-propyl-2(*E*),6(*E*)-octadienoate (202). A mixture of aldehyde **201** (0.615 g, 2.6 mmol) and (carbethoxyethylidene)triphenylphosphorane (1.12 g, 2.9 mmol) in dry CH_2Cl_2 (15 mL), was refluxed for 6 h under an argon atmosphere. The solvent was

evaporated *in vacuo* and the resulting slurry was diluted with hexanes (20 mL), filtered through a pad of celite and the precipitate rinsed with portions of hexanes (5 X 20 mL). The solvent was evaporated *in vacuo* and the oil chromatographed using ethyl acetate/hexanes (1/9) as the eluant to give geometrically pure **202** (0.700 g, 82%) as a clear oil. IR (film) 2930, 2869, 1709, 1649, 1453, 1366, 1268, 1091cm^{-1}; mass spectrum, CI *m/z* (isobutane, rel intensity) 331 (M^++1, trace amount), 223 (100), 149 (20); ^{1}H NMR ($CDCl_3$, ppm) 7.40 (5H, m), 6.75 (1H, t, *J*=7.0 Hz), 5.42 (1H, t, *J*=6.5 Hz), 4.50 (2H, s), 4.17 (2H, q, *J*=7.0 Hz), 4.05 (2H, d, *J*=6.5 Hz), 2.30 (2H, q, *J*=8.0 Hz), 2.15 (2H, t, *J*=8.0 Hz), 2.02 (2H, t, *J*=7.0 Hz), 1.84 (3H, d, *J*=1.0 Hz), 1.40 (2H, sex, *J*=7.5 Hz), 1.28 (3H, t, *J*=7.0 Hz), 0.87 (3H, t, *J*=7.5 Hz); ^{13}C NMR ($CDCl_3$, ppm) 168.0, 143.0, 141.3, 138.3, 128.2 (2C), 127.9, 127.6 (2C), 127.4, 121.8, 71.9, 66.2, 60.2, 35.1, 27.1, 21.5, 14.1, 13.9, 12.2. Anal. calcd. for $C_{21}H_{30}O_3$: C, 76.31; H, 9.16. Found: C, 76.19; H, 9.04.

8-(Benzyloxy)-2-methyl-6-propyl-2(*E*),6(*E*)-octadien-1-ol (203). To a solution of **202** (0.50 g, 1.5 mmol) in THF (10 ml) at 0 °C under argon was added dropwise DIBAL-H (3.75 mL, 3.75 mmol, 1.0 M in THF). The mixture was stirred for 2 h, poured into 25 ml of 25% aqueous tartaric acid solution and extracted with Et_2O (4 X 20 mL). Standard work-up followed by chromatography using ethyl acetate/hexanes (25/75) as the eluant gave **203** (0.41 g, 95%) as a clear oil. IR (film) 3415, 2958 (bm), 1710, 1659, 1452, 1378, and 1070 (m) cm^{-1}; mass spectrum, CI *m/z* (isobutane, rel intensity) 289 (M^++1, trace amount), 271 (3), 253 (2), 241 (trace amount), 213 (2), 181 (25), 163 (100), 135 (5), 123 (20); ^{1}H NMR ($CDCl_3$, ppm) 7.30 (5H, m), 5.39 (2H, m), 4.50 (2H, s), 4.02 (2H, d, *J*=6.5 Hz), 3.97 (2H, s), 2.16 (2H, m), 2.08 (2H, m), 2.02 (2H, t, *J*=7.0 Hz), 1.66 (3H, s), 1.37 (2H, sextet, *J*=7.5 Hz), 0.86 (3H, t, *J*=7.5 Hz); ^{13}C

NMR ($CDCl_3$, ppm) 143.7, 138.4, 135.0, 128.2 (2C), 127.7 (2C), 127.4, 125.3, 121.4, 71.9, 68.5, 66.3, 36.2, 32.5, 25.9, 21.6, 13.9, 13.5. Anal calcd. for $C_{19}H_{28}O_2$: C, 79.11; H, 9.79. Found: C, 79.42; H, 9.90.

8-(Benzyloxy)-1-chloro-2-methyl-6-propyl-2(*E*),6(*E*)-octadiene (204). To a solution of NCS (0.180 g, 1.35 mmol) in dry CH_2Cl_2 (10 mL) at 0 °C under argon atmosphere was added dropwise DMS (0.15 mL, 2.0 mmol) and the slurry cooled to -20 °C. To this was added dropwise alcohol **203** (0.262 g, 1.0 mmol) in CH_2Cl_2 (5 mL). The mixture was warmed to 0 °C and after 6 h poured into water (25 mL) then extracted with Et_2O (4 X 30 mL). Standard work-up followed by chromatography using ethyl acetate/hexanes (5/95) as the eluant gave **204** (0.250 g, 93%) as a clear oil. IR (film) 2930, 2868, 1453, 1377, 1263, 1090, 1027, 736, and 697 cm^{-1}; mass spectrum CI (isobutane, rel intensity, major isotopes) 289 (M^++1, 5), 271 (3), 253 (7), 201/199 (33/100), 163 (43); ^1H NMR ($CDCl_3$, ppm) 7.30 (5H, m), 5.52 (1H, t, *J*=6.5, Hz), 5.40 (1H, t, *J*=7.0 Hz), 4.50 (2H, s), 4.04 (2H, d, *J*=6.5 Hz), 4.00 (2H, s), 2.17 (2H, m), 2.07 (2H, m), 2.00 (2H, t, *J*=7.5 Hz), 1.56 (3H, s), 1.37 (2H, sex, *J*=7.5 Hz), 0.86 (3H, t, *J*=7.5 Hz); ^{13}C NMR ($CDCl_3$, ppm) 143.3, 138.5, 131.8, 130.2, 128.2 (2C), 127.6 (2C), 127.4, 121.7, 71.9, 66.3, 52.2, 35.8, 32.6, 26.4, 21.6, 14.0, 13.9.

1-(Benzyloxy)-7-methyl-3-propyl-3(*E*),6(*E*)-nonadiene (205). To cuprate $Me_2CuMgBr(I)$, at -78 °C under argon, prepared from CuBr-DMS (0.335 g, 2.0 mmol) and MeMgI (1.1 mL, 4.0 mmol, 3.0 M in THF) in THF (10 mL) at -40 °C for 1 hr, was added dropwise allylic chloride **204** (0.25 g, 0.86 mmol) in THF (5 mL). After 0.5 hr, saturated NH_4Cl/NH_4OH (25 mL) was added and extracted with Et_2O (4 X 25 mL). Standard work-up followed by chromatography using ethyl acetate/hexanes (5/95) as the eluant gave **205**

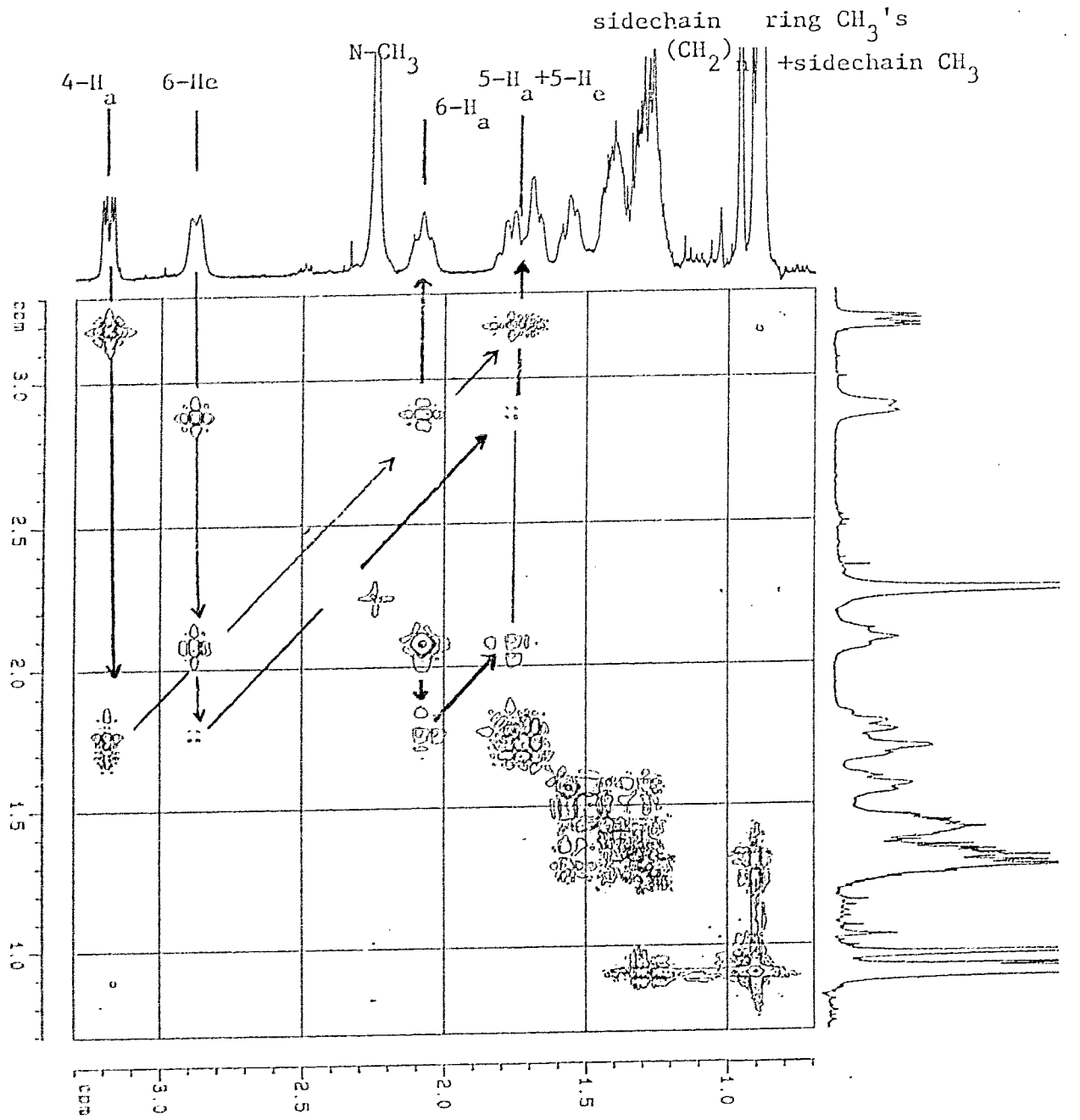
(0.238 g, 97%) as a clear oil. IR (film) 2930, 2870, 1664, 1454, 1376, 1203, 1072, 1007, 734, and 697 cm^{-1}; mass spectrum, CI *m/z* (isobutane, rel intensity) 286 (M^++1, trace amount), 179 (100); ^1H NMR ($CDCl_3$, ppm) 7.30 (5H, m), 5.40 (1H, t, *J*=7.0 Hz), 5.10 (1H, tq, *J*=6.5, 1.2 Hz), 4.50 (2H, s), 4.03 (2H, d, *J*=6.5 Hz), 2.05 (8H, m), 1.58 (3H, s), 1.38 (2H, sextet, *J*=7.5 Hz), 0.97 (3H, t, *J*=7.5 Hz), 0.86 (3H, t, *J*=7.5 Hz); ^{13}C NMR ($CDCl_3$, ppm) 144.3, 138.8, 137.1, 128.3 (2C), 127.8 (2C), 127.5, 122.7, 121.4, 72.0, 66.5, 36.9, 32.8, 32.4, 26.6, 21.8, 15.9, 14.1, 12.8. Anal. cacld. for $C_{20}H_{30}O$: C, 83.85; H, 10.56. Found: C, 84.01; H, 10.58.

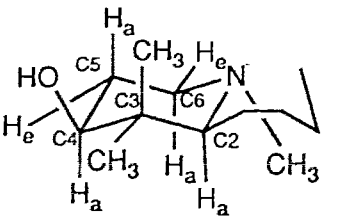
7-Methyl-3-propyl-3(*E*),6 (*E*)-nonadien-1-ol (206). To $EtNH_2$ (~5 mL) at -78 °C under argon containing Li (25 mg) was added benzyl ether **205** (0.220 g, 0.77 mmol) in THF (2 ml). After 10 min, blue colour reappeared, was added solid NH_4Cl (0.200 g) and the excess Li removed with forceps. The mixture was diluted with water (10 mL) and extracted with Et_2O (3 X 25 mL). Standard work-up followed by chromatography using ethyl acetate/hexanes (2/8) as the eluant gave alcohol **206** (0.148 g, 98%) as clear oil. IR (film) 3330, 2930, 2871, 1664, 1455, 1378, 1001 cm^{-1}; mass spectrum, CI *m/z* (isobutane, rel intensity) 196 (M^++1, trace amount), 179 (100); ^1H NMR ($CDCl_3$, ppm) 5.42 (1H, t, *J*=7.0 Hz), 5.10 (1H, tq, *J*=6.5, 1.2 Hz), 4.15 (2H, d, *J*=7.0 Hz), 2.20-1.90 (8H, m), 1.59 (3H, s), 1.40 (2H, sextet, *J*=7.5 Hz), 0.98 (3H, t, *J*=7.5 Hz), 0.89 (3H, t, *J*=7.5); ^{13}C NMR ($CDCl_3$, ppm) 143.8, 137.2, 123.8, 122.5, 59.2, 36.8, 32.6, 32.3, 26.6, 21.9, 15.9, 14.0, 12.8. Anal. calcd. for $C_{13}H_{24}O$: C, 79.52; H, 12.33. Found: C, 79.34; H, 12.22

7-Methyl-3-propyl-2(*E*),6(*E*)-nonadienyl acetate (49): To alcohol **206** (0.100 g, 0.51 mmol) in CH_2Cl_2 (5 mL) was added pyridine (2.0

mL), acetic anhydride (0.10g, 1.0 mmol) and few crystals of DMAP. The mixture was stirred overnight (10 hr), poured into ice-cold water and extracted with Et_2O (4 X 20 mL). Standard work-up followed by chromatography using ethyl acetate/hexanes (5/95) as the eluant gave **49** (0.120 g, 99%) as a clear oil. IR (film) 2932, 2873, 1742, 1460, 1369, 1231 and 1023 cm^{-1}: mass spectrum, CI *m/z* (isobutane, rel. intensity) 238/239 (M^++1, trace amount), 179 (M+-$C_2H_3O_2$, 100); EI *m/z* (rel intensity) 178 (4), 149 (9), 135 (9), 121 (12), 108 (7), 93 (11), 83 (60), 82 (29), 81 (20), 79 (20), 68 (21), 67 (31), 55 (100), 53 (15). ^1H NMR ($CDCl_3$, ppm) 5.35 (1H, t, *J*=7.0 Hz), 5.08 (1H, tq, *J*=6.0, 1.2 Hz), 4.58 (2H, d, *J*=7.0 Hz), 2.15-2.02 (9H, m), 1.98 (2H, t, *J*=7.5 Hz), 1.59 (3H, s), 1.40 (2H, sex, *J*=7.5 Hz), 0.97 (3H, t, *J*=7.5 Hz), 0.89 (3H, t, *J*=7.5 Hz); ^{13}C NMR ($CDCl_3$, ppm) 170.9, 146.1, 137.3, 122.3, 118.7, 61.2, 36.8, 32.7, 32.3, 26.4, 21.8, 21.0, 15.9, 13.9, 12.7. Anal. calcd. for $C_{15}H_{26}O_2$: C, 75.58; H, 10.99. Found: C, 75.36; H, 11.20.

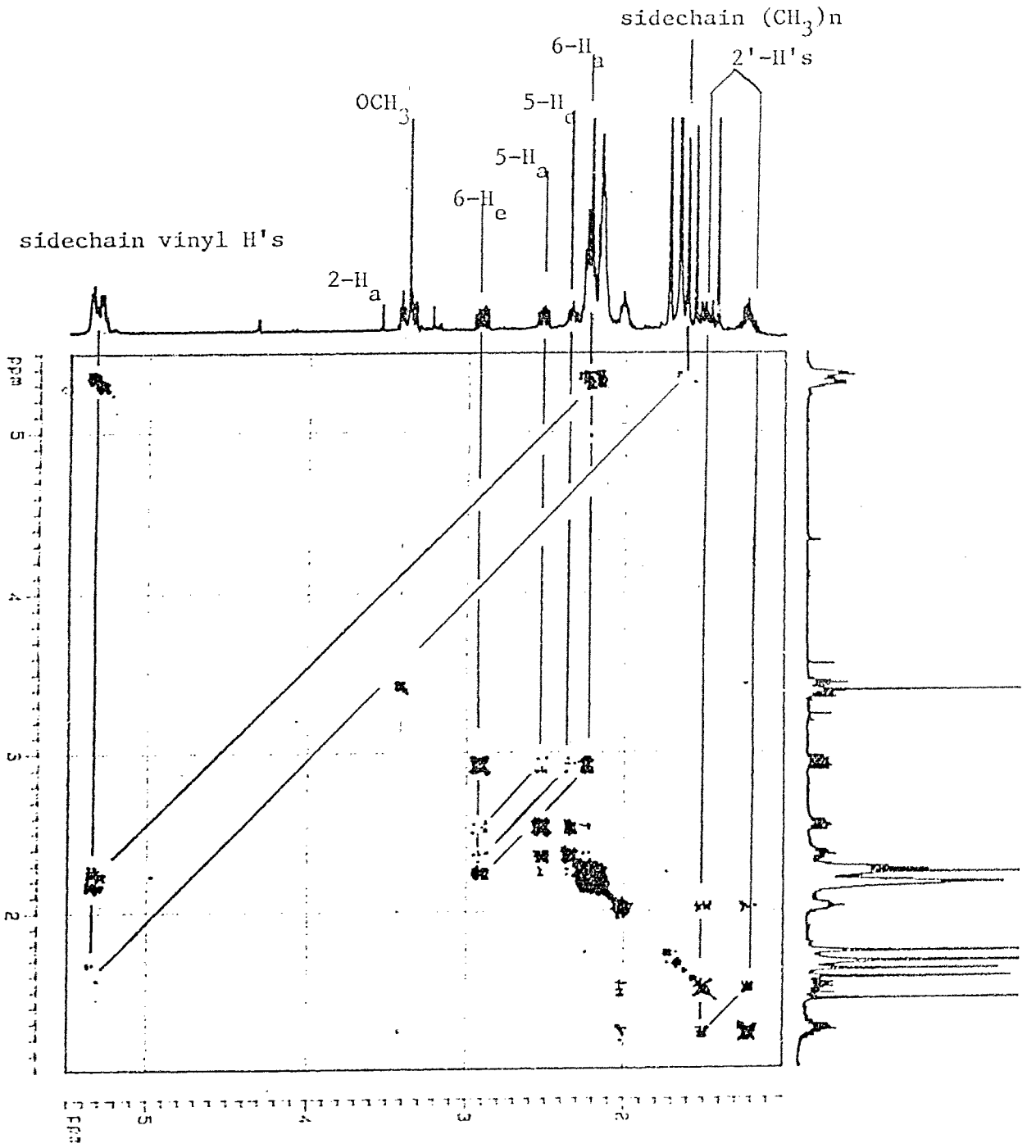
E: 2-Dimensional ^{1}H NMR Spectra of Compounds 35, 129 and 150

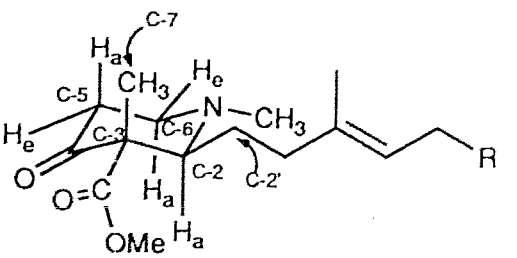




35

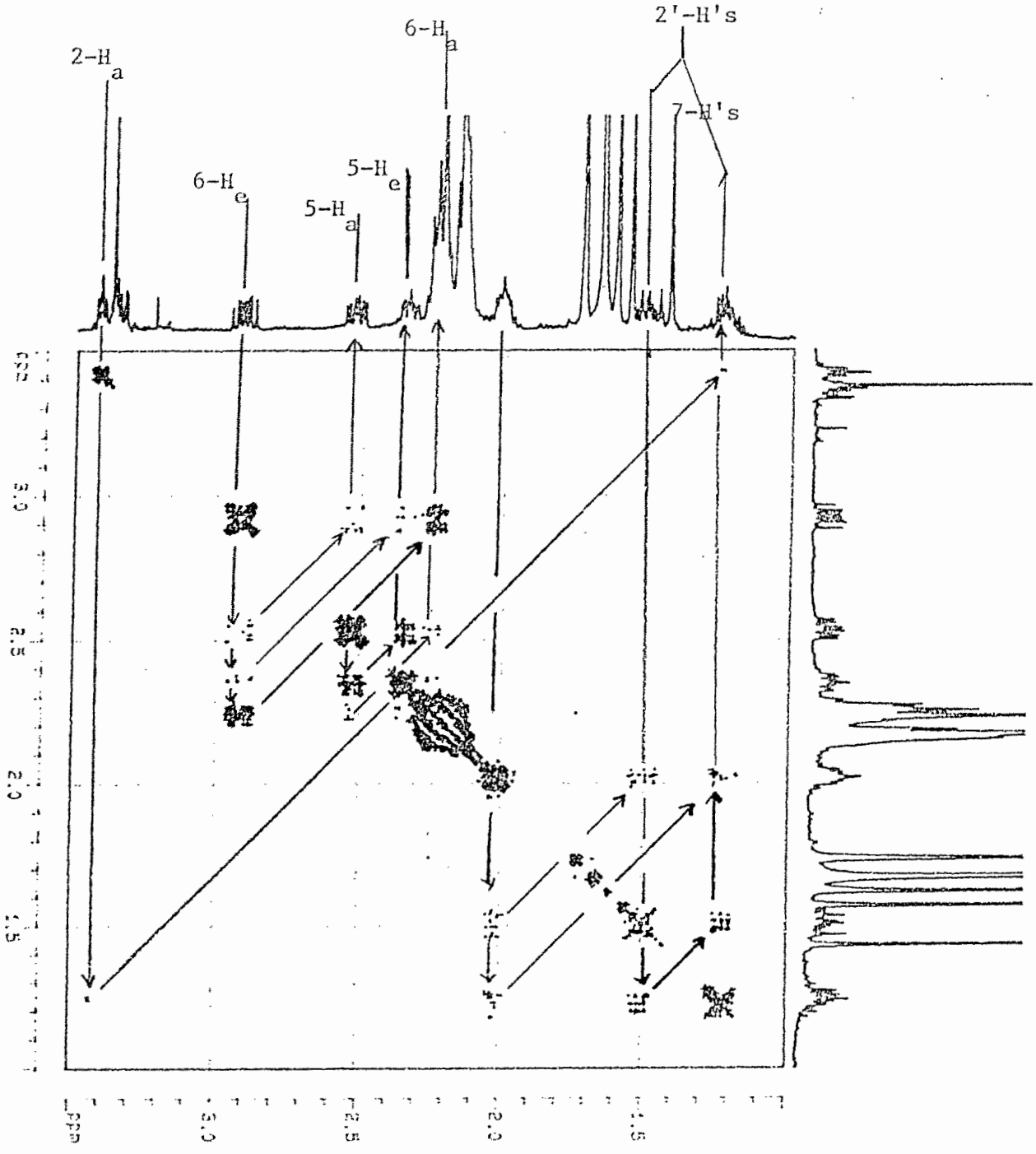
Figure 7-1: 400 MHz COSY spectrum of **35**.

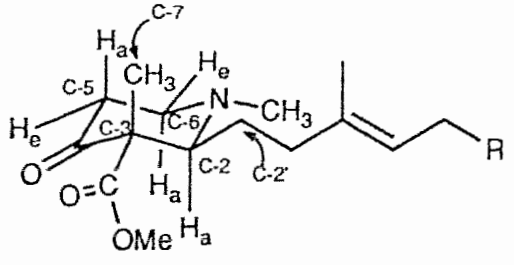




129

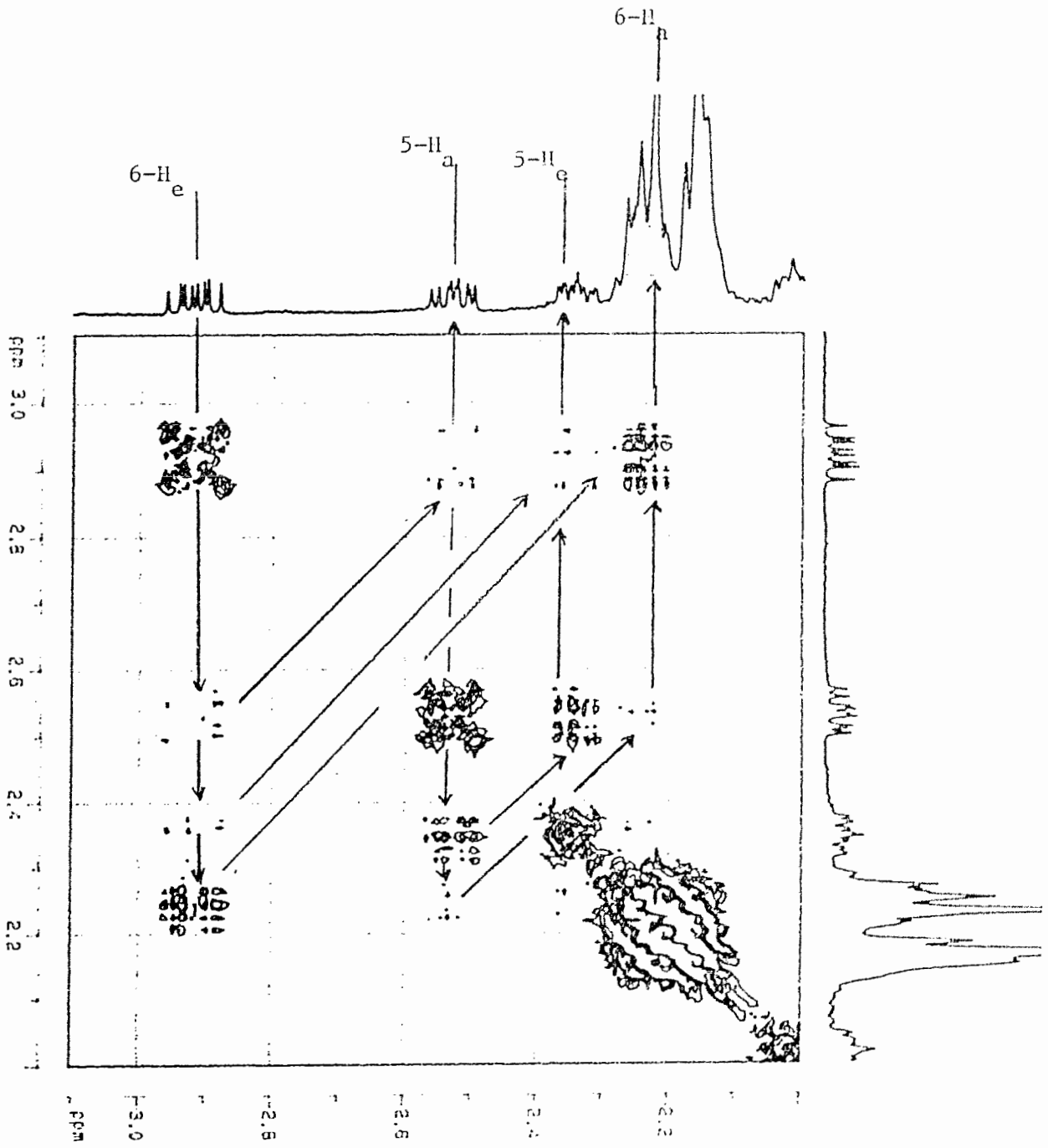
Figure 7-2a: 400 MHz COSY spectrum of **129**.

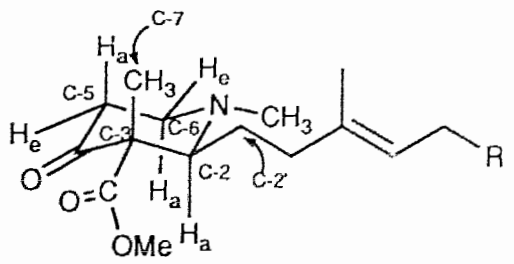




129

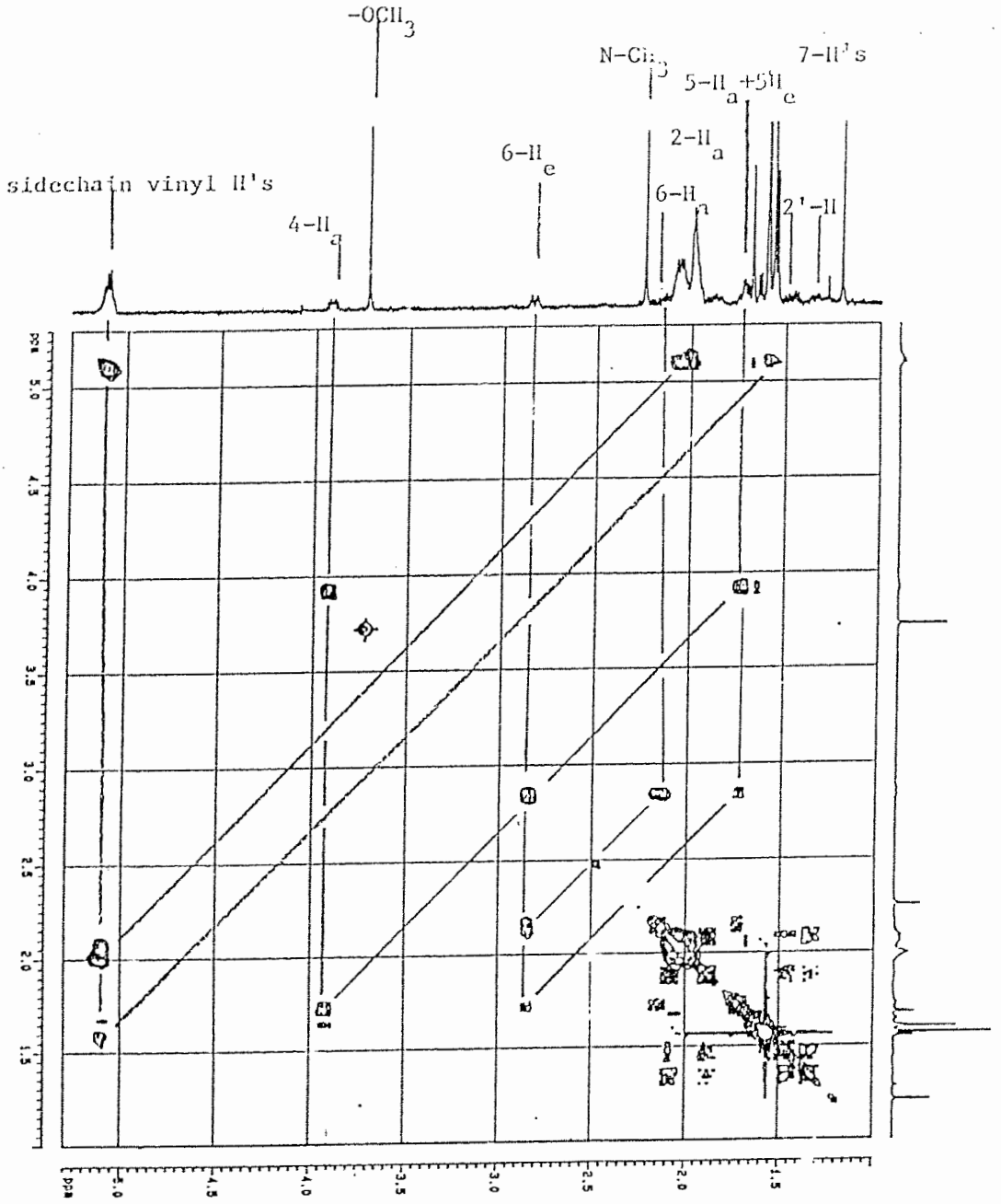
Figure 7-2b: 400 MHz COSY spectrum of **129** (expanded region 3.5 ppm to 0.0 ppm).

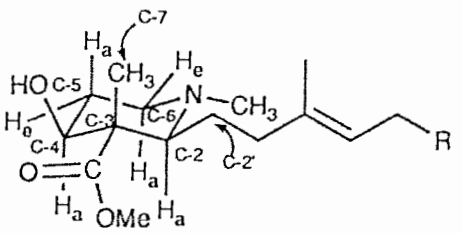




129

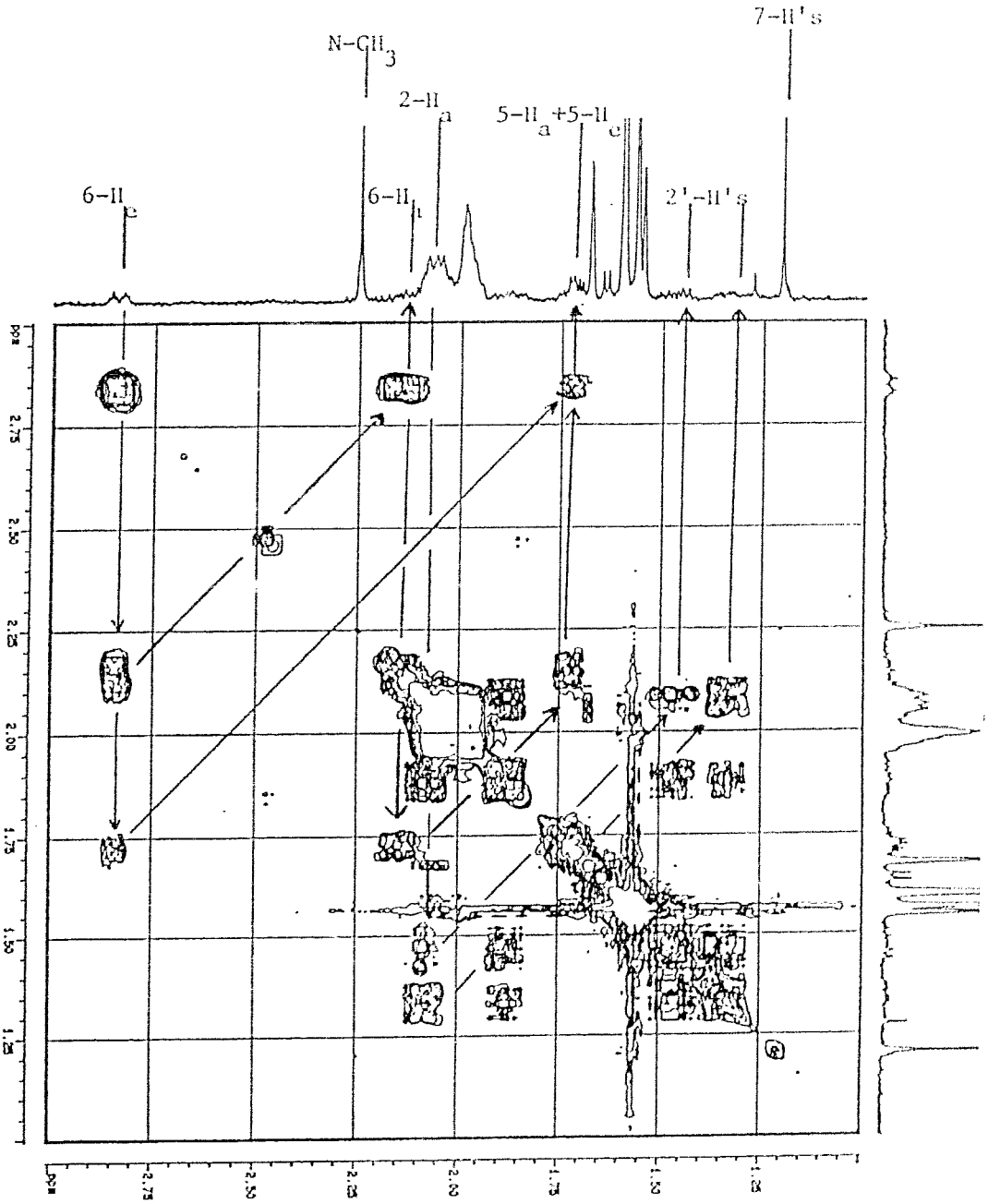
Figure 7-2c: 400 MHz COSY spectrum of **129** (expanded region 3.0 ppm to 2.0 ppm).

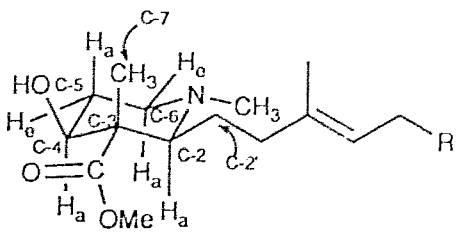




150

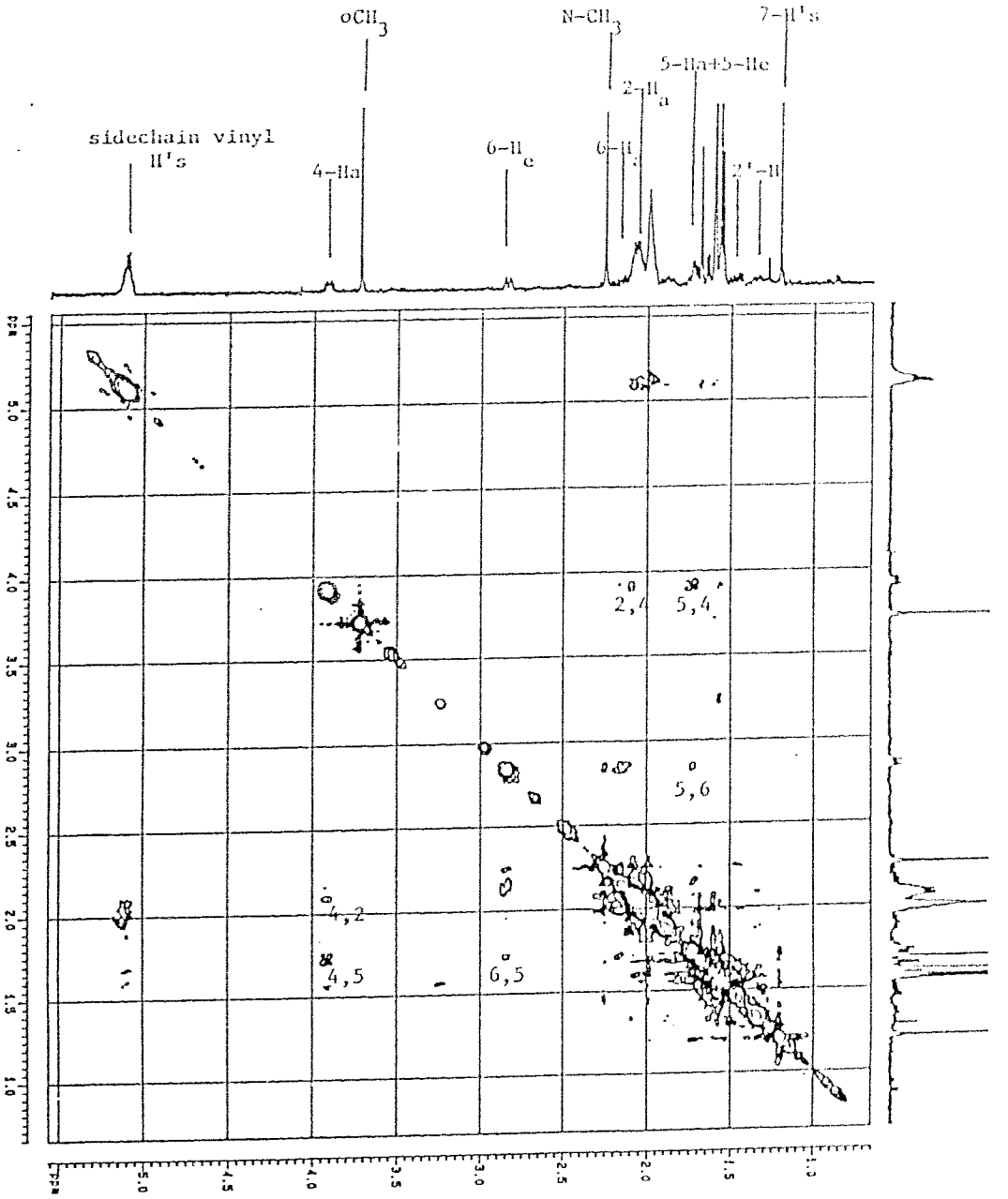
Figure 7-3a: 400 MHz COSY spectrum of **150**.





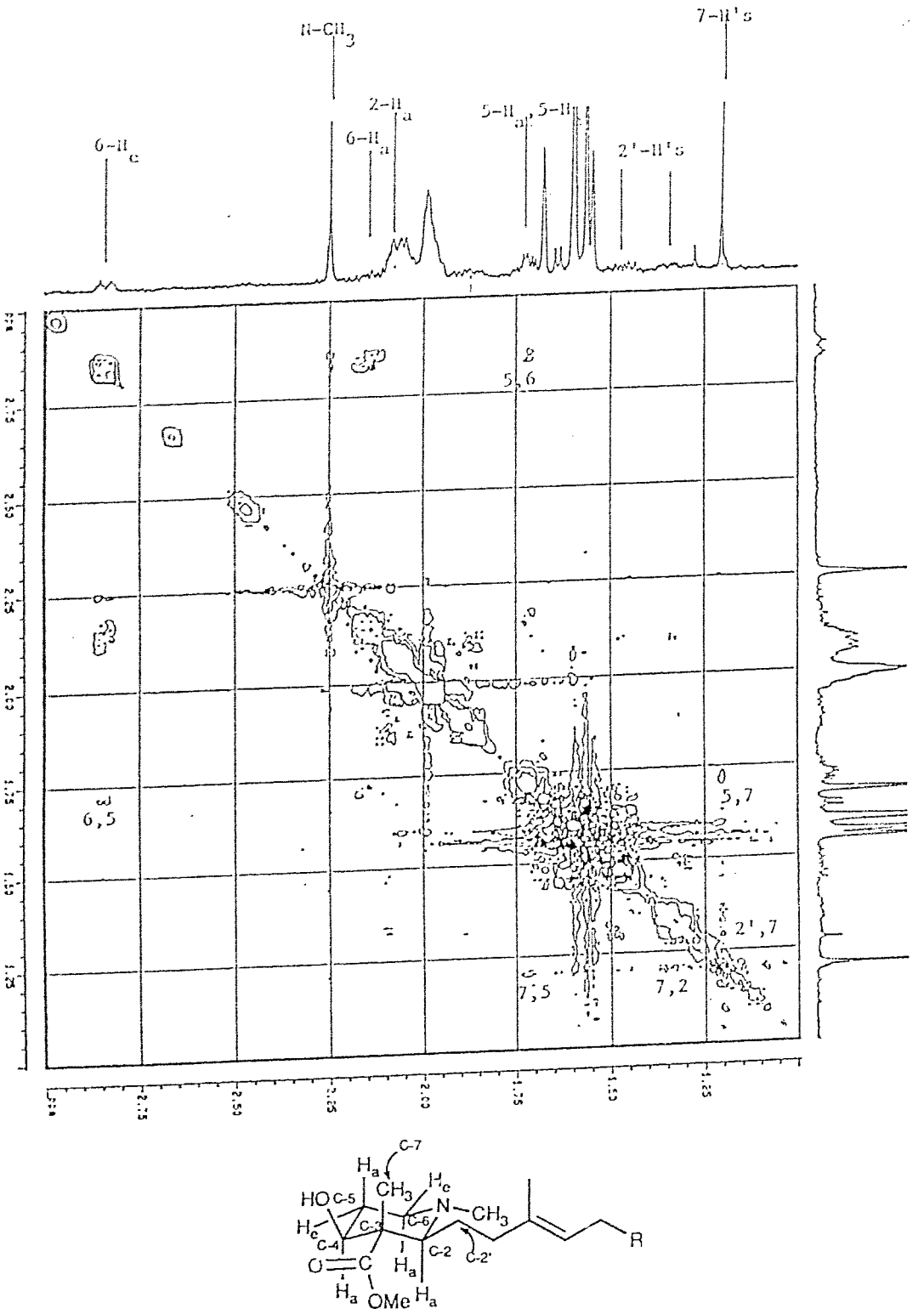
150

Figure 7-3b: 400 MHz COSY spectrum of **150** (expanded region 1.00 ppm to 3.00 ppm).



150

Figure 7-3c: 400 MHz NOESY spectrum of **150.**



150

Figure 7-3d: 400 MHz NOESY spectrum of **150** (expanded region 3.00 ppm to 1.0 ppm).

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